

Vasif Hasirci
Nesrin Hasirci

Fundamentals of Biomaterials



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Vasif Hasirci
BIOMATEN Center of Excellence in
Biomaterials and Tissue Engineering, and
Department of Biological Sciences
Middle East Technical University
Ankara, Turkey

Nesrin Hasirci
BIOMATEN Center of Excellence in
Biomaterials and Tissue Engineering, and
Department of Chemistry
Middle East Technical University
Ankara, Turkey

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Foreword

Biomaterials World

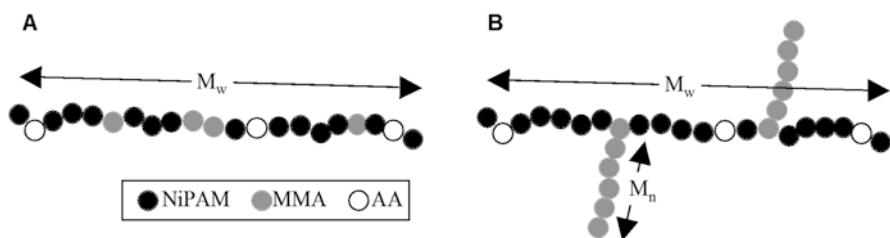
It was probably during occasional accidents or periodic wars that foreign materials found their way into the human body. Imagine the surprise of victims who discovered that it was not the end of “life” to have a foreign object stuck in our bodies and that one probably continued to function reasonably well with a foreign implant. We must have gradually learned to accept or even take advantage of foreign materials in our body. While ancient references are abundant on early use of materials to restore bodily functions, it was the last century that witnessed our scientific mastery of the art of biological replacement using materials, where the word *biomaterials* became common in scientific literature. Early impetus was provided by materials that had no biological basis, namely, metallic implants. To physicians’ amazement, it was possible to implant *structural solids* such as steel into specific body cavities, and it would remain in place, maintain its integrity, and support the function of the body, albeit only mechanically. If such synthetic materials with no biological equivalent can function in a highly dynamic biological milieu, physicians must have gotten excited about the possibilities of developing more realistic, integrating, and biomimetic materials. Today, we find ourselves with an exciting array of materials that *can get the job done* in different parts of our body.

It was not surprising that we originally turned to *metallic implants*, the basis of our modern tools, to support the mechanics of body parts. It was a successful approach since we did not ask metals to do too much, just to be stable and transmit environmental loads at the location we placed them. However, our requirements changed over time; we designed the metals over the years to create more demanding mechanical properties, ones that match the microenvironment in which they are found. Special surfaces were requested so that the interface with tissues is continuous. We are even asking the metals to undergo degradation, that is, to dissolve and diffuse away, not to leave their footprint. However, it did not take long for the inquisitive minds to wonder about alternative and possibly improved biomaterials.

Believing that Mother Nature knows best, we turned our attention to *materials derived from nature*, especially other living organisms. Hoping to catch the *elixir* of living tissues, we implanted living matter to supplement required biological functions. It is exciting to transmit the “substance of life” from one individual to another

in need. Of course, there is no reason to limit ourselves to using the elixir of other humans. If unique architectures and intricate geometries exist in other organisms, such as corals and bamboos, surely we can take advantage of such sophistication without waiting for the painstakingly slow evolutionary process in our bodies. However, it can be laborious to prepare them in such a way that they are acceptable for today's medicine. The undesired components or less than perfect geometries are bound to complicate the desired outcomes. One can still emulate Mother Nature by creating the chemical entities that are the building blocks; why use hard-to-come-by living bone when one can prepare the gist of the bone, arguably hydroxyapatite, and augment the required function using this basic element. Being a mechanically resilient and chemically inert *ceramic* facilitated its easy preparation and propelled hydroxyapatite to the forefront of natural ceramics. Placing the right formulation of hydroxyapatite in a location that requires *de novo* mineralization or integration with existing mineral should provide the necessary guide. Mind you such an approach is bound to miss important clues that may help in repair; the bone after all is not pure hydroxyapatite, and other *carbonaceous macromolecules* such as collagen do contribute to the required physiological functionality. It is possible to improve the materials by supplementing with additional components and creating *composite biomaterials*, but there is a practical limit to our manufacturing capabilities, and it becomes harder and harder to design material with all of the biological components in an optimal configuration.

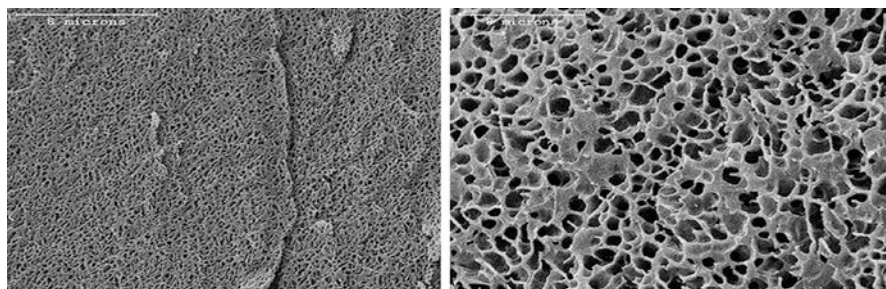
Synthetic *polymeric biomaterials* may provide some relief in this endeavor since they can be tailored at will and their architecture and physicochemical properties can be controlled exquisitely. The possibility of creating endless configurations of macromolecules with today's advanced chemical schemes has propelled polymers to the forefront of biomaterials.



Synthetic materials can be controlled from compositional (a) or architectural (b) perspectives. Here a synthetic polymer with three different building blocks is shown in linear and graft form with controlled molecular sizes

If one aims to develop the ideal substitute for a bodily function, one is tempted to build a living tissue that matches the intended application, with physical features to “fill” the space, biochemical efficiency to undertake the chemistry of life, and regenerative capacity to enable a permanent presence at the site. One has to rely on living cells in this case, and we are obliged to do everything in our power to create a suitable environment to form the *engineered tissue*. Our full arsenal has to be deployed, be it designer materials for scaffolding, biochemical supplements for

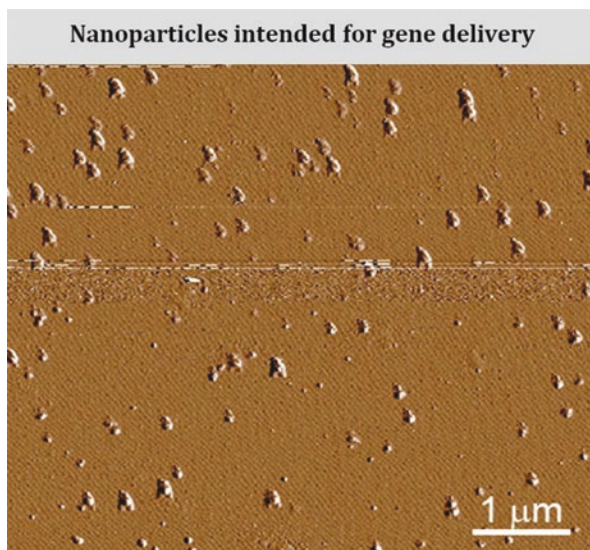
desired cellular physiology, different cell types mimicking the regenerative niche, and even static and dynamic forces to provide a familiar medium to the cells. Employing cells from unnatural origins (such as skin cells for neural tissue) and molecular agents not typically found in our physiological milieu (such as fluorescent proteins) is fair game in this endeavor.



Scaffolds prepared with controlled pore sizes and intended for organoid culture

While engineers are busy developing new materials, biomedical scientists and clinicians are contributing to our understanding of *biomaterial behavior in contact with tissues*. The last five decades have been revealing what happens when materials come in contact with living tissue with the overall goal of defining *biocompatibility*. We have a fairly good grasp of the interaction of macromolecules in bodily fluids with biomaterials, especially *interactions with blood components*, which in turn paved the way for understanding cellular events at the interface of tissues and biomaterials. Revealing the nature of cells at this interface, especially their activation state, and linking the cellular activity to materials chemistry have allowed us to design better materials for medicinal use. The spectrum of material applications has accordingly grown in successive years, and a lively biomedical industry has sprung from the unmet need and fundamental understanding of biomaterial behavior. Almost every organ and tissue, be it *soft* or *hard tissue*, benefited from this endeavor.

Irrespective of the approach one devises, materials have and will become a central pillar in medicine. While the pharmaceutical industry is stubbornly focused on mitigating healthcare problems with soluble molecules (drugs), one can appreciate that not all problems can be solved in this way and that not all drugs can be administered by conventional routes. Biomaterials that can *control the release or delivery of drugs* have provided unique solutions in some diseases. Specialty materials are needed for this purpose, and scientists have recognized this fact and are actively developing new materials on a daily basis. Feedback-controlled delivery systems have been the dream for patients, who would rather allow intelligent systems to control their medication, and biomaterials establishing the foundation of *enzyme and related biosensors* are paving the way for intelligent medicines. *Materials precisely controlled and engineered at nanoscale*, a technical capability we did not have until the last two decades, are particularly becoming critical in today's biomaterials and impacting every aspect of new material development.



Nanoparticles intended for gene delivery

While we strive to understand biomaterials and develop new ones, one should be mindful of two broad issues irrespective of the specific utility of the biomaterials: First is *doing good* (i.e., efficacy), which will give us an opportunity to improve the status quo and restore good health. Biomaterials must provide a clinical benefit to the patient. One should not be afraid to ask our materials to do the impossible, so that a drive for significant improvements in the performance of our biomaterials continually exists. Second is *doing no harm* (i.e., safety). Nature has a way of reminding us of the limitations of our good intentions, and this is no exception when it comes to developing lifesaving biomaterials. Degradable biomaterials that leave no trace of the original biomaterial and release products eliminated harmlessly are the best way to ensure safety. Striving toward efficacious and safe biomaterials is our *ethical* obligation.

University of Alberta
Edmonton, AB, Canada

Hasan Uludağ

Foreword

Ocular Biomechanics: Past, Present, and Future

Biological tissues possess highly complex mechanical behavior. Their resistance to mechanical actions increases with both deformation and deformation rate and with aging and also changes with medical history such as exposure to diabetes and high blood pressure or surgery and injury. The resistance to mechanical actions also depends on the microstructure of the tissue and in particular how the collagen and elastin fibers, which form the main load carrying components of the tissue, are oriented and cross-linked together. This complex picture has made it necessary to test tissue in all possible forms that could arise in physiological conditions and while preserving it in relevant humidity, temperature, and connectivity states. Experience has shown us that deviating from these states, which are expected in vivo, can lead to mechanical properties that do not represent the tissue's true behavior.

Fifteen years ago, and in one of the keynote speeches in the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), it was observed that ocular biomechanics was still in the middle ages. Comparing the few estimates of mechanical resistance to loading (stiffness) of human corneal tissue revealed differences that were as high as 4000%. A common analogy used at the time was a bridge whose deformation under a particular load was somewhere between an inch and a meter. This incredible level of uncertainty was untenable and meant that any effort to design medical devices that interact mechanically with the eye was almost impossible.

A quick analysis of available studies revealed the reasons for this huge difference in property estimates. Tests were conducted in different temperatures, under different deformation rates (or strain rates), and using tissues obtained from different parts of the cornea. More importantly, while some tests used tissue strips surgically removed from corneal buttons, others used whole corneal buttons or even whole eye globes inflated with internal fluid pressure simulating the natural intraocular pressure, to which in vivo eyes are subjected. These tests showed that most alterations of test conditions, away from what is experienced in vivo, lead to significant variations in mechanical properties and negatively affect the reliability of results.

This realization resulted in concerted efforts to obtain the behavior of ocular tissue while simulating the in vivo conditions as much as possible. However, this drive

has had its own serious challenges. Ideally, ocular tissue should be tested intact, with no surgical interference, supported in a way that simulates the soft tissue surrounding the eye, and kept in the temperature and humidity expected in vivo. This aim has been quite difficult to achieve with challenges in experimental setup and subsequent analysis. These challenges have meant that the experimental studies on ocular tissue conducted over the past 15 years have been repeated over and over again, every time with some improvements in the test methods in never-ending attempts to approach in vivo conditions and hence improve the reliability of results.

A further major development has been the realization that the collagen fibers of ocular tissue played a major role in the mechanical behavior of the tissue and should therefore be used to guide or control the distribution of mechanical stiffness and anisotropy across the ocular globe. Initially, attention was given to the cornea for its importance in the planning of refractive surgery, corneal implants, and design of contact lenses and to the posterior sclera, the main site of damage in glaucoma. Later, attention expanded to cover the whole cornea, limbus, and sclera. X-ray scattering quickly became the method of choice because of its accuracy and reliability, and a number of recent studies have used it successfully to characterize the microstructure—in particular the collagen fiber density and anisotropy—across the whole ocular globe in healthy and highly myopic eyes. This information has enabled for the first time a high level of control of the regional variation of mechanical properties over the outer tunic of the eye.

With a better understanding of the tissue's microstructure and the new methods to experimentally test intact eye globes, it has become possible to produce material properties representing the regional variation of stiffness and anisotropy, the nonlinear hyperelastic behavior of the tissue, and its hysteresis and strain rate dependency. The ability to embed these properties in numerical models of the eye has been a major challenge but has allowed reaching an unprecedented level of accuracy in predictions. Numerical models can now represent refractive surgery procedures (such as LASIK, SMILE, and PRK) and noncontact tonometry, leading to highly accurate predictions of behavior and paving the way for future planning tools. Other future steps will likely include expansion of the test program to include unhealthy eyes—including those with diabetes, keratoconus, and long-term exposure to glaucoma—and consideration of cases where the microstructure of the eye has been changed in eyes undergoing surgery.

This last aspect is particularly challenging as it requires changing the microstructure of the eye in consideration of the effects of surgical cuts. This work relies on experimental evidence showing that collagen fibers change orientation with changes in strain distribution, which are caused by surgical incisions. The ability to represent these changes in the microstructure during the simulation of surgery will, for the first time, enable modeling the changes taking place in the behavior of the eye following surgery. A logic development of this work will be the conversion of predictive tools—based on numerical analysis—into planning tools that could be used in clinical practice.

With these developments, it is expected that ocular biomechanics will move to the forefront of healthcare in ophthalmology, enabling the customization of

treatments for individual patients and creating much needed planning tools to assist with improving the outcome of surgeries, corneal implants, and intraocular pressure measurement using tonometry.

University of Liverpool
Liverpool, UK

Ahmed Elsheikh

Preface

Starting from the First World Biomaterials Conference held in 1980 in Baden near Vienna, we were involved in the development of the biomaterials field. We carried out research and gave courses on this new expanding interdisciplinary science soon after this meeting. In 2016, we attended the Tenth World Biomaterials Conference in Montreal, Canada, which was strikingly larger, more colorful, and with very different research topics developed over the years.

Several years ago, in one of the European Society for Biomaterials meetings, during a talk with the representative of Springer, a discussion took place about writing a book to teach undergraduate and graduate students of this field using a material that is uniform in its flow, language, and depth since there was a need for a book to recommend to our students and follow during a one-term course. Springer liked the idea, because they apparently felt that there was a need for a book not edited, not written by many authors who contributed a chapter. Since then we started putting our course material into the form of a book. It progressed at a slow pace because without sabbatical leaves and with very time-consuming academic activities of teaching, research, and supervision of students, not much time was left to write the book.

When writing this book, we targeted graduate or senior undergraduate students who have completed or about to complete their formal degree studies, interested in the medical applications field but the edited books and journals were too detailed for them to make an entry to the field. So, we planned the book to give the students from biological sciences, chemistry, materials engineering, medicine, and other similar disciplines the fundamentals of the biomedical materials field so that in the following semesters and years these students would have a common background to read and study together.

During this time, the biomaterials field in the world advanced significantly. We now have tissue engineering, regenerative medicine, microtissues, tissue models, microfluidic systems, nanomaterials, intelligent systems, and patient-specific implants produced by 3D printing, which were also introduced in this book.

During the years of the writing process, some of our students provided significant help in drawing figures, finding references, materials, images, copyrights, and so many things we did not think that we would be dealing with. We especially thank G. Bahcecioglu, T. Endogan, D. Sezlev, and S. Alagoz for their contributions in the

earlier phases, and M. Ermis Sen and D. Tamay for their significant contributions during the final drawing, editing, and proofing stages of the book.

We are grateful to our friends Prof. Hasan Uludag for reviewing the text, and both him and Prof. Ahmed Elsheikh for the vignettes which give different perspectives of the field.

We hope this small drop in the large pool of biomaterials will do its contribution to those interested in the field because it actually will be an important step on the road to improving human health.

Ankara, Turkey
October 2018

Vasif Hasirci
Nesrin Hasirci

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1.1 Vitruvian Man

Marcus Vitruvius Pollio was a Roman architect and engineer of the first century BC. Vitruvius is one of the first people who looked at the human body as an object that has dimensions and proportions and regarded it as a symbol of harmony that he observed between the various human body parts. The Vitruvian Man diagram which Leonardo da Vinci has drawn (Fig. 1.1) was inspired by Marcus Vitruvius and has now come to symbolize the biomedical field maybe because the biomaterials scientists and engineers are trying to create a harmony between the natural organs and tissues and their artificial counterparts that we prepare in the lab.

1.2 The Need for Biomaterials and Biomedical Devices

Human tissues and organs fail to perform ideally due to genetic makeup, age, sickness, or accidents. Some of these disorders are treated by the use of bioactive agents called drugs. Others, however, could not be rectified by provision of drugs and require the use of materials and devices. For example, when a patient has diabetes type I, administration of insulin can be used to control the blood glucose level, but in the case of a malfunctioning kidney, the abnormal blood urea levels cannot be controlled by drugs. In this latter case, the problem is solved by transplanting a kidney from a healthy donor of matching tissue type, or in the absence of a transplantable organ, the kidney is supported through artificial means, for example, by routine dialysis of the patient's blood in a dialysis machine. If a dialysis machine is used, the removal of the excess water, metabolic waste, and toxic compounds in the blood is achieved by filtration of the blood against counter current flow of a basically salt solution called the dialysate (contains Na^+ , K^+ , Ca^{+2} , Mg^{+2} , acetate ion, colloidal iron, glucose). The goal here is to remove the waste products while retaining all the essential constituents of the blood. Thus, the dialysis machine used in this example is a biomedical device constructed of biomaterials.

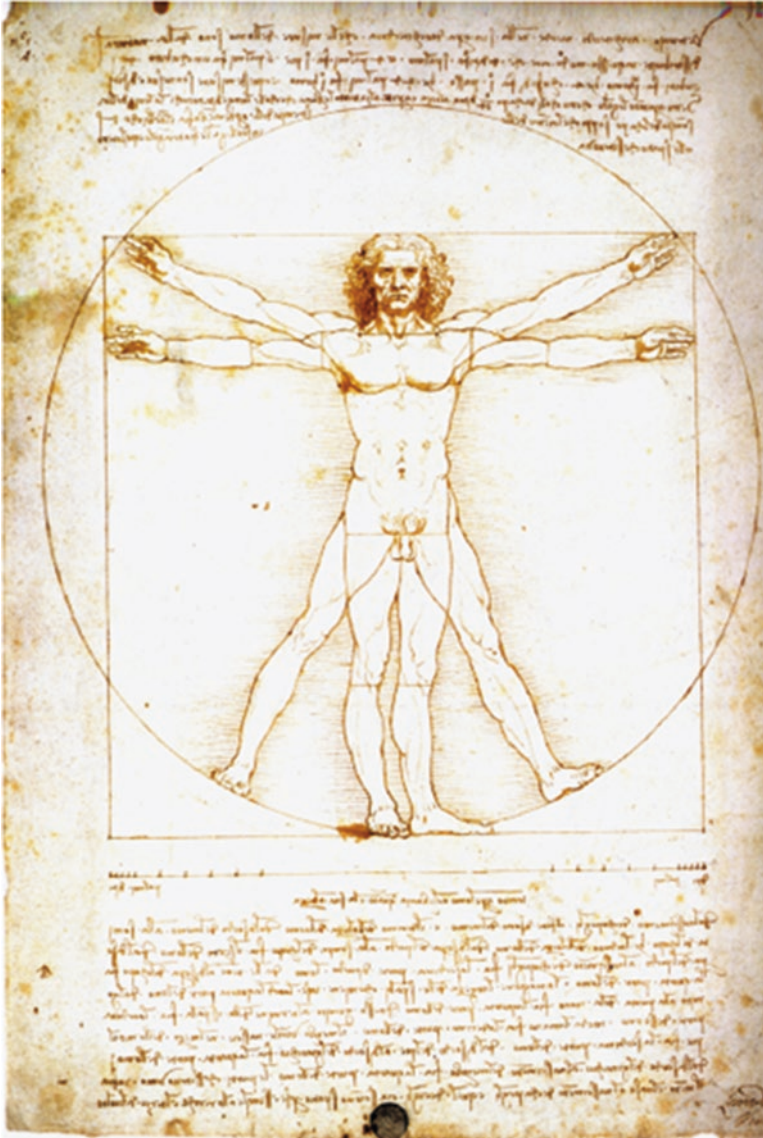


Fig. 1.1 Vitruvian man, drawn by Leonardo da Vinci and inspired by Marcus Pollio [1]

Human kind has strived since the early ages to live a longer and healthier life and searched for materials and tools to achieve these goals. He either found the tools or some models in nature, or he developed or modified them further to be able to use them for his own benefit (Fig. 1.2). This is how the scientific and technological discoveries are made. In our age, detection of diseases became easier with the

Fig. 1.2 A prosthetic leg [2]



development of diagnostic kits, faster and more effective treatment can be achieved with the use of novel drugs, damaged or injured tissues can be repaired with the use of new materials and technologies, and as a result longer and healthier living conditions are attained.

Living things are constituted of billions of cells that come together to create a very complex organism. The bodily functions are sustained as a result of the billions of simultaneous and/or consecutive reactions which take place in harmony, triggering one another, and in unison with all the organs and systems.

We do not pay special attention to what is happening in our body during a normal day. We do not know when the digestive process in the stomach ends; we do not voluntarily control the rhythm of our heart beat, our vision, or our hearing. The organs and tissues which work so hard are also very sensitive, delicate, fragile, and susceptible to be damaged if they are not properly cared for.

When we are hurt, or when our system does not function properly due to disease, a variety of treatment approaches are applied to remedy the situation. The dysfunctional organ could be treated by introduction of some materials into the body such as tooth fillings used in the treatment of tooth cavities, transplantation of healthy tissues as autografts from the patient's own body, or allografts from another human donor or xenografts from another mammal such as in the case of kidney transplants. On the other hand, the damaged tissues could be supported by high technology products and biocompatible materials such as the hip joints, heart valves, and stents developed in the lab by researchers (Fig. 1.3).

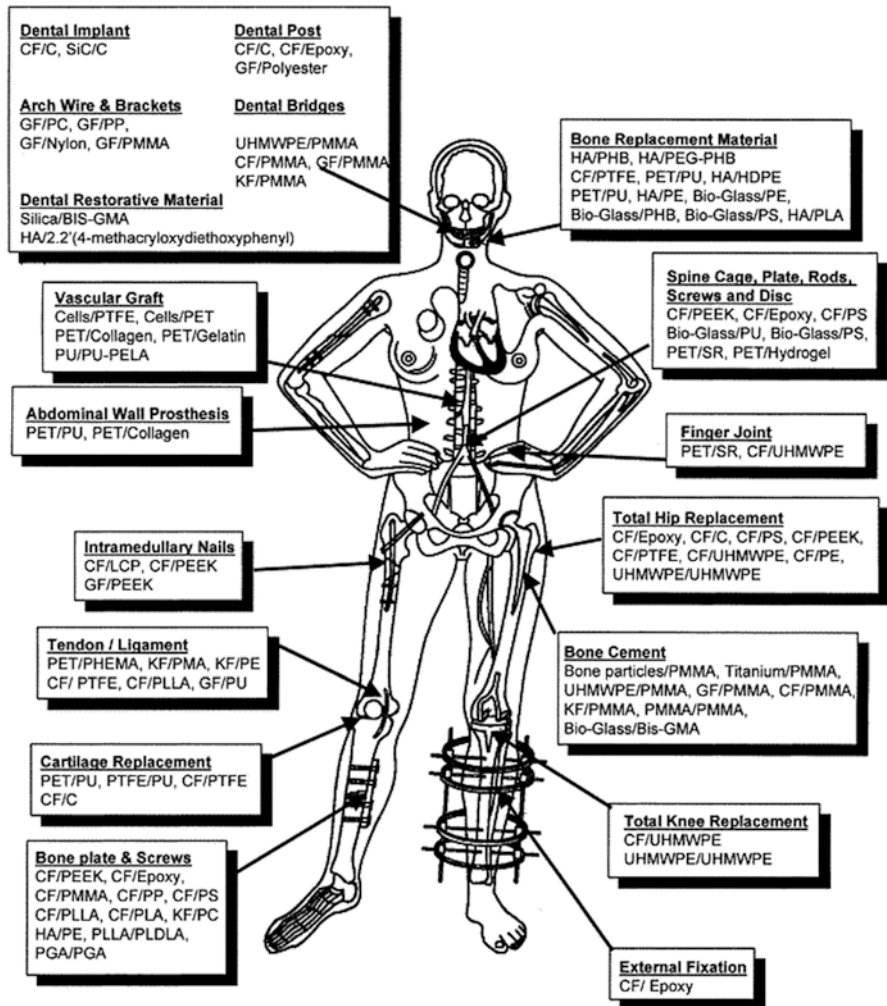


Fig. 1.3 Some biomaterial applications [3]

1.3 Historical Development of Biomaterials

In the broad sense of the word, natural or synthetic materials used to support or completely take over the function of the nonfunctional tissues in the human body are called biomaterials. The complete or partial replacement of damaged or diseased organs and tissues have improved the quality of life and prolonged the average life expectancy, and this further increased the interest in the biomaterials field.

Even though the biomaterials discipline is a new interdisciplinary field, its applications date back to thousands of years BC. The glass eyes, metal noses, and ivory teeth discovered on the Egyptian mummies are good examples of this. The



Fig. 1.4 Tlailotlacan woman from ancient Teotihuacan with dental implants [4]

legendary, iron-based hooks and wooden legs of the pirates are also well-known examples. The skull of the Tlailotlacan woman from ancient Teotihuacan (Mexico) who was between 35 and 40 years old when she died about 1600 years ago carried dental implants in the form of encrusted mineral stones as substitute teeth (Fig 1.4).

Similarly, the bronze and copper bone substitutes dating to years BC and designed to be implanted in the human body should also be classified as biomaterials. Especially the implants made of copper were used until mid-nineteenth century due to the lack of better materials. Copper was eventually replaced by stainless steel. The use of gold in dentistry dates back to 2000 years ago. Hippocrates mentions in his writings about gold wires being used as sutures to sew tissues together. The devices prepared from cadaver bones and ivory around 1880s for use in orthopedics also classify as biomaterials.

In the old ages, damaged tissues and organs would quickly become infected and gangrenous and were surgically removed. The rate of success in these surgical procedures was quite low due to lack of sterilization. Around the 1870s Joseph Lister showed the importance of sterilization, and after its use in the operating theaters, the surgical procedures became more successful. Historical treatments dating before sterilization are depicted in the following figure (Fig. 1.5).

1.4 Some Firsts in the Biomaterial Field

Biomaterials have been in use since the dawn of history without being called that. Among the many examples, the iron dental implant found in 200 AD can be considered as a recent one (Nature, Feb 1998). One of the most exciting observations that



Fig. 1.5 Historical examinations and treatments [5, 6]

eventually led to the birth of a completely new field was that of Perspex (PMMA) splinters or shards in the eyes of World War II gunmen who were located in the bubble turrets of B17 bomber planes. Dr. Ridley, a surgeon, noticed that these splinters were inert. He then bought a sheet of Imperial Chemical Industries (ICI) Perspex and machined it in the form of lenses and started the intraocular lens (IOL) industry. These Perspex lenses were used on cataract patients whose natural lenses had become occluded with age and thus lost their transparency impairing their vision.

On a similar line, Sir John Charnley designed the first total hip implant with ball headed femoral stem and cups of Teflon. They were a disaster. When he replaced the polymer with ultrahigh molecular weight polyethylene (UHMWPE), the results were so good that his hip implant was reportedly as good as any modern hip in terms of clinical life. They lasted for 10–15 years. At the end of World War II, another medical inventor, Dr. Wilhem Kolff, took sausage casings made of cellulose, hooked them up to a washing machine, and dialyzed the blood of kidney patients saving them up to a certain death. In time the properties that were required of a biomaterial were clearly identified. Table 1.1 shows the development of biomaterials use in time.

As can be seen in Table 1.1, the earlier biomaterials used were metals, especially those which were to be noncorrosive such as gold and platinum. The risks of infection were very high due to the absence of any awareness of sterilization. Sterilization improved the success rate of the treatments immensely. Developments in the kind of materials available and use of stainless steel and alloys increased the success rates further. It was not until the 1940s that the polymeric implants started to be used. Controlled polymerization of certain chemicals and advances in the field made the use of rubber elastic and glassy polymers more plausible. With the use of polymers in the form of fiber medical grade textiles, vascular grafts became possible. Rubber elastic polymers were developed in addition to natural rubber with controlled properties, and blood interfacing elastic materials became possible. Bone cements are basically rigid polymers formed in situ, and are used in the stabilization of implants such as total hip implants. Composites, especially carbon-based materials, revolutionized the field with the inertness of the products which is very important for blood-interfacing applications. The total implantable heart of the 1980s was made possible by using a variety of materials: polycarbonate (polymer) casing, pyrolytic

Table 1.1 A historical look at biomaterials

Year	Application	Inventor
600 BC	Nasal reconstruction	Shushruta
Eighteenth to nineteenth century	Use of iron, gold, silver, and platinum wires and nails in the stabilization of bone fractures	
1860–1870	Use of sterilization in surgical procedures	J. Lister
1893–1912	Use of stainless steel nails and plates in fracture fixation	W. A. Lane
1912	Development of corrosion resistant Vanadium stainless steel alloys for medical applications	W. D. Sherman
1926	Use of screws in the repair of femur neck fracture	E. W. Hey-Groves
1926	Preparation of molybdenum-containing noncorrosive alloys	M. Z. Large
1931	Design of metal nails for use in the fractures of femur necks	M. N. Smith-Petersen
1936	Development of Vitallium stainless steel alloys	C. S. Venable W. G. Stuck
1938	First total hip prosthesis	P. Wiles
1940	Use of acrylics as cornea substitutes	M. J. Dorzee A. Franceschetti
1944	Development of hemodialysis systems	W. J. Kolff
1946	Use of polymers with appropriate mechanical properties in hip prostheses	J. Judet R. Judet
1952	Vascular grafts with textile materials	A. B. Voorhees, A. Jaretzta, A. H. Blackmore
1953	Application of intravascular balloons	A. Kantrowitz
1958	Use of acrylic bone cement in total hip prosthesis	J. Charnley
1958	First successful heart stimulation	S. Furman G. Robinson
1960	Heart valve application	A. Starr M. I. Edwards
1980s	Artificial heart application	W. J. Kolff et al.
1980s	Computer controlled devices, electrodes, intelligent materials	
1990s	Tissue engineering, development of artificial tissues and organs	
2000s	Nanobiomaterials	

carbon heart valve, stainless steel rims of the valves, Dacron cuffs of the valves, and hexyne rubber (polymer) for the pneumatic system to empty the ventricles. The 1990s saw the advent of tissue engineering which employed biodegradable materials and cells (stem, mature, autologous, allogenic). The recent decade has seen the developments in surface treatments, drug delivery, and imaging through the use of nano-sized biomaterials and nanotechnological approaches previously used in the electronics industry.

1.5 Definition of Biomaterials

What is expected of biomedical materials changed in time mainly because of the developments in chemistry, materials science and engineering, and biological sciences, and these changes were reflected in their definition. One of the earliest formal definitions of the word *biomaterials* was “a systemically and pharmacologically inert substance designed for implantation within or incorporation with living systems” and was coined by the Clemson University Advisory Board for Biomaterials in 1976. This definition, however, did not take into account the more recent bioactive agents or biological entities (cells, cell fragments, proteins, nucleic acids, hormones, growth factors, or drugs) carried by biomaterials or biodegradable systems. In time, several other definitions were proposed.

The European Society for Biomaterials (ESB) had two consensus conferences; in the ESB Consensus Conference I (1999), biomaterial was defined as a “non-viable material used in a medical device, intended to interact with biological systems.” It was later refined in 2005 at the Consensus Conference II as “material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body.”

These definitions need to be modified as follows to compensate for the missing aspects: “Biomaterials are substances implanted within or used in conjunction with the body, designed to have properties closely matching that of the biological system, be stable enough for the aimed use, have appropriate levels of bioactivity and are designed to partially or completely fulfill the functions of the diseased, damaged or malfunctioning tissues and organs.”

What the most recent conference on “Definitions in Biomaterials” which convened in Chengdu (China) during 11–12 June 2018 will bring to definitions should be closely followed through the newest update on this matter.

1.6 Properties of Biomaterials

Biomaterials due to their intended use in a very complex environment need to fulfill various requirements. The most important and general ones are that they should:

1. Be biocompatible (nontoxic, non-carcinogenic, non-allergenic, etc.)
2. Have physical properties (e.g., density, form, porosity, surface roughness topography) comparable to those of the tissue it replaces or is implanted in
3. Have appropriate mechanical properties (compressive, tensile, shear, impact)
4. Have appropriate service lives (stable for life or degrade within a matter of days or weeks depending on the goal)
5. Have chemical properties similar to that of tissues (e.g., hydrophilic or hydrophobic, have similar functional groups)
6. Be processable and sterilizable without difficulty
7. Have appropriate bioactivity (mostly inert, but could have induction or conduction activities or carry bioactive agents if needed)
8. Be economical and available

1.7 Biomaterial Sources

The biomaterials used in solving human health problems are derived from a number of sources. These are (1) natural materials, (2) synthetic polymers, (3) metals, (4) ceramics, and (5) composites. The following table summarizes some of the properties and uses of biomaterials (Table 1.2).

The sources of the biomaterials are presented in Table 1.2. These are natural materials, synthetic polymers, ceramics, metals, and composites. Natural materials can be considered as biological polymers and also decellularized tissues. The major difference is that decellularized tissues already have the geometry and texture of the original tissue, whereas the biopolymers are solids which has to be processed into the form needed for the application. The biopolymers presented as examples in the table are of plant (cellulose), animal (collagen, hyaluronic acid, chondroitin sulfate), insect (silk), microorganism (polyesters), crustacean (chitosan is a derivative of chitin), and algae (alginate) origin. Since they all are made by organisms, enzymes and most of the time templates are involved in their production. Thus, their properties are generally highly controlled. They, however, have a major disadvantage: there are also enzymes that can hydrolyze and degrade them. As a result, they all are degradable in the biological system, or in other words, they are biodegradable. Their sources are abundant and generally quite inexpensive. Since they are polymeric materials, they are not highly crystalline, and as a result not strong enough for most load-bearing applications. One major advantage is that their chemistry and mechanical properties are very similar to those of the tissues and therefore quite compatible with the biological system. Important applications of those are soft tissue replacements, including tissue engineering, wound dressings, and cartilage substitutes.

The synthetic polymers are, like the biological ones, not highly crystalline, and therefore, they do not have high strength. Some, however, like ultrahigh molecular weight polyethylene (UHMWPE) or poly(L-lactide), can crystallize significantly (35–55% for UHMWPE [7] and 55% for PLLA [8]) and have significant strength. Although there are hydrophilic ones, in general most of the synthetic polymers are hydrophobic (e.g., PMMA, PVC, Teflon, Dacron, PE), and therefore their properties are not similar to biological tissues and biopolymers. As a result, their interaction with the tissues and tissue growth on or in these biomaterials is limited. Also as a result of their hydrophobicity and chemistry, most are not degradable. In the last decades, some degradable polymers were synthesized and used especially in the production of scaffolds for tissue engineering applications. Polymeric materials whether biological or synthetic can be processed into complex shapes under mild processing conditions, and this gives them a great advantage over other biomaterials. When not cross-linked (not as a network) polymers deform under load, they cannot resist abrasion or shear forces. When cross-linked, however, they become elastic as natural rubber, and this makes them suitable for use in applications where cyclic or continuous flexing is required. Very high cross-link density makes them as tough as metals.

Ceramics are a completely different category of materials. They do not chemically degrade, corrode, or conduct heat or electricity. They are inert, hard and brittle,

Table 1.2 Types and sources classification of biomaterials and their comparison

Material type	Examples	Advantages	Disadvantages	Applications
Natural materials (biopolymers)	Cellulose, collagen, hyaluronic acid, chondroitin sulfate, chitosan, microbial polyesters, silk, alginate	Abundance, low cost, mechanical properties similar to tissues	Degradable, evoke immune responses, cannot resist high processing temperatures and char or denature	Tissue engineering, wound dressings, sutures, artificial skin, fluid for vitreous humor for cataract surgery and for cartilage defects
Synthetic polymers	Polymethyl methacrylate (PMMA), polyvinylchloride (PVC), Teflon, Dacron, Nylon, poly(L-lactide) (PLLA), polyhydroxybutyrate-valerate (PHBV), polydimethylsiloxane (PDMS), polyurethanes, polyethylene (PE), polypropylene (PP), polytetrafluoroethylene (PTFE)	Degradable, no corrosion, density similar to soft tissues, ease of processing, ability to form complex shapes, malleable	Degradable, low strength, flows under stress and sometimes with high temperature, low resistance to impact, low resistance to wear	Soft tissue implants, drug delivery systems, contact lenses, bone plates, bone cement, dental fillings, tissue engineering
Ceramics	Aluminum oxide, titanium dioxide, hydroxyapatite	Inert, mimic the biological inorganic tissues, high compressive strength	High density, brittle, difficult to produce reproducibly	Hard tissue implants used for high compression sites (tooth crowns, femoral heads)
Metals	Stainless steel, titanium, cobalt-chromium alloys	High tensile and compressive strength, high resistance to wear and impact	Corrosion, high density, difficulty in processing, complex shapes, release ions in the biological fluid	Bone plates, screws, pins, staples, joints
Composites	Metal-ceramic, polymer-ceramic, metal-polymer	Novel properties	Vary with specific composite	Hip implants, carbon fiber reinforced ligaments, tendons, screws, pins and plates

strong under compressive forces, and have high density. With these properties, they are more similar to hard tissues like bone and teeth in the body. Their processing conditions are not mild, and their large-scale production or formation of complex shapes is not easy. Tooth crowns are ideal sites for their use because of their being subjected to compressive loads during chewing and sudden changes of temperature experienced during eating.

Metals, unlike the earlier materials (polymers and ceramics) discussed so far, have no direct counterpart in the body. However, there is a great need for them as the main load bearers because metals have the capacity to withstand tensile, compressive, shear stresses, and impact. No other biomaterial type can do that. Metals, however, are highly reactive and prone to oxidation, such as corrosion. They also release ions from their grain boundaries, and this might have undesirable consequences such as toxicity. Oxidation might lead to a highly tenacious oxide film like in the case of titanium oxide layer forming on titanium and, therefore, acts as a passivation layer which stops corrosion and release of ions. This is why titanium is preferred as a surface material rather than being used in the bulk. Metals are crystalline and have metallic bonds; these make them good conductors of heat and electricity. Metals are therefore useful as signal-conducting materials in implanted sensors and pacemakers but are not good as surfaces in dental implants where the material is subjected to variations in temperature. Due to the pliability of the metals, they are often used as wires or staples to close wounds and stabilize bone fractures in addition to carrying load.

Composites are combinations of two or more materials which form an integrated structure combining the properties of its components to produce a much improved product. Properties of composites are difficult to generalize because there are so many different combinations of materials to make composites. Heart valves of pyrolytic carbon-coated graphite and carbon fiber-reinforced bone plates or tendons and hydroxyapatite-coated implants are some examples. In the case of the heart valve, the graphite core has low density to make the valve light, and the pyrolytic carbon is glassy, hard, and inert. The composite valve is therefore both inert in the very difficult environment of the cardiovascular system where the main problem being blood clotting and blood element adsorption on the valve, and also light enough to be moved with the physiological pressure applied on the valve during opening and closing of the passages. Hydroxyapatite-coated stainless steel, titanium, or cobalt-chromium alloy implants are strong due to the presence of the metal, but attachment of the bone or bone cement onto the implant is significantly improved when the surface is coated with a material that has a composition similar to that of the mineral component of the bone, calcium phosphate.

With the developments in chemistry (especially in polymer chemistry and organic chemistry), materials engineering, cell and molecular biology, and medicine, biomaterial use increased rapidly over the years, and today it is a multibillion-dollar business. An estimate of the total US Healthcare Market was larger than \$1 trillion in 1998. The US market for biocompatible materials was valued at \$ 22.2 billion in 2007 and was projected to grow with a compound annual growth rate of 6.9% to reach \$30.9 billion in 2012 [9]. Tissue replacements constituted the largest

Table 1.3 Certain biomedical devices, materials, and amounts used

Device	Biomaterial	Implants per year USA
Intraocular lenses (IOL), contact lenses	PMMA, silicone	2.0 million (2018)
Hip and knee prostheses	Titanium, stainless steel, high density polyethylene (HDPE)	860,080 (2017)
Vascular grafts	Teflon (polytetrafluoroethylene, PTFE), Dacron (polyethylene terephthalate, PET)	400,000 (2016)
Heart valves	Pyrolytic carbon, graphite, reconstituted tissue	182,000 (2018)
Catheters	Silicone, PVC, PEU, Teflon	Millions
Percutaneous devices	Titanium, silicone, PVC	>25,000
Stimulatory electrodes	Platinum, iridium, gold	>25,000

segment with a value of nearly \$11.7 billion in 2007. This represented 52% of the total biomaterials market value. The tissue replacements were projected to grow at a rate of 6.6% to reach over \$16 billion in 2012 [10]. The number of implants introduced per year in the USA show the size of the market (Table 1.3).

The use of ophthalmologic devices appear to be the most frequent application. Contact lenses are not implanted but daily removed by the user, whereas the intraocular lenses which are mostly applied to cataract patients are implanted to replace the natural lens of the eye (Table 1.3). With the aging population, the need for hip and knee prostheses keeps increasing in the elderly population due to arthritis and other causes, and they are implanted at a rate of more than 200,000 per year. The young population also needs these implants as a result of damages caused by traffic and sports injuries. Cardiovascular diseases affect a large fraction of the population, and among the biomaterials used vascular grafts and heart valves constitute an important segment. Even though there are autograft possibilities for the vascular grafts and xenografts (mainly of porcine origin) for the heart valves, biomaterials developed as alternatives function successfully. The most used among all is the catheters which are employed for short periods but for many different fluid transfer applications that their numbers run into millions. The budget involved in the use of these implants is on the order of billions of US dollars.

1.8 Biocompatibility

Biocompatibility is one of the most crucial properties that a biomaterial or a medical device should possess. It was defined by Williams (1999) at the ESB Consensus Conference I as “the ability of a material to perform with an appropriate host response in a specific application.” American Society for Testing and Materials (ASTM) defined it as a “comparison of the tissue response produced through the close association of the implanted candidate material to its implant site within the host animal to that tissue response recognized and established as suitable with control materials” [11].

Table 1.4 Tests for biological evaluation of medical devices (ISO 10993)

ISO codes of biocompatibility tests	
ISO 10993-1:2009	Evaluation and testing
ISO 10993-2:2006	Animal welfare requirements
ISO 10993-3:2003	Tests for genotoxicity, carcinogenicity, and reproductive toxicity
ISO 10993-4:2002	Selection of tests for interactions with blood
ISO 10993-5:2009	Tests for in vitro cytotoxicity
ISO 10993-6:2007	Tests for local effects after implantation
ISO 10993-7:2008	Ethylene oxide sterilization residuals
ISO 10993-8:2001	Selection and qualification of reference materials for biological tests
ISO 10993-9:1999	Framework for identification and quantification of potential degradation products
ISO 10993-10:2010	Tests for irritation and delayed-type hypersensitivity
ISO 10993-11:2006	Tests for systemic toxicity
ISO 10993-12:2012	Sample preparation and reference materials
ISO 10993-13:1998	Identification and quantification of degradation products from polymeric medical devices
ISO 10993-14:2001	Identification and quantification of degradation products from ceramics
ISO 10993-15:2000	Identification and quantification of degradation products from metals and alloys
ISO 10993-16:1997	Toxico-kinetic study design for degradation products and leachables
ISO 10993-17:2002	Establishment of allowable limits for leachable substances
ISO 10993-18:2005	Chemical characterization of materials
ISO 10993-19:2006	Physicochemical, morphological, and topographical characterization of materials
ISO 10993-20:2006	Principles and methods for immunotoxicological testing of medical devices

In practice, biocompatibility necessitates that a biomaterial does not induce an adverse reaction in the body, but it does not need to be inert; it should cause a suitable response if necessary. For example, a bone implant is expected to function without inciting a negative response from the bone. However, most bone plates lead to a depression in the bone tissue onto which they are fixed. This is obviously not biocompatible even though the implant does not incite any carcinogenic, allergic, or immune responses. A PMMA contact lens which does not transmit sufficient oxygen and causes hypoxia in the corneal tissue under the lens is not completely biocompatible, however, a poly(hydroxyethyl methacrylate) (PHEMA) contact lens that allows plenty of oxygen is more biocompatible. In order to test the biocompatibility of a material, a complex series of tests involving in situ, in vitro, in vivo, and clinical testing are carried out according to standards defined by “The International Organization for Standardization (ISO) for Biological Evaluation of Medical Devices” (ISO 10993) (Table 1.4). The selection of the test types and other conditions require some expertise in the field in order not to perform excessive numbers of tests spending large sums. Details of biocompatibility testing are covered in Chap. 11.

1.9 Conclusion

In this chapter, we covered the materials used in medical applications; the history and progress of prostheses and biomaterial usage; the properties, advantages, and disadvantages of different materials; and finally, the market value of some medical devices.

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2.1 General Properties

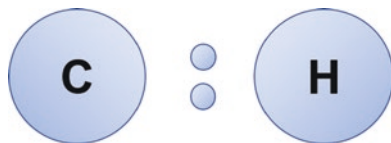
Properties of the solids are very important for the biomaterials field because all biomaterials including metals, ceramics, and the very soft ones like hydrogels used in contact lenses, cell printing, or tissue engineering applications are solids. Soft biomaterials also need to be studied like the less hydrated and much harder biomaterials such as pyrolytic carbon used in the heart valves or hydroxyapatite of bone-like implants.

2.2 Basic Bonding Types

Materials used in the biomaterials field are basically solids. As a consequence of this, properties of solid materials need to be known in detail. The major factor which defines properties of these materials is the type of bonding which holds them together. Their polarity, solubility, degradability, density, mechanical properties, etc. all depend on the type of bonding that form the solid material.

2.2.1 Covalent Bond

Covalent bonding differs from ionic bonding in that the electrons forming the bond are almost equally shared between two atoms. Covalent bonds are observed in non-metals. Atoms forming covalent bonds have vacancies in their valence energy levels, and upon bonding, they share and, therefore, gain electrons and achieve stable configuration. Sharing produces low-energy (stable) electron arrangements due to full outer shells like those of the inert elements as is in hydrogen (Fig. 2.1).

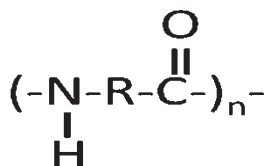
Fig. 2.1 Covalent bond

Due to the sharing of the electrons and localization of electrons being in between the atoms bonded, covalent molecules have the following properties:

- Electrons are tightly bound to atoms and shared by atoms.
- They do not conduct electricity and heat.
- Their molecules are not charged.
- Their melting and boiling points are low.

For example, polyethylene which is a polymer extensively used in the biomaterials field, has covalently bonded carbon atoms ($-C-C-$) in its backbone and consists of thousands of $-CH_2-$ groups bonded to each other. Polyethylene is not known to be a good conductor of electricity or heat, and it does not have any charged or functional groups to react with other molecules. As a result, it is inert.

Most biological molecules have backbones of covalent linkages. For example, proteins (polypeptides) have the following polyamide chemistry where the $N-H$, $N-C$, $C-H$, $C=O$, etc. bonds are all covalent bonds:

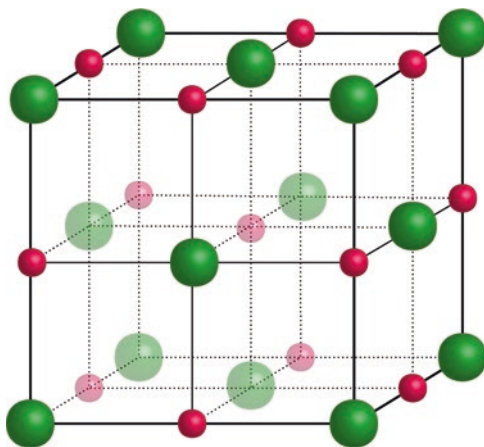


Same thing is true for other biological polymers such as polysaccharides and polynucleotides.

2.2.2 Ionic Bonds

Atoms of a metal can easily give up their outer shell valence electrons to nonmetallic atoms. In doing so, both elements gain inert gas configuration and therefore become stable. Upon donating or receiving electrons, they become ions. A typical example is the alkali halide sodium chloride ($NaCl$). Sodium has an atomic number of 11 and when it donates an electron it becomes a positively charged ion (a cation) which has 10 electrons like the inert gas neon. Chloride, on the other hand, receives an electron and becomes negatively charged (an anion) with the electronic configuration of inert gas, argon. Thus, two constituents having different charges with inert gas configuration electrostatically attract each other and form a stable crystal structure. In ionic compounds, unlike covalent bonding, the electrons are not equally shared between the bonding atoms but are transferred from one to the other.

Fig. 2.2 Ionic bonded positively and negatively charged atoms occupy alternate positions on the crystal lattice



Ionic bonding energies range between 600 and 1500 kJ mol⁻¹ are relatively large and therefore the melting temperatures of ionic compounds are high.

Model of an ionic crystal that has a face-centered cubic (fcc) organization are presented in Fig. 2.2. Typical examples of fcc are sodium chloride (NaCl), potassium chloride (KCl), silver bromide (AgBr), potassium bromide (KBr), lead sulfide (PbS), magnesium oxide (MgO), and iron oxide (FeO).

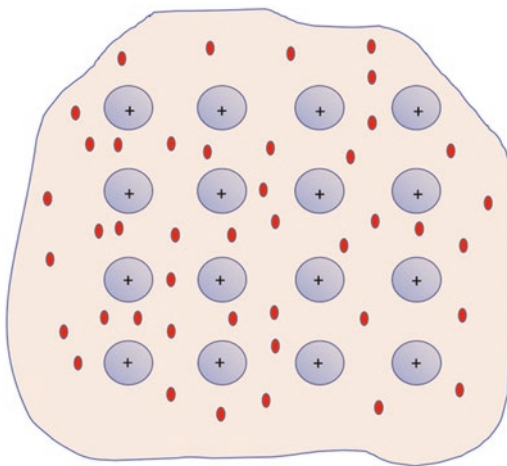
The melting and boiling points of ionic compounds are high because a large amount of thermal energy is required to separate the ions which are bound by strong electrical forces. Solid ionic compounds do not conduct electricity because there are no free or mobile charged particles, and no free electrons. Most ionic compounds are hard because the ions are bound strongly to each other forming a crystal lattice, and these ions cannot be easily displaced. Ionic compounds are generally brittle because a displacement of the ions with respect to each other is irreversible and thus lead to a permanent change of position of atoms with respect to each other. If force is applied, they fracture.

Among the materials which have ionic bonds are ceramics, polymers, biological molecules, and composites. Of these only the ceramics are made solely of ionic bonds. The directionality and the strength of these bonds reflect on the properties of these molecules. For example, ceramics are brittle and hard; do not conduct electricity; withstand compression, but not tension; have high density; and are inert. Some of these properties make ceramics invaluable for some biomaterials applications such as enamel and middle ear implants.

2.2.3 Metallic Bond

A metallic bond is found in metals and alloys. Metallic materials have a few valence electrons that are not bound to any particular atom in the solid and are more or less free to move throughout the entire metal crystal. This leads to the notion of an electron cloud being present in the structure, and positively charged cations are placed in this negatively charged cloud. Even though the rest of a crystal is positively

Fig. 2.3 Metallic bonds consist of positively charged metal atom nuclei and a cloud consisting of electrons randomly orbiting in space between and around the nuclei



charged, there is no distinct repulsion between the metal atoms constituting the lattice structure. There is no directionality in the bonding. All these make the metals highly conductive (Fig. 2.3).

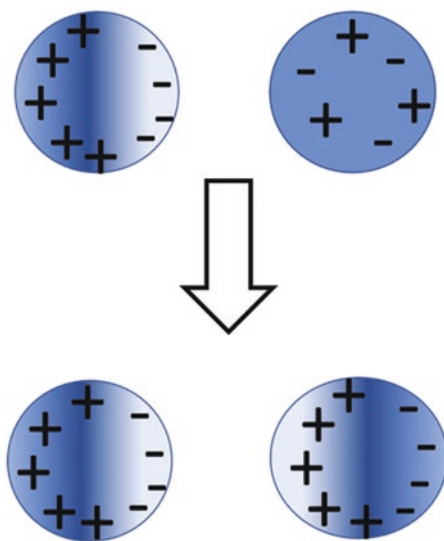
The crystalline structure of metals makes them strong in compression and tension, but unlike ceramics they are pliable and malleable and thus not brittle. This increases the applicability of metals in the biomedical field. They are used in load-bearing applications such as orthopedic implants in the form of plates, wires, rods, nails, screws, etc. They are also used in signal- and electricity-transmitting applications such as transducers, sensors, pacemaker, and sensor cables.

2.2.4 van der Waals Bonds

Secondary bonds like van der Waals bonds are weak in comparison to the covalent and ionic bonds but play an important role in the form and interactions of biological macromolecules. These partially positive and negative charges form due to the instant movements of the electron shells of the atoms (Fig. 2.4). They result from Coulombic attraction between the positive and negative regions of an atom or molecule. As a result they are present in all molecules, but their contribution to the final attraction between atoms may be negligible in comparison to the other stronger bond types, but when their number is significant even the van der Waals bonds make a difference. They are found between induced (not permanent) dipoles; between induced dipoles and dipoles of polar molecules; and between polar molecules.

These groups are found in almost all materials, and they alone cannot give strength to a material, but they help large molecules to take certain forms essential for their activity or loss of it.

Fig. 2.4 van der Waals interactions



2.2.5 Hydrogen Bonding

This bonding always involves a hydrogen-containing group and a group that has an atom with a lone pair of electrons such as nitrogen (N), sulfur (S), fluoride (F), and oxygen (O) which is not involved in bond formation. During this bonding the electron pair of the donor is shared between the donor and the hydrogen of the other molecule or group. As a result, the bond becomes directional. Like van der Waals bonds, they are weak, but when their number is significant as in the DNA (deoxyribonucleic acid), RNA (ribonucleic acid), and polypeptides, they have a great impact on the conformation and the other properties of the molecule (Fig. 2.5).

Like van der Waals bonds, hydrogen bonds cannot alone make a material solid, but they are essential in helping to retain form and in defining the reactivity of biomaterial surfaces. Examples of some materials having different types of bonds are given in Table 2.1 with bond energies and the melting temperatures.

2.3 Physical Form

The types of bonding involved in the formation of a molecule have an impact on its solid state form. The bond types mentioned help keep the solids as they are, but the form of the solid is very crucial in the performance of a biomaterial. They can be in the form of fibers, sheets, foams, spherical (micro and nanoparticles), and as biomaterials with engineered surfaces.

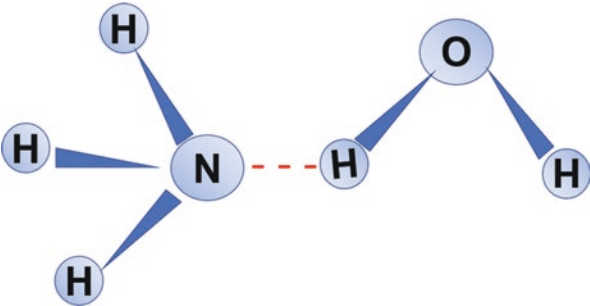


Fig. 2.5 Hydrogen bond

Table 2.1 Bond types, energies, and melting temperatures of various materials

Bond type	Substance	Bond energy		Melting temperature (°C)
		kJ mol^{-1}	ev/atom, ion or molecule	
Ionic	Sodium chloride, NaCl	640	3.3	801
	Magnesium oxide, MgO	1000	5.2	2800
Covalent	Silicon, Si	450	4.7	1410
	Carbon, C (diamond)	713	7.4	>3550
Metallic	Mercury, Hg	68	0.7	−39
	Aluminum, Al	324	3.4	660
	Iron, Fe	406	4.2	1538
	Tungsten, W	849	8.8	3410
van der Waals	Argon, Ar	7.7	0.08	−189
	Chlorine, Cl ₂	31	0.32	−101
Hydrogen	Ammonia, NH ₃	35	0.36	−78
	Water, H ₂ O	51	0.52	0

Adapted from Callister, 1994 [1]

2.3.1 Fibers

Among the most important forms that a biomaterial should take is the fiber form (Fig. 2.6). All three major material groups lend themselves to form fibers but with extremely different properties and performances. For example, a polymeric rod can easily be made by melting and extrusion at relatively low temperatures. Metals on the other hand require a much higher temperature to be processed into a fiber. Ceramics are the most difficult to process into fibers because of the ionic nature not allowing the ceramic to flow at low temperatures. They are produced by drawing from the melt, by spinning, or by extrusion. Some important ceramic fiber types are alumina, magnesia, zirconia, silicon carbide, and carbon fiber. As ceramics are crystalline and strong under compression, so are the fibers. They withstand high temperatures and forces but are brittle unless made into composites.

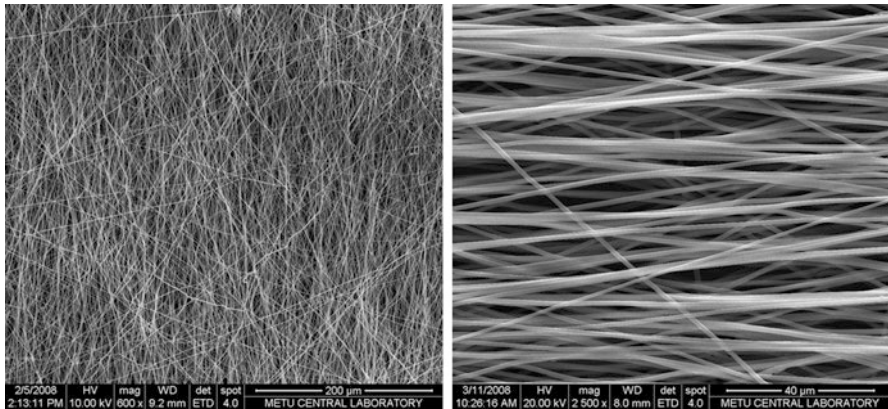


Fig. 2.6 Nano-microfibrous structures [2]

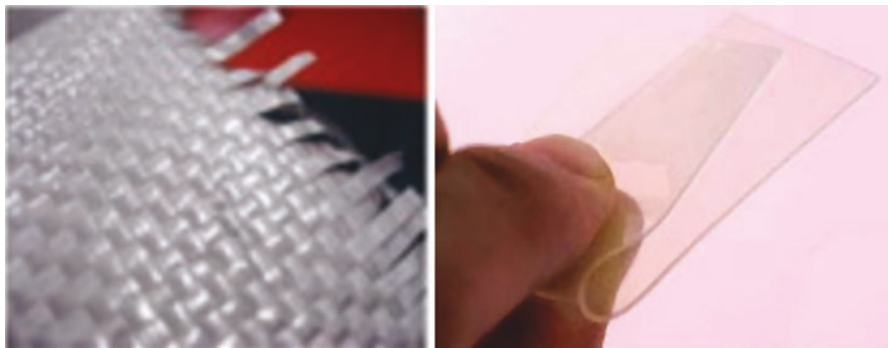


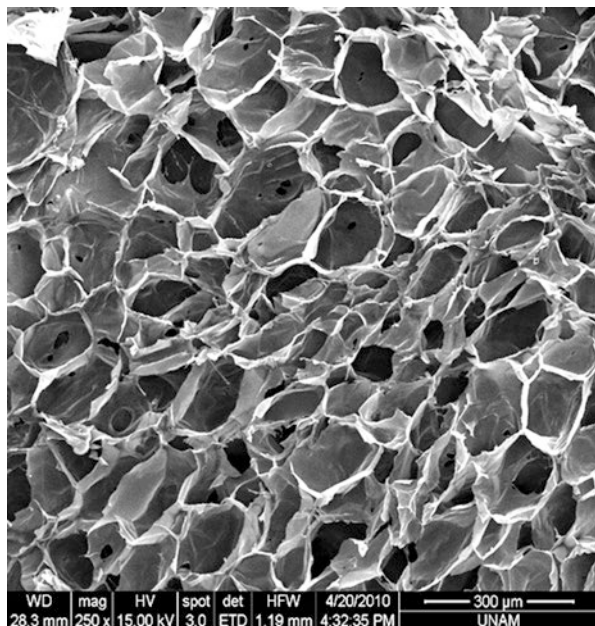
Fig. 2.7 Biomaterials in sheet form: (a) PLGA [3] and (b) bacterial cellulose with acrylic resin [4]

Polymeric fibers are easily prepared by extrusion, drawing from melt or solution. The temperatures needed are not excessive since most polymers melt at around or below 200 °C. Their fibers are more easily prepared, economical, widely available, and therefore more commonly used than those of the other two materials. In the daily life of the humans, polymeric fibers find more use. Some of the important ones are nylon, polyesters, aramides (e.g., Kevlar), and polyurethane fibers (Spandex).

2.3.2 Sheets

Obtaining sheets of polymers, metals, and ceramics is not as difficult as fiber forming (Fig. 2.7). The processing conditions are more easily attainable because they do not require high technology. For polymers in addition to melt compression, polymerization in sheet form and casting from solutions are the methods available. By adding extra steps, the sheets of polymers can be produced in porous or nonporous

Fig. 2.8 Biomaterials in foam (sponge) form [5]



forms to serve the final purpose. Ceramic sheets are more difficult to obtain because of the stringent conditions under which the ceramics are processed.

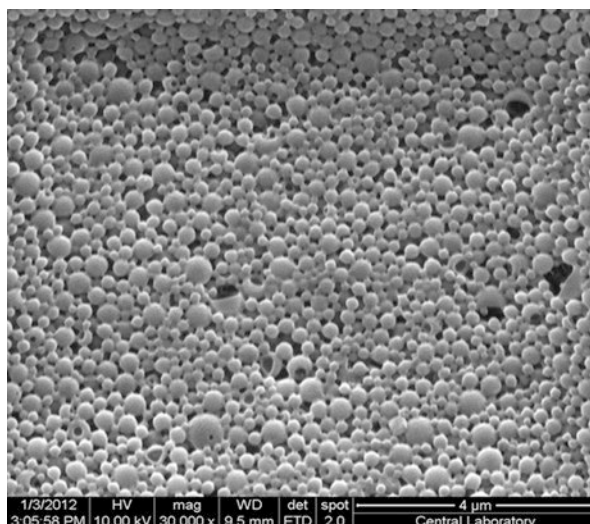
2.3.3 Foams

Foams or sponges are very frequently used in the biomaterials field (Fig. 2.8). The most common use is in tissue engineering where cells of the targeted tissue or stem cells are seeded onto these porous structures which preferably are biodegradable. Interconnectedness of the pores is required so that the cells growing in the pores can move around, fully occupy the structure, and modify the environment to suit their biological needs. For these applications, sponges need to have pores in the range of 100–300 μm . Some other applications where porosity is needed are the interfaces where the integration of the biological system with the implant is desired. These could be porous surfaces like those on metal hip implants or in implants where ceramics are used as bone or dental implants.

2.3.4 Spherical Biomaterials

Spherical biomaterials are called spheres or capsules depending on whether they are full or hollow in the center (Fig. 2.9). Spherical biomaterials are especially important in drug delivery applications where the micro- or nanospheres or capsules are loaded with bioactive agents and then introduced to the body within capsules or

Fig. 2.9 Biomaterials in spherical form [6]



injected to release their contents preferably at the target to be treated. Micro- and nano-size, targeted drug delivery vesicles are commonly used for cancer therapy.

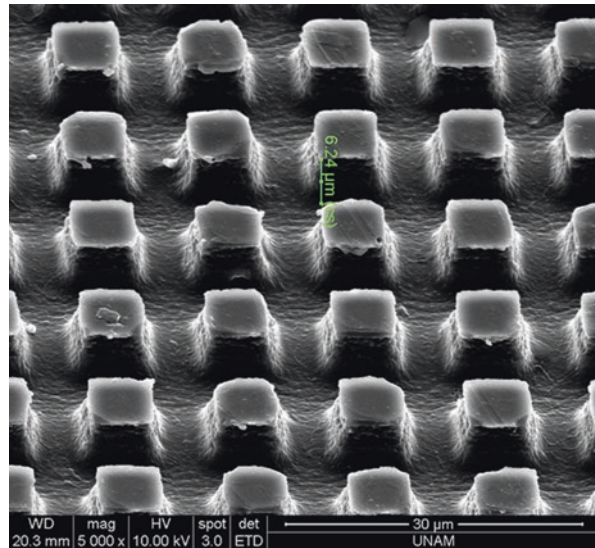
2.3.5 Tubular Biomaterials

Biomaterials can also be processed in the form of tubular structures. A wide variety of applications employ tubular biomaterials as catheters, cannulas, tubes of extracorporeal devices, nerve guides, drug eluting systems with impermeable walls. They are not permeable because they need to deliver their contents fully at the target tissue or if they serve as coats they have to separate their contents and the biological environment. On the other hand, stents and vascular grafts are among the semipermeable tubular biomaterials used in the treatment of a variety of impairments. In the case of these devices, permeability is needed generally to achieve infiltration of the tissue into the implant to achieve stable anchorage of the device.

2.3.6 Biomaterials with Engineered Surfaces

Engineered surfaces having a certain design and surface topography or functionalized by tethering certain molecules are important as biomaterials. The material surfaces having micro- or nano-designed decorations are used to study cell-biomaterial interactions and modified to improve implant-tissue attachment properties (Fig. 2.10). The ability to modify surfaces at such low dimensions is a relatively recent capability learned from the developments in the microelectronic industry. Biological information is gathered through physically and chemically

Fig. 2.10 Biomaterials with engineered surfaces [7]



modified surfaces by studying adsorption and conformational changes of biological molecules on these engineered surfaces. Meanwhile, biomaterials are also modified by immobilizing active molecules onto these surfaces in order to create antibacterial or antithrombogenic implants or surfaces with enhanced cell adhesion capacity.

2.4 Important Properties

Solids are the main implant material types that we use today. In order to be able to use them, their properties have to be known before and after processing so the right kind of processes and materials can be selected. The following are some major properties of the solids that need to be determined prior to any biomedical application.

2.4.1 Mechanical Properties

Materials experience a variety of forces. These are mainly tension, compression, shear, and a combination of these, such as shear in compression applied simultaneously as experienced by the knees of a tennis player when hitting a backhand. *Tension* is when a force is applied on a sample in opposite directions to stretch or elongate it. For example, tension is the force our arm is subjected to when lifting or carrying a weight. *Compression* is the force applied on a material in order to decrease its length. For example, compression is the force when our femur is subjected to body weight when we take a step.

A mechanical tester set to do tension and compression testing is shown in Fig. 2.11. It is the kind of force applied to our feet when we are standing up. In order

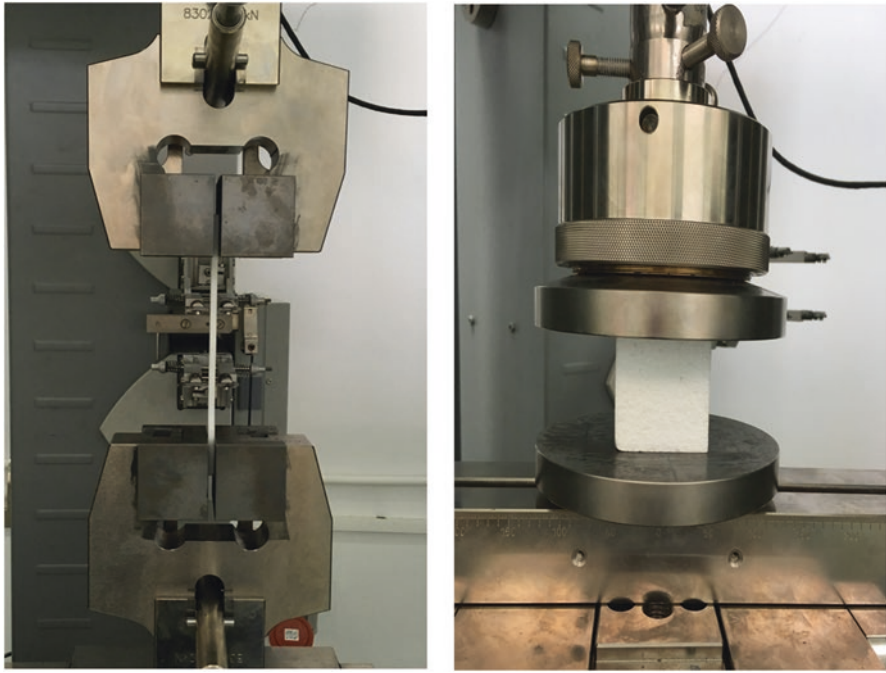


Fig. 2.11 Mechanical tester set to determine tensile and compressive properties

to be able to identify the appropriate materials in designing biomaterials, a system of objective and therefore quantitative comparison is needed. *Shear stresses* occur within a material when external forces are applied along parallel lines in opposite directions or on a substance in a tangential manner.

Some of the most important parameters needed to measure mechanical properties are stress and strain, and they are defined as follows:

$$\text{stress}(\sigma) = \text{force}(\text{N}) / \text{area}(\text{m}^2) \quad (2.1)$$

The unit for stress is N/m^2 or Pascal (Pa).

$$\text{strain}(\epsilon) = \text{change in length}(\text{m}) / \text{original length}(\text{m}) \quad (2.2)$$

As a result, strain is unitless. Sometimes strain is expressed as percent deformation ($\epsilon\%$).

In order to compare the mechanical properties of different materials, a relation between the applied stress and the response of the material is required. It is observed that some materials respond to forces as a spring does, extend when the two ends are forced apart, and contract when the force is removed. Materials that behave like an ideal spring are called elastic materials, and their behavior is expressed as:

$$\sigma = E\epsilon \quad (2.3)$$

This is analogous to Hooke's Law, the physics law developed for springs:

$$F = -kx \quad (2.4)$$

where F is the force, x is the displacement, and k is the spring constant.

Thus, the higher the force applied, the more is the displacement. The stiffer (or thicker) the spring (higher k value), the less is the extension with a given force. When the force is too high, then the material snaps. For these materials, the constant, E , is called the Modulus of Elasticity or Young's Modulus which is an intrinsic property of the material. Meanwhile, a structural property like *stiffness* is influenced by the geometry of the specimens.

Some materials are fluids and flow under force and cannot recover their original shape when the force is removed. If the change of strain of a fluid within unit time (or strain rate) with the stress applied is linear, it is called a Newtonian fluid, but if nonlinear then it is called a non-Newtonian fluid. So assuming a Newtonian behavior for the viscous fluid, the expression that can be used becomes:

$$\sigma = \eta d\epsilon/dt \quad (2.5)$$

where σ is the stress, η is the viscosity of the fluid, and $d\epsilon/dt$ is the strain rate.

It is known that some materials do not snap when the force is too high, they just extend further, but when the force is released, they cannot recover the extension, and retain some of the extension. This kind of materials are called plastic materials due to their ductility. When the whole behavior of the plastic material is considered, it is observed that to some level of force they respond as elastic materials, and beyond a certain value, they flow and act as plastic materials before eventually breaking (or failing). When a material behaves in this fashion, it is called a viscoelastic material, and its behavior is best represented as in the stress-strain curves in Figs. 2.12 and 2.13.

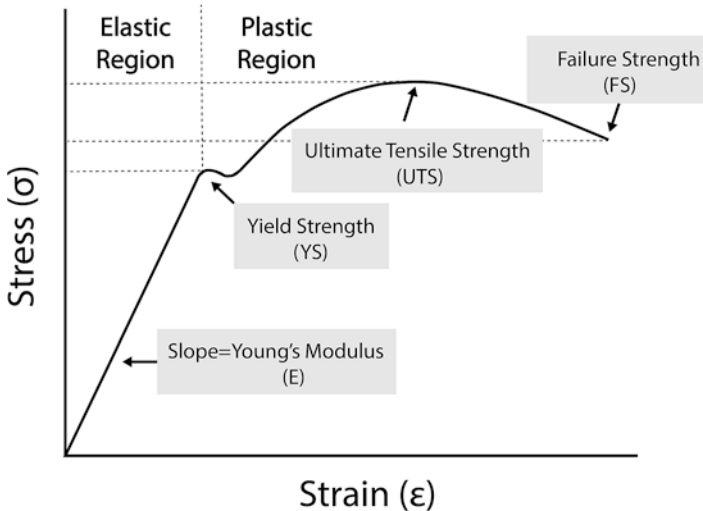


Fig. 2.12 Tensile stress-strain curve of a typical viscoelastic material, a polymeric sponge

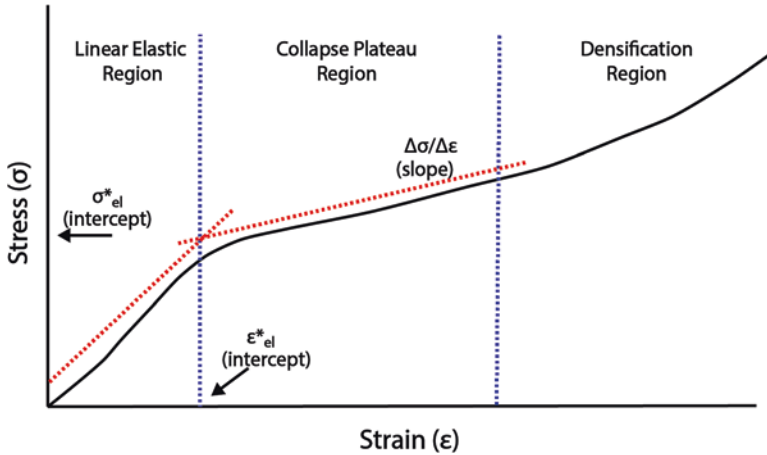


Fig. 2.13 Compressive stress-strain curve of a viscoelastic material, a polymeric sponge

The important points on the stress-strain curve of the viscoelastic material are:

Elastic region: The region of the plot where the material behaves as a spring, where the deformation is reversible.

Plastic region: The region of the plot where the material behaves as a plastic material, where the strain is not fully recoverable.

Young's modulus (E): The slope of the elastic region.

Yield stress (Y_S): The point where the elastic region ends and the plot starts deviating from linearity.

Ultimate tensile stress (UTS): The highest stress reached in the plot. This is the highest stress that the material can withstand.

Failure (or fracture) stress (FS): This is the stress where the material breaks.

In addition, more information can be obtained from a stress-strain curve. One of them is the *toughness*, a value obtained as the area underneath the stress-strain plot of a material (Fig. 2.14).

When the tested material shows a highly nonlinear stress-strain relationship, then there is difficulty in determining the yield stress, and therefore instead of the yield strength, by convention, a straight line is drawn from 0.2% strain parallel to the original plot and the point it intercepts the test line is accepted as the yield stress (Fig. 2.14). This is just for design purposes; otherwise it has no physical meaning.

An interesting observation in the stress-strain plots is the dip just after the yield point. It appears as if the load required to strain a material is suddenly much lesser than earlier stages. This is a result of the experimental procedure. The instruments which record the strain upon application of stress, calculate the stress as the force applied divided by the initial cross-sectional area of the sample. At a certain point in the test, where the sample starts getting thinner, a phenomenon called *necking* takes place, decreasing the cross-sectional area and, therefore, decreasing the stress needed for further deformation (Fig. 2.15). The uncorrected curve is called the *engineering stress*, whereas the corrected curve is *true stress* curve.

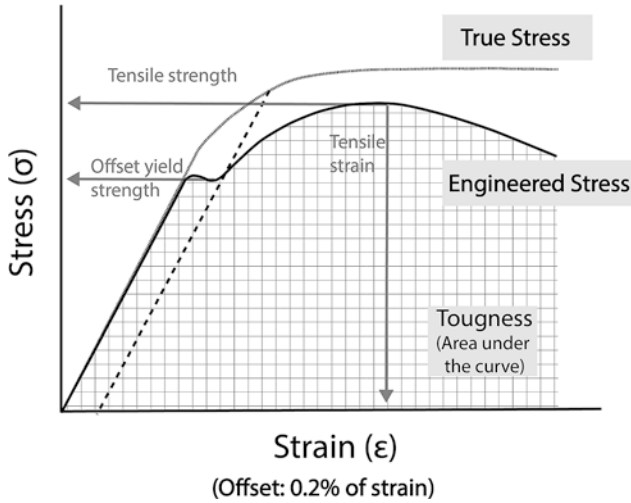
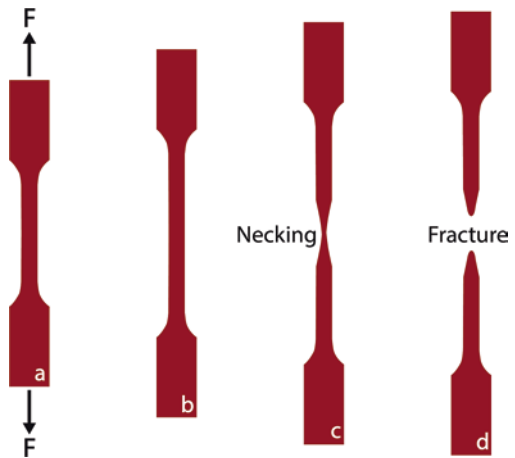


Fig. 2.14 Engineering stress-strain curves

Fig. 2.15 Dog bone samples for tensile testing



In the load-displacement graph (Fig. 2.16), lines with a higher elastic moduli represent stiffer materials, and the one ending without entering a non-linear, plastic phase is brittle. The lines that extend beyond the linear region represent viscoelastic materials.

Ductility and malleability are terms sometimes used interchangeably, but actually they are different. *Ductility* is the property of being able to stretch, bend, or be drawn out into a wire. *Malleability* is the property of a material to deform under compression such as stamping, hammering, forging, pressed, or rolling into sheets. Both these properties indicate viscoelastic materials that present elastic and plastic regions in their stress-strain curves.

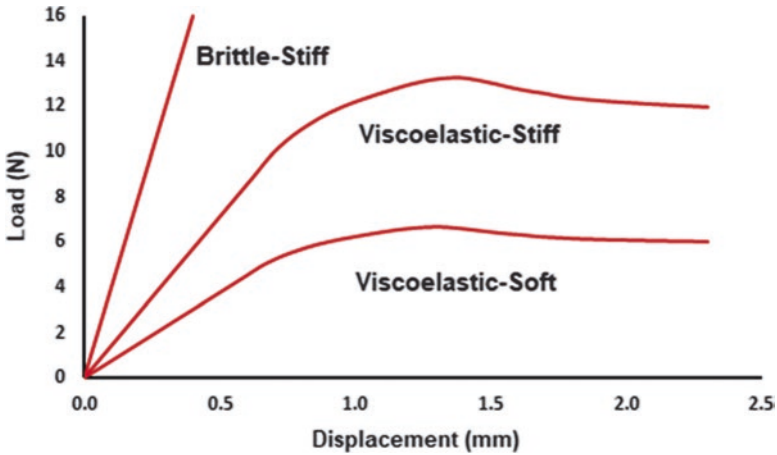


Fig. 2.16 Brittle-viscoelastic, stiff-soft material mechanical behavior

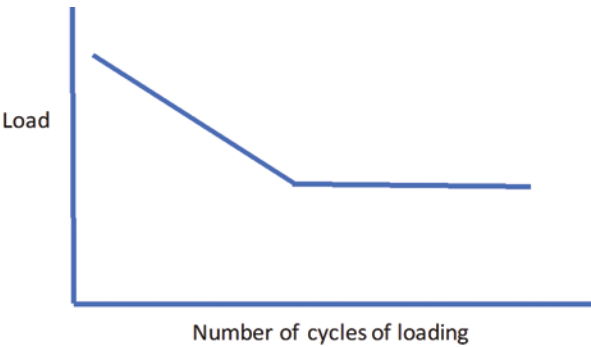


Fig. 2.17 Fatigue observed after cyclic application of load

Fatigue is another property associated with materials. When a cyclic process is applied, a damage such as a crack or a dislocation is created which eventually progresses into a failure after a certain number of times. It is like continuously bending and relaxing a plastic ruler. After a while the ruler develops an irrecoverable damage observable from the outside. Eventually it breaks. The failure is observed to be after lesser number of cycles if the load applied is increased. In fatigue, the damage is localized and cumulative. Theoretically below a certain load, a material is immune from fatigue regardless of the number of cycles (Fig. 2.17). *Impact strength* is the ability of a material to withstand a striking force application. In other words it is the maximum force applied in the form of an impact, not a steady force.

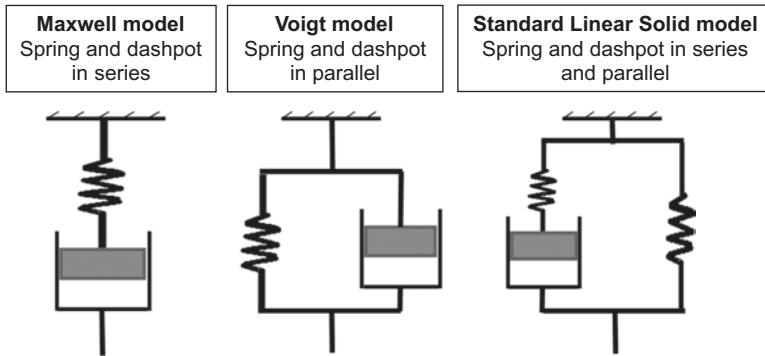


Fig. 2.18 Maxwell, Voigt, and standard linear solid model

2.4.2 Viscoelasticity

In order to be able to select materials for biomedical applications, one has to have quantitative data about the properties of the available materials. For viscoelastic materials this is difficult because the material at hand is neither a spring nor a viscous fluid but behaves rather like a mix of the two. Therefore, models were developed to analyze the behavior of a viscoelastic material under stress. Models have elastic (represented by a spring) and viscous fluid (represented by a dashpot) elements combined with each other in a variety of ways to approximate the behavior of the viscoelastic material. These include the Maxwell, Voigt (Kelvin) and the Standard Linear Solid models (Fig. 2.18).

In the *Maxwell model*, one spring and one dashpot are connected in a series fashion, one connected directly to the other. A test that is done with this model to learn about the material involves suddenly extending the whole system and then keeping this length constant (constant strain). Obviously in the instantaneous pull, the spring will expand, and then it will try to retract expanding the dashpot. Thus there will be a constant strain shared by the two elements. The stress applied is gradually decreased as the dashpot pulls itself out of the viscous fluid it contains. Eventually the stress becomes zero. When the strain rate is calculated (it actually is zero because after the instantaneous pull there is no change in the strain) from the contributions of the spring and the dashpot, Eq. (2.6) is obtained. When this equation is solved, it shows that stress is decreased exponentially with time (Eqs. 2.7 and 2.8):

$$d\varepsilon_{\text{total}}/dt = 1/E(d\sigma_{\text{spr}}/dt) + \sigma_{\text{dp}}/\eta \quad (2.6)$$

$$\sigma/\sigma_0 = e^{-(E/\eta)t} \quad (2.7)$$

$$\sigma = \sigma_0 e^{-t/\tau} \quad (2.8)$$

where τ is equal to η/E and is called the “relaxation time” to represent the relaxation of the stress applied. It can be seen from this equation that if the relaxation time is short in a material, then it means its stress will decrease in a short while.

In the Voigt model, one spring and one dashpot are connected to each other in parallel. The test of the sample is done as follows: first the whole system is extended (both the spring and the dashpot are subjected to the same strain), and the system is allowed to retract. Of course, the moment the system is let loose, the stress on the whole system becomes zero, but the strain gradually decreases as time progresses, and eventually recovers the whole strain. In this model the spring tries to retract, but the dashpot resists this move and thus retards the recovery of strain. From this model “retardation time” is measured using initially the relation below that gives the stress which is zero the moment the system is allowed to retract (Eq. 2.9):

$$\sigma = E\varepsilon + \eta(d\varepsilon / dt) \quad (2.9)$$

The equation obtained is about the strain that is being recovered (Eq. 2.10).

$$\varepsilon = \varepsilon_0 e^{-(E/\eta)t} = \varepsilon_0 e^{-t/\lambda} \quad (2.10)$$

where λ is equal to η/E and is called the “retardation time” to represent the retardation caused by the dashpot on the retraction efforts of the spring.

Standard Linear Solid model has more than two units including both parallel and serial combinations. The relation between stress and strain are given as follows:

$$\text{For parallel components: } \sigma_{\text{Total}} = \sigma_1 + \sigma_2 \quad \text{and} \quad \varepsilon_{\text{Total}} = \varepsilon_1 = \varepsilon_2$$

$$\text{For series components: } \sigma_{\text{Total}} = \sigma_1 = \sigma_2 \quad \text{and} \quad \varepsilon_{\text{Total}} = \varepsilon_1 + \varepsilon_2$$

2.4.3 Electrical Properties

Electrical properties of materials are presented by their conductivity and piezoelectricity. The electrical properties of the materials are also dictated by the bond types they have within their structure. Covalent-bonded and ionic-bonded structures have their electrons fixed between them, and therefore it is not expected that these electrons would leave the atoms and conduct electricity even though they might be crystalline. When the material types are considered, it cannot be expected that polymers and ceramics to be conductors of electricity due to their bond types. They generally are used as insulators or as inert coatings. In the case of metals, however, it is different. The crystalline structure has positive-charged nuclei in the lattice points of the crystal, whereas the outer shell electrons of the atoms freely move about and form an electron cloud. It is these electrons which make conductivity possible. Metals conduct electricity, and as a result all the conductivity needs in a bio-medical device are solved using metals and metal alloys of various types.

2.4.4 Thermal Properties

For a solid material, there are a number of important thermal parameters to know. It is especially important from the point of view of a biomaterials researcher because as one learns about these properties, the selection of materials for a specific biomedical application becomes more systematic and sound. These parameters are:

- Melting-freezing temperature (T_m)
- Glass transition temperature for polymers (T_g)
- Decomposition temperature (T_d)
- Heat of fusion (ΔH_f)
- Molar heat capacity or specific heat (C_p)
- Linear coefficient of expansion (α)

Melting temperature is the temperature at which the solid atoms or molecules fully separate from each other under action of heat added to the system. Glass transition temperature is a temperature for macromolecules at which the chains start showing some mobility but are unable to disentangle from each other. It is also the temperature at which a glassy polymer softens into a rubbery form. Glass transition temperature of a macromolecule is much lower than its melting temperature. For example, for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with 8% 3-hydroxyvalerate content, the glass transition temperature is -1.1°C , but the melting temperature is around 159°C [8]. Decomposition temperature is the temperature some polymers start to degrade because they cannot melt. As an example, for such polymers one can consider the cross-linked structures like those of the thermoset polymers, the polymers which harden upon heating instead of softening or melting.

Heat capacity is the amount of heat energy 1 g of material needs to absorb to raise its temperature by 1°C . In other words, a material heats up faster if its heat capacity is smaller. What determines the heat capacity is the material type (wood, metal, ceramic, etc.) and the form it is in.

Heat of fusion is the amount of heat energy needed to melt a unit amount of solid material. Linear coefficient of expansion is the fractional change in the dimension of a material with a unit change in its temperature. Material dimension changes with temperature because an increase in temperature leads to an increased vibration of the atoms in a material, and this in return increases separation distance of adjacent atoms.

Linear coefficient of expansion (α):

$$\alpha = dl / l \cdot \Delta T \quad (2.11)$$

where dl is the dimension change, l is the original dimension, and ΔT is the temperature difference. Linear expansion coefficients of some materials are given in Table 2.2.

Table 2.2 Some examples of linear coefficient of expansion

Substance	Coefficient of linear expansion $\times 10^{-6}$ (m/m °C)
Aluminum	23.0
Brass	18.9
Copper	16.8
Iron	11.4
Lead	29.4
Nickel	12.8
Silver	18.8
Steel	13.2
Tin	26.9
Zinc	26.3
Enamel	11.4
Dentin	8
Porcelain	4
Amalgam	25
Acrylic resin	90

2.5 Conclusion

In this chapter, the fundamental properties of solid materials are presented. Chemical bonds between atoms constituting a material, the states and physical forms of solid materials, their mechanical, viscoelastic, electrical and thermal properties were introduced and discussed. Using this knowledge, we gain a better sense of the properties that a medical product should have based on its intended use as a tissue substitute. For example, should a skin graft be thermostable and biodegradable? Should it be in sheet or sponge form? What kinds of mechanical properties should it have? These questions can all be answered using the information provided in this chapter.

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3.1 General Properties

Metals are generally hard, opaque, shiny, malleable, ductile, and conductive materials. Organization of the atoms in solid metals is generally close-packed, having crystal structures like body-centered cubic (bcc), face-centered cubic (fcc), or hexagonal close-packed (hcp). Outer shell electrons of the atoms are delocalized and free to move and form a kind of cloud around atoms. Meanwhile atoms stay together due to the electrostatic interactions created among each other. This kind of bond is called *metallic bond*. Since the outer shell electrons are not strongly bonded to the total structure, metals can easily lose them in chemical reactions and form cations. Electrostatic interactions among cations and anions form salts which are soluble in aqueous media. Metals can form alloys by mixing with other metallic elements at the molecular level. The main purpose of forming alloys is to enhance some properties of the metal such as make it less brittle, harder, and more resistant to corrosion or have a more desirable color and luster. Metals have several properties that are specific to them, including malleability, which allows the shaping of metal into implants, and ductility, which refers to the ability to draw out metal in the shape of wire and is an important property in allowing the manufacture of intramedullary rods, screws, and long stems.

Due to their higher density and strength compared to polymers, metals and metal alloys are extensively used as surgical and dental instruments, biomedical devices, implants, joint replacements, and skull plates. An orthopedic implant is a device manufactured to replace a missing joint or bone, or to support a damaged bone. Among the most common types of medical implants are pins, rods, screws, and plates used to anchor fractures, and they are mostly made from metals.

As every medical device and implant, metals used in the clinics should fulfill some requirements, such as they should have high biocompatibility, high mechanical strength, high wear resistance, and high corrosion resistance. Metals are preferable in the production of load-bearing devices such as hip joints and femur plates, due to their high modulus of elasticity and yield strength. Under load, they do not deform and do not lose their shape easily. One of the most well-known alloys of metals is stainless steel which contains iron mixed with chromium, nickel, molybdenum, and carbon and also commonly used in medical applications. Metals currently used in manufacturing implants mostly contain Fe (iron), Cr (chromium), Co (cobalt), Ni (nickel), Ti (titanium), Ta (tantalum), Mo (molybdenum), V (vanadium), and W (tungsten). By combining several metallic elements together, alloys with improved properties can be prepared beyond those of a single element. The alloys used in orthopedic surgery need to have certain specific properties. Because the implant is bathed in body fluid, a low rate of corrosion and relative inertness is essential. Metallic implants are used for two primary purposes: one of them is used to replace a portion of the body such as joints, long bones, and skull plates, and the other is used as fixation devices to stabilize the broken bones. All alloys have a modulus of elasticity significantly higher than that of the bone. This mechanical incompatibility causes implants to be structurally stiffer than the bones. Alloys with elastic moduli closer to the bone may cause less stress shielding and are therefore more appropriate in many applications.

This chapter reviews the most commonly used metallic biomaterials, such as pure Ti and Ti alloys (e.g., Ti-6Al-4 V), Co-Cr alloys and stainless steel (iron alloy), and novel metallic biomaterials currently attracting attention, such as, bioresorbable magnesium alloys and Ni-Ti shape memory materials.

3.2 Medical Applications of Metals

In the periodic table, although about 80% of the elements are metals, only a few of them can be used in medical applications due to their limited biocompatibility caused by corrosion. The first modern applications go back to the nineteenth century where metals are applied for the fixation of the long bones. Most operations were, however, not successful since there was no sterilization technique applied to materials prior to medical applications. After the 1860s when Lister's antiseptic technique started to be used, subsequently, the success rate of metallic implants was increased.

In orthopedic applications metals are used as artificial joints, plates, wires, and screws and in dentistry as braces and dental implants. Metals are also used in the production of cardiovascular and neurosurgical devices, such as artificial heart valve cages, staples, and stents. The high conductivity of the metals makes them preferable in the production of electronic parts of the implantable medical devices. The

Table 3.1 Advantages and disadvantages of the metals in medicine

Advantages	Disadvantages
High strength Fatigue resistance Wear resistance Ease of fabrication Ease of sterilization Economical	Corrosion High density High modulus Metal ion sensitivity Toxicity

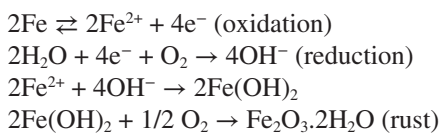
Table 3.2 Metallic biomaterials and application areas

Type	Application area
Stainless steel	Internal fixation materials (bone plates, screws, pins, nails, etc.) Total hip implants
Cobalt alloys	Total joint implants Dentistry
Titanium alloys	Total hip implants (stem and cup) Dental implants Pacemakers
Nickel-titanium alloys (nitinol)	Orthodontic dental arch wires Vascular stents Catheter guide wires Orthopedic staples and clips
Magnesium alloys	Biodegradable implants
Tantalum alloys	Wire sutures Radiographic markers

advantages and disadvantages of the metals in medicine can be summarized as given in Table 3.1.

Table 3.2 summarizes application areas of some metals.

The main disadvantage of the metals is corrosion in the aqueous body environment. In general, all metals corrode in this media and may cause some undesirable effects such as chronic allergy and toxic reactions in the post-implantation period. Corrosion is an electrochemical reaction which causes deterioration of a metal and converts it from uncharged metallic form to charged ionic form as oxide, hydroxide, or sulfide depending on the environment. In water metals ionize creating metal ions and electrons. These are the active species and take part in a variety of oxidation-reduction reactions. During corrosion, the metal (Fe) is oxidized in aqueous media to Fe^{2+} and then to Fe^{3+} ($\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$) which is rust:



In this oxidation-reduction reaction, the noble metal behaves as cathode, and the parts which are stress points, distorted parts (if any), or lower-oxygen regions behave as anode. While corrosion resistance determines the long-term success of a metallic implants, different parts of the body have different pH values and oxygen concentrations. Therefore, an implant that performs well in one region of the body may suffer an unacceptable amount of corrosion in another, due to acidic erosion and oxidation.

Under normal conditions, most human body fluids have a salt concentration of around 0.9% mostly of sodium chloride (NaCl) and some other trace ions in addition to molecules such as amino acids, sugar molecules, and a range of soluble proteins and polysaccharides. There are also traces of debris and cellular material that can result from adhesions onto implants. The extracellular body fluids have a nearly neutral pH value (7.2–7.4 at 37 °C and 1 atm pressure). However, the pH value of body fluid may fall to 3 or 4 when there is inflammation caused by surgery or injury, due to inflammatory cell secretions. Combined with fluctuations in ionic strength in relation to high blood pressure, or due to ion deposits, and mechanical stress, the human body presents an aggressive environment for any implant (Fig. 3.1). Furthermore, the internal partial pressure of oxygen is about one quarter of atmospheric oxygen pressure. In addition, there are tissue-specific pH values significantly different than 7.4. For example, the pH in the stomach, small intestine, and large intestine are around 1, 6, and 8, respectively.

While less reactive in terms of oxidation, lower oxygen actually accelerates corrosion of metallic implants by slowing down the formation of protective passive oxide films on the metal surfaces once an implant is broken or removed. Ideally, corrosion resistance should be such that the release of metal ions from a metallic implant will be minimized in the harshest conditions of the body and remain at a satisfactorily low level over a long service period (more than 30 years) under normal physiological conditions.

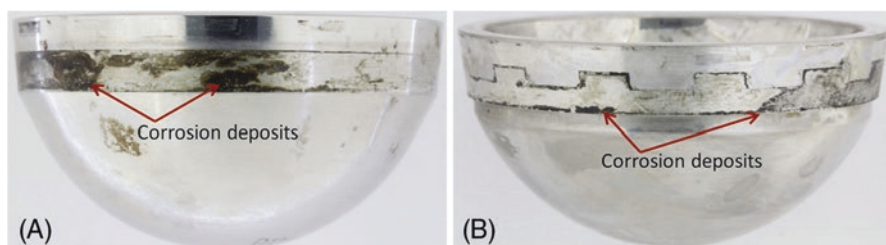


Fig. 3.1 A corroded cup of a total hip joint implant [1]

3.3 Types and Properties of Biomedical Metals

While some pure metals have excellent characteristics for use as implants, most metal implants are made from alloys which are formed from two or more metals. By combining few different metals, a new material can be obtained that has a good balance of the desired characteristics. The most common metal alloys used in orthopedic implants are stainless steel, cobalt-chromium alloys, and titanium alloys. Recently magnesium-containing alloys with biodegradable properties and tantalum-based alloys with radiographic properties are in demand. The following sections summarize the properties of the metals used in medical applications.

3.3.1 Stainless Steel

Stainless steel is a very strong iron alloy, and its first introduction to humans for fracture treatments has started at the beginning of the 1900s with the application of “Sherman vanadium steel.” Stainless steel is most often used in implants that are intended to repair fractures, such as orthopedic implants, joint replacements, surgical and dental instruments, bone plates, bone screws, pins, rods, and coronary stents. Stainless steel is mostly iron and contains other metals such as chromium (at least 10.5%, w/w), cobalt, molybdenum, and carbon (less than 1.2%, w/w) which are added to make it more resistant to corrosion. Chromium, especially, is a very reactive element and is essential in preventing “rusting” (oxidation) of the stainless steel. If the amount of chromium is more than 10.5%, an adherent and insoluble film is instantaneously formed on the surface which prevents the further diffusion of oxygen and prevents the oxidation of the iron in the matrix.

There are many different types of stainless steel. Type 316 is an austenitic chromium-nickel-molybdenum-containing stainless steel. Surgical stainless steel alloys (316L) is made with varying amounts of iron, chromium, and nickel with addition of lower amount of carbon (Table 3.3). The carbon content of 316 is about 0.08%, whereas 316L is about 0.03%. In austenitic stainless steels, carbon and chromium react to form chromium carbides (Cr_{23}C_6) and precipitate at grain boundary regions which are more susceptible to corrosion. Low carbon content below 0.03% prevents carbide formation and corrosion. The presence of low amounts of carbon diminishes corrosion, and decreases adverse tissue responses and metal allergies. Nickel is the essential element present in 300 series of stainless steel and adds strength, ductility, toughness, and nonmagnetic property to the material.

Table 3.3 Compositions of different stainless steels

Stainless steel	Composition (% w/w)						
	Cr	Ni	Mo	Mn	C	Si	Fe
316	16–18	10–14	2–3	2.0 max	0.08 max	1 max	Balance
316L	16–18	12–15	2–3	2.0 max	0.03 max	1 max	Balance
317	18–20	11–15	3–4	2.0 max	0.08 max	1 max	Balance
317L	18–20	11–15	3–4	2.0 max	0.03 max	1 max	Balance

Stainless steel is preferred for plates and screws, but not for weight-bearing implants and for extended periods. The chromium-containing steels have a chromium oxide layer on the surface which results in passivation and increases resistance to corrosion.

3.3.2 Cobalt-Chromium Alloys

Cobalt-chromium (Co-Cr) alloys have two basic elements, up to 65 w/w % Co and 35 w/w % Cr. Molybdenum (Mo) can be also added to obtain finer grain sizes which result in higher strength after casting or forging (Table 3.4). These alloys have high strength, temperature endurance, and wear resistance, and therefore, they are used in a variety of joint replacement implants, as well as in some fracture repair implants that require a long service life. Commonly used areas are dental and orthopedics, as cemented total hip or in knee arthroplasty (Fig. 3.2). These alloys are among the most widely used metals in knee implants. Co-Cr alloys are especially useful where high stiffness or a highly polished and extremely wear-resistant material is required. In orthopedic implants, it is usually composed of cobalt with chromium, molybdenum, and traces of other elements.

Table 3.4 Composition of cobalt-chromium alloys (balance cobalt)

Sample	Composition (% , w/w)					
	Cr	Mo	Ni	Fe	C	Si
Co-Cr alloy	26–30	5–7	1 max	0.5 max	0.35 max	1 max
ASTM F75	27–30	5–7	0.5 max	0.75 max	0.35 max	1 max

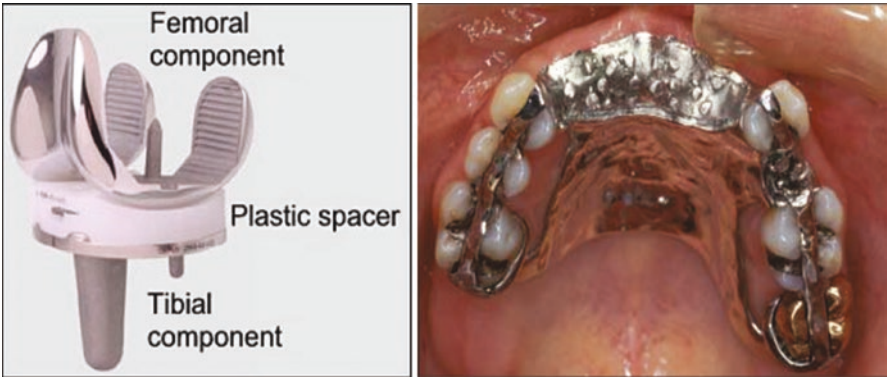


Fig. 3.2 Use of metal alloys as biomaterials. (a) Co-Cr in knee joints [2], (b) Vitallium in dentistry [3]

During the early 1930s, an alloy called “Vitallium” which has a weight by weight composition of 30% Cr, 7% W, and 0.5% C (the balance is cobalt) was used in the preparation of dental castings, and later it was adopted for artificial joint applications. The implants are produced generally by investment casting where a ceramic mold is used. These alloys are among the least ductile compared to iron- or titanium-based alloys. Therefore, it is not easy to manufacture intramedullary rods and spinal instrumentation from these alloys.

3.3.3 Titanium Alloys

Titanium and its alloys are mostly preferred due to good mechanical strength, relatively low density (4.5 g/cm^3), excellent corrosion resistance, and remarkable biocompatibility. Additionally, the elastic nature of titanium and titanium alloys is lower than that of the other metals used in knee implants. Because of this, the titanium implant acts more like the natural joint, and as a result, the risk of some complications like bone resorption and atrophy is reduced. Titanium and titanium alloys have great corrosion resistance, making them inert biomaterial (which means they will not change after being implanted in the body). Titanium is classified based on its composition. Pure titanium is called “commercially pure titanium” (cp-Ti) and can be found in four grades. Pure titanium is generally used in implants where high strength is not necessary, such as in making layers of metal fibers bonded to the surface of an implant to allow bone ingrowth or to allow the cement to bond to an implant better for stronger fixation. The most used titanium alloy in knee implants is Ti-6Al-4V which is Grade 5 and has aluminum and vanadium contents of 6 and 4%, respectively. The mechanical properties along with the composition of some Ti alloys are given in Table 3.5.

Oxygen content in cp-Ti has a significant influence on the mechanical properties. As can be seen from Table 3.5, as the oxygen content increases from Grade 1 to Grade 4, yield and tensile strengths also increase. Titanium has two crystallographic forms, one of which is alpha phase, and it has a hexagonal close packed (hcp) crystal structure at temperatures up to 883°C . At this temperature, alpha phase transforms to beta, which has a body centered cubic (bcc) crystal structure. Titanium alloys can also have a mixture of these and form α - β alloys. α Alloys are produced by alloying titanium with elements stabilizing the α phase, whereas β alloys are produced by alloying titanium with β -stabilizing elements. Alpha stabilizers are Al, Ga, and Sn, while beta stabilizers include V, Ta, Mo, Nb, W, Cr, Fe, Co, Ni, Cu, and Mn. α - β Alloys are the ones that contain balanced amounts of α and β stabilizers. Ti-6Al-4V is an α - β alloy because it contains Al, which is an alpha stabilizer, and V, which is a beta stabilizer.

High biocompatibility of titanium is attributed to naturally occurring surface oxide layer, the thickness of which is variable between 5 and 20 nm. Titanium alloys are used in orthopedic and dental implants, hip joint replacements, etc. The major limitation of titanium is its chemical reactivity with other materials at elevated temperatures. This property has necessitated the development of nonconventional

Table 3.5 Composition and mechanical properties of Ti alloys

Sample Ti alloy	Elements (% max)						Mechanical properties			
	C	O	N	H	Fe	Ti	Young's modulus (GPa)	Yield strength (MPa)	Tensile strength (MPa)	
Grade 1	0.10	0.18	0.03	0.015	0.20	Balance	103	170	240	
Grade 2	0.10	0.25	0.03	0.015	0.30	Balance	103	275	345	
Grade 3	0.10	0.35	0.05	0.015	0.30	Balance	103	380	450	
Grade 4	0.10	0.40	0.05	0.015	0.50	Balance	104	485	550	
Grade 5 (Ti-6Al-4V)	0.08	0.13	0.05	0.0125	0.25	Balance	110	795	850	

refining, melting, and casting techniques. As a result, titanium alloys are quite expensive.

Titanium-based alloys have excellent properties for use in porous forms for biological fixation of prostheses. The most common alloy is Ti6Al4V (which has Ti with 6% Al and 4% V by weight), but newer alloys are coming into use. They are the most flexible of all orthopedic alloys. Because of the lower modulus of elasticity than cobalt-based alloys or surgical stainless steel, titanium-based alloys have not been considered as a reliable material for use in a cemented hip replacement. They are also less dense than most other orthopedic alloys and have a modulus of elasticity closer to that of the bone.

3.3.4 Tantalum

Tantalum is a pure metal which has a remarkable resistance to corrosion, and excellent physical and biological characteristics. It is a very hard, malleable, ductile, and one of the most unreactive metals. It is extremely stable at temperatures lower than 150 °C, is corrosion resistant, and has excellent biocompatibility. The resistance to corrosion is the result of the formation of a protective layer created by oxides of tantalum on the surface of the metal.

Tantalum has been used in making biomedical implants either in its commercially pure form or as an alloying element in titanium alloys. It has also been used as coating on other metallic devices due to its stable surface oxide layer, such as in 316L stainless steel, to improve the corrosion resistance of the substrate and enhance its biocompatibility.

Tantalum is used in artificial hips, knees, and other joints. Pins, screws, staples, and other devices used to hold bones together are also made of tantalum alloys. Tantalum based sheets, plates, rods, and wires are also used in the production of prosthetic devices for humans such as skull plates, meshes to repair the bone defects after cancer surgery, suture clips, and stents for blood vessels. Although the mechanical properties of tantalum are inferior to other metals, it has many other biomedical applications. Because of its high density (16.6 g/cm³), it has been used in radiography as a marking agent for diagnostic purposes. Some properties of tantalum are given in Table 3.6.

Tantalum can be fabricated in a highly porous form which has a modulus of elasticity closer to that of the bone than stainless steel or the cobalt-based alloys. These highly porous structures can be used as coats because they can be conducive to bone ingrowth. The size of the pores makes this material very suitable for bone ingrowth (Fig. 3.3).

Zimmer Inc., USA, produces porous implants applicable for use in the construction of hip and dental implants. The structure and stiffness of the trabecular metal material are similar to that of the trabecular bone and are fabricated by coating a vitreous carbon skeleton with tantalum through a proprietary chemical vapor deposition coating process. Tantalum exhibits a crystallographic growth on the vitreous carbon surface of the interconnecting struts that form the material.

Table 3.6 Mechanical properties of tantalum

Young’s modulus (GPa)	185
Yield strength (MPa)	138
Tensile strength (MPa)	207

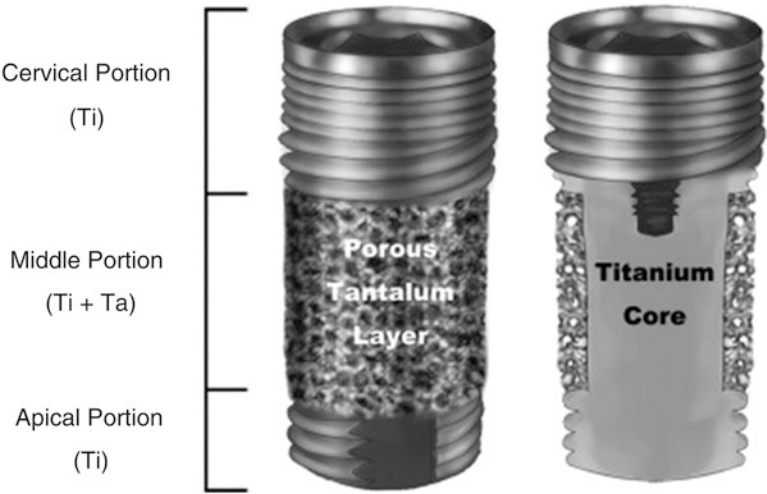


Fig. 3.3 Dental implant with a titanium core and a porous tantalum layer [4]

Trabecular metal technology (TMT) differs from sintered-bead surfaces, titanium plasma-sprayed surfaces, titanium fiber mesh, and titanium foam in the high degree of interconnected porosity (up to 80%) and the uniformity of its pore size and shape. In contrast to conventional bone-to-implant contact achieved by non-porous surfaces, TMT’s interconnected pores is designed for biological ingrowth into the pores.

One other application of tantalum is in the manufacture of capacitors, and the smallest hearing aids and pacemakers are likely to have a tantalum capacitor. Tantalum capacitors experience an extremely low failure rate, making them suitable for use in medical equipments, including hearing aids and devices like pacemakers that should not randomly fail.

3.3.5 Nickel-Titanium Alloy (Nitinol)

This is a shape memory alloy of nickel and titanium, where the two elements are present almost in equal atomic ratio. The name *nitinol* is the acronym for **nickel-titanium Naval Ordnance Laboratory**, where this alloy was discovered. It has been used in the manufacture of endodontic instruments in recent years. Nitinol alloys have greater strength and a lower modulus of elasticity compared with stainless steel alloys. Nitinol wires have super-elastic behavior, and they return

to their original shape upon unloading the force that caused deformation and by performing an appropriate heat treatment above its phase transformation temperature. Heating to this temperature leads the alloy to change from its low-temperature monoclinic martensitic structure to the high-temperature cubic austenitic structure. Deformation is normally carried out at relatively low temperature, whereas shape memory occurs upon heating (Fig. 3.4). These properties are of interest in endodontology as they allow construction of root canal instruments that utilize these favorable characteristics to provide an advantage when preparing curved canals.

Materials that have been found to recover significant amount of deformation in addition to nickel-titanium alloys (nitinol) are some copper-based alloys, such as Cu-Zn-Al and Cu-Al-Ni.

A shape memory alloy is polymorphic, that is, it may have two crystal structures (or phases), and the shape memory effect involves the transformation between them. In Nitinol, the two distinct crystalline phases are, namely, martensite and austenite. Low-temperature stable phase is martensite (monoclinic distorted crystal structure), whereas the high-temperature stable phase is austenite (body-centered cubic) (Fig. 3.5).

Upon cooling, austenite transforms to martensite spontaneously. Mechanical properties of these two phases are given in Table 3.7. Degree of transformation depends on temperature. In addition, martensite is heavily twinned, so that under the influence of an applied stress, it can easily be deformed (i.e., given shape). Furthermore, when the stress is removed, the deformed shape is retained at this temperature. Finally, upon heating to initial temperature, martensite reverts back to its original size and shape. This transformation from deformed martensite to austenite occurs within a temperature range, between the temperatures denoted by A_s (austenite start) and A_f (austenite finish).

Corrosion resistance of nitinol is debatable. Although most scientific literature suggests that it is corrosion resistant, some studies have shown that Ni leaches out from the alloy and causes toxic effects in the surrounding tissue.

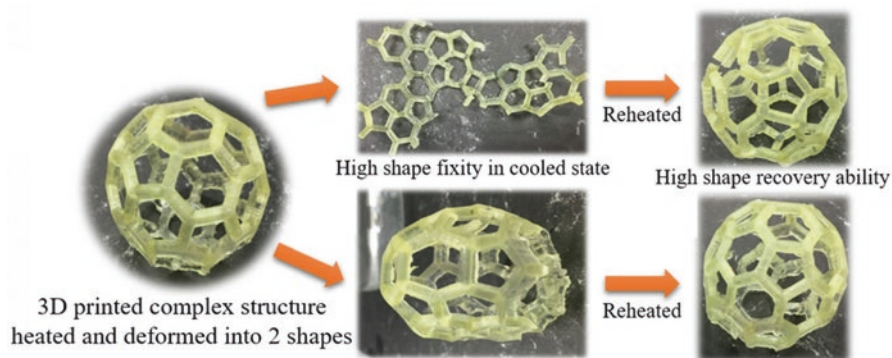


Fig. 3.4 Shape memory effect as shown with a 3D-printed polymeric structure [5]

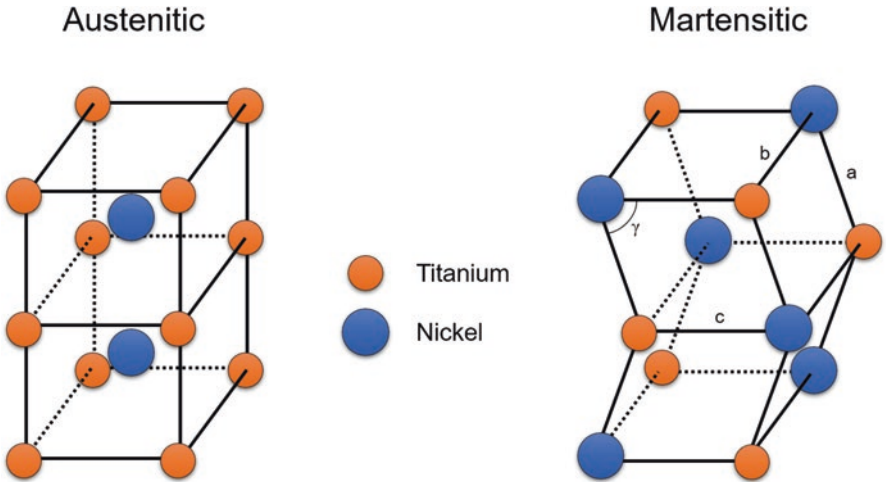


Fig. 3.5 Austenitic and martensitic crystal structures of shape memory alloy Nitinol

Table 3.7 Mechanical properties of nitinol in two different phases

Property	Austenite phase	Martensite phase
Young’s modulus (GPa)	75–80	30–41
Yield strength (MPa)	195–695	70–140

Nitinol is used to manufacture devices for dental, orthopedic, and cardiovascular applications. The most commonly used devices are catheter tubes, dental files, guide wires, arch wires, gallstone retrieval baskets, filters, needles, and other surgical instruments (Fig. 3.6).

3.3.6 Magnesium-Based Biodegradable Alloys

Magnesium (Mg) is well known for its light weight (density is 1.7 g/cm³) and biodegradability. Density, elastic modulus, yield strength, and fracture toughness are close to that of the bone. Mg is present naturally in the bone. In fact, it is estimated that approximately 50% of the total magnesium is stored in the bones. However, rapid corrosion of the pure metal limits its use in load-bearing applications. Magnesium degrades within the first few weeks after implantation in vivo as shown below:

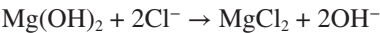
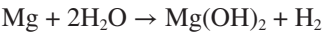




Fig. 3.6 Orthodontic dental wires [6] and vascular stents [7]

Hydrogen evolved during the reaction is of significant concern; it can accumulate as gas bubbles under the skin. Patients with such issues are treated by removing the gas with a needle. In order to improve corrosion resistance, a variety of elements such as Al, Zn, Mn, and rare earth elements were alloyed with magnesium. Magnesium alloys showed improved corrosion properties when compared to those of pure Mg. However, the degradation products of these alloys should still be carefully investigated as the release of metal ions may cause toxic, allergic, and carcinogenic effects in the body.

3.4 Surface Properties of Metal Implants for Osseointegration

The term “osseointegration” refers to the direct structural and functional connection between the host bone and the surface of the load-bearing artificial implant. Typically, an implant is considered to be osseointegrated when there is an absence of movement between the implant and the bone under normal conditions of loading following a defined healing period. Various factors determine the progress toward osseointegration, including the implant’s material properties, form and surface characteristics, applied mechanical load, surgical technique, location, and local quality of the host bone. The final goal is to reach an interface matrix, equivalent to the bone in its structure, composition, and biomechanical properties, to withstand early mechanical loading.

For successful hard tissue implantation, osseointegration is crucial, and this mostly depends on surface properties. The metal surface and the natural bone are not really compatible, and both have completely different properties. In these cases, modification of the surface of the implant either chemically, morphologically, or physically is necessary. There are some methods for improving surface properties of metal implants. Physicochemical methods generally increase the surface energy, while morphological modifications create rougher

surfaces. Bone cells can easily attach to the rough surfaces and have a positive effect on healing process.

There are some techniques for modification of metal implants in order to achieve better osseointegration. The main modification techniques are summarized below:

1. TPS (titanium plasma spray): At very high speeds and very high temperatures, titanium particles (40 μm and smaller) are sprayed on the surface of the titanium implant producing a rough titanium surface into which the bone tissue newly deposited by the cell can penetrate and attach.
2. SLA (sand-blasted, large grit, acid-etched): Sand particles are sprayed on the implant surface to make macro grids and acid etched to produce micro-scaled grids (2–4 μm). Usually hydrochloric acid or sulfuric acid is used for the etching process. With SLA technique, large, medium, or small grid rough surfaces are obtained.
3. HAp-CaP-coated implants: Hydroxyapatite (HAp) and other calcium phosphate (CaP) salts are bioactive ceramics. They are usually used in implants as coating materials because of their positive effect on osseointegration. CaP salts increase the bone formation, and they improve implant fixation in the bone. HAp is the most stabilized form of CaP salts in the body; usually other CaP salts are highly soluble in the body. There are many methods used in HAp coating. Some of the basic methods are dip coating-sintering, hot isostatic pressure, plasma spray, immersion coating, electrostatic spraying, and biomimetic coating.
4. Electropolished (oxidized): The surface of the implant is anodized. Electrochemical anodic oxidation is performed on titanium surface. With this technique, micro-pits are formed on the implant surface.
5. TiO_2 grit-blasted: In this method, TiO_2 is blasted on implant surface with several ways such as plasma spray.
6. Machined surface: In this method, the surface roughness is created by machining of the surface.

Each of these methods creates some alterations on the surface properties and leads to better osseointegration of the metal with the body.

3.5 Conclusion

Metals are among the earliest biomaterials used in human health applications especially as load-bearing implants. In this chapter, the properties of metals, which make them very important as load bearing, thermally and electrically conductive implants, are explained from a chemical and materials science point of view. The advantages and disadvantages of various metals and their alloys ranging from those in use for over a century to those introduced more recently were discussed. Metals are here to stay for the foreseeable future.

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4.1 General Properties

Ceramics are inorganic materials that are composed of metallic and nonmetallic elements which are bonded to each other with ionic or covalent bonds. Ionic bonds are strong and directional, and therefore ceramics have melting temperatures higher than those of metals and polymers which have metallic or covalent bonds, respectively. Ceramics are produced from materials in powder form by application of heat (sintering). They are hard, strong, and brittle. Since they do not have any free electrons, they are poor conductors of heat and electricity. There are numerous combinations of the metallic and nonmetallic compounds, and the most commonly known nonmetallic groups are oxides, hydrides, carbides, phosphates, sulfides, and silicates. Aluminum oxides, calcium phosphates, and titanium nitrides are in this class. Carbon-based materials such as carbon, graphite, diamond, and graphene are sometimes classified as members of the ceramics group, but in this book, they will be presented in another chapter (Chap. 6).

Ceramics are now extensively used in dentistry, in the production of orthopedic implants for the spine, and particularly in total hip implants due to their resistance against compression and wear. There are also bioceramics produced in various complex porous and moldable forms. In the USA, the estimated number of spinal fusion surgeries is about 300,000, and this is much more than the hip, knee, and shoulder implants combined [1]. Meanwhile, porcelain types of ceramics have been used in dentistry as tooth crowns because of their stability in body fluids, high compressive strength, and good esthetic appearance.

The ceramics can be classified as follows:

- Clay: These are the naturally occurring ceramics that contain minerals in the form of fine powders and have a certain plasticity (e.g., kaolinite ($\text{Al}_2(\text{Si}_2\text{O}_5)(\text{OH})_4$), a geological deposit).
- Glasses: This type of ceramics can be amorphous glasses (silica-based ones) or crystalline glasses (e.g., the polycrystalline Pyroceram®).

- **Cements:** These ceramics are used as binders to hold two or more hard body components together (mostly calcium silicates).
- **Refractories:** These are the types of ceramics which are very difficult to fuse, corrode, or draw. They can resist high temperatures and cannot be melted for processing or shaping (e.g., alumina, silica, high purity oxides, graphite).
- **Abrasives:** These ceramics are very hard and are therefore used for polishing purposes (e.g., diamond, silicon carbide, silica sand).
- **Advanced ceramics:** These ceramics are produced in different forms for certain applications to carry the desired properties (e.g., nanotubes, fibers, particles) and are basically crystalline materials with rigorously controlled composition (e.g., SiC, Si₃N₄, WC).

Ceramics are more expensive compared to other materials such as metals, polymers, and composites. Meanwhile, they have some inherent advantages such as being biologically inert, not producing any wear debris, and having high resistance to corrosion, compression and high temperature, and ability to be engineered to closely match the properties of the natural bone. The reason which makes them so resistive to the environmental conditions is the very strong chemical bonds formed between the metal and nonmetal groups of the ceramics. The high wear resistance and the chemical inertness make them preferable in bone replacements in the highly corrosive [environment](#) of the [human body](#). Ceramics are known as nonconductive even though some advanced ceramics can be designed to conduct electricity as the metals do. Although the use of Plaster of Paris (CaSO₄·H₂O) goes back to 1892, research on new ceramics with high biocompatibility and properties closely mimicking the natural bone tissue gained importance in the 1960s. The use of calcium phosphate in human surgical procedures became popular in the 1980s. Hydroxyapatite (or hydroxyl apatite) (Ca₁₀(PO₄)₆(OH)₂) is a calcium phosphate ceramic and constitutes the main mineral component of the bone and dentin.

4.2 Manufacturing Ceramics

In the production of all types of ceramics, the main materials are in powder form; in addition there are additives such as binders and stabilizers. After mixing and forming a homogeneous paste, a desired shape is given by pressing, extruding, injection molding, or casting. Then the shape obtained is fired (sintered) at high temperatures (ca. 1800–2000 °C), where the particles melt and fuse and form a hard, dense material. Simple shapes using ceramics can also be produced by hot pressing where forming and firing steps are combined. In this case, the mix of powders are heated and pressed simultaneously so that the final form is achieved under pressure. Another method is chemical vapor deposition (CVD) where the precursor gases are deposited on the heated substrate under controlled temperature and pressure. Some ceramics such as silicon carbide (SiC) or silicon nitride (Si₃N₄) with superior physical properties can be produced in this fashion. Porous ceramics can be produced by applying reaction bonding technique in which the powders are linked to each other

by linkers via chemical reactions. After giving the shape, the binders are burned, and their places remain as pores. Bioactive glass with its interconnected porosity has added advantages when used as a hard tissue prosthesis. The porous structure supports tissue in growth and improves implant stability through this biological fixation.

4.3 Structural Compositions of Ceramics

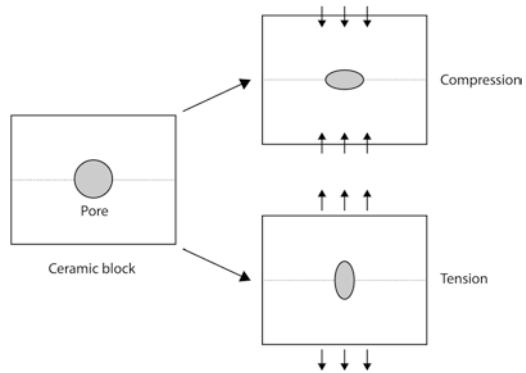
Ceramics can have different structural compositions like A_mX_n where A represents the metal and X the nonmetal; m and n are constants which show the simplest integer mol numbers present in the ceramic structure. If the positively charged metallic atom and the negatively charged nonmetallic atom are about the same size, the resultant structure becomes a simple cubic structure (e.g., CsCl). If the positive ions are smaller than the negative groups, a face-centered cubic (fcc) structure forms. Positive ions can be fitted in the tetragonal or the octagonal spaces within this fcc. Al_2O_3 and Cr_2O_3 belong to A_2X_3 type structures where the oxygen ions form hexagonal close-packed structures and positive ions fill in roughly two thirds of the octahedral sites while leaving the rest vacant.

Hardness of the materials is quantified based on Mohs scale devised by Friedrich Mohs in 1812 as presented in Table 4.1. Among the ceramic materials, the softest one is talc ($Mg_3Si_4O_{10}(OH)_2$), and it is graded as scale 1, that is an absolute hardness of 1 Moh. The hardest ceramic is diamond (a 3D tetrahedral network in a face centered cubic lattice of carbon that is graded as scale 10 and has an absolute hardness of 1500). Mohs' relative hardness of apatite crystals is 5, and the value for the tooth enamel which contains the highest mineral content in the body is estimated to be about 5 in this scale. Another hardness measurement system was proposed in 1920 as the Vickers scale which is derived from the applied force over a certain area of the material. A square base pyramid-shaped diamond indenter is applied; the indentation on the surface of the material is measured and converted to a hardness value with a unit kg-force per surface area.

Table 4.1 Hardness scale of ceramics [2]

Mohs hardness	Mineral	Vickers (kg-f/mm ²)
1	Talc ($Mg_3Si_4O_{10}(OH)_2$)	27
2	Gypsum ($CaSO_4 \cdot 2H_2O$)	61
3	Calcite ($CaCO_3$)	157
4	Fluorite (CaF_2)	315
5	Apatite ($Ca_5(PO_4)_3(OH^-, Cl^-, F^-)$)	535
6	Orthoclase ($KAlSi_3O_8$)	817
7	Quartz (SiO_2)	1161
8	Topaz ($AlSiO_4(OH^-, F^-)_2$)	1567
9	Corundum (Al_2O_3)	2035
10	Diamond (C)	10,000

Fig. 4.1 Crack propagation in ceramics



Ceramics are hard to shear. In order to shear, the plane of atoms should slip past each other when a force parallel to the surface of the plane is applied. In ceramics, slip is very difficult because atoms with the same charge repel each other, and this makes slippage very difficult. If too high a force is applied, then they break. Therefore, ceramics are brittle and hard to creep at ambient temperatures. However, ceramics have very high flaw sensitivity; they are very sensitive to notches or micro cracks because of stress concentration that occurs at the tips of cracks. Here, stress concentrated at the edges of the cracks may be 100 or 1000 times higher than the stress applied (Fig. 4.1), making the ceramics brittle and decreasing their tensile strength to much lower values than the normal. On the other hand, if the ceramics could be made free of flaws (cracks, pores), then they would become extremely strong. For example, flaw-free glass fibers have tensile strengths of about 7 GPa, which is twice that of steel.

The tensile strength of ceramics is lower than their compression strength. When a compression force is applied to a ceramic material which might have micron size irregularities as pores or cracks, these defects are closed under the force. In case of tension, however, these defects behave as stress concentrators and cause propagation of the cracks or break at those points.

4.4 Advanced Ceramics

An advanced ceramic is described as “an inorganic, nonmetallic (ceramic), basically crystalline material of rigorously controlled composition and manufactured with detailed regulation from highly refined and/or characterized raw materials giving precisely specified attributes” by the 1993 Versailles Project on Advanced Materials and Standards (VAMAS) [3].

Advanced ceramics are also called engineering ceramics. They are produced with a method similar to traditional ceramics, by mixing and sintering the powdered materials but in general by using high purity synthetic precursors instead of raw natural materials. The process is carried out with high precision in highly controlled cleanrooms, and the final product has highly controlled porosity, grain size, and if

required, whiskers and fibers. There are regulations for the processing and quality of the products, and the products are carefully characterized. Densification of advanced ceramics is generally achieved by reactive-liquid sintering or solid-state sintering. One of the challenging points in sintering is the submicron size of the particles. Small particles produce highly homogeneous mixtures and make the diffusion of the particles easier compared to larger particles. Small particles with small grains have larger surface area-to-volume ratio and densify easily. They have superior functionality such as superconductivity, extreme toughness, or resistance to very high temperatures especially when designed for a specific application.

4.5 Bioceramics

Ceramics have some preferable properties over the metals such as resistance to heat, chemicals and corrosion, lower density, hardness and stiffness, and ease of modification, and therefore they are commonly used in medical applications especially in the treatment of hard tissue as bones and teeth. The class of ceramics used to support, repair, or replace diseased, damaged, or missing parts of the musculoskeletal system are called bioceramics. Bioceramics are generally used in orthopedic and dental applications with tailorable biological responses. They can be produced in different forms as microspheres, as thin coatings on metallic implants, or as porous or resorbable scaffolds for use in tissue engineering applications. Composites of ceramics with polymers can be used as scaffolds for bone tissue engineering or as cements. Bioceramics generally stimulate bone growth when used as scaffolds, avoid blood clotting on the surfaces on heart valves, and lower the friction in joint prostheses, and they can carry and deliver bioactive agents such as growth factors or drugs.

In cases where a ceramic material is used as an implant material, the fixation of the implant to the tissue can take place in different ways.

Classes of fixation of bioceramics can be summarized as follows:

1. **Morphological Fixation:** Attachment of the implant to the tissue occurs by cementing the device in the tissue or by press fitting into the defect. Single crystal or polycrystalline Al_2O_3 can be examples of implants fixed in this fashion.
2. **Biological Fixation:** In this approach, porous and inert ceramics are fixed by bone ingrowth into the pores and mechanically attach the bone and the material. As examples, polycrystalline Al_2O_3 and hydroxyapatite coat on a porous metal can be stated.
3. **Bioactive Fixation:** Surface reactive ceramics can attach directly to the bone via chemical bonds. Examples for this group are bioglasses, glass ceramics, functionalized ceramics, bioactive glass ceramics and hydroxyapatites.
4. **Fixation of Resorbable Implants:** The ceramics fixed with this approach are designed to slowly degrade and be eliminated from the body, while the newly forming bone replaces the ceramic. Calcium sulfate and various calcium phosphates including tricalcium phosphate are some examples of this category.

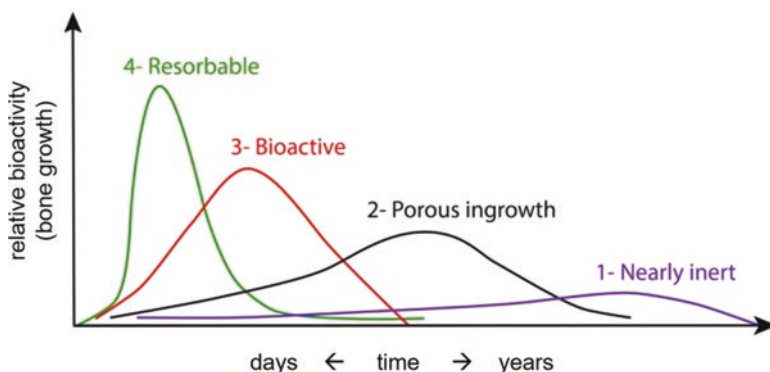


Fig. 4.2 Stability and bioactivity of calcium phosphate salts

The relative bioactivity of the ceramics depends on the reactions which occur between the implant material and the molecules existing in the biological tissue environment. Resorbable ones degrade hydrolytically under the action of water and the ions present (Fig. 4.2). After a certain period, they are completely removed from the region and the body. Meanwhile, dense, nonporous, and inert ceramics can stay in the body without showing any biological activity or causing any adverse reactions in the body.

4.5.1 Examples for Bioceramics

Bioceramics have many superior properties such as high compressive strength, wear and corrosion resistance, and ability to serve as a polish or abrasive agent, and they can be prepared to be bioinert or bioactive. On the other hand, they also have some disadvantages such as high modulus of elasticity, low tensile strength, low fracture toughness, and difficulty of fabrication.

Bioinert bioceramics do not lead to chemical or biological reactions in the biological media and therefore can maintain their physical and mechanical properties in the host. They have reasonable fracture toughness and are generally used as structural support implants such as bone plates, bone screws, and femoral heads (Fig. 4.3).

4.5.2 Alumina

Alumina is one of the most commonly used bioinert, biocompatible and highly stable bioceramic. It has low fracture toughness, high compressive strength, high hardness, and high abrasion and wear resistance. Alumina coatings yield smooth surfaces. Meanwhile, it may have a problem in adhering to the tissue interface and therefore may lead to interfacial loosening. The stable crystal structure of Al_2O_3 is hexagonal where aluminum ions occupy the octahedral interstitial sites. Its



Fig. 4.3 Implants made from alumina (Al_2O_3) [4]

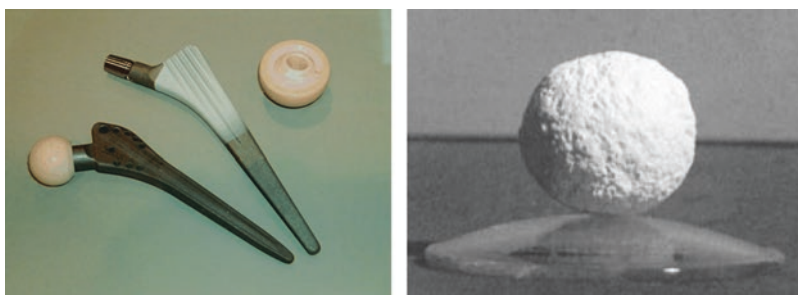


Fig. 4.4 Alumina head in hip joints (left) [5] and artificial eye implant for use after enucleation and before ocular prosthesis application (right) [6]

application areas include orthopedics such as the femoral head, joint, knee prosthesis, bone screws and plates, porous coating for femoral stems, dental crowns, bridges, and dental implants (Fig. 4.4). The main source of alumina is bauxite and native corundum.

4.5.3 Zirconia (ZrO_2)

Zirconia, like alumina, is one of the inert bioceramics. It is obtained from mineral zircon, a gemstone of many colors. Zircon is the most popular form of the zirconium mineral, and it turns into zirconium when it is chlorinated twice and then precipitated with sulfides or hydroxides, and finally it is calcined to its oxide. Zirconia has several advantages over other ceramic materials due to the transformation toughening mechanisms operating in their microstructure that can be detected in components made using them. Addition of some oxides such as MgO , CaO , CeO ,



Fig. 4.5 A zirconium based ceramic dental product [7]

and Y_2O_3 stabilizes the tetragonal crystal structure of zirconia. It has a high mechanical strength and fracture toughness. It is produced generally by hot press or by hot isostatic press processes. Zirconia is generally used in orthopedic applications such as femoral head, joint replacement, artificial knee, screws and plates, as well as dental crowns and bridges (Fig. 4.5). The main application of zirconia ceramics is in total hip replacement (THR) ball heads. Zirconia has superior wear resistance and is therefore preferred over ultrahigh molecular weight polyethylene (UHMWPE).

4.5.4 Calcium Phosphate Ceramics (CPC)

Calcium phosphate is naturally present in bone structure, and therefore calcium phosphate salts were successfully applied in replacing and augmenting bone tissue for many years. Calcium phosphate bioceramics have bioresorption and bioactivity properties. Calcium phosphate can be in many different forms, and the most widely used calcium phosphate-based bioceramics in medical applications are hydroxyapatite (HAp) and β -tricalcium phosphate (β -TCP). Their bioactivity depends on their chemical structure and degree of crystallinity. The nonstoichiometric apatite structures containing CO_3^{2-} and HPO_4^{2-} ions are highly soluble and resorbable. Similarly, tricalcium phosphate is more easily resorbed than stoichiometric apatites such as HAp. The types and the calcium to phosphate ratios of various calcium phosphate ceramics are presented in Table 4.2.

Hydroxyapatite chemistry is similar to natural apatite structure present in the natural bone. Ca to P ratio in hydroxyapatite is 1.67, higher than many phosphate ceramics. In biological media, HAp ceramics react with the ions present in the body fluid and form a surface apatite coat which induces protein adsorption and cell attachment and lead to bone formation and resorption of the biomaterial. The chemistry, composition, and crystallinity of the calcium phosphate ceramics determine their solubility and resorption rate.

Table 4.2 Calcium phosphate ceramics [8]

Compound	Chemical formula	Abbreviation	Ca/P (mol)
Monocalcium phosphate	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	MCP	0.5
Monocalcium phosphate monohydrate	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	MCPM	0.5
Dicalcium phosphate	CaHPO_4	DCP	1.0
Dicalcium phosphate dihydrate	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	DCPD	1.0
Octacalcium phosphate	$\text{Ca}_8(\text{HPO}_4)_2 (\text{HPO}_4)_4 \cdot \text{H}_2\text{O}$	OCP	1.33
Amorphous calcium phosphate	$\text{Ca}_x(\text{PO}_4)_y \cdot n\text{H}_2\text{O}$	ACP	1.1–1.5
Alpha-tricalcium phosphate	$\alpha\text{-Ca}_3(\text{PO}_4)_2$	$\alpha\text{-TCP}$ and	1.5
Beta-tricalcium phosphate	$\beta\text{-Ca}_3(\text{PO}_4)_2$	$\beta\text{-TCP}$	1.5
Calcium-deficient hydroxyapatite	$\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x} \cdot (\text{H}_2\text{O})_{2-x}$	CDHA	1.5–1.67
Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	HAp; HA	1.67
Fluorapatite	$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$	FAP	1.67
Oxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6\text{O}$	OAP	1.67
Tetracalcium phosphate	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	TTCP; TetCP	2.0

Calcium phosphate ceramics can be prepared as powders, tissue engineering scaffolds, self-setting bone cements, and as coating materials for metals and heart valves to prevent blood clotting. They are used as repair materials for bone damaged by trauma and diseases, as filling materials after resection of bone tumors, as ocular implants, as materials to repair maxillofacial and dental defects, and as herniated discs and fusion of vertebra. These ceramics are also used as nano- or microparticles and can be loaded with bioactive agents to be used as drug delivery devices. Similarly, hydroxyapatite can be used as a coating material for metallic implants, in bone repair and augmentation, in bone replacement, and as synthetic bone grafts (Fig. 4.6).

4.5.5 Bioactive Glasses (Glass Ceramics)

Glass ceramics are crystalline materials with various compositions and different properties. In the production of a specific composition, a controlled nucleation usually with two-stage heat treatment is used. Crystals of small, uniform size are obtained. Bioactive glasses are generally silica-based having SiO_4^{4-} groups, and have a place in orthopedic and dental prosthetic applications. They can be prepared as dense or porous glasses. They have excellent mechanical properties. Studies carried out both in vivo and in vitro have shown that many bioactive glasses are non-toxic. Bioactive glasses form strong chemical bonds with tissue and are therefore used in fixation of implants in the skeletal system.

Bioglasses are composed of minerals and elements that occur naturally in the body (SiO_2 , CaO , Na_2O , H , and P_2O_5), and the molecular proportions of calcium and

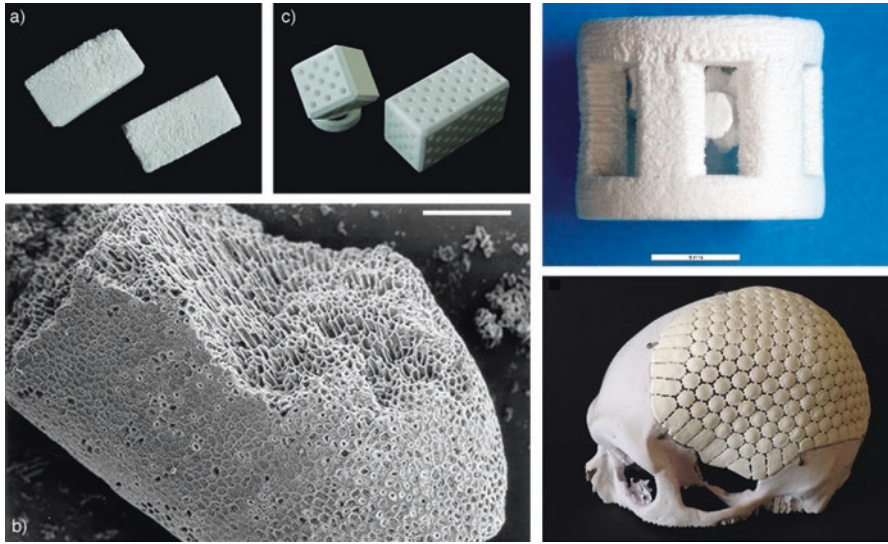


Fig. 4.6 Biodegradable ceramics [5, 9] (a) Cerabone (hydroxyapatite), (b) Aligipore (hydroxyapatite), (c) Cerasorb (synthetic phase-pure β -TCP), (d) 3D printed DCPA/monetite, (e) Craniomosaic (DCPA- 3D-printed titanium mesh)

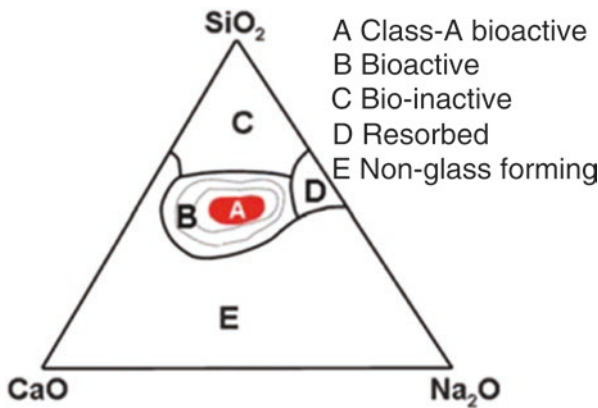


Fig. 4.7 Stability of bioactive glasses containing oxides of Si, Ca and Na based on their composition [10]

phosphorous oxides are similar to those in the bones. The chemical composition (w/w %) of the original bioglass 45S5 is 45% silica (SiO_2), 24.5% calcium oxide (CaO), 24.5% sodium oxide (Na_2O), and 6% phosphorous pentoxide (P_2O_5). In 45S5, the first number, 45, shows mass percent of SiO_2 where the last number, 5, shows the ratio of $\text{CaO}/\text{P}_2\text{O}_5 = 5:1$. If the ratio of $\text{CaO}/\text{P}_2\text{O}_5$ is less than 5, the glasses do not form bonds with bone (Fig. 4.7).

Table 4.3 Composition of bioglass and glass ceramics

Bioglass sample	Composition (mol%)				
	SiO ₂	P ₂ O ₅	CaO	CaF ₂	Na ₂ O
45S5	45	6	24.5		24.5
45S5F	45	6	12.25	12.25	24.4
45S5.4F	45	6	14.7	9.8	24.5
42S5.6	42.1	2.6	29.0		26.3
46S5.2	46.1	2.6	26.9		24.4
49S4.9	49.1	2.6	25.3		23.8
52S4.6	52.1	2.6	23.8		21.5
55S4.3	55.1	2.6	22.2		20.1
60S3.8	60.1	2.6	19.6		17.7

Ions present in the body fluids are mineralized on the surface of the bioglass implants and stimulate osteoblast adhesion, differentiation, and bone growth. The typical compositions of the bioglass and bioceramics are summarized in Table 4.3.

Depending on the combinations of silicon, calcium, and sodium oxide levels, the properties of the bioglass varies significantly and can be adjusted for a specific targeted application. Stable soda lime silica glass formulations have a SiO₂ content of 65% or more. This is essential since higher silicon amounts add durability and resistance to moisture. As the SiO₂ decreases, the stability of the bioglass decreases. It is possible, however, to stabilize glass surfaces by the incorporation of multivalent metal ions that form protective films on the glass when exposed to water or body fluids. Bioglasses have the potential to form a dynamic surface when in contact with H₂O, due to alkaline components Na₂O and CaO, the ions that can be exchanged in aqueous media. Addition of P₂O₅ to soda lime silica composition stabilizes the surface layer and results in a high rate bioactivity. Meanwhile, during the dissolution of the glass matrix, P₂O₅ is also released into the solution together with CaO which leads to formation of hydroxyapatite. Bioglasses can be bioinert, bioresorbable, or bioactive.

Studies indicated that SiO₂ is the main component responsible for apatite formation in simulated body fluid (SBF). However, silica glass and crystalline silica (quartz) showed no tendency to form an apatite layer on their surfaces when soaked in SBF for prolonged durations. Based on these findings, it may be concluded that the intrinsic properties of silica gel, silica glass, and crystalline silica and their ability to stimulate apatite formation is different. One main characteristic of amorphous silica gel is that it forms a layer containing silanol groups (Si-OH) on its surface when immersed in aqueous media. The concentration of silanol groups on silica gel surfaces was found to be much higher than on silica glass or crystalline silica. Various forms of silica glass are shown in Fig. 4.8.

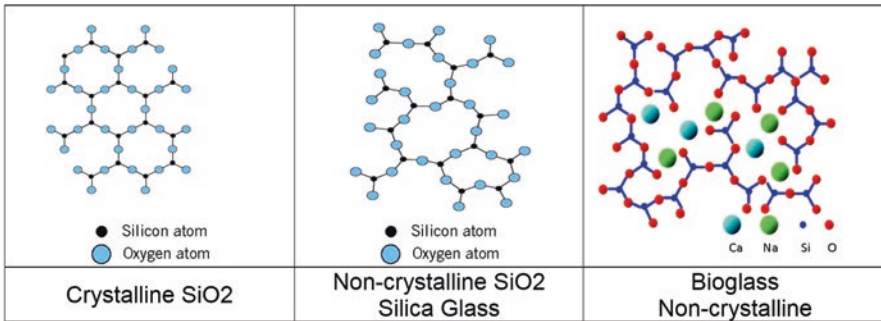


Fig. 4.8 Various forms of SiO₂ [11]

4.6 Ceramics, Bioglasses, and Composites for Biomedical Applications

Ceramics and bioglasses are used as powder, fiber, or tissue engineering scaffolds in medical applications, especially in dental and orthopedic treatments as well as composites with polymers and metals. Composites are the substances that contain two or more distinct constituent materials or phases on a microscopic or macroscopic scale. In composites the distinct phases are separated on a scale larger than the atomic, and the properties such as mechanical strength or elasticity are significantly altered in comparison with those of a homogenous material. Bone itself is a composite of collagen (organic phase), hydroxyapatite (inorganic ceramic phase), and other constituents in which the organic phase gives the elasticity and inorganic phase gives mechanical strength. The compressive and tensile strengths of cortical bone are in the range 130–180 MPa and 50–150 MPa, respectively. For porous cancellous bone these values are much lower and estimated as 4–12 MPa for compressive strength and 1–5 MPa for tensile strength.

In bioceramics, depending on their composition, the strength values vary in the range 500–4000 MPa for compression and 50–500 MPa for tension. For example, one of the strongest ceramics, alumina, the compressive and tensile strength values are about 4500 MPa and 350 MPa, respectively. For pyrolytic carbon these values are about 500 MPa for both cases.

The shape of the constituents can be particulate (with small size), fiber (with one long dimension), and platelet or lamina (with two long dimensions). The degree of adhesion of the reinforcing materials to the matrix is an important factor in the performance of composites.

For the composites to be used as biomaterials each constituent of the composite should be biocompatible, and the interface between constituents should be tight and should not degrade in the biological environment. The most common applications of ceramic containing composites are:

1. Dental filling composites (e.g., resin matrix with silica, quartz, or bioglass particles)

2. Bone cements (e.g., bone particle, calcium phosphate or carbon fiber-reinforced polymethyl methacrylate (PMMA))
3. Porous implants (e.g., polymeric scaffolds loaded with hydroxyapatite)
4. Fibrous and particulate composites for dental and orthopedic implants (Fig. 4.9)

Bioceramics have become crucial materials in our modern healthcare treatments. Their composition, microstructure, molecular composition, and surface properties vary depending on the type and process and allow them to be tailored for a specific requirement of a tissue.

Table 4.4 summarizes the types, chemical composition, and application areas of various ceramics used in medical treatments.

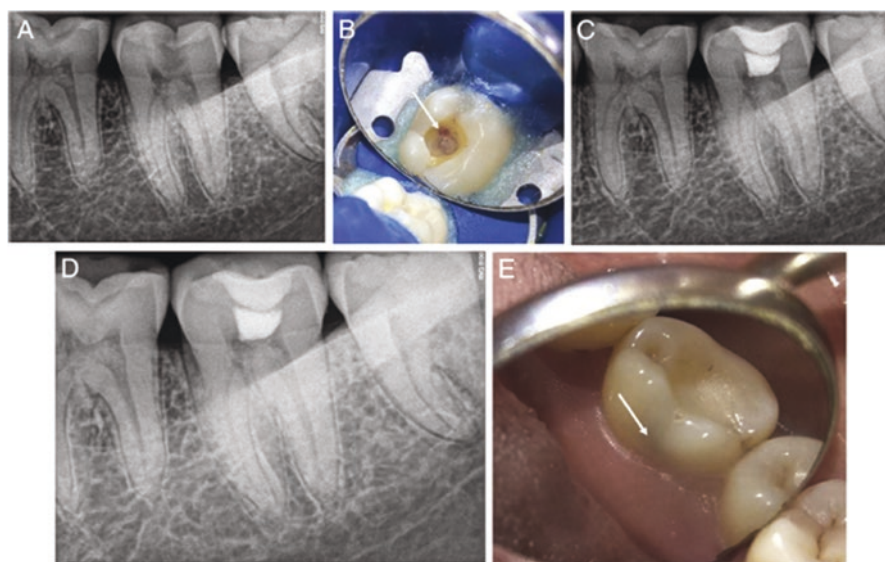


Fig. 4.9 Bioceramics used as fillers in dental applications [12]

Table 4.4 Bioceramic use and application areas

Material	Chemical composition	Application
Alumina	Al_2O_3	Joint replacement Dental implants
Zirconia	ZrO_2	Joint replacement
Tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$	Bone grafts, bone repair, bone augmentation, surface coating on metals
Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	
Bioactive glasses	$\text{SiO}_2\text{-Na}_2\text{O-CaO-P}_2\text{O}_5$	Bone replacement
Porcelain		Dental restoration
Zinc phosphates		Dental cements
Carbons (Pyrolytic carbon)		Coating for blood contacting devices
Rare earth element doped bioglasses		Therapeutic drug delivery devices

4.7 Conclusion

In this chapter, ceramics, a very important class of biomaterials were introduced. Ceramics are important because of their inertness, rigidity, compressive strength, stability, and resistance against heat and electricity conductivity. No other type of biomaterial has similar properties, making ceramics indispensable. A number of well-accepted examples of ceramics and ceramic-polymer composites, such as teeth and bones, which occur in nature were discussed and examples of the properties, production, and classification of ceramics in use within the biomaterials field were presented.

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5.1 Types of Polymerization Reactions

The chemical reaction in which high molecular weight molecules are formed from monomers is known as polymerization reaction. Polymerization can proceed according to two different mechanisms, *chain growth* (or addition) and *step growth* (or condensation) polymerization. A distinction can be made between condensation and addition mechanisms of polymerization; in condensation polymerization, two functional groups bond with each other, generally by releasing a small molecule such as H_2O , while in addition polymerization, double bonds of monomers react without releasing any molecule. However, there are some condensation reactions where no molecule release occurs such as polyurethane polymerization. Meanwhile, these two methods are not exclusive; both approaches can be used in the polymerization of the same monomer, or the same polymer can be prepared by two or more different monomers through both of these approaches, as long as suitable groups are available for individual polymerizations. Nylon 6 is a polymer with repeating units $-NH(CH_2)_5CO-$ and can be synthesized either from 6-aminocaproic acid by condensation or from caprolactam by addition polymerization [1]. There are also some new polymerization techniques such as click polymerization, atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer polymerization (RAFT) which became popular in the last decades. In these techniques, some special catalysts and agents are used to obtain specially designed polymers with controlled molecular weights (Fig. 5.1).

5.1.1 Chain Growth (Addition) Polymerization

In chain growth polymerization, reactions occur by addition of monomer molecules to each other via unsaturated (double) bonds. The most important group of chain growth polymerizations is a combination of vinyl monomers such as ethene (ethylene), propene, styrene, and vinyl chloride which form polyethylene, polypropylene,

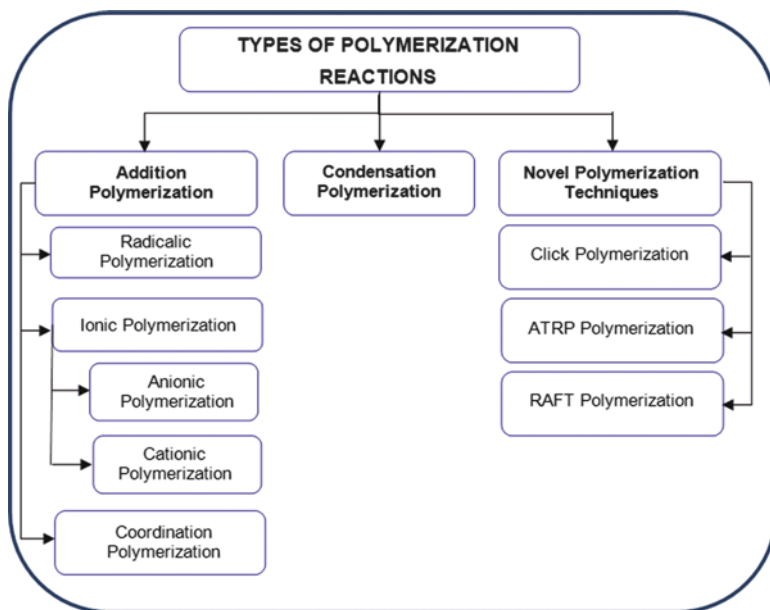
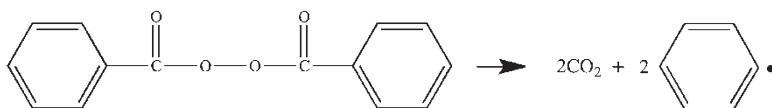


Fig. 5.1 Types of polymerization reactions

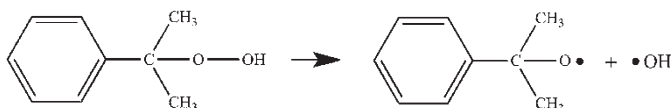
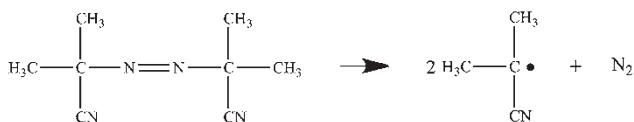
polystyrene, and polyvinylchloride, respectively. In chain growth polymerization, a *reactive center* such as a radical, an anion, or a cation must first be created on a molecule which has a double bond. New monomer molecules are then added successively to this active molecule creating a new active center for further additions. This continues until some other reaction or event terminates the reaction. Termination produces “dead” polymer which has no activity to react further. In these reactions, chain growth must be initiated and usually involves a termination reaction. The initiating species can be a chemical molecule (as azo compounds, peroxides, etc.) or any other physical source (as heat or electromagnetic radiation). These initiators create a radical, anion, or cation on the monomer. Depending on the type of initiation, chain growth polymerizations can be classified as radical, anionic, cationic, or coordination polymerization. All chain growth polymerizations share three common steps: initiation, propagation, and termination.

The most common chain growth polymerization is free radical polymerization. Most radical polymerizations need an initiator to produce the first active radical and thus start the chain of addition reactions. The most common initiation reaction is the thermal decomposition of molecules containing weak bonds, e.g., peroxides ($-O-O-$) or azo compounds ($-N=N-$).

Some examples of initiators used for chain growth mechanisms are given in Fig. 5.2. The radicals formed then react with the monomers. Once initiated, a chain will grow by repeated additions of monomer molecules with simultaneous creation of a new radical site. This propagation is very fast, so very long polymer chains will form at the earliest stages of the reaction. Termination can occur by disproportionation or combination.

Benzoyl Peroxide (Bz_2O_2)

Cumyl Hydroperoxide

 $\text{N,N}'$ -Azobisisobutyronitrile (AIBN)

Electromagnetic Radiation



Redox Initiators

**Fig. 5.2** Examples of free radical initiation reactions

In case of disproportionation, polymer chain ends through transfer of either an electron or an atom from one to the other and both chains become dead polymers. Meanwhile, in combination, two active ends connect to each other, and in this type of termination, chain length will be the sum of the two growing chains.

For ionic polymerizations, the reactive center may be a negatively charged carbanion or a positively charged carbonium ion generated by intermolecular transfer of an electron or proton, respectively. Depending on the charge, the overall reaction is called anionic or cationic polymerization. However, in ionic polymerizations, termination by combination of two chain carriers with one another cannot occur because charges with the same sign repel one another. As a result, reactive centers may be left behind when all the monomers are used up. Therefore, these polymers are named as “living polymers.”

In coordination polymerization monomers have double bonds, and their polymerization is initiated by attachment of a monomer molecule to a metal complex. The polymer grows by successive insertion of monomer molecules to the growing chain. Monomer approaches the empty orbital of the metal center and therefore assumes a certain orientation which leads to the formation of “stereospecific polymers.”

5.1.2 Step Growth Polymerization

Step growth polymerization involves a reaction between two different functional groups of monomers. Functional groups can undergo a condensation reaction where the polymerization is called step growth polymerization. Most condensation reactions result in the elimination of a small molecule like water or methanol. It applies to monomers with functional groups such as $-\text{COOH}$, $-\text{COOR}$, $-\text{COOOC}-$, $-\text{COCl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{CHO}$, $-\text{NCO}$, epoxy, etc. Reactions can be carried out either by using one type of monomer which has two kinds of reactive groups (as A–B) or two different monomers each with one type of reactive group (as A–A and B–B). In condensation polymerization, reactive groups on the two ends of each monomer react with one another. The reactions are usually similar to the low molecular weight organic chemistry reactions such as esterification (formation of a bond between an alcohol and a carboxylic acid) or amide (formation of a bond between an amine and a carboxylic acid) formation. A growing chain has reactive groups at each end of the chain, and when two chains join, the length of the chain instantly becomes larger. Some examples of commercially important step growth polymerization reactions are presented in Fig. 5.3.

In chain growth polymerization, the completion of polymerization of an individual chain is very rapid, and a high molecular weight product is obtained even at the very beginning. The amount of monomer in the medium decreases slowly with time. However, in step growth polymerization, the increase in molecular weight of the product is slow because growing chains add to each other; thus depletion of the monomers occurs at the beginning. However, unlike chain polymerization, large molecules do not form at the beginning, and the molecular weight of the final polymer is usually not as high. Chain growth polymerization is generally rapid, and it is moderately or highly exothermic. On the other hand, step growth polymerization is usually slow, limited by the equilibrium, and slightly exothermic.

5.1.3 Click Polymerization

Click polymerization reactions are fast and irreversible reactions in which specific polymers such as highly stereoregular, regioselective, linear, or hyperbranched polymers with high efficiency and with required functionalities are produced under mild reaction conditions. The first click reactions were carried out by Sharpless and co-workers in the studies of Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reactions [2]. In these reactions, the efficiency of the reaction is almost 100%, and there are almost no side products. The polymerization technique can be achieved by using different catalysts. The types of these reactions can be Cu(I)-catalyzed azide and alkyne polycycloaddition, 1,3-dipolar polycycloaddition of nitrile N-oxides and azides, thiol-based click polymerizations, or Ru(II)-catalyzed click polymerizations. A scheme for click reactions is given in Fig. 5.4.

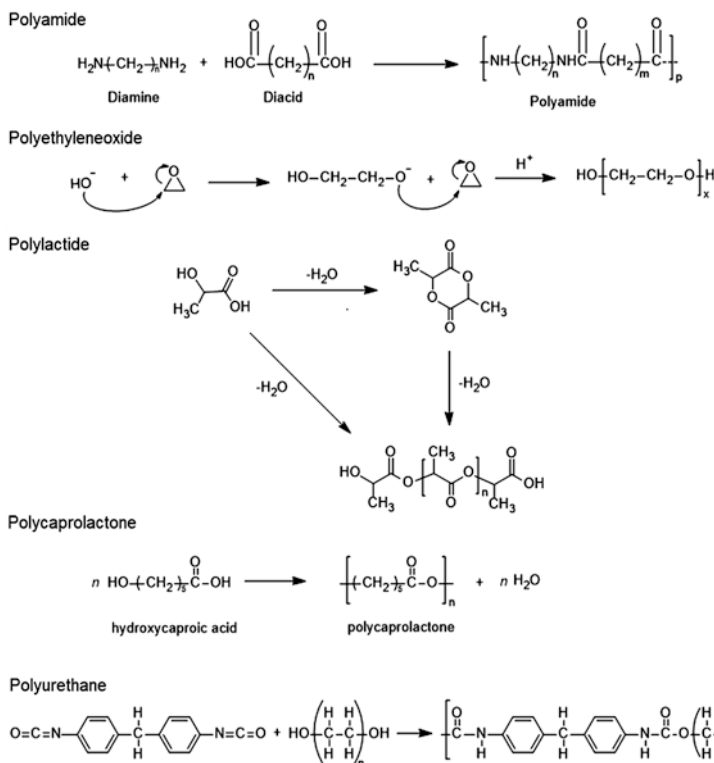


Fig. 5.3 Examples of important step growth polymers

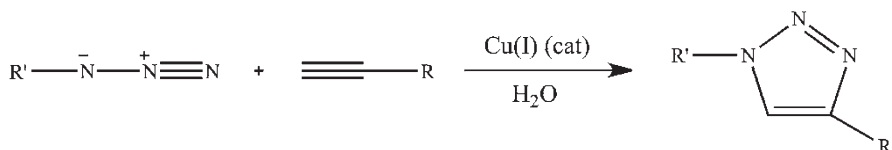


Fig. 5.4 Click reactions

Click polymerization is widely used in the synthesis of poly(triazole)s (PTAs) where the main chain contains some heteroatoms besides C-C bonds and this adds some extra properties to the chains similar to those of the biological macromolecules (such as proteins). It is also possible to introduce certain properties such as polarity, flexibility, or electrical charge to the polymers and tissue engineering scaffolds via click polymerization. Click chemistry has therefore found a wide range of applications especially in bioconjugation. It was also used in surface modifications of nanoparticles, modification of backbone structure of polymers, synthesis of hyperbranched polymers and dendrimers, and synthesis of hydrogels.

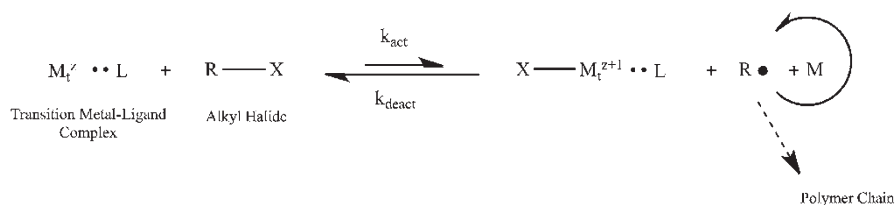


Fig. 5.5 Mechanism of metal complex-mediated ATRP

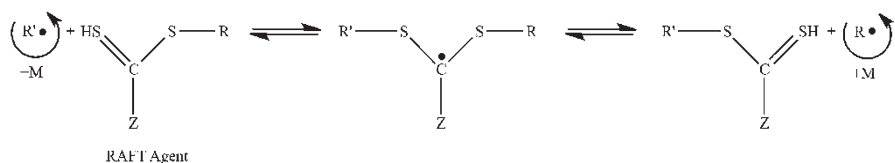


Fig. 5.6 Mechanism for reversible addition-fragmentation chain transfer (RAFT)

5.1.4 ATRP Polymerization

Atom Transfer Radical Polymerization (ATRP) is a highly controlled radical polymerization and uses alkyl halide initiators and transition metal complex catalysts. Monomers like styrene, methyl acrylates, and acrylonitriles can be polymerized with the required molecular weight. Transition metal complexes such as Cu, Fe, Ru, Ni, and Os can be used as catalyst. In the reaction, equilibrium is established between the neutral dormant species and their activated form [3]. The radicals created go to polymerization reaction by adding monomers, while there is reversible halogen transfer between the dormant molecule and growing chain and the transition metal complex (Fig. 5.5). ATRP reactions are complex reactions. The transition metal complex has two or more oxidation states during the reaction, and the initiators have one or more radically transferable atoms or groups. Therefore, reaction medium and the environmental parameters affect the equilibrium among all species very easily. By RAFT process, preparation of well-defined block and graft copolymers or telechelic polymers and oligomers which have di-end functional groups is possible. Synthesis of polymers with well-defined compositions is possible by using telechelic polymers.

5.1.5 RAFT Polymerization

Reversible addition-fragmentation chain transfer (RAFT) polymerization is a reversible-deactivation radical polymerization. These reactions use RAFT agents which are generally thiocarbonylthio compounds (dithioesters, thiocarbamates, xanthates, etc.) as chain transfer agents (Fig. 5.6). It is possible to obtain polymers with designed complex structures such as star, comb-like, brush, dendrimers, linear, branched, or cross-linked. The process is not complex, and it is a radicalic

polymerization reaction and only needs the use of RAFT agents. The important point is its applicability to water-soluble polymers and copolymers. By using hydrophilic polymers, it becomes possible to prepare hydrogels which can be used in medical area as cell or drug carriers [4].

5.2 Polymerization Techniques

Polymerization reactions can be carried out in various media. Some of them are presented in the following sections.

5.2.1 Bulk Polymerization

The simplest technique and the one that produces a polymer with highest purity is bulk polymerization. Only a monomer, an initiator soluble in the monomer, and a chain transfer agent to control molecular weight are used. This technique has a high yield, ease of polymer recovery, and even the option of casting the product starting with the polymerization mixture. It, however, has limitations such as the difficulty of removing unused monomer trapped in the polymer and the exothermically produced heat during polymerization. The heat produced by free radical polymerizations is quite high, and about 42–88 kJ mol⁻¹ is released during the reaction. This kind of heat release becomes a serious problem in the application of bone cements.

5.2.2 Solution Polymerization

This polymerization reaction takes place in a solvent. The heat evolved during the polymerization process can be dissipated into the organic or aqueous solvent. The obvious disadvantage of solution polymerization is the need for a solvent recovery system, but the resultant products are usually more homogeneous and pure. Many free radical and ionic polymerizations can be conducted in solution. Important water-soluble polymers that can be synthesized in an aqueous solution include polyacrylic acid (PAA), polyacrylamide (PAAm), polyvinyl alcohol (PVA), and poly(N-vinylpyrrolidone) (PVP). On the other hand, poly(methyl methacrylate) (PMMA), polystyrene (PS), polybutadiene (PB), poly(vinyl chloride) (PVC), and poly(vinylidene fluoride) (PVF) are polymers that can be prepared in organic solvents.

5.2.3 Suspension Polymerization

Suspension polymerization is a heterogeneous polymerization technique where there are organic and aqueous phases. Heat removal can be improved when the polymerization is conducted in a suspension because the droplets have a high

surface area-to-volume ratio making heat dissipation easier. In suspension polymerization, which is also called “bead” or “pearl” polymerization, a water-insoluble monomer and an initiator are present in a continuous aqueous phase. Droplets of monomer containing the initiator, typically 100 nm to 5 mm in diameter, yield spherical polymer beads of this size. The size of the particles can be controlled by adjusting the volume fraction of the monomer, stirring speed of the medium, and the stabilizer concentration.

5.2.4 Emulsion Polymerization

Emulsion polymerization is also a heterogeneous reaction with two phases. In addition to water and monomer, the reaction medium contains a water-soluble initiator, a chain transfer agent to control polymer molecular weight, and an emulsifier (surfactant), a molecule such as a salt of a long fatty acid chain. Under these conditions, the hydrophobic monomer molecules form large droplets that are stabilized by the surfactant molecules. Polymerization starts in the aqueous phase, and the growing polymer is stabilized by emulsifier. As the polymerization proceeds, monomer droplets get smaller, and the polymer chains are stabilized in the form of micrometer-size particles. Polymer particles produced need purification.

5.3 Polymer Types

Polymers to be used in medical applications should be compatible with the biological media, blood, tissues, or organs. They should not have any toxic, allergic, or carcinogenic effects. Some common addition and condensation polymers used for medical purposes are given in Table 5.1.

5.3.1 Linear, Branched, and Cross-linked Polymers

From a structural perspective, some polymers are linear, some are branched, and some others are cross-linked (Fig. 5.7). Linear polymers, such as polyethylene, polyvinyl chloride, Nylon 66, and polymethyl methacrylate, are long chains consisting of monomers connected end to end. A branched polymer can be visualized as tree branches. These branched polymer structures do not connect with other polymer molecules. Some examples of branched polymers include star polymers, comb polymers, brush polymers, and dendrimers. A cross-linked polymer, sometimes called network polymer, is one in which different chains are connected to each other. Essentially, the branches are connected to other branches at the ends and form an infinite molecular weight network. A polymer chain that is linked to a neighboring chain is said to be cross-linked. These polymer structures are shown in Fig. 5.7.

Table 5.1 Some common polymers used in the medical area

Addition polymers		
Name(s)	Formula	Monomer
Polyethylene (PE)	$-(CH_2-CH_2)_n-$	Ethylene; $CH_2=CH_2$
Polypropylene (PP)	$-[CH_2-CH(CH_3)]_n-$	Propylene; $CH_2=CHCH_3$
Poly(vinyl chloride) (PVC)	$-(CH_2-CHCl)_n-$	Vinyl chloride; $CH_2=CHCl$
Poly(vinylidene chloride) (Saran A)	$-(CH_2-CCl_2)_n-$	Vinylidene chloride; $CH_2=CCl_2$
Polystyrene (PS)	$-[CH_2-CH(C_6H_5)]_n-$	Styrene; $CH_2=CHC_6H_5$
Polyacrylonitrile (PAN, Orlon, Acrilan)	$-(CH_2-CHCN)_n-$	Acrylonitrile; $CH_2=CHCN$
Polytetrafluoroethylene (PTFE, Teflon)	$-(CF_2-CF_2)_n-$	Tetrafluoroethylene; $CF_2=CF_2$
Poly(methyl methacrylate) (PMMA)	$-[CH_2-C(CH_3)(CO_2CH_3)]_n-$	Methyl methacrylate; $CH_2=C(CH_3)CO_2CH_3$
Poly(vinyl acetate) (PVAc)	$-(CH_2-CHOCOCH_3)_n-$	Vinyl acetate; $CH_2=CHOCOCH_3$
Condensation polymers		
Name(s)	Monomer 1	Monomer 2
Polyamide	Dicarboxylic acid	Diamine
Polyamide	Amino acid	Amino acid
Polycarbonate	Bisphenol	Phosgene
Polyester	Dicarboxylic acid	Diol, Polyol
Polyimide	Tetracarboxylic acid	Diamine
Polysiloxane	Dichlorosilane	Water
Polysulfone	Bisphenol	Dichlorophenylsulfone
Polyurethane	Diisocyanate	Diol, polyol
Polyphenylene oxide	2, 6-Dimethyl phenol	Oxygen (via oxidative coupling)

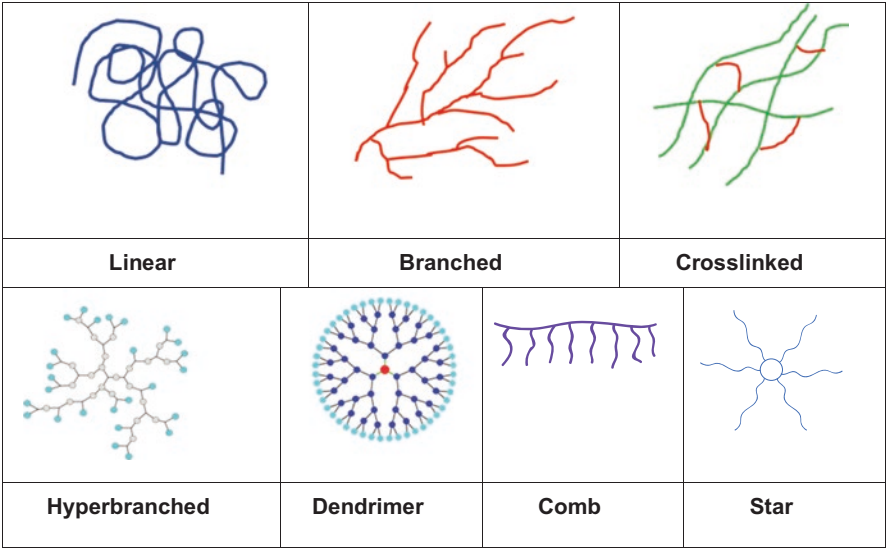


Fig. 5.7 Different polymer geometries

5.3.2 Thermoplastics, Thermosets, and Elastomers

Polymers are commonly defined by their thermal properties, especially their response against heat. Thermoplastics are polymers composed of independent, linear, or branched molecules which can melt (e.g., polyethylene, PMMA) upon heating above their melting temperature and processed (molded, extruded) by heating. Thermosetting polymers are those with unsaturation or other reactive groups which upon heating form covalent links between chains. They cannot be remelted once solidified (e.g., silicone elastomer) [5]. If the number of these bonds between the chains, the cross-links, is few, the polymer acts as an elastic material and stretches reversibly upon application of tensile forces. These materials are called elastomers.

5.3.3 Hydrogels

Hydrogels are the most attractive tissue engineering scaffolds due to their structural and functional similarities to the natural extracellular matrices (ECM). Hydrogels are interconnected networks consisting of hydrophilic (or amphiphilic) polymers that are made insoluble via cross-linkages between separate polymer chains and thus have infinite molecular weight [6]. In many cases, hydrogels are produced by covalent linkages to yield long-term service lives, but weaker and reversible linkages like those using ions or hydrogen bonds are also possible. Hydrogels can be formed starting from water-soluble monomers and polymers. When the gel formation is aimed through cross-linkage, the cross-linking reagents used must contain at least two functional groups and should be water soluble to ensure proper mixing of the reactants. Radical polymerization is the most widely used method in the synthesis of covalently cross-linked hydrogels where vinyl monomers and multivinyl group carrying cross-linkers such as ethylene glycol diacrylate (EGDA) are used.

Alternatively, modified polymers can be prepared by adding methacrylic acid or its derivatives to the end groups or the repeating units. Radicals can be generated via the reaction between the oxidizing (e.g., a persulfate) and reducing reagents (e.g., N,N,N',N'-tetramethyl ethylenediamine, TEMED). Photoinitiators, such as 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651), 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-propanone (Darocur 2959), and Eosin Y, photochemically (e.g., UV) decompose to generate free radicals [7]. Created radicals immediately take part in the cross-linking reactions through the vinyl groups on the cross-linkers or the polymers. Radically cross-linked hydrogels have been prepared from a variety of monomer/polymer sources, including methacrylic acid (MMAc), 2-hydroxyethyl methacrylate, N-vinylpyrrolidone, N-isopropylacrylamide, dextran, hyaluronic acid (HA), (hydroxyethyl)starch, poly(vinyl alcohol) (PVA), oligo[poly(ethylene glycol) fumarate] (OPF), and poly(ethylene glycol) (PEG). If the initiator and the wavelength of the light are carefully tuned, photocrosslinking allows for in situ cell encapsulation in a spatial and temporal fashion with minimal DNA damage and cell toxicity [8].

In addition, functional groups are also used in the cross-linkage of biological molecules like proteins and polysaccharides especially using the carboxylic acid ($-\text{COOH}$) and amine (NH_2) groups available on the molecules. One such group with ability to be cross-linked is lysine (HOOC-CHR-NH_2), an amino acid where the R group is $-(\text{CH}_2)_4\text{-NH}_2$, and it is cross-linked by molecules like glutaraldehyde as cross-linker of two amino groups on separate protein chains.

Similarly, in the presence of an appropriate activating reagent, such as N,N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC), polymers carrying carboxylic acid groups can be cross-linked by molecules with amine groups, forming amide bonds at the cross-linking points. The carbodiimide chemistry has been frequently employed to stabilize reconstituted collagen gels by the coupling reaction between the carboxylic acid groups (in glutamic acid and aspartic acid residues) and lysine amines, forming zero length cross-links. This method has been successfully applied to fabricate transparent, strong hydrogels as corneal substitutes [9].

5.4 Properties of Polymers

In any biomaterial application, the composition and structure of a candidate biomaterial relevant to the properties under consideration should be evaluated. The thermal properties of a biomaterial are very important as the physical, electrical, mechanical, chemical, and biological properties. Thermal properties provide information on stability of form, effect of sterilization methods, processing and shaping, and storage. The thermal and mechanical properties of some biodegradable polymers are summarized in Table 5.2.

5.4.1 Conducting Polymers

Conducting polymers have electrical properties similar to those of metals and inorganic semiconductors [10]. Research on conducting polymers for biomedical applications expanded significantly upon discovery that these materials could be

Table 5.2 Thermal and mechanical properties of some biodegradable polyesters

Polymer	T_g (°C)	T_m (°C)	Tensile strength (MPa)	Tensile modulus (MPa)	Elongation at break (%)
Poly(L-lactic acid) (PLA)	54– 59	159– 178	28–50	1200–3000	2–6
Polyglycolic acid (PGA)	35	210	–	–	–
Poly(3-hydroxybutyrate) (PHB)	1–2	170	36	2500	2
Poly(3-hydroxybutyrate-co- 3-hydroxy valerate) (PHBV)	2	145	20	1100	17
Polycaprolactone (PCL)	–60	60	15	400	80

biocompatible and used in a variety of biomedical applications such as biosensors. It was later discovered that upon electrical stimulation, cellular activities such as cell adhesion, migration, DNA synthesis, and protein secretion could be manipulated [11]. These are especially important in nerve, bone, muscle, and cardiac cell studies where activity is dependent significantly on electrical impulses. Among these, polypyrrole (PPy) and polyaniline (PANI) have been used in nerve regeneration research. The success of neural tissue engineering is mainly based on signal transmittance along the axons of the cells, and thus conductivity is a tool for activation of cell growth as well as in the measurement of the level of healing or regeneration. For example, during regeneration, neurite outgrowth could be induced and measured as a result of the conductivity of a nerve guide [12].

Polypyrrole is a well-known conducting polymer and has been found to enhance nerve regeneration by electrical stimulation [13]. The structures of conducting polymers polythiophene, polyaniline, and polypyrrole are shown in Fig. 5.8. Polyacetylene is the first documented conducting polymer; polypyrrole, polythiophene, poly(3,4-ethylenedioxythiophene), and polyaniline are the most commonly used conducting polymers. Blends of PPy/PDLLA/PCL were used to bridge a gap of 8 mm in a rat sciatic nerve study and were shown to promote nerve cell proliferation and axon regeneration when electrical stimulation was applied. The mobility in the operated limb of the rats was gradually restored over a 2-month period. Moreover, the immunohistological analysis and transmission electron microscopy of harvested implants demonstrated the presence of newly formed myelinated axons and Schwann cells similar to that of native nerve [14].

Most conducting polymers have a number of important advantages in biomedical applications including biocompatibility and ability to release bioactive agents (drugs, growth factors) on demand. These unique characteristics are useful in many biomedical applications. Although they are at the experimental stage, intense research is continuing on the use of conductive polymers in the design and production of devices such as biosensors, tissue engineering scaffolds, neural probes, and drug delivery systems.

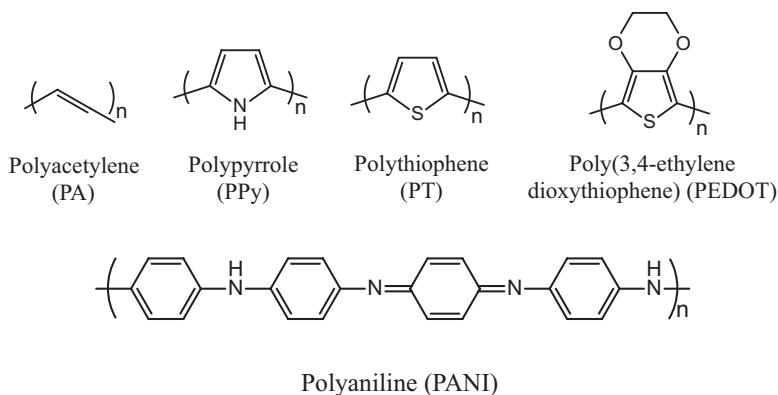


Fig. 5.8 Chemical structures of commonly used conductive polymers

5.4.2 Shape Memory Polymers

In the recent years, biomaterials called “stimuli responsive,” “intelligent,” or “smart” are increasingly being considered for numerous clinical applications. The shape memory polymers (SMP) undergo some structural changes (e.g., form, dimension) when exposed to external stimuli such as temperature or pH changes, or exposure to electromagnetic radiation (UV) or magnetic forces. These physical or chemical changes of the structures make these polymers very useful in on-demand drug delivery and minimally invasive procedures.

Different swelling levels in solutions with different pHs is used to design responsive drug delivery systems. For example, a drug can be loaded when the material is in its most swollen form, and then it can be introduced to a medium where it shrinks, thus decreasing the rate of release. Or the reverse can be done, and when the pH change is made, the release becomes faster leading to “on-demand” release. Although there are quite a large number of studies on SMPs, only a few are successfully translated to commercial medical devices.

5.4.3 Degradation/Deterioration

Degradable polymeric biomaterials are preferred candidates for developing temporary therapeutic devices such as 3D scaffolds for tissue engineering and controlled release drug delivery vehicles. As a result, a wide range of natural or synthetic biodegradable polymers are continuously being searched and investigated for biomedical applications. One important reason for the consideration of biodegradable materials over biostable ones for biomedical applications is the long-term compatibility risks observed with many of the current stable implants and the issues related with revision surgeries [15]. Besides the recent novel biomedical technologies such as tissue engineering, regenerative medicine, gene therapy, controlled drug delivery, and bionanotechnology, all require biodegradable materials in their preparation. The properties of a biodegradable material vary with time, and their degradation products can have different levels of compatibility issues compared to the starting parent material. Some of the important properties required of biodegradable polymers are as follows [16]:

- The polymer should not cause any acute or chronic inflammatory response after implantation in the body.
- The degradation time and change in the properties of the polymer should match the healing of the tissue.
- The degradation products should not be toxic; they should be metabolized and cleared from the body.

Degradable biomaterials have bonds that are cleavable under normal physiological conditions, and in order to obtain a biodegradable material, hydrolytically and/

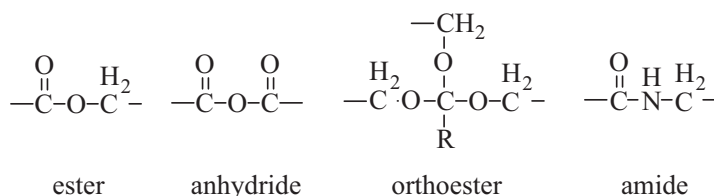


Fig. 5.9 Molecular chain structures sensitive to hydrolysis by water and/or enzymes

Table 5.3 Parameters influencing the degradation rate of polymers

Structural parameters	Chemical composition Molecular weight and its distribution Presence of polar and ionic groups Presence of hydrophilic functional groups Presence of branches or side chains
Physical parameters	Shape and surface roughness of the device Inhomogeneity of the material Presence of micro or macro cracks on the device Processing and thermomechanical history
Environmental factors	Sterilization process Implantation site Adsorbed or absorbed chemicals Ionic strength and pH of the media Hydrolytic environment Enzymatic environment

or enzymatically degradable bonds such as ester, anhydride, orthoester, amine, etc., should be present in the material (Fig. 5.9).

Hydrolytically degradable polymers have hydrolytically labile chemical bonds in their backbone. The functional or chain backbone groups susceptible to hydrolysis include esters, orthoesters, anhydrides, carbonates, amides, urethanes, ureas, and others [17, 18]. Hydrolytic degradation is possible throughout the body as it is abundant in the tissues. Most polymers, especially those produced by biological systems, are enzymatically degradable, but for the biomedical field, degradation by the enzymes of the human body is our main focus. In the body, enzymatic hydrolysis can take place especially at sites where the specific hydrolytic enzymes have a concentration above a threshold. The degradation rate of hydrolytically cleavable bonds can be increased substantially by enzymes, and therefore, the degradation rate differs significantly at different sites in the body (Table 5.3).

Degradation involves breakage of bonds, a process which can take place at the side chains or the backbone of the polymer chains. In the latter case, oligomers and monomers are formed. On the other hand, erosion is material loss arising in the polymer (even without degradation). Two mechanisms exist for the hydrolytic biodegradation of polymers: (i) bulk degradation that happens throughout the structure of the polymeric product, and (ii) surface erosion, where water cannot penetrate the bulk and its action stays limited to the surface. Polyanhydrides, for example, degrade

Table 5.4 Hydrolytically and enzymatically degradable polymers as biomaterials

Hydrolytically degradable polymers	Enzymatically degradable polymers
Poly(α -esters)	Proteins and poly(amino acids)
Poly(glycolic acid) (PGA)	Collagen
Poly(lactic acid) (PLA)	Gelatin
Poly(lactide-co-glycolide) (PLGA)	Cyanophycin
Polydioxanone (PDS)	Poly(ϵ -L-lysine)
Polycaprolactone (PCL)	Poly- γ -glutamic acid (γ -PGA)
Bacterial polyesters	Silk fibroin
Poly(3-hydroxybutyrate)(PHB)	Poly(L-glutamic acid) (L-PGA)
Polyurethanes	Poly(aspartic acid) (PAA)
Poly(ester amide)	Elastin
Poly(orthoesters)	Albumin
Polyanhydrides	Fibrin
Poly(anhydride-co-imide)	Polysaccharides
Cross-linked polyanhydrides	Hyaluronic acid (HA)
Poly(propylene fumarate)	Chondroitin sulfate
Poly(alkyl cyanoacrylates)	Chitin and chitosan
Polyphosphoester	Alginate acid

by surface erosion. The device gets smaller over time, and if it is loaded with drugs, the drug is released as the surface degrades. By altering the material, the rate of degradation of the delivery system and therefore the rate of drug release can be controlled. Bulk degradation occurs when the rate of water penetration is faster than the rate of degradation. Initially the water penetrates the device and begins degrading the entire device. This causes the device to fragment into smaller pieces. Polyesters such as PLA, PGA, and copolymers of PLA/PGA undergo mainly bulk erosion, i.e., the polymeric matrices degrade all over their cross section and have erosion kinetics that are nonlinear [19, 20]. This kind of degradation does not just depend on the chemistry of the device but also on the form. Thin fibers or porous products degrade faster than solid blocks of material.

Poly(α -ester)s are thermoplastic polymers with aliphatic ester linkages in their backbone that can be hydrolyzed (Table 5.4). All polyesters are theoretically degradable as esterification is a condensation reaction and, as such, chemically reversible in water. However, aliphatic polyesters with relatively short chains between ester bonds can degrade over a time frame appropriate for most biomedical applications. For biodegradable polymers, water diffusion is normally faster than hydrolysis. Therefore, water penetrates the polymer and initiates the bulk erosion process. The hydrolysis process leads to chain scission. Ester hydrolysis can be both acid and base catalyzed.

In addition to hydrolytic degradation, most naturally occurring polymers can also undergo enzymatic cleavage. The rate of *in vivo* degradation of enzymatically degradable polymers, however, varies significantly with the site of implantation because the concentration of the relevant enzymes varies with the site in the body. Rate of degradation can be controlled by chemical modification of these polymers.

Table 5.5 Application areas of polymers

Polymer	Application area
Polyethylene	Joints, birth control spirals
Polyethylene terephthalate	Blood vessels, heart valves, coatings
Polyalkyl sulfone	Oxygenator membranes
Polytetrafluoroethylene	Blood vessels, oxygenator membranes
Polycyanoacrylate	Tissue adhesive
Polyvinyl chloride	Blood bags, medical tubing
Polymethyl methacrylate	Hard contact lenses, intraocular lenses, dental fillings, bone cements
Polyhydroxyethyl methacrylate	Soft contact lenses, burn dressings, drug release systems, prosthesis coatings
Polyvinylpyrrolidone	Plasma extender
Polyacrylamide	Electrodes
Polyglyceryl methacrylate	Eye prosthesis
Polyvinyl alcohol	Plasma extender, drug release systems
Cellulose, cellulose acetate	Dialysis membranes
Polycarbonate	Dialysis membranes
Polyurethane	Heart supporting systems, skin grafts
Polysiloxanes	Oxygenator membranes
Polysilicones	Soft tissue prostheses, heart support systems, burn dressing, coatings
Pyrolytic carbon	Heart valve, dental fillings

Hydrolytically degradable synthetic polymers are generally preferred as implants due to their much lower site-to-site and patient-to-patient variation compared to enzymatically degradable polymers [21]. Extensive research has been done over time to custom designing of biodegradable polymer systems with predictable erosion kinetics such as drug/gene delivery vehicles or scaffolds for tissue engineering.

One of the parameters that influences the rate of degradation is surface area; the higher the surface area, the higher is the rate of degradation. The number of hydrolytically cleavable bonds in a macromolecule also affects the hydrolysis rate. The molecular weight and polydispersity as well as the crystallinity and morphology of the polymers are important factors in the biodegradation of polymer.

The degradation of semicrystalline polymers proceeds in two phases; in the first phase, the amorphous regions and then, in the second phase, the crystalline regions are hydrolyzed [22, 23].

Areas of application of some polymers in the medical sector are presented in Table 5.5.

5.5 Conclusion

Polymers are very versatile materials, and, in this chapter, the various types of polymerization reactions, polymerization techniques, and properties of polymeric materials used in the medical area were discussed. The properties and forms of

polymers range from very soft to hard, elastomeric to rigid, and porous to fibrous. Polymers can be processed into many different physical forms, such as fibers, sheets, tubes, membranes, and plates. They are also very economical and well-suited to further modifications, depending on the requirements of the targeted biomedical device. Their ease of processing, high biocompatibility, and adjustable degradability make polymers the preferred materials in many medical applications. As such, they are used in the production of artificial heart valves, blood vessels, blood oxygenators, contact and intraocular lenses, catheters, controlled release devices, dental implants, dialysis membranes, drainage tubes, heart valves, and orthopedic and dental support materials.

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6.1 General Properties

Carbon is an element found abundantly in the Earth's crust and in the human body. The various bonding capabilities enable it to form so many different varieties of compounds including the many gases, liquids, and solids. The carbon compounds constitute the nutrients, the organic energy sources, the building materials for plants, and many other molecules in the body. Since all living species are hydrocarbon based, carbon basically is the element of life if water is the molecule of life. Carbon-derived compounds like diamond, graphite, and graphene are made of only one element, and the method of their production is different than the commercially available ceramics since the melting temperature of carbon is very high.

The carbon-based materials are very diverse; even though they are formed only of carbon, they can be very soft like graphite and very hard like diamond. This difference originates from the chemistry and the organization of carbon atoms; they can be amorphous or highly ordered crystalline forms. They have the ability to form molecules with a broad range of properties, and this makes carbon an indispensable element for the biomaterials field (Fig. 6.1).

6.2 Pyrolytic Carbon (PC)

Pyrolytic carbon is obtained by thermal decomposition of gaseous hydrocarbons at high temperatures. Making of the coal in the traditional way is also a method of pyrolysis. Within graphite, one s-orbital and two p-orbitals undergo a sp^2 hybridization, and the geometry is trigonal planar. Pyrolytic carbon has a structure similar to that of graphite, which consists basically of sp^2 -bonded carbon atoms. In both compounds the carbons form hexagonals which constitute layers. These layers are stacked on top of each other and held together by weaker interlayer bonds. Pyrolytic carbon differs by the lower size of the layers and the disorder of the layers which

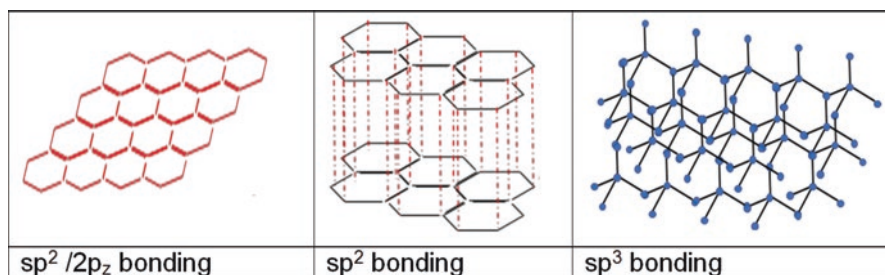


Fig. 6.1 Structures of (a) graphene sheets, (b) graphite, and (c) diamond

lead to distortions within and between layers. Due to the lack of perfect layering, shear forces cannot slide layers past each other, and this gives pyrolytic carbon the glassiness and improved durability compared to graphite. Some mechanical properties of pyrolytic carbon are the following: tensile strength, 100–120 MPa; flexural strength 300–400 MPa; compressive strength 450–550 MPa; and the modulus of elasticity 23–29 GPa [1].

The properties of pyrolytic carbon (PC) films are determined by the mobility of the carbon fragments on the growing surface (graphite) at the time of their deposition. The higher the mobility, the larger is the crystallite size and the better is the crystallite orientation. The crystallites of high quality PC are well aligned relative to each other and to the substrate surface. As a result of good crystallite alignment and high compaction, the PC can have densities as high as 2.22 g/cm³, which is close to that of graphite (2.26 g/cm³). The density of PC varies markedly with processing conditions including deposition temperature and gas pressures.

The parallel layers of the crystallites have crosslinks between them. If the crosslinking is minimal, it forms lubricating graphite, and the high level of crosslinking forms pyrolytic carbon. Graphite is stable under ambient temperature and pressure and can be converted into diamond at high temperature and pressure.

General advantages of pyrolytic carbon are high strength, high wear resistance, and therefore durability, biocompatibility (initiation of no adverse responses in the body), and hemocompatibility (no blood clotting, in other words, thromboresistance). In its applications in the biomedical field such as heart valves, small orthopedic joints (fingers), and spinal inserts, pyrolytic carbon generally is used as a coating on implant materials prepared by pyrolysis of hydrocarbon vapor carried with a hydrocarbon carrier gas. In the case of heart valves, it is deposited in a fluidized bed on graphite shaped in the form of the heart valve to render it inert, strong, light, and hemocompatible.

A typical orthopedic implant production process involves thermal decomposition of hydrocarbons by introduction of a graphite substrate into a chamber heated to 1200–1500 °C. A hydrocarbon carrier gas, such as propane, propylene, acetylene, and methane, is introduced into the chamber in the absence of oxygen where the typical decomposition of the hydrocarbon to carbon dioxide and water does not take

place. The heat causes breakage of the C-H bonds and releases carbon atoms which are then deposited onto the graphite core of the orthopedic device. The pyrolytic carbon coating thickness could vary with the application: in one study, the thickness of the LTI pyrolytic carbon layer deposited onto a graphite substrate designed for use as a femoral head component was 500 μm [2]. Another method of production of pyrolytic carbon is to heat synthetic organic fibers under vacuum [3]. Operation at pressures as low as 10^{-2} atm is common because the production of carbon black, a carbonaceous material somewhat similar to soot and used as a filler in tyres, is minimized. Instead, an increase in the density and ordering of crystallites in the carbon deposit is observed [4].

6.3 Graphite

Graphite exists as a layered material consisting of parallel layers of hexagonal arrays with sp^2 -bonded carbons. The layers are bonded by weak $2p_z$ linkages (Fig. 6.2). The unit cell dimensions are $a = b = 2.456 \text{ \AA}$ and $c = 6.694 \text{ \AA}$. The carbon-carbon bond length in the layer is $1.418 \text{ \AA}$, while the interlayer spacing is significantly larger ($3.347 \text{ \AA}$). Graphite does not bear any net charges because in its natural form, no reactive ions or groups exist within the hexagonal layers.

Graphite can take in various atoms, molecules, metal complexes, and salts between the layers to form “graphite intercalation compounds.” Graphite can also be modified to form graphene oxide (graphite oxide), graphite intercalated compounds (GICs), and expanded graphite (EG). Some properties of graphite are presented in Table 6.1.

Graphite, due to its intrinsic properties, can be used in many applications in the biomedical field. Even though uncharged, graphite is a conductor due to the p electrons that are delocalized over the atomic sheets of carbon and therefore can be used as a conductor. In addition, the weak $2p_z$ bonding between the sheets in graphite

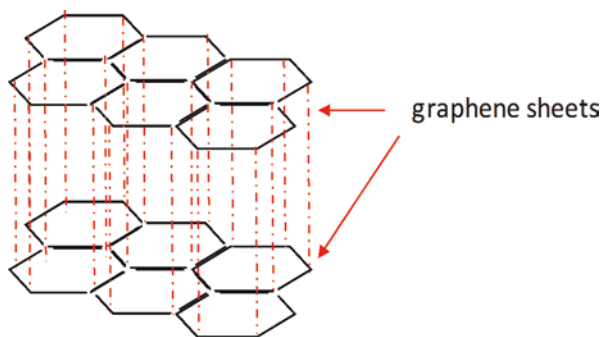


Fig. 6.2 Graphite structure showing constituent graphene sheets

Table 6.1 Properties of graphite [5]

Property	Value
Density	2.26 g cm ⁻³
Elastic modulus	1 TPa (in plane)
Strength	130 GPa
Resistivity	50 Ω cm (in plane)
Thermal conductivity	3000 W m ⁻¹ K ⁻¹ (in plane); 6 W m ⁻¹ K ⁻¹ (z-axis)
Thermal expansion	10 ⁻⁶ K ⁻¹ (in plane); 29.10 ⁻⁶ K ⁻¹ (z-axis)

makes its powders suitable for use as a lubricant. As a result of the same bonding type, it can cleave perfectly in one direction, and its thin flakes are flexible but inelastic. Graphite is one of the softest materials (hardness level 1–2 Mohs) due to its layered structure, unlike the other pure carbon compound, the diamond, which is the hardest mineral known to man (10 Mohs). In addition, it is quite a dense structure too (density 2.2 g cm⁻³).

Thrombosis on prostheses coated with graphite was studied in the 1960s, and they were found to be non-thrombogenic, and it was also found that graphite-based endoprostheses were generally nontoxic and produced no immunological reactions. When treated with glow discharge, its nonreactivity changes; the compound becomes hydrophilic, and this makes the surface more reactive and favorable for protein adsorption [6].

The hexagonal crystal structure of graphite formed by the sp² σ bonds is actually graphene layers or carbon layer planes, bonded together in between the planes by π bonding. The most common crystal form of graphite consists of stacks in the order ABABAB [7]. The rhombohedral form of graphite has a stacking sequence of ABCABC but constitutes only a minor fraction of well-crystallized graphites.

The defects, particularly vacancies, are the main microstructural feature that affect the strength of brittle materials such as graphite the most. Under the influence of the applied tensile or shear stress, microscopic defects grow and eventually lead to the failure by fracture. This, in addition to weakness against shear forces, makes graphite mechanically fragile, which constitutes a disadvantage in many applications.

6.4 Active Charcoal (Activated Carbon)

Activated carbon is an amorphous solid with very high porosity and a very large internal surface area. It has the capability to adsorb molecules from both the liquid and gas phase and is therefore used in purification and cleaning processes including the biomedical field. It can be produced from many organic natural carbon-based materials such as wood, nutshells (coconut, pecan, etc.), and lignite coal. The process is basically activation of charcoal, and it is done by heating it to 600–1200 °C in the presence of oxidizing gases such as CO₂, steam, or air, or it is impregnated with chemicals such as acids (phosphoric acid) or bases (potassium hydroxide), or

salts (zinc chloride), and then heated to 450–900 °C. Each approach has its advantages and disadvantages. Activated charcoal can be processed into powder, granular, and pellet forms. On the average, the specific surface area (SSA) of the material can range from 500 to 1400 m²/g [8].

The total pore volume is about 0.71 cm³/g. The complex interior of activated charcoal consists of pores with different diameters: micropores (diameters < 2 nm), mesopores (diameters 2–50 nm), and macropores (diameters > 50 nm) [9]. Micropores constitute the major portion of the structure of the AC.

Activated carbon is a modified graphite-like structure and has microcrystallites formed during the carbonization process, and these are disrupted during the activation process causing free valences which are very reactive. The presence of impurities also influences the microcrystalline structures.

Of the various activated carbon materials, fibers have the narrowest distribution of pore sizes and nanopores. The dominance of the carbon nanopores in the fibers makes them attractive for various applications.

High specific surface area (SSA) in activated carbon fibers is created by controlling the temperature, the time for activation, and the precursor materials. The SSA is usually measured using adsorption isotherms of N₂ gas at 78 K or CO₂ gas at 195 K.

Removal of poisonous substances from the body by passing the blood through an activated charcoal bed in an extracorporeal circuit was first described by Yatizidis in 1964 [10, 11]. Since then, many investigators have used hemoperfusion through activated charcoal and have shown the removal of a number of drugs and toxins. Some researchers coated the charcoal surface by polymeric materials after plasma glow discharge application to enhance the compatibility while not affecting adsorption capacity [12, 13].

6.5 Graphene

The discovery of C₆₀ Buckminsterfullerene, a ball of carbon molecules 7 Å in diameter, paved the way to a whole new chemistry and physics of nanocarbons [14].

Graphene is the basic building block for all graphitic materials because its origin is the honeycomb hexagonal sp² carbon layer, but to be able to substitute for the 2p_z bond, it forms double bonds and therefore, creates an unsaturated structure. Graphene is the thinnest, the strongest, and the lightest material known. It can be found in single and multiwalled tubular form (single-walled carbon nanotubes, SWCNT; multiwalled carbon nanotubes, MWCNT), or it can be in 2D sheets which are called graphene layers (Fig. 6.3).

Graphene has excellent mechanical, electrical, thermal, and optical properties. Until 2004, graphene was considered to be thermodynamically unstable and hence theoretically impossible to exist in free state, but after the discovery of free-standing graphene, interest of material scientists in it grew exponentially. It can be oxidized to graphene oxide (GO) or functionalized using amines, carboxyls, PEG, PEI, etc. [16] (Fig. 6.4).

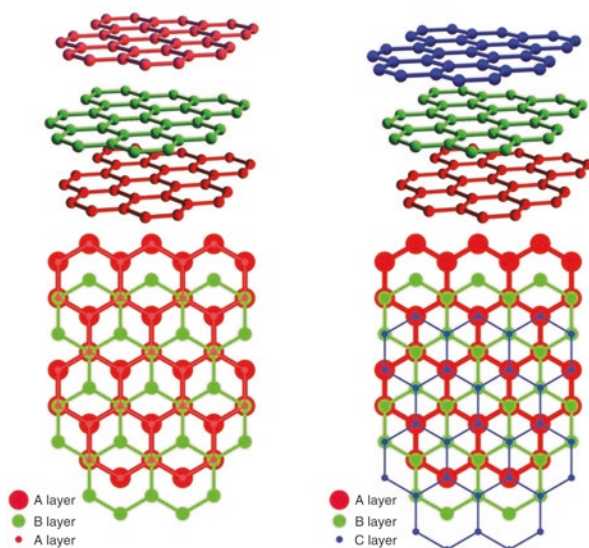


Fig. 6.3 Graphene layers in ABA and ABC crystals form graphite [15]

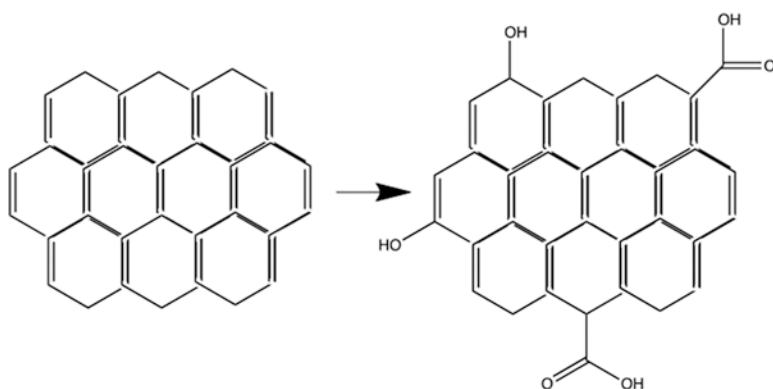


Fig. 6.4 Graphene and graphene oxide

Modified graphenes are being considered for various biomaterial applications, and their biocompatibility is therefore studied. When tested *in vitro* with mammalian cells and cell lines, modified graphenes lead to a certain degree of cell viability loss (up to 60% or more) through apoptosis and necrosis. Cell membrane damage and cell deformation occur. It is suggested that they cause physical damage on membranes upon direct contact, resulting in the release of intracellular contents. This membrane damage is a result of the blade like action of sharp graphene crystals. *In vivo* results were observed to depend on their physicochemical properties, concentration, exposure duration, route of administration, and the test animals used. Most studies report no occurrence of adult animal death, even for GBMs

(glioblastoma multiforme) with doses up to 20 mg kg^{-1} . In one study modified graphenes were reported to kill half the population when injected i.v. into mice. When administered i.v. they generally accumulate in the RES (reticuloendothelial system) mainly in the liver, spleen, kidney, and lung. Zhang et al. [17] stated that GO introduced i.v. had a low uptake by the reticuloendothelial system (RES), a rather long circulation time, caused no pathological changes, and no damage to erythrocytes indicating its hemocompatibility.

Obviously these are cases when the graphene and derivatives are used as small particles or flakes rather than as a coat on or as a component in an implant. Most studies show that the toxic effect of the fillers is reduced when incorporated in biomaterials, due to decrease of direct biological interactions and inability of the cells to endocytose these particles or get damaged mechanically by their sharp edges. On the contrary, hydrophobic carbon materials decrease adhesion of blood elements, favor albumin adsorption, and decrease platelet activation. As a result they appear to be non-thrombogenic.

6.6 Carbon Nanotubes

Carbon nanotubes are graphene surfaces of only carbons forming hexagonal lattices in cylindrical form, and the diameters of single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs) are typically in the range 0.8–2 nm and 5–20 nm, respectively (Fig. 6.5). They have very high aspect ratios; their lengths may be as long as several microns. They were first identified by Iijima in 1991 [19] using a high resolution TEM (transmission electron microscope). Iijima observed multiwalled carbon nanotubes (MWCNTs) with diameters in the nanometer range. Several years later, single-walled carbon nanotubes (SWCNTs) were identified by Loiseau et al. (1996) [20].

The structure of carbon nanotubes was initially explored using high resolution TEM and scanning tunneling microscopy (STM) confirming that the nanotubes are continuous cylinders of a single atomic layer of crystalline graphite organized in a honeycomb lattice. This is very much a graphene sheet rolled into a tube.

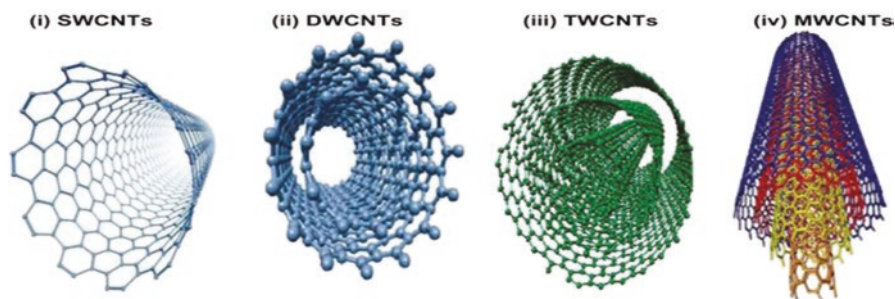


Fig. 6.5 Single, double, triple, and multiwalled carbon nanotubes [18]

Carbon nanotubes like graphite have the same sp^2 bonding, but unlike graphite their curvature apparently adds some sp^3 character to the C-C bonds. The mechanical properties of this material are extremely high. The cross-section of the walls of MWNTs have an elastic modulus approaching 1 TPa and a tensile strength of 100 GPa. These values are about ten times higher than any industrial fiber. Deng et al. (2011) have found that the modulus of SWNTs blended with PVA is in the range 530–700 GPa which is still very impressive [21]. MWNTs are typically metallic and can carry currents of up to 109 A cm^{-2} [22]. The conductivity of as spun CNT fibers is around 595.2 S cm^{-1} at room temperature, and its variation with temperature shows a semiconductive behavior from 300 to 75.4 K. Spun carbon nanotube (CNT) fibers, neat and well-aligned spuns from arrays of millimeter long CNTs, had a very high conductivity of around 595.2 S cm^{-1} at room temperature. Their semiconductive behavior varies in the temperature range 300–75.4 K. The electrical resistivity of individual CNTs was found to be as low as $10^{-6} \Omega \text{ cm}$ [23].

The basic properties of nanotubes such as a very high longitudinal flexibility factor, a Young's modulus similar to that of diamond, a high mechanical resistance to stretching (several hundred times greater than the most resilient steel), the highest heat conductivity of all known materials, a very high length-to-diameter ratio, and a large surface area make CNT useful for many applications in the biomedical field, too.

CNTs are considered for use as biosensor components and medical device parts because their dimensions and chemistry are quite suitable to work with biomolecules such as nucleic acids (DNA, RNA) and proteins. The impetus behind their use is (i) large surface area and (ii) possibility for chemical modification and (iii) for creating ordered structures that can be “read” easily. CNTs also allow fluorescent and photoacoustic imaging and are very useful in localized therapy via exposure to near-infrared radiation.

6.7 Carbon Products as Coating Materials

The fate of a biomaterial is determined by its reaction with the biological environment [24]. Amorphous carbon and diamond-like carbon (DLC) are known as bioinert film materials causing no toxic reactions in the living organism [25, 26]. The hardness, low coefficient of friction, high resistance to wear and corrosion, and the bioinert character of carbon films make them ideal surface finish materials for biomedical implants like heart valves [27], catheters, drainage tubes or polymer contact lenses [28]. DLC is an amorphous hydrogenated carbon and has excellent mechanical, tribological, and biological properties. Due to its amorphous nature, some elements, such as Si, F, N, O, W, V, Co, Mo, and Ti can be added into the structure [26].

DLC is often preferred for use in blood-contacting devices (stents and heart valves) because of its excellent blood compatibility and antithrombogenic properties, smoothness, and inertness and also in load-bearing joints because of high wear resistance. Coronary artery stents and heart valves lead to platelet activation during contact with blood and also release metallic ions which might lead to enzyme inhibition. These are important factors in triggering thrombosis.

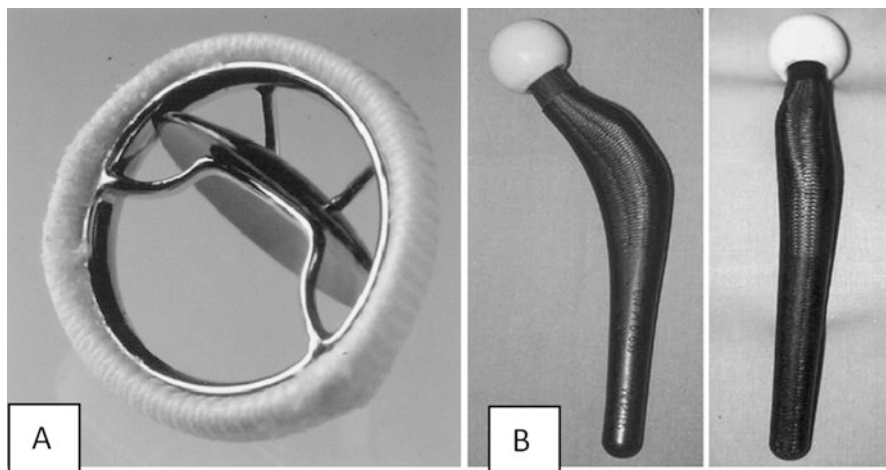


Fig. 6.6 Carbon-coated biomaterials. (a) Tilting pyrolytic carbon disk heart valve with Co-Cr housing [29] and (b) total hip implant with carbon-coated stem [30]

Carbon coating of metallic stents and heart valves has been suggested as a remedy for these situations [20], and DLC-coated artificial heart valves and stents are already commercially available (Fig. 6.6).

In bone healing being osteoconductive, or better yet, osteoinductive is an important property of the fixation devices and implants. Pyrolytic carbon and glassy carbon have been shown to be osteoinductive, even though they are mechanically unstable [20].

Low coefficient of friction, high wear resistance, and hardness are properties needed to improve the service life of total hip or knee implants. In the standard design of a hip replacement, the contact point is the one between a metal or a ceramic head and an UHMWPE (ultrahigh molecular weight polyethylene) cup (Fig. 6.6b). They are designed to serve for life, and their failure is mainly a result of debris formation due to polyethylene erosion during the movement of the femoral head and the acetabular cup against each other. DLC is recommended as coatings to prevent such wear problems due to the smoothness and the hardness of the material [20].

Du et al. (1998) [31] studied the morphological behavior of osteoblasts on DLC and amorphous carbon nitride deposited on silicon substrates and showed that the cells were able to attach, spread, and proliferate on these surfaces without apparent impairment of cell physiology.

A hip simulator was used by Tiainen (2001) [32] to study the mechanical properties of tetrahedral amorphous carbon (ta-C) film deposited by filtered pulsed arc discharge method, and they showed that the wear rate of ta-C-coated metal polyethylene joints was decreased almost 100 times after this procedure. The corrosion rate in a biomimetic saline solution at 37 °C for 2 years was also found to be significantly lowered [33]. In vitro studies showed that DLC coatings significantly reduced the release of metal ions and diminished platelet activation; therefore, lower degrees

of acute and subacute thrombosis were expected [34, 35]. Among coated heart valves tested in clinical trials, the most bio- and hemocompatible material was found to be pyrolytic carbon, in a bulk solid or a coating form [36, 37].

However, serious controversy remains about the effect of the DLC coating on restenosis. Clinical tests revealed that DLC coating of stents did not significantly improve restenosis problems [38]. This appears to be contrary to the expectations based on the excellent hemocompatibility of DLC coating reported in many in vitro and in vivo tests [22].

In brief, it can be stated that carbons are important biomaterials as bulk materials or as coats due their unique properties which make them essential components of especially composite biomaterials, and they are used as implants for acetabular cup, femoral head for hip joints, elbow joint, shoulder joint, toe joint, teeth, and transcatheter implants.

6.8 Conclusion

The human body is carbon-based, and in this chapter, the types and properties of carbon materials, such as pyrolytic carbon, active carbon, diamond, graphite, and graphene, are introduced, and some examples of those used in medical applications are presented. These materials can be used in ways ranging from adsorptive materials used in blood purification to hemocompatible surface coatings. With so many important uses, carbon materials are indeed indispensable.

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Building Blocks of the Human Body

7

7.1 General Properties

The human body can be considered to be a combination of very complex groups of systems which function smoothly. When this organization is examined from the constituent molecules upward toward the systems, the lowest layer is amino acids, nucleotides, saccharides, and lipids. Upon their combination, proteins and enzymes, polynucleotides, polysaccharides, and lipid structures are formed. These, in return, form the cells, tissues, organs, organ systems, and finally, the living organism, the human body.

Nucleic acids are responsible of the genetic information preservation, copying, and transfer. Polysaccharides are especially found as coats or layers (e.g., mucous layer) or highly viscous fluids or gels which help form and preserve the shape of soft tissues, serve as lubricants in tissues like the cartilage of the knee or the vitreous humor (the fluid that fills the anterior and posterior chambers of the eye), and also serve as energy depots. The lipids are also energetic molecules; they serve as the main component of cell membranes as well as constituting the lipid tissue. All these molecules come in contact with the biomaterials upon implantation as they are part of the tissue and also some of them (not necessarily from human sources) could be used as biomaterials and in biomedical systems, and therefore, their properties and interactions need to be well understood.

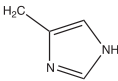
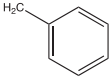
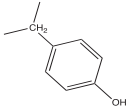
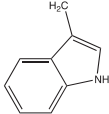
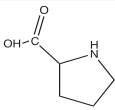
7.2 Proteins

Proteins are polypeptides; therefore, they are in macromolecular form, but these macromolecules need not be fully covalently bonded single, linear polymers, but instead, they can be constituted of a small number of polymers physically or ionically bonded to each other to form larger aggregates. Proteins are formed from 20 basic amino acids binding to each other with covalent bonds called peptide bonds. They can be considered as beads of 20 different colors forming a necklace

(Table 7.1). Actually two new and rare amino acids, Selenocysteine (Sec) and pyrrolysine (Pyl), are now added to the list of known amino acids making the current total 22.

The number and sequence of the amino acids (the beads) are very crucial for their physical and chemical properties and for their function. What makes an amino

Table 7.1 Main natural amino acids found in the human body

Amino acid name	Abbreviated name	R Group
Positive charged		
Arginine	Arg	$\text{CH}_2\text{--CH}_2\text{--CH}_2\text{--NH--CH(NH}_2)_2$
Histidine	His	
Lysine	Lys	$\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--NH}_2$
Negative charged		
Aspartic acid	Asp	$\text{--CH}_2\text{--COOH}$
Glutamic acid	Glu	$\text{--CH}_2\text{--CH}_2\text{--COOH}$
Polar but uncharged		
Serine	Ser	$\text{--CH}_2\text{--OH}$
Threonine	Thr	--CH(OH)--CH_3
Asparagine	Asp	$\text{--CH}_2\text{--CO--NH}_2$
Glutamine	Glu	$\text{--CH}_2\text{--CH}_2\text{--CO--NH}_2$
Hydrophobic, uncharged		
Alanine	Ala	--CH_3
Valine	Val	$\text{--CH(CH}_3)_2$
Isoleucine	Ileu	$\text{--C(CH}_3)_2\text{--CH}_2\text{CH}_3$
Leucine	Leu	$\text{--CH}_2\text{CH(CH}_3)_2$
Methionine	Met	$\text{--CH}_2\text{--CH}_2\text{--S--CH}_3$
Phenylalanine	Phe	
Tyrosine	Tyr	
Tryptophan	Trp	
Special cases		
Glycine	Gly	--H
Cysteine	Cys	$\text{--CH}_2\text{--SH}$
Proline	Pro	

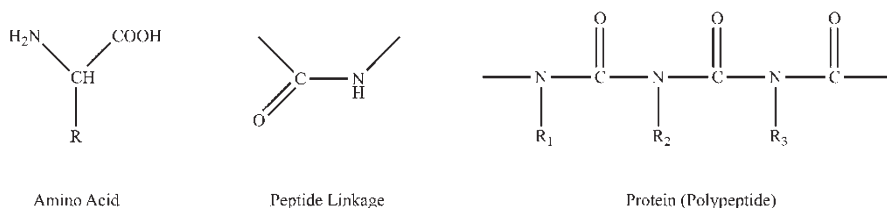


Fig. 7.1 Amino acids and their linkages leading to protein formation

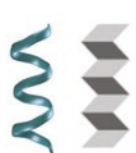
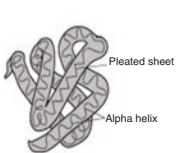
Primary Structure	Secondary Structure: α -helix and β -sheet	Tertiary Structure	Quaternary Structure
			

Fig. 7.2 Levels of organization of a polypeptide [1]

acid different than the others is the “R” group in the middle of the molecule attached to the central carbon (Fig. 7.1). The electronic nature of the amino acids (polarity, charge, charge density, etc.) arises from these R groups and may lead to the formation of regular structures. These regular regions on the chains constitute the crystalline portions of the proteins. There are basically two major ways of organization of polypeptide chains: α -helix and β -sheet (Fig. 7.2).

Proteins can be described by four structures with increasing complexity and size: primary, secondary, tertiary, and quaternary structures.

Primary structure is the linear string of amino acids attached to each other with covalent peptide linkages and gives the sequences of the amino acids. The linear chains can branch off or even cross-link when the R groups have appropriate functional groups such as $-\text{SH}$, $-\text{NH}_2$, or $-\text{COOH}$. This structure is important because the eventual 3D conformations of the whole protein will be determined to a large extent by the interactions between or within chains, and these are made possible by the presence of suitable R groups at appropriate distances from each other.

The secondary structure defines the spatial organization of the two main crystalline conformations called alpha helix (α -helix) and beta-sheet (β -sheet) and the unorganized (amorphous) regions in between them. Alpha helix is a right-handed helical structure or in other words if one places the right hand with the thumb pointing up the direction of the fingers shows the movement on an alpha helical structure. The amino acids of this structure are located on the outside of the helical staircase. In an alpha helix, the coil makes turns climbing up the whole structure, and every turn is constituted of 3.6 amino acids covalently bonded to each other. At every three turns of the helix, the 1st and the 11th amino acids are superimposed if viewed along the axis of the helix. These amino acids all make hydrogen bonds among

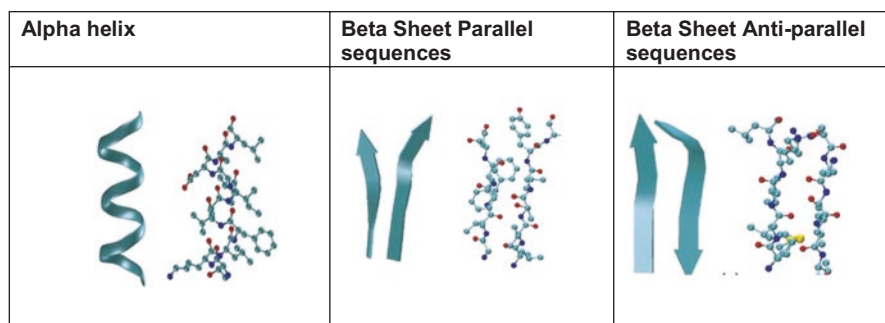
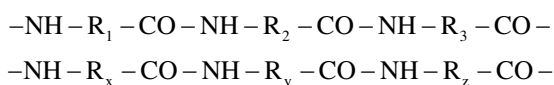


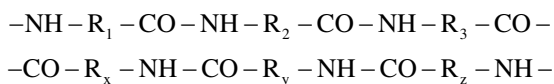
Fig. 7.3 Alpha helix and beta sheet forms of the polypeptide. Beta sheet parallel and antiparallel sequences [2]

themselves within the chain and stabilize the structure. In the end, a tubular, stable, spring-like structure is formed.

Beta-sheet is formed by polypeptide molecules zigzagging along a direction (according to two-fold symmetry) with every third amino acid coming on top of the first and where several polypeptide sequences of the same polypeptide chain are organized parallel or antiparallel to each other. Thus, the main direction of the chains is the same, but the organization of the atoms in the linear chain could be the same or opposite as shown in the example below (Fig. 7.3). The organization of the functional groups or NH_2 and CO is different. In the parallel sequence, the strands are positioned in the same direction:



and in the antiparallel sequence, the strands are in opposite directions:



Tertiary structure is the last conformation that a single polypeptide chain can take. It is a result of the interactions between the $-\text{R}-$ groups and the peptide bonds within the chain. These interactions lead to alpha helices, beta-sheets, various folds and turns, and even rings which are then connected by amorphous (no distinct conformation) regions. Hydrogen bonds, ionic bonds, disulfide linkages, and hydrophobic bonds (those between the hydrophobic groups which are forced to avoid water molecules and are pushed together) all keep this final form together (Fig. 7.4). The tertiary structure can also be seen as one of the dimers of Fig. 7.5 that constitute the quaternary form.

Quaternary structure forms when some specific tertiary structures of proteins come together to form an upper level of organization. This conformation is brought together by several independent polypeptide chains held together by covalent and weaker bonds. Thus, the quaternary structure is an aggregate of several identical or

Fig. 7.4 Tertiary structure (line presentation)

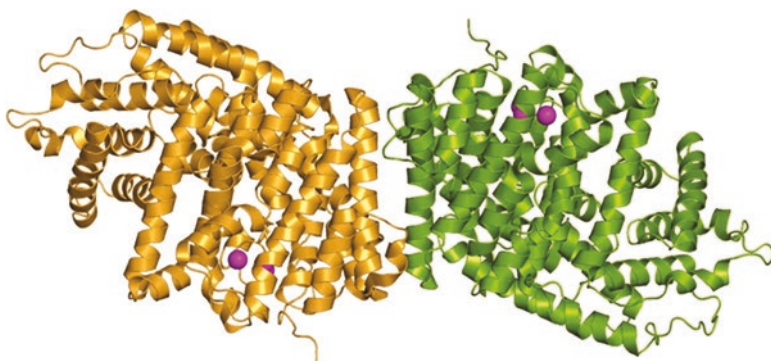
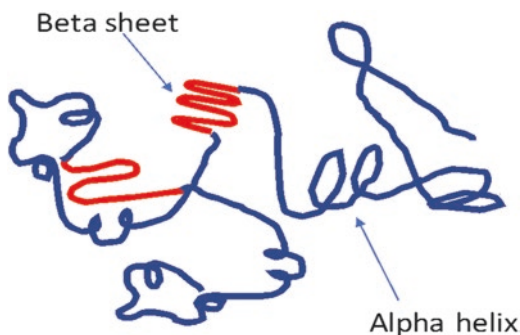


Fig. 7.5 Quaternary structure of isoprene synthase [3]

different tertiary structures (Fig. 7.5). A typical and common example is that of hemoglobin which is constituted of four identical, globular, tertiary structures. As another example, one of the most abundant proteins in the body, collagen, is constituted of three linear chains which are similar but different in their composition. The important aspect of the collagen quaternary structure is that being in that form imparts a new property that the individual left-handed helical (opposite of the alpha helix of the tropocollogens which is right handed) tertiary molecules do not have. Quaternary structure of the collagen molecule is a right-handed triple helix (constituted of three left-handed helices) which is held together by hydrogen bonds and other weak bonds and is very hydrophobic and stiff making it a strong structural material that none of its individual chains have. Similarly the oxygen-carrying capability of the hemoglobin is a property of the quaternary structure and is not observed in the individual tertiary structures.

7.3 Polynucleotides: DNA and RNA

DNA and RNA are not directly important for the biomaterials field as structural materials yet, but they are macromolecules with which the biomaterials interact (e.g., in gene delivery for therapeutic purposes) and they are essential for the living

Fig. 7.6 Double-helical structure of the DNA [4]

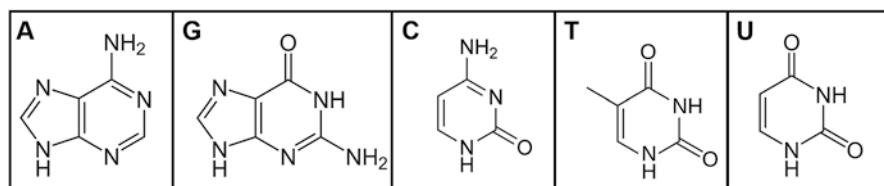
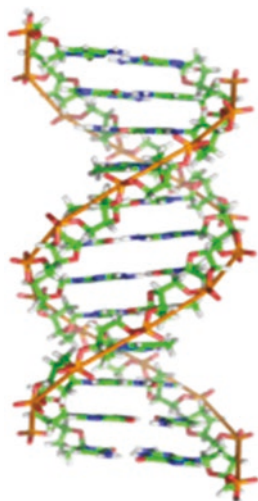
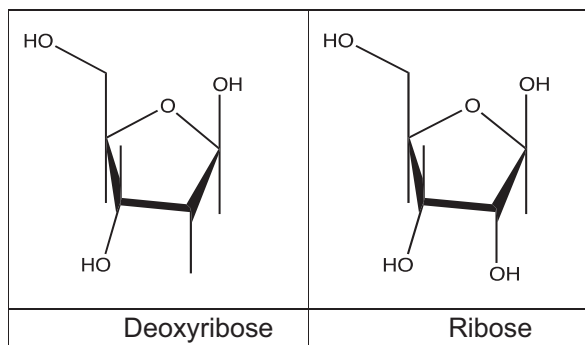


Fig. 7.7 The bases of the DNA and RNA: adenine, guanine, cytosine, thymine, and uracil

system for which the biomaterials are designed to help heal. DNA has a double helix structure and carries the main genetic information of the human body (Fig. 7.6). RNA (and from there proteins) is produced from certain lengths of the DNA used as the template. This process is crucial for the functionality of the cells and the organism. DNA is also directly replicated (copied) to be transferred to the next generation. Any errors during this replication stage are called mutations. The likelihood of such errors can be better appreciated when it is remembered that a typical human DNA is about 2 m long. The main components of DNA are four molecules called the bases or the nucleotides: adenine (A), guanine (G), cytosine (C), and thymine (T). Unlike the polypeptides which are formed by the direct bonding of amino acids together, the bases are linked to each other through intermediate molecules, a saccharide molecule, the deoxyribose, and a phosphate group. DNA is made up of two chains complementary to each other and linked with hydrogen bonds. The complementarity between bases is through pairing of A with only T, and G with only C (Fig. 7.7).

For clarity in understanding the events involving the DNA strands, the chains are assigned direction based on the bonding sites on the deoxyribose (Fig. 7.8). The chain end that has a phosphate group bonded to the fifth carbon of the deoxyribose is called the 5' end and the other end for this chain has an -OH group bonded to the

Fig. 7.8 Sugar molecules of the DNA and RNA



third carbon of the deoxyribose making it the 3' end. If a chain is in the 5'–3' direction, then its complementary strand is antiparallel to it and is labelled 3'–5'. Since the two strands are complementary if one strand starts with a 5' end, then its counterpart should have the 3'. DNA, like the alpha helix of the polypeptide chains of the proteins, has a right-handed helix conformation. Every turn of the DNA helix, however, has 10.4 nucleotide pairs, and every base is 3.4 nm higher than the previous one. The wrapping of the two strands around each other leads to the formation of two types of grooves in the double helix structure: the large and small grooves.

In contrast, the RNA is single stranded and made as a complementary copy from the DNA on which it is produced and has four bases like DNA, but these are A, G, C, and uracil (U) instead of T of DNA.

RNA is critical in the functionality of the organism because the polypeptides, proteins, and enzymes are produced by its translation or by its use as a template for polypeptide production. Another difference between the DNA and the RNA is in the saccharide molecule used in binding the phosphates and the base; it is a ribose instead of a deoxyribose. The RNA is less stable than the DNA due to its being a single-stranded molecule. It is also shorter since the code for the proteins does not take up the whole DNA sequence. There are three classes of RNA each with its own role in the functionality of the organism. These are mRNA (messenger RNA), tRNA (transfer RNA), and rRNA (ribosomal RNA).

mRNA is directly synthesized on the DNA strand and crosses from the nucleus into the cytoplasm to the ribosomes for the synthesis of the coded protein. tRNA is also involved in the protein synthesis where it carries amino acids to the mRNA for the formation of the protein. rRNA is also involved in the protein synthesis but not as directly as the m- and tRNA since it constitutes the backbone or the skeleton of the ribosomes on which the protein synthesis is carried out (Fig. 7.9).

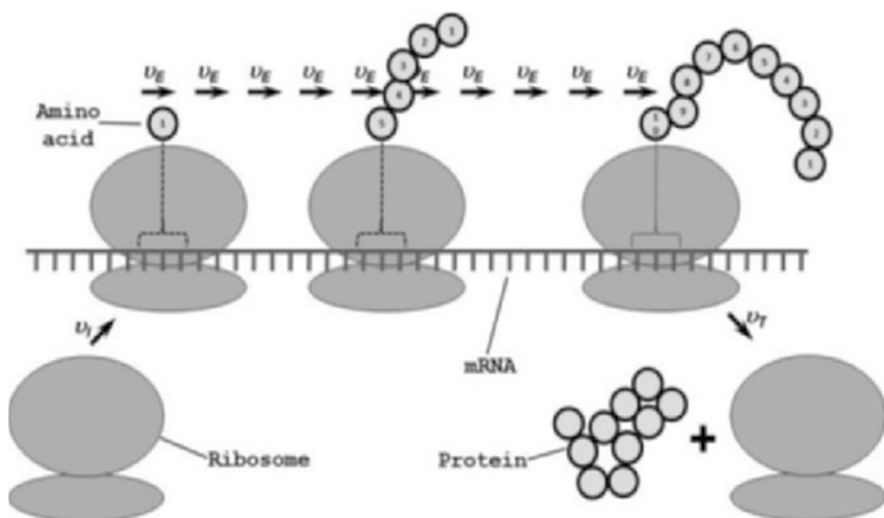
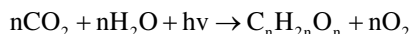


Fig. 7.9 Protein synthesis [5]

7.4 Polysaccharides/Carbohydrates

Carbohydrates are carbon-, hydrogen-, and oxygen-based molecules whose main function is to provide energy to the cells in addition to serving as structural materials, and in this latter role, they are of importance in the biomaterials field. They are simply represented as $C_nH_{2n}O_n$ since they are produced through photosynthesis according to the following reaction:



As a consequence of their polar hydroxyl groups, they are hydrophilic. Glucose ($C_6H_{12}O_6$) is the most abundant monosaccharide in the body. Since it is also found in the blood, it is also called the blood sugar. The main saccharides in the body have either five or six carbons, and as a result they are known as pentoses and hexoses, respectively. Disaccharides are formed by the condensation, by removal of one water molecule when OH groups of two monosaccharides react. For example, sucrose is obtained in this way from glucose and fructose (Fig. 7.10), maltose is a disaccharide of two glucose molecules, and lactose (the saccharide in milk) is formed from the reaction of glucose and galactose. In return, entry of one water molecule leads to the hydrolysis of the disaccharide.

Polysaccharides are formed from the condensation of many saccharides; both glycogen and starch are products of condensation of many glucose molecules and are used as glucose storage molecules in animal and plant cells, respectively (Fig. 7.11).

Glycosaminoglycans (GAGs, mucopolysaccharides) are linear polysaccharides formed by repeating disaccharides. The saccharides found in these molecules may

Fig. 7.10 Formation of sucrose from glucose and fructose by condensation reaction

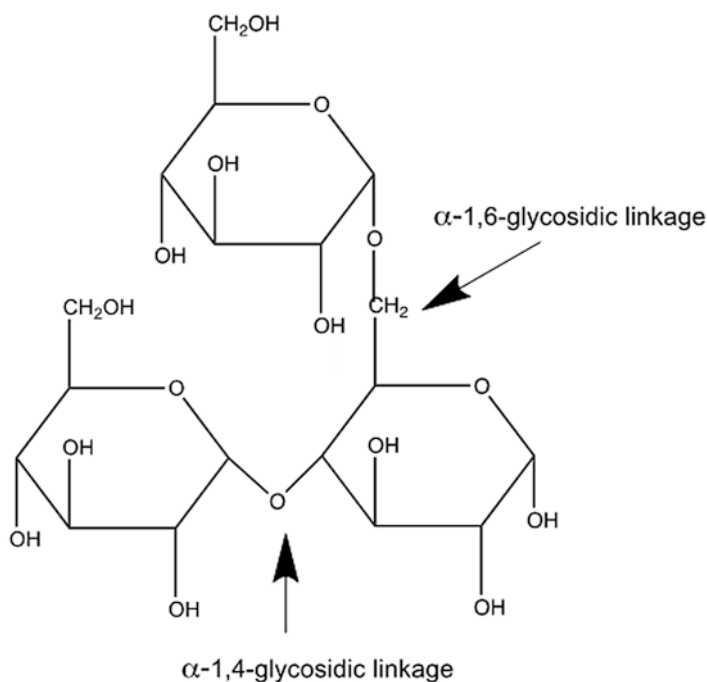
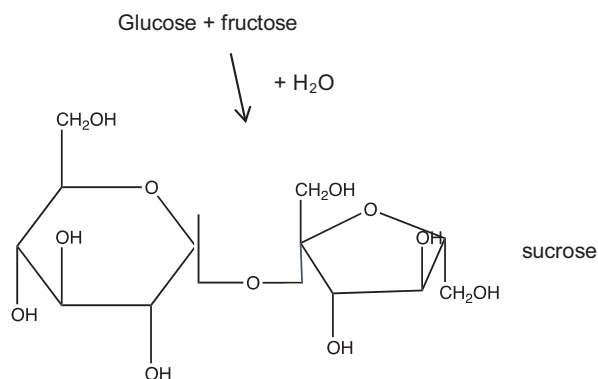


Fig. 7.11 Linkages in polysaccharides

possess acidic groups as well as sulfated and hydroxyl or amino groups which give them negative charges and amine group becomes protonated and provides a positive charge. These charges impart significant hydrophilicity on these molecules.

Hyaluronan is a GAG, consisting of disaccharides of two modified glucoses, glucuronic acid and N-acetylglucosamine. Under physiological conditions the first saccharide has a negative charge, while the second has a positive charge. Heparin is a hydrophilic glycosaminoglycan found in the granules in the mast cells and is quite

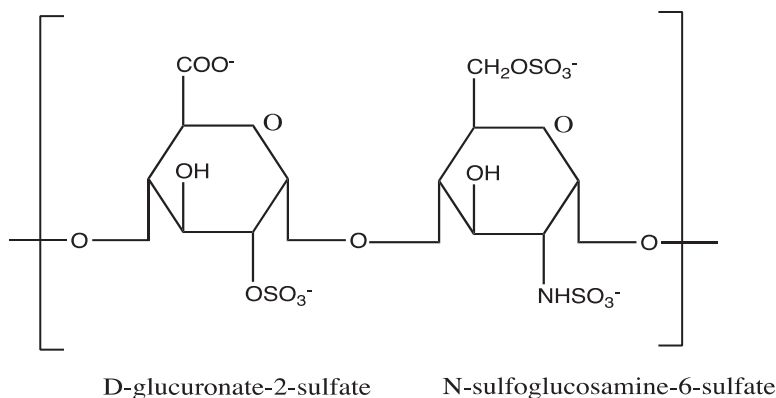


Fig. 7.12 Chemical structure of heparin

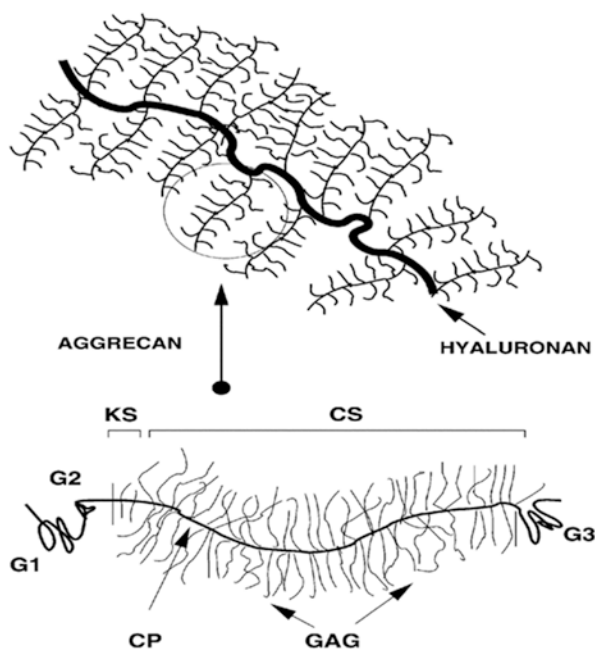


Fig. 7.13 A proteoglycan structure [6]. *KS* keratan sulfate, *CS* chondroitin sulfate, *G* are globular domains. *CP* is the core protein to which the GAGs are bound

similar to heparan sulfates except that heparin has more sulfate groups. When released into the blood, heparin binds antithrombin and prevents blood coagulation. Therefore, it is used as a drug to dilute the blood and to prevent clot formation (Fig. 7.12).

Some heparan sulfate GAGs are covalently bonded to membrane-bound proteins. For example, syndecan heparan sulfate proteoglycan carries a transmembrane

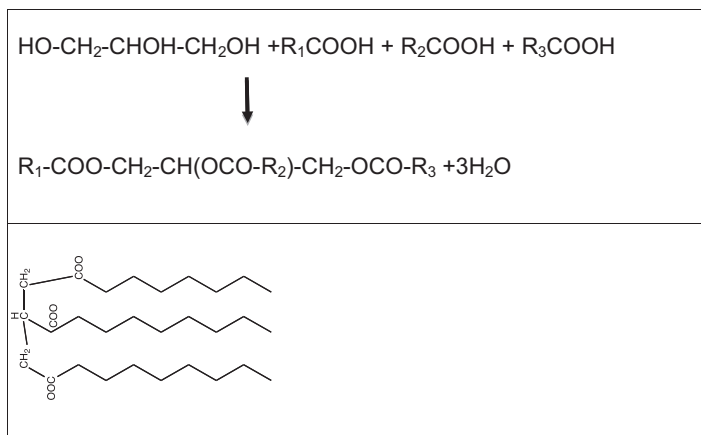


Fig. 7.14 Formation of fats from glycerol and fatty acids

α -helix. Proteins involved in binding or signal transmission at the cell membrane recognize heparan sulfate chains and bind to them. For example, some small protein molecules called growth factors bind more to the membranes due to their ability to bind to heparan sulfates.

Proteoglycans are GAGs bonded to proteins through serine amino acids (Fig. 7.13). An aggrecan molecule contains around 100 chondroitin sulfate glycosaminoglycan (CS-GAG) chains, each of which has 20–60 disaccharides and is covalently bound to a 300 kDa linear protein chain. Aggrecan consists primarily of these CS-GAGs. Heparan sulfate, another GAG, is a disaccharide of glucuronate and N-acetylglucosamine and binds to a protein originally bound to the membrane to form the proteoglycan. Due to their highly hydrophilic nature, the cell membrane is covered by a hydrated layer of polysaccharides carrying a negative charge.

7.5 Lipids

The word lipid defines more than one type of molecule; these are fats, waxes, phospholipids, steroids (e.g., cholesterol), and similar molecules. Fats form from the condensation of glycerol (a three-carbon polyol with one OH on each of its carbon atoms) and three fatty acids, losing three water molecules in the process (Fig. 7.14). The fats are also called the triglycerides because of the binding of three fatty acid molecules and are very hydrophobic. This property is especially a result of the hydrophobic tail of the fatty acids. The fatty acid molecule has ionic (carboxylic) head group which is changed during the condensation process making the final compound much less hydrophilic. The hydrophobic tails might be saturated or unsaturated, and as a result they can be kinked or straight (Fig. 7.15). The straight hydrophobic chains can align with each other in an aqueous environment, while the kinked/unsaturated chains lead to a looser packing in water.

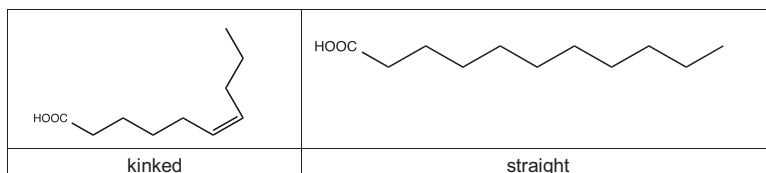
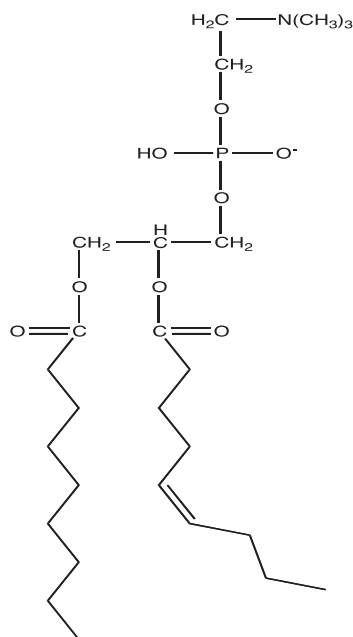


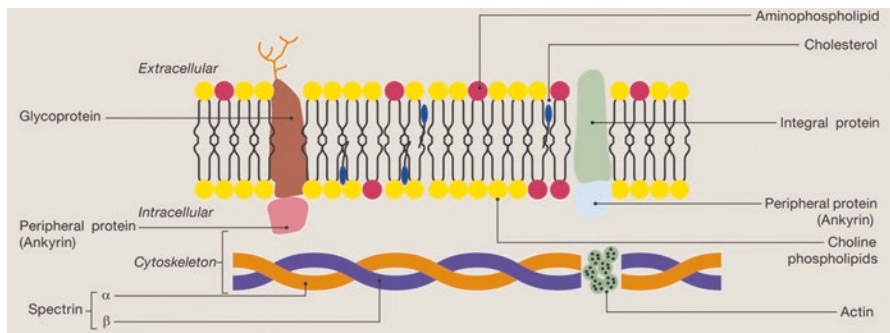
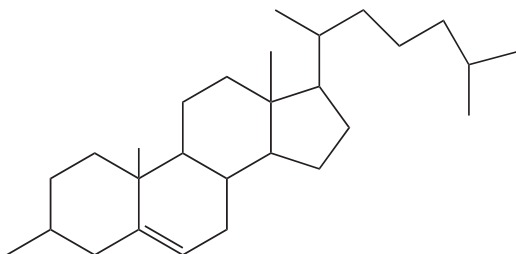
Fig. 7.15 Straight and kinked hydrophobic chains

Fig. 7.16 Phosphatidyl choline with an unsaturated tail



7.5.1 Phospholipids

Phospholipids play a very important role in the human body, form the cell membranes, and are quite similar in structure to the fats. They are the main molecules that constitute the cell membranes, and therefore, they are very crucial in cell-material interactions and biocompatibility. They, too, have a glycerol in their structure, but only two of the glycerol OH groups are bound to the fatty acids, and the third is linked to a phosphate-containing molecule. For example, this phosphate group could be bound to a choline moiety, and upon binding to the fatty acids, the formed molecule is phosphatidyl choline. The fatty acid tails are hydrophobic, but at the phosphate end (the polar head), there are the oxygens of the phosphate along with the functional groups of the head group (e.g., choline) making it ionic or highly polar depending on the pH of the medium. The hydrophobic tails are bent permanently (kinked) when unsaturated, and this results in difficulty in the packing of this molecule into an organized structure (like cell membrane) and causes an increase in

Fig. 7.17 Cholesterol**Fig. 7.18** The positioning of cholesterol in the cell membrane [7]

the fluidity of the structure (Fig. 7.16). The polar head and the nonpolar tail lead to a surfactant-like molecule which is partially soluble in both water and in oil, and it can nicely place itself at the interface between them, as it does in the cell membrane.

Figure 7.16 shows a typical phospholipid molecule. It has a phosphatidyl polar head with unsaturated tails with a kink and therefore, an obstruction against proper organization of the phospholipids side by side. However, not all phospholipids have unsaturated tails.

7.5.2 Cholesterol

Cholesterol is another important molecule that is largely found in the cell membrane and is involved in the synthesis of vitamin D. Cholesterol has a planar structure constituted of three hexagons and a pentagon (Fig. 7.17). These four fused rings form the main frame of the molecule. One of the major roles of cholesterol is to define the stability and the rigidity of the cell membrane. In figure Fig. 7.18, the positioning of cholesterol in the cell membrane is presented. Within this structure, the relationship between the phospholipids, cholesterol, and transmembrane proteins are shown.

Here, cholesterol extends parallel to the phospholipids only on external or internal side of the lipid bilayer (shown in blue). Cholesterol constitutes about half the

composition of the cell membrane constituents (or 20%, w/w). Cholesterol is not only found in cell membranes but also in the composition of membranes of organelles within the cells. For example, in the mitochondria and endoplasmic reticulum, cholesterol constitutes 3% of the weight. In the last decade, studies on the organization of lipids in the membrane have revealed lipidic structures called lipid rafts. These appear to be regions where there is increased presence of cholesterol and sphingolipids, and they act as separate phases within the membrane where the lipids are more than in the rest of the membrane and therefore less fluid. They play roles in signal transduction [8].

7.6 Some Important Structural Molecules

Natural materials are very commonly used in medical applications to treat the damaged parts of the tissue or organs. Proteins like collagen and gelatin, as well as saccharides as chondroitin sulfate and hyaluronic acid are the ones widely applied for these applications. The following section will provide some information about these structures.

7.6.1 Collagen

One of the most important molecules in biomaterials and tissue engineering is collagen. The main reason for this is that it is the most abundant protein in the body and is found in many different locations in different roles. There are 20 different types of collagens. Collagen's fundamental structural element is the tropocollagen which is a right-handed helix.

Their stability is a result of the hydrogen bonds between the three chains and the cross-links formed between them through the amino acid lysine. Their main components are generally presented as Gly-Pro-X which are glycine (the smallest amino acid whose R group is H), proline (or hydroxyproline derived from proline, a rigid, ring structured, hydrophobic amino acid), and X is any hydrophobic or hydrophilic amino acid. Glycine is essential for the stability of the triple helix because for the triple helix to wind three left-handed helices into one tight right-handed helix, small amino acids are needed to make the twists, and that is why at every third position there is a glycine. Proline and hydroxyproline, due their ring structure, push the individual chains into a helical form with their rings extending outward.

Table 7.2 Composition of collagen [9]

Amino acid	Collagen α -1 chain	Collagen α -2 chain	Collagen (% number)
Alanine	115	130	6.59
Glycine	329	381	23.77
Proline	230	231	9.79

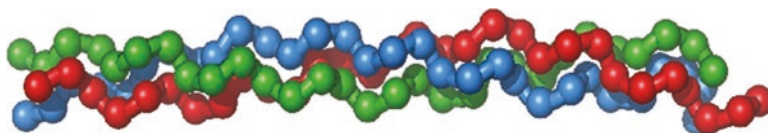


Fig. 7.19 The triple helix of collagen [10]

Table 7.3 Major collagen types and their most abundant sites in the body

Type	Location in the body
I	Most abundant collagen in the human body found in the skin, tendons, and bones
II	Mainly associated with the cartilage (articular and hyaline) and constitutes almost 50% of all the protein in the cartilage
III	Mainly found in the walls of the arteries, intestines, and the uterus
IV	Mostly in the basal lamina, in the lens of the eye, and in the kidney
V	Mostly in the interstitial tissue and in the placenta

Hydroxylysine, a derivative of lysine, is also present in the structure to achieve the cross-links between the three chains.

In a typical human type I collagen, it is reported that there are 1069 amino acids in α -1 chain and 1366 in the α -2 chain. Out of these, major amino acids are presented in Table 7.2. Most abundant among the 20 amino acids is glycine.

Tropocollagen units are about 300 nm long and 1.5 nm in diameter and are arranged in a line to form an individual left-handed helix, and these helices are aligned side by side. The polar heads of individual tropocollagens are not opposite each other but are staggered by 70 nm. This creates the well-known striated electron micrographs. The typical collagen fibril diameter is about 50 nm (Fig. 7.19).

Due to the hydrophobicity of the constituent amino acids (Gly, Pro, Hypo), collagen is a very hydrophobic molecule and is insoluble in the aqueous media. When solubilized, collagen cannot retain its original stiffness, hydrophobicity, and strength. However, for use in the biomaterial applications, it has to be removed from the body by solubilization, and therefore, the extracted collagen is never as strong as the original molecule embedded in the tissue. Even though there are 20 types of collagens in the body, the major types of collagens are five, and they are localized specifically in some tissues and cause the main differences between them (Table 7.3).

7.6.2 Gelatin

Gelatin is obtained from partial hydrolysis of collagen type I. The collagen source could be skin (cow hide), bones, connective tissues, fish skins, bones and fins, sea urchin, jellyfish, and intestines of some animals. Gelatin as a result of the thermal denaturation or disintegration of the hydrolysis process has a different conformation than collagen, has a much higher solubility, and is soluble in polar solvents including water unlike the parent molecule collagen. The chemical composition of gelatin is dependent on the source and is slightly different in each isolate. The collagen

triple helix molecular weight is ca 100 kDa, while that of gelatin that is formed by the breaking of the covalent cross-linkages of collagen is in the range of 40–90 kDa, and it is polydisperse [11]. During bovine spongiform encephalopathy (BSE) epidemic, gelatin and gelatin-based products were suspected to transmit the virus, but combined acid demineralization and lime treatments decreased the infectivity by about 1000-fold. Other treatments like UHT sterilization, washing, filtration, ion exchange, and other chemical treatments also were reported to decrease the SE activity. Another advantage of gelatin is that it is nonantigenic making it more suitable in biomedical device applications.

The increased solubility of gelatin however is a disadvantage when it is considered for biomaterial and tissue engineering applications. The solution to this problem is to cross-link the polypeptide using molecules such as glutaraldehyde, formaldehyde, EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide)/NHS (N-hydroxysuccinimide), genipin, dehydrothermal treatments, and electromagnetic radiation (UV, gamma). Not all treatments are suitable for all conditions; genipin stains the product blue; radiation and aldehydes are damaging to cells. In order to have the optimum cross-linkage conditions, a careful analysis of the targeted application and reaction medium should be made.

7.6.3 Elastin

Elastin is the major structural protein of those tissues which require rapid extension and complete recovery. Elastin contributes to the important structural, mechanical, and biological properties of the extracellular matrix. Elastic fibers are mainly composed of elastin. Elastin is, after collagen, the other critical protein found in the body, and the main amino acid that gives it its elasticity is lysine (and its derivatives desmosine and isodesmosine). Other amino acids are glycine (one third of all the amino acids), valine, alanine, and proline. Similar to collagen forming from tropo-collagens, elastin is formed from tropoelastins which are hydrophilic. Elastin has a molecular weight around 72 kDa. Its importance lies in the fact that it is made by cross-linking of soluble tropoelastin molecules via the lysine derivatives with catalysis by lysyl oxidase, and as a result it forms an insoluble, durable, and elastic macromolecule. Desmosine and isodesmosine are formed by oxidative deamination of three out of every four lysine side chains, which then condense to yield these two compounds (Fig. 7.20). The flexible nature of elastin is the main reason why it is found particularly in elastic tissues such as arteries (especially aorta) lungs, ligaments (nuchal ligament), the skin, bladder, and cartilage (Fig. 7.21).

7.6.4 Keratin

Keratins are fibrous, tough, and insoluble structural proteins and are basically found in two forms: alpha and beta. The human hair (along with wool, horns, nails, claws, hooves) is made of α -keratins, while β -keratins are found in the feathers, scales, and

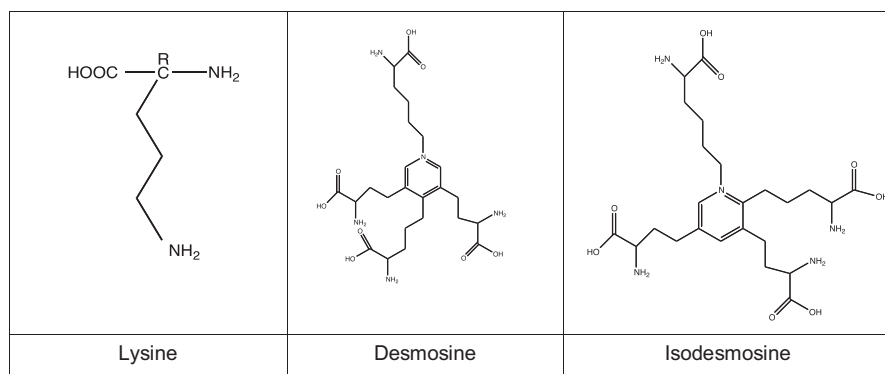


Fig. 7.20 Lysine and its derivatives desmosine and isodesmosine

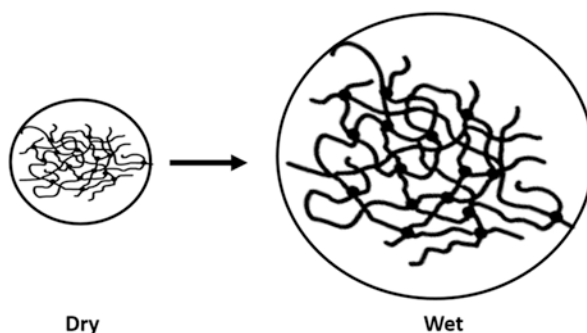


Fig. 7.21 Flexibility of elastin is a result of the cross-links formed between the amino groups by the action of enzyme lysyl oxidase

shells of animals. The alpha-keratins are named as such due to the main structural component being organized in α -helical strands, which forms superhelical structures. The beta-keratins are composed of beta-sheets twisted together and stabilized by disulfide linkages. The more flexible and elastic keratins have fewer interchain disulfide linkages than the stiffer keratins. Keratins contain a high proportion of glycine and alanine, both of which are very small in size, and this and their hydrophobic nature allow it to be tightly packed.

In silk fibroin, which is also a β -keratin, glycine and alanine constitute ca. 75% of the amino acids, followed by serine (ca 10%). The chains in the beta-sheets are antiparallel. In addition to intra- and intermolecular hydrogen bonds between the beta-sheets, keratins are further stabilized with disulfide linkages through the cysteines they have in their structure.

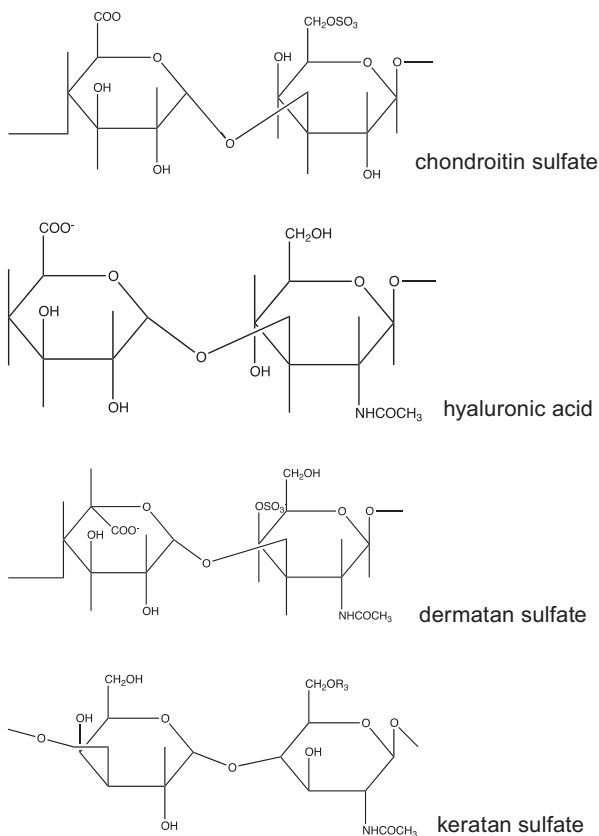


Fig. 7.22 Chondroitin 6-sulfate, keratan sulfate, heparin, dermatan sulfate, and hyaluronate [12] (R: H, SO_3^{2-})

7.6.5 Chondroitin Sulfate

Chondroitin sulfate is a linear polymer formed by 40–100 disaccharide units of glucuronic acid and N-acetylgalactosamine. Frequently galactosamine's C4 and C6 OH groups or glucuronic acid's C2 and C3 OH groups are replaced with sulfate groups. For example, in the formation of chondroitin 6-sulfates, the OH at N-acetylglucosamine's sixth carbon is replaced with a $-\text{OSO}_3$ group. Chondroitin sulfates are found as proteoglycans; in other words, they are bound covalently to proteins and form brushes or branches (Fig. 7.22).

In the cartilage, the highly organized and negatively charged sulfates of chondroitin sulfate repel each other electrostatically, and this makes them resist compression. Decrease of chondroitin sulfate amount in the cartilage leads to osteoarthritis. That is why glucosamine and chondroitin sulfate are frequently used in cartilage treatment.

Fig. 7.23 Dermatan sulfate or chondroitin sulfate B consists of L-iduronate and N-acetyl-D-galactosamine-4-sulfate disaccharide units

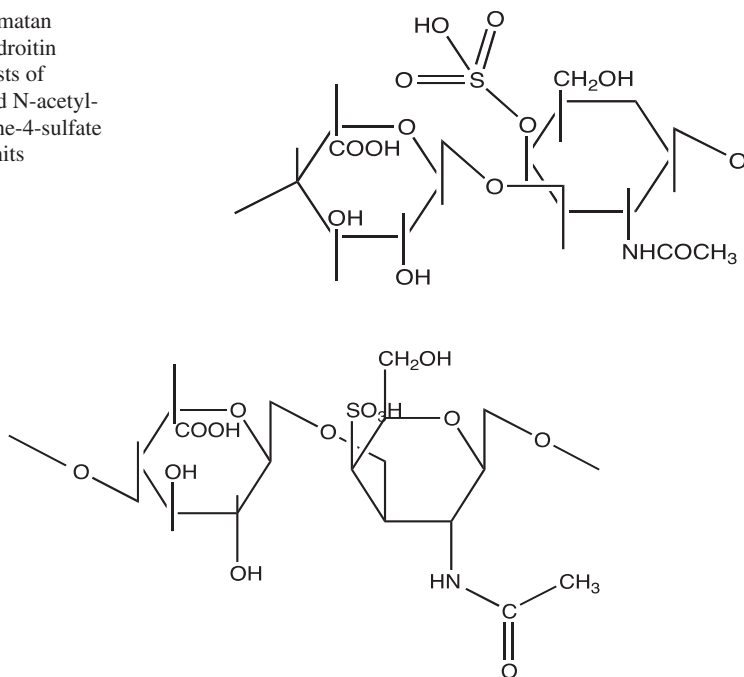


Fig. 7.24 Chondroitin 4-sulfate is composed of glucuronic acid and N-acetylglucosamine disaccharide repeating units

7.6.6 Dermatan Sulfate

Dermatan sulfate, also known as chondroitin sulfate B, is a glycosaminoglycan found in many mammalian tissues such as blood vessels, heart valves, and especially the skin. Its repeating unit is a dimer of L-iduronate (many are sulfated) and N-acetylglactoseamine-4-sulfate with a $\beta(1, 3)$ linkage. Its role as an important soluble component of the extracellular matrix is becoming more clearly understood. The secondary structure and especially the conformational flexibility of the iduronate residue is believed to play a key role in defining its wide range of biological specificities (Fig. 7.23).

Chondroitin sulfate has three isomers that differ in the orientation of the carboxylic acid or the sulfate groups: chondroitin sulfate A (chondroitin 4-sulfate), chondroitin sulfate B (dermatan sulfate), and chondroitin sulfate C (chondroitin 6-sulfate). Chondroitin sulfate A is also called chondroitin 4-sulfate because of the position of the sulfate group and is found in the cartilage, bones, and cornea (Fig. 7.24). Chondroitin sulfate is abundant in the skin and is also found in heart valves, tendons, and arterial walls. Its molecular weight ranges from 15 to 40 KDa. Chondroitin 6-sulfate is found in the cartilage, umbilical cord, and tendon. Due to the highly viscous solutions they form, they serve as lubricants, too.

7.6.7 Hyaluronic Acid

Building blocks of hyaluronic acid are β -D-glucuronic acid and N-acetylglucosamine (2-acetamido-2-deoxy- β -D-glucose). The first monosaccharide has its OH group at position six replaced with a carboxylic acid (COOH) and thus can easily ionize to yield a negative charge. The second monosaccharide has an N-acetyl group (NH-COCH₃) which is highly polar. Hyaluronic acid is the principal polysaccharide found in the body fluids such as synovial fluid, vitreous humor of the eye, skin, cartilage, and Wharton's jelly of the umbilical cord. In the umbilical cord, it has a molecular weight of 6 MDa and is found in solution as solvated spherical molecules with a diameter of around 200 nm.

Its external sources are mainly rooster comb, bovine vitreous humor, bovine synovial fluid, human umbilical cord, and *Streptococcus bacterium* (as a recombinant production source). HA derived from rooster combs reported to yield 7.5 mg hyaluronan/g. Alternative sources such as *Bacilli* and *Escherichia coli* are also being used [13]. High molecular HA (200–400 KDa) was found to be chondroinductive (induces cartilage formation) [14], and its oligomers were angiogenic (increasing vascularization of tissues) [15]. Besides, HA is also involved at sites where the cells are quite mobile, such as during tissue development and wound healing. It is produced in large amounts during increased cell mobility, and when the activity decreases, it is removed by hydrolysis through the action of enzyme hyaluronidase. It also is an important component of the synovial fluid and serves as a lubricant.

7.7 Conclusion

In this chapter, the building blocks of the body, mainly proteins, polynucleotides, polysaccharides, and lipids were discussed. Their chemical structure, composition, organization, and functions in biological media were described. These details are important for a biomaterial scientist, since in the production of materials to mimic or to substitute for human tissues, these building blocks serve both as the guide and as the constituent materials. For example, in making a soft tissue replacement such as a skin graft, we need to know the materials that can be used to construct a bilayer skin substitute. The knowledge gained in this chapter will be helpful in the design and construction of such products.

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8.1 General Properties

Composites are materials which contain more than one component with different physical, chemical, and structural characteristics, and each component contributes to the final product to reach a desirable composition and property. Research on engineered composites was started in the 1960s, and the first composites were designed for automobile and aerospace industries to produce tough, mechanically strong, and stable materials the performance of which exceeds the requirements. The definition of a composite is “combination of two materials in which one of them serves as the reinforcing phase (in the form of fibers, sheets, or particles) embedded in the second material, the matrix.”

The main purpose of making composites is to manufacture reinforced materials, and therefore materials like glass fibers, particulate carbon black, and carbon nanotubes are added into the solid matrix which is mostly a polymer. In a composite, the important point is that each component should keep its identity as a different phase, should be distinctly separated from each other, and contribute with its specific property to the matrix material and create the desirable end product with enhanced properties. Metal alloys are not composites although they contain different metal atoms because there are no distinctly separated phases. Some examples of the reinforced industrially used composites present in the market are molding plastics loaded with fillers, rubber tires containing carbon black, cemented carbides containing carbide particles, asphalt, and concrete. In the medical area, the composites are formed by the combination of polymers and ceramics, polymers and metals, and metals and ceramics or a combination of several of these composites to be used in the treatment of the damaged tissues. For example, polymer coated-metals are used as heart valves, polymers combined with hydroxyapatite particles are used in the production of bone tissue or bone tissue engineering scaffolds, and polymeric nanoparticles and fibers are combined with magnetic particles to be used as intelligent drug delivery devices.

8.2 Limitations of Composites

Composites are produced to enhance certain properties of materials for some desired application, but there are a number of limitations in the production of composites:

- **Anisotropic properties:** The properties of many important composites show differences depending on the direction the measurement is made. For example, a material may possess a higher elastic modulus in x direction than in y or z directions. This property may be an advantage or a disadvantage depending on the targeted application.
- **Effects of chemicals:** Many composites may show degradation, dissolution, or corrosion when they are subjected to chemicals or solvents. The attack of chemicals may be less detrimental than it would be on the fresh, unused material, but still this may be sufficient to prevent the final products from being used for a certain application.
- **Laborious production:** Many composites are produced and shaped by slow and complex methods.
- **Expensive production:** Many composite materials are expensive partially because of the value of starting materials but mainly because of the expense of production.

8.3 Biomedical Composites

Scientists continuously try to produce new composites with different physical characteristics and mechanical properties to meet the requirements for medical applications. A number of biomedical composite studies have focused on dental and orthopedic implants. The goal is to enhance the stiffness, strength, and biocompatibility, to make the product more suitable for the host location and to achieve proper interactions with the surrounding tissue.

In the nature, there are many composites such as the bone, cartilage, teeth, and skin. The bone consists of organic molecules, mainly collagen, and inorganic materials such as hydroxyapatite, a calcium phosphate, produced in the body by bone cells. Organic components give the elasticity, and the inorganic components provide the mechanical strength. In natural composites, there are hierarchically ordered structures, and in general particulate or fibrous components of different sizes are dispersed in a porous or fibrous matrix. The properties of the final composite material depend on the chemical composition and physical form of each constituent and the interactions at the interfaces between these constituents. The minor component should be homogeneously distributed within the matrix and create a product with uniform properties throughout the structure. It is possible to produce a large variety of composites with different properties using the same starting materials. By controlling the composition, organization, and order of introduction into the final product, the desired properties can be obtained. The shape of the components (particle, fiber, platelet) added into the matrix and their volume fractions have a significant

control on the properties of the final product. The most commonly used heterogeneous constituents are classified in three groups: particles (have no long dimension, can be spherical, ellipsoidal, polyhedral, or irregularly shaped), fibers (have one long dimension with size in the range nm to mm), and platelets (have two long dimensions with regular or irregular shapes) as shown in Fig. 8.1.

The morphology, orientation, direction, and homogeneity of the added components affect the properties of the composites. The addition of the components can be in different ways such as unidirectional, layer-by-layer, and random, as shown in Fig. 8.2.

Composites can be divided into three groups depending on their matrix:

1. *Polymer matrix composites (PMCs)*: Matrix is a polymer such as thermosetting resins or thermoplastic polyesters. Some examples are polylactides or polyurethanes which have properties enhanced with addition of carbon fibers or ceramic particles like HAp.
2. *Ceramic matrix composites (CMCs)*: Matrix is a ceramic such as Al_2O_3 or SiC where metallic- or carbide-type fibers or particles are added as the second phase.
3. *Metal matrix composites (MMCs)*: Matrix is a metal, and the added components can be ceramics or metals, such as the cemented carbides or cermets. Cermets are combination of materials formed from ceramic (cer) and metallic (met) components.

8.4 Polymer Matrix Composites (PMCs)

Polymers are materials highly preferred in medical applications since they have versatility and ease of synthesis and are economical. They may be modified according to demand by adding some ceramic or metallic particles or polymeric fibers. For example, for tissue engineering purposes, biodegradable polymeric composite scaffolds can be engineered as hard tissue or soft tissue applications by adding TCP (tricalcium phosphate), selenium, or zeolite.

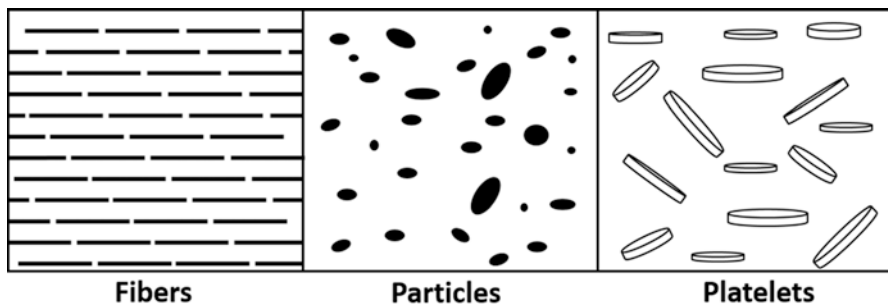


Fig. 8.1 Shapes of some basic composite ingredients: fibers, particles, and platelets embedded in polymer matrix

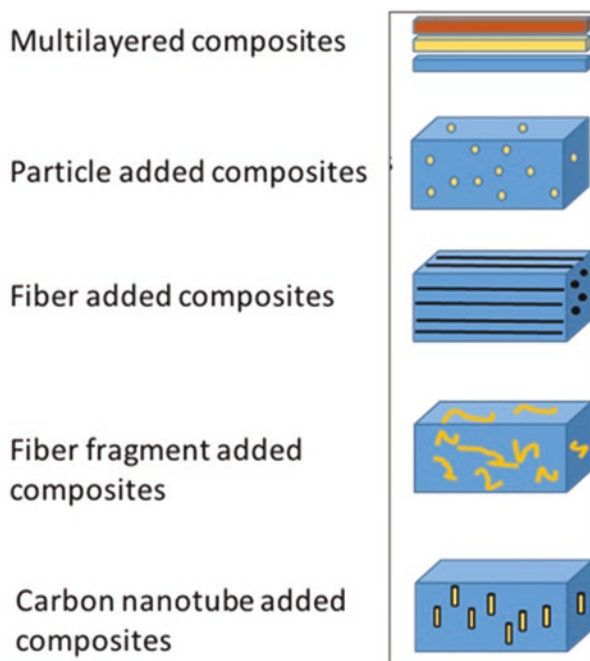


Fig. 8.2 Various components introduced to the composite matrix

For biomedical composites, simple definitions and classifications are not easy. Although composites are solid structures with two or more components with different physical and chemical properties, the goal of making composites used in medical applications may not be to enhance the mechanical properties of the product. For example, in some cases the composites can be designed to be soft and biodegradable, and this is achieved by adding some ingredients to the matrix. Among such ingredients are proteins, growth factors, or cells that are added for bioactivity. In these cases, medical composites are divided as vital-avital and avital-avital composites depending on the presence of cells (the first case) in the composite. For example, a polymeric scaffold loaded with cells can be considered as a “vital-avital composite” since there is more than one component, each has its own properties, and one of them is living or possesses biological activity. On the other hand, a polymeric scaffold loaded with HAp powder is classified as an avital-avital composite. Table 8.1 shows some examples for this type of scaffolds.

Most of the commercially important composites are polymer matrix composites (PMCs), and they generally are impregnated with various types of fibers, particles, or platelets as the second phase. Addition of carbon black or polymeric fibers into the matrix reinforces the mechanical properties of the polymer. From the biological point of view, polymer-ceramic composites are highly similar to the natural bone tissue which is composed of collagen (polymeric) fibrils and hydroxyapatite (ceramic) crystals.

Table 8.1 Some commonly used polymer matrix composite biomaterials

Class	Type
Composites with cells	Poly(L-lactic acid) (PLLA) with fibroblasts Polyglycolic acid (PGA) with fibroblasts Poly(LD-lactic acid) (P-LD-LA) with stem cells Polytetrafluoroethylene (PTFE) with endothelial cells Polycaprolactone (PCL) with stem cells PLLA with hydroxyapatite (HAp) and stem cells
Resorbable composites	Polyethylene glycol (PEG)/HAp Collagen/polyethylene (PE) Polyhydroxybutyrate (PHB)/HAp Collagen/tricalcium phosphate (TCP) PLA/PGA PGA/PGA fiber PGA/carbon fiber PLA/carbon fiber PLLA/PLDLA PCL/HAp Alginate/HAp Collagen/HAp Gelatin/HAp Fibroin/HAp
Nonresorbable composites	Polyetheretherketone (PEEK)/carbon fiber Ultrahigh MWpolyethylene (UHMWPE)/HAp UHMWPE/carbon fibers UHMWPE/polymer fibers PTFE/carbon fiber PP/carbon fiber Polymethyl methacrylate (PMMA)/PE PMMA/bone powder PMMA/HAp PMMA/alumina PMMA/bioglass PMMA/glass fiber PMMA/carbon fiber Polyethylacrylate (PEA)/TCP Polystyrene (PS)/polymer fiber PS/TCP Polyurethane (PU)/polymer fiber PU/HAp PU/TCP PU/carbon fiber

8.5 Ceramic Matrix Composites (CMCs)

Ceramic matrix composites (CMCs) have a ceramic component both as the matrix and the minor component phases where the added phase could consist of ceramic fibers. Carbon and carbon fibers are also considered ceramics, and both, the matrix and fibers, can be carbon based. The most commonly used ceramic matrix material or the ceramic fibers are made of carbon, silicon carbide (SiC), silicon nitride,

aluminum oxide (Al_2O_3), mullite ($\text{Al}_2\text{O}_3\text{-SiO}_2$), and glasses. Ceramic fibers can be amorphous or crystalline and should be stable at high temperatures ($>1000^\circ\text{C}$) since the CMC production takes place above this temperature.

Since the conventional ceramics like alumina, zirconia, silicon nitride, or carbide are not strong against impact forces due to cracks or voids (bubbles), they are combined with additives. Thus the CMCs are strengthened against cracking problems. Particles, fibers and platelets can be embedded in the ceramic matrix in order to decrease or prevent crack formation and propagation and to increase toughness. Carbon fibers are the most commonly used reinforcing materials in the industry. In prostheses and other medical applications where esthetic concerns are not important, carbon fiber-based composites can be used. In some applications, in order to increase the interactions between the composite components, polymers are added during the manufacturing stage. CMCs represent an attempt to retain the desirable properties (as high stiffness, hardness, compressive strength, etc.) of ceramics and compensating for their weaknesses (low toughness and bulk tensile strength, susceptibility to thermal cracking). CMCs enhance properties like fracture toughness, thermal shock resistance, and dynamical load capability of pristine ceramics. Meanwhile, the orientation of the fibers affects the mechanical strength. While highly aligned fibers are preferred for anisotropic stress application, random distribution of fiber orientation is more suitable for isotropic loading.

Thermal and electrical properties of CMCs vary depending on the components. Increase in the electrical properties can be seen in the presence of carbon fibers, while effective insulation can be observed for oxide ceramic composites since they have highly porous structures. Their stability at high temperatures and their light weight make them preferable in the production of space vehicles and gas turbines.

Bioglasses can also be considered as ceramic biocomposites. Some novel bioactive glasses were developed for medical applications. Sintered Na-containing and borate-based ones are two of them. Some elements such as Sr, Zn, or Cu can be introduced to the glass structure. These structures enhance formation of bone and vascular tissue and differentiation of stem cells to osteoblasts and have adjustable degradation rates. Although trace elements have positive effects, high amounts may cause toxicity, and care must be applied to preserve safe levels. The polymers PLLA and PDLA are commonly used bioceramics especially in Bioglass® production.

8.6 Metal Matrix Composites (MMCs)

With the metal matrix composites (MMCs), the matrix is metal, and the other component can be a metal, ceramic, carbon, or organic compound in the form of particles or fibers. The property of metal is enhanced in the desired direction by addition of the second phase. In case of strengthening, generally light metal matrices such as aluminum, magnesium, or titanium are preferred, and when resistance to high temperature is required, metals such as cobalt and cobalt-nickel alloys are used.

In general, stability of MMCs at high temperatures is much better than that of PMCs but lower than CMCs. Similarly, fabrication and production costs of MMCs

are higher than PMCs but lower than CMCs. MMCs do not absorb moisture and are stable upon exposure to ultraviolet radiation and therefore have certain advantages over polymeric composites.

Reinforcement materials such as monofilament wires, and carbon or silicon carbide fibers can be added to metallic matrices. In order to prevent possible reactions among the materials, the surface of the reinforcement materials can be coated. For example, composites with high strength and low density can be prepared by addition of carbon fibers coated with nickel boride (Ni_2B) or titanium boride (TiB_2) in aluminum. Mechanical properties such as friction coefficient, wear resistance, and thermal conductivity can be enhanced with addition of the fibers or particles into the matrix. Orientation of the fibers affects the strength of the final product, and in general continuous fibers provide the highest mechanical strength by transferring load properly from matrix to the reinforcing component. The aspect ratio of the reinforcement material is also important since the strength also depends on this property. MMCs can be produced by blending of powders, addition of constituents in molten metal, coating of metal vapor on metal, or in situ fabrication by unidirectional solidification of one component in a matrix.

Biodegradable metals are becoming important in the production of short term implants. The most important requirement of these materials is that the soluble and insoluble (particulate) degradation products and ions released should not cause any undesirable effects and side reactions. Controlled release of some trace elements essential for the biological metabolism can be achieved with such systems. One of the most studied metals in this category is magnesium and its alloys. Magnesium is a light element that has good mechanical strength, good conductivity, and high biocompatibility, and magnesium ions are required for many biological pathways. Biodegradable cardiovascular stents of magnesium and magnesium composites are recently being produced, and their in vivo performance is investigated.

Cermets are ceramic (cer) and metal (met) containing composites as stated before. They have a high fraction of ceramic (up to 90%) distributed in a metallic matrix. Metal matrices are generally constituted of low density metals such as aluminum, magnesium, titanium, and chromium. Ceramic components of the cermets are mainly alumina, boron, carbon, and silicon carbide. Bonding between these two phases can be enhanced by the slight melting achieved at elevated temperatures used in processing.

8.7 Constituents and Classification of Biocomposites

The most important aspect of biocomposites is their purity and biocompatibility of their constituents. Therefore, the processing and production steps should be carried out under standardized, clean conditions, and the products should have medical grade purity. Some materials approved for diagnostic and therapy purposes and used in biocomposite preparation are summarized in Table 8.2.

Table 8.2 Constituents of biocomposites

Matrices	Fibers	Particles
Thermoplastics	Polymers	Inorganic
Polyolefins	Polyesters	Glass
Polypropylene (PP)	Polyolefins	Alumina
Polyethylene (PE)	Aromatic polyamides	Silver
Polycarbonates	(aramids)	Gold
Polyesters	UHMWPE	Organic
Polysulfones	PTFE	Polyacrylate
Poly(etherketones)	Bioresorbable polymers	Polymethacrylate
Thermosets	Poly(lactide (PLA)	PLA
Polyacrylates	Polyglycolide (PGA)	PGA
Polymethacrylates	Copolymers of PLA and	PLGA
Polyesters	PGA (PLGA)	
Polyaldehydes	Collagen	
Silicones	Gelatin	
Inorganic	Silk	
Phosphate ceramics	Inorganic	
Hydroxyapatite	Carbon	
Calcium carbonate	Glass	
Glass ceramics	Hydroxyapatite	
Carbon	Tricalcium phosphate	
Stainless steel		
Titanium		
Resorbable polymers		
Poly(lactide (PLA)		
Polyglycolide (PGA)		
Copolymers of PLA, PGA		
(PLGA)		
Poly(hydroxybutyrate)		
Alginate, chitosan, collagen		

8.8 Bone Structure: A Natural Composite

Natural bone is a very good example of a natural composite material. It is made of organic and inorganic components where the organic component is mainly protein in the form of a collagen fiber matrix, and the inorganic component is hydroxyapatite (HAp). Collagen consists of tropocollagen units and forms fibers. In the bone there are other organic molecules such as glycosaminoglycans, osteocalcin, osteonectin, and bone sialoproteins, too. Inorganic component HAp is a calcium phosphate compound with the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and makes up about 70% of the bone dry weight. It is present as small crystal plates in the size range 5–40 nm embedded in the collagen component. Combination of these two dissimilar materials with different properties (HAp crystals with high hardness and collagen fibers with high toughness) with a highly organized design results in the impressive properties of the bone whose strength is much higher than that of individual materials.

The bone has microscopically two different types of structures. Woven (trabecular) bone is one type that is immature bone with unorganized collagen fibers, and the

other, the lamellar (cortical) bone is the mature bone which has a highly regular, parallel alignment of collagen. Similar differences in the organization of the mineral component is also apparent at the macroscopic level. Initially the rapid production of osteoids by osteoblasts leads to unorganized collagen formation, but as the bone grows, it forms the “organized concentric sheets” structure. Lamellar bone has parallel collagen fibers in a layer where they are in opposite directions in subsequent layers. This plywood-type organization gives strength to the bone and high resistance against torsion. Thus, the bone is a very good example of a composite system designed by nature (Fig. 8.3).

Histological examinations with light and fluorescence microscopy show the osteocytes embedded in the matrix surrounding the osteons and the interaction of osteocytes through their extensions called filopodia (Fig. 8.4).

One important property of the bone is its responsiveness to the mechanical forces applied. It can remodel itself according to the level and the direction of the forces. In order to resist high forces, a dense bone structure develops via proliferation of bone cells. The bones of the patients staying in the bed for long periods lose their density since almost no force stimulates the cells to produce gravity resisting bone. The load exerted on a hip joint is about three to four times higher than the body weight during walking or running and may increase by up to ten-fold during jumping. The number of cycles of load application on the femur during walking is about one million per year, and the bone resists all the compressive, tensile, shear, or cyclic forces applied onto it. The average elastic modulus of femur is 17 GPa. Although the bone has a high mechanical strength, its resistance to impact forces is low and can easily fracture or break. Still, composite nature of the bone makes it an ideal material for load bearing and protection of human tissues against environmental forces. Mechanical properties of some hard tissues are summarized in Table 8.3.

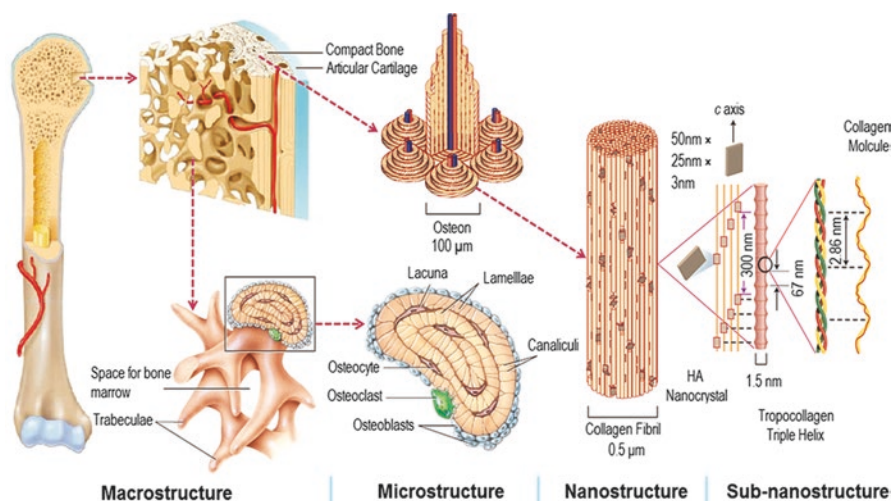


Fig. 8.3 Bone structure and organization [1]

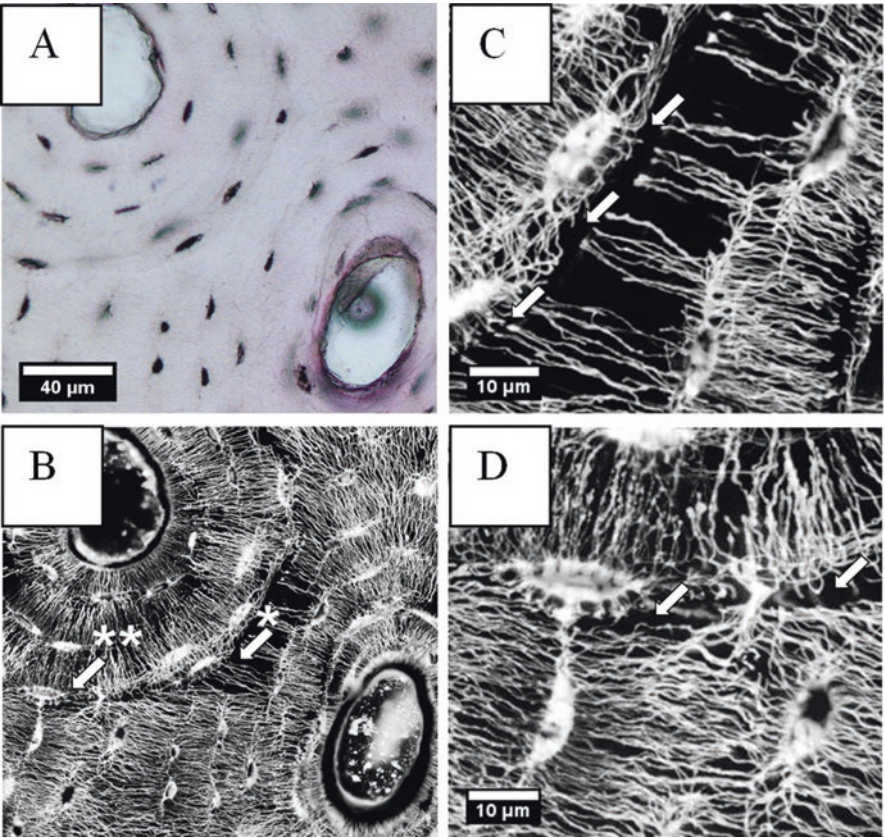


Fig. 8.4 Histology of the bone. (a) Rhodamine staining, (b–d) Confocal images (close-ups *:c, **:d) *c) the cement lines disrupt the osteocytic network (arrows); **d) orientation of canaliculi and bone lamellae [2]

Table 8.3 Mechanical properties of hard tissues [3, 4]

Type	Tensile strength (MPa)	Compressive strength (MPa)	Elastic modulus (GPa)
Tibia	140	159	18.1
Femur	121	167	17.2
Humerus	130	132	17.2
Cervical	3.1	10	0.23
Lumbar	3.7	5	0.16
Enamel	10	384	84.3
Dentin	39.3	297	11.0

8.9 Orthopedic Implants

In orthopedic implants, the mismatch of hardness, stiffness, and density between the bones and the implants is the main problem. Cortical bone is dense (10% porosity) and carries the main load. Spongy bone is porous and acts as shock absorber between the bone and soft tissue. Since their stiffnesses are different, the stress distribution may not be homogeneous, and the sharing of the applied load between the bone and implant material may not be equal. In general, metallic implants carry the majority of the load leaving a very low fraction to the bone so that bone regeneration and the healing process are delayed leading to increased porosity in the bone structure (bone atrophy). This is called “stress shielding” or “stress protection.” In order to have proper regeneration of the bone tissue and fast healing, it is essential to adjust the stiffness of the implant material to the host bone tissue value.

Some other undesirable effects of metal implants are ulcer formation, skin diseases, and immune response. For example, nickel causes skin problems such as dermatitis, aluminum causes epileptic problems and Alzheimer’s disease, cobalt causes anemia, and chromium affects the central nervous system. These effects are more severe in cases where the implant is close to the vital organs like the kidney and liver.

Polymers are preferred in the production of orthopedic implants because of their lower stiffness, but their low modulus and easy deformation under stress limit their widespread use for load-bearing applications. Therefore, reinforced polymer composites containing metallic, ceramic, or polymeric fibers or particles are preferable materials demonstrating the required properties for many applications. Mechanical strength of the bone is anisotropic with a higher value in the longitudinal direction than in the transverse. Polymeric composite materials can be made to mimic these properties by organizing the fibers in the polymeric matrix. Polymer matrix composites have advantages over the metals such as not corroding, not releasing ions from the matrix, not showing any magnetic property, not having metallic fatigue failure, and not leading to some allergic tissue reactions which the metals show. Polymer composites can be preferred over ceramics because of their higher fracture toughness. Another property is the adjustable radio-opacity of the polymeric composites. Solids such as metals and ceramics are radio-opaque and block imaging of tissues during X-ray examinations. With polymeric composites, by adjusting the fraction of the radio-opaque component, it becomes possible to adjust radio transparency. The most common polymer for total hip and knee replacements is ultrahigh molecular weight polyethylene (UHMWPE). The creep and fatigue resistance of UHMWPE can be further enhanced by addition of fibers.

For hard tissue applications, the most commonly used materials are metals and ceramics due to their compressive strengths. However, the corrosion problem of metals and the difficulty in the production of ceramics make the polymer composites the most preferable materials. In hard tissue applications, mechanical strength and bone-bonding property of the material are important, and polymeric composites prepared with metal or ceramic particles can increase the strength and toughness of the materials and reinforce the interaction between the bone and the implant

material. Effective bonding with the bone makes the implant more stable at the implantation site and thus more acceptable by the host tissue. Bone bonding ability makes the material “bioactive.” Total hip replacement is one of the most frequently performed operations. The service life of these implants is roughly limited to 15 years. Sometimes genetic diseases, osteoarthritis, injury, or wear of the implant can cause its failure, and replacement of the implant by a revision surgery becomes essential.

The application areas and the types of composites considered for use as hard tissue supports or implants can be summarized as follows:

- *Bone fracture repair* (the cast materials for bandage include woven cotton fabrics and plaster of Paris)
- *Bone plates* (common material for composites – carbon fiber fabric and epoxy matrix, carbon/PEEK)
- *Total knee* (UHMWPE reinforced with carbon fiber or UHMWPE itself)
- *Ankle, hip, and other joint replacements* (UHMWPE reinforced with carbon fiber or UHMWPE itself)
- *Skull (cranial) reconstruction* (common material for composites – carbon fiber fabric and epoxy matrix, carbon/PEEK)
- *Dental applications* (crowns from carbon fiber reinforced epoxy composites, glass fiber reinforced polyester)

8.10 Surface Modifications: A Route to Composites

The surface chemistry and topography of biomedical implants are important since the first reactions take place at the implant-host tissue interface. As mentioned previously, ceramics such as alumina or zirconia are preferred because of their high wear resistance, inertness, and biocompatibility, while metals such as titanium or cobalt are preferred because of their very high load-carrying ability. Meanwhile, these materials do not show the required bone-forming capacity and osseointegration. In these cases, surface modification is required to increase the cell efficiency and tissue interaction of the material and to prevent the fibrous capsule formation around it. Coating of metallic surfaces also prevents the allergic reactions often caused by the release of nickel, cobalt, or chromium ions into the biological environment. A commonly applied technique is to coat the implant with HAp or bioactive glasses. This type of coating enhances tissue integration and promotes fixation of the implant, as well as preventing corrosion of the metal. HAp coating can be made by plasma spraying or by deposition from a solution. Another surface modification approach is formation of oxide film on metals. This also prevents corrosion and release of ions. There are also cementless total hip arthroplasties where the implant surface is modified and made porous so that new bone tissue can grow in and stabilize the implant (Fig. 8.5).

Carbon coating of the metallic surfaces is also frequently applied. One specific type of coat material is pyrolytic carbon which is obtained by heating hydrocarbons



Fig. 8.5 The cementless stem and acetabular cup of a total hip implant [5]

at their decomposition temperatures and is widely used for heart valves or hip joints to make the systems compatible with the host tissue.

Long-term success of the implants depends partly on the initial mechanical stability of the implants. Titanium and its alloys are used widely because of their biocompatibility, rapid passivation (oxidation) of their surfaces, high corrosion resistance, and low elastic modulus which is close to that of the bone. In order to create a high friction surface to increase physical interlocking at the implant-bone interface and to enhance bone-bonding activity of the titanium implants, porous or rough structures on the surface of the implant have been developed with the use of plasma-spraying, grid-blasting, and fiber-metal or bead-sintering methods. For example, HAp is then coated on the porous implant surface to promote direct bonding between the implant and the bone because of its good osteoconductivity.

In some cases, such as blood-contacting stents or catheters, it is necessary to passivate the surface to reduce adsorption of certain types of protein and to prevent thrombus formation. For this purpose, the surface of the implant can be coated with an inert biological molecule such as albumin.

8.11 Tissue Engineering Scaffolds

Biodegradable polymeric composites are used in scaffold (cell carrier) preparation for bone and cartilage tissue engineering purposes. Polylactides, polyglycolides, and their copolymers produce acidic compounds when they degrade, and therefore their degradation products cause inflammatory reactions due to low local pH. The presence of calcium phosphate in the structure can buffer the environment since its degradation produces basic ions. HAp crystals are very stable and do not dissolve, but there are other soluble calcium phosphate crystals as beta-calcium phosphate. The presence of HAp or different apatite crystals increases cell attachment and proliferation and increases osteointegration and the healing process. The most commonly used scaffold composites are calcium-containing inorganic compounds such as $\text{Ca}_3(\text{PO}_4)_2$, CaCO_3 , or HAp (in powder, flake, or fiber forms) or bioglass

(nano- and microparticles) added to synthetic and biological polymers such as PLLA, PLGA, PE, PCL, chitosan, collagen, and fibrin. Introduction of inorganic compounds to the composite material enhances the mechanical properties of the scaffolds and accelerates osteointegration by enhancing cell attachment especially in hard tissue engineering applications.

8.12 Conclusion

In this chapter, composites – materials formed by bringing together two or more components to obtain an improved product – and their use in medical applications were discussed. In general, one component is the matrix, the continuous phase, and the other(s) is added into the matrix in different forms. The matrix can be polymeric, ceramic, or metallic, while the minor component can be added as a particle, fiber, layer, or in any other form to enhance the desired physical, chemical, and structural properties of the biomaterial. Best properties of two or more components frequently lead to very successful biomedical products.

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Fundamentals of Human Biology and Anatomy

9

9.1 Fundamentals of Human Biology and Anatomy

The human body has different structural levels of organization, starting with molecules and macromolecules at the lowest level and increasing in both size and complexity to cells, tissues, organs, and eventually to the systems that make up the whole organism.

In the human body, macromolecules such as polypeptides (proteins and enzymes), polynucleotides, and polysaccharides are as important as small molecules (H_2O , CO_2 , O_2 , N_2) that constitute them or interact with them. Actually between the molecules and the cells are the organelles that are the components of the cells, and without forming the cells, they do not have the properties of a living entity. Cells are the smallest independent units of life because all life depends on the many chemical activities of cells. Some of the basic functions of the cells are growth, metabolism, and reproduction.

Tissues are made up of many similar but different cells that perform a certain function. The various tissues of the body are divided into four groups. These are epithelial, connective, nervous, and muscle tissues. Epithelial tissues constitute the outer layer of skin, lining of the organs, blood and lymph vessels, and the body cavities. Connective tissues connect and support the rest of the body. They constitute most part of the skin, bone, and tendons. Muscle tissues produce movement through their ability to contract and extend, and among the several types of muscle tissues are the skeletal, smooth, and cardiac muscles.

Organs are a highly integrated collection of two or more kinds of tissue that work together to perform a specific function. For example, stomach is made of a number of tissues to digest the food taken in, in order to convert it into building blocks for reconstitution of the tissues and also to generate energy.

Systems are groups of organs that work together to perform a major function. An example is the cardiovascular system whose main function is to circulate the blood in the body to provide the required nutrients to the tissues and the cells. All the organ systems working together make up the whole organism.

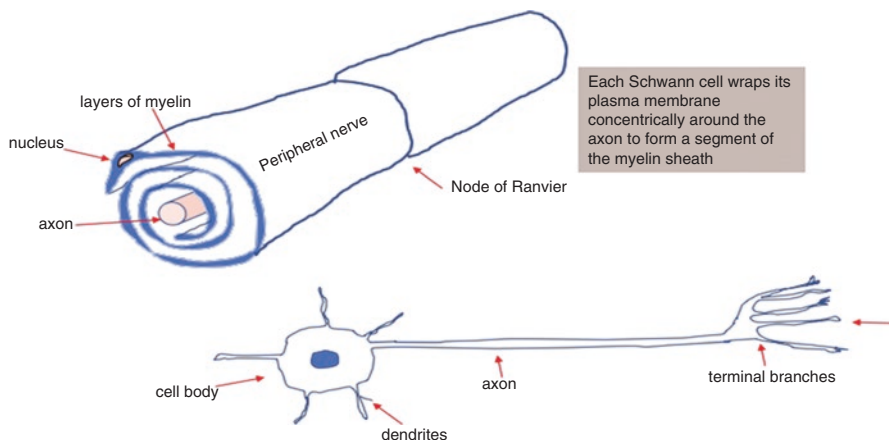


Fig. 9.1 Schematic presentation of peripheral nerve organization

As an example of the complex organization, nervous system can be studied. This system provides the main mechanism for the transmission of various types of stimuli between the nervous, muscle and the sensory tissues. The nervous system has two main components: central nervous system (CNS) and peripheral nervous system (PNS). CNS consists of the brain and the spinal cord, while the PNS is the nervous system component that connects the muscles at the extremities and the spinal cord (Fig. 9.1). There are a variety of cells that make up the nervous system. Nerves are cables that link the CNS with sense organs and muscles for input and output of information.

9.2 The Cell

In unicellular (single-celled) organisms, a single cell performs all life functions and can maintain life alone. It functions independent of any other cell. On the other hand, multicellular organisms, including the human beings, have various levels of organization. Individual cells in such multicellular organisms perform certain specific functions assigned to them alone or in small groups, and on the whole, they all work together in unison for the benefit of the entire organism. The mammalian cell has a highly organized structure. It has organelles which have certain specific functions and compartments. These include the nucleus which contains the genetic information carried in the DNA and serves as the main control system of the cell. There are also mechanisms established to transfer this information as information packets to get cellular activities performed by the other organelles and molecules (Fig. 9.2).

The mitochondria serves as the power plant of the cell, the Golgi apparatus is the site where glycoproteins and lipoproteins are assembled. The smooth and rough endoplasmic reticulum is involved in protein synthesis and also in the transport of

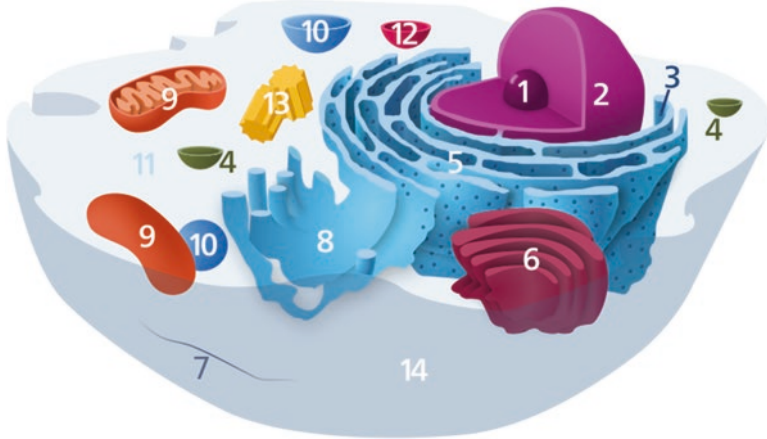


Fig. 9.2 The mammalian cell. (1) Nucleolus, (2) nucleus, (3) ribosomes, (4) vesicle, (5) rough endoplasmic reticulum, (6) Golgi apparatus, (7) cytoskeleton, (8) smooth endoplasmic reticulum, (9) mitochondrion, (10) vacuole, (11) cytosol, (12) lysosome, (13) centriole, and (14) cell membrane [1]

molecules within the cell. Lysosomes contain proteolytic enzymes to metabolize endocytosed foreign particulates and materials. The cell shape and mobility are the main responsibilities of the cytoskeleton, a very complex structure which is also involved in mechanical and chemical signal transduction between the nucleus and the cell membrane. The cell is protected by a membrane that surrounds the organelles and the cytoplasm, controls the transference of molecules and fluids in and out of the cell, and preserves the homeostasis (tonicity, pH). Membrane is also involved in various other functions such as absorption of metabolites, secretion of waste products along with other cellular products such as proteins. It is involved in attachment onto the extracellular matrix or substrates and in the communication between cells. The membrane is actually a very dynamic structure consisting of a double layer of phospholipids in which proteins, glycoproteins, lipoproteins, and cholesterol are embedded.

9.3 Tissues

Tissues are organization of various cells into a more complex structure which we call the organs to carry out a specialized function. Same tissue groups can be organized in different ways and are found in organs which carry out completely different functions. For example, epithelial tissues are found in the skin, cornea of the eye, and the lungs, among others.

In the vertebrates, the tissues are derived from the three original layers of the embryo: the *ectoderm* (outer layer) gives rise to the skin and tissues of the nervous system; the *mesoderm* (middle layer) forms the muscles, bones, and many of the

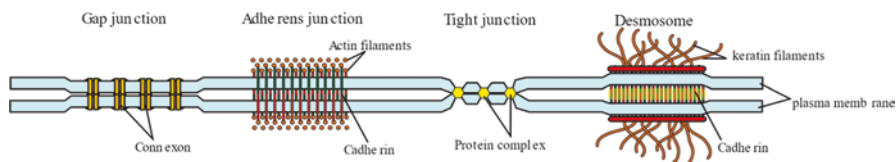


Fig. 9.3 The junction types between the cells [2]

organs involved in reproductive, urinary, and circulatory activities; and the *endo-derm* (inner layer) gives rise to the lining of the digestive tract and organs like the lungs which are derived from it.

The cells that form the tissues are attached to each other by a variety of attachment sites, called junctions, according to the tissue they constitute. These are tight junctions, adherens junctions, gap junctions, and desmosomes (Fig. 9.3).

Tight junctions perform two vital functions: they tie the cells together, and they also limit the transfer of compounds into the tissue. Thus, forcing the molecules to cross the cells by diffusion or active transport. The tight junctions between the cells of the epithelial tissue restrict the movement of the membrane proteins between the apical (facing the external medium or the cavity) and basolateral (facing the basal membrane) surfaces of the cell. Adherens junctions are present as narrow bands connecting adjacent cells forming strong mechanical attachments in between. Gap junctions serve as narrow (1.5–2 nm diameter) intercellular channels. These are especially useful in the transfer of ions which normally cannot easily pass through the hydrophobic bilipid layer membrane to get in and out of the cells which is important for communication via macromolecule exchange.

Gap junctions contribute to changes in the membrane potential between the cells because ions can pass through them causing potential differences between the two sides of the cellular barrier. In addition to ions, they also allow the passage of small molecules (ca.1 kDa). Desmosomes are patches that also hold the neighboring cells together. They are attached to the intermediate filaments of the cytoskeleton in the cytoplasm, which are connected to the nuclear membrane. Carcinomas are cancers of epithelia, and the cells of carcinomas do not have desmosomes. This is considered to be the reason why the carcinoma cells can metastasize (move between tissues).

9.3.1 Epithelial Tissues

The outer and inner surfaces of the body (such as the skin and the gut, respectively) are lined with epithelium. The inner surfaces of all the tubes, ducts, circulatory system, and the glands of the body are also lined by the epithelium. The shapes of epithelial cells vary from cuboidal to squamous (like pancakes) and to columnar (like columns). The major function of all epithelia is to form a barrier. Thus, epithelial cells are always connected with each other, and there are special junctions that join them. Generally, the intercellular space between epithelial cells is very limited.

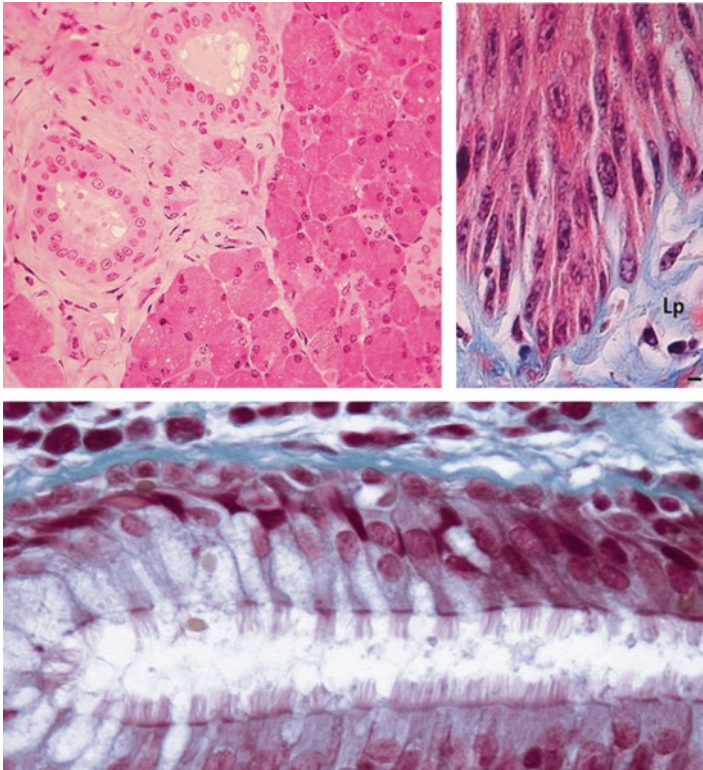


Fig. 9.4 Epithelial tissue: cuboidal (gland) (left) [3], squamous (right) [4], and columnar (with cilia) (bottom) [5]

The functions of epithelia differ markedly. Although all form a barrier, some are much more impermeable than others. The epidermis of the skin is almost an impermeable barrier. On the other hand, the epithelium lining the intestine, while providing a barrier, must also absorb nutrients. Some epithelia have a secretory function (e.g., stomach, glands), and some are actively involved in the synthesis of a large number of metabolic substances (e.g., the endothelial cells lining blood vessels, pancreas, thyroid gland) (Fig. 9.4).

9.3.2 Connective Tissues

Connective tissue consists of individual cells scattered within an extracellular matrix and serves as a framework or a mechanical support system upon which the epithelial tissues rest, and nerve and muscle tissues and blood vessels are localized within or pass through this tissue. Unlike the cells of epithelium, these cells are not directly attached to one another. Connective tissue functions not only as a mechanical support for other tissues but also acts to connect other tissues. In addition, the

main cell types of the immunological defense are concentrated in the connective tissue. Connective tissue is derived from mesenchymal layer of the embryo, while epithelial tissue is derived from the ectoderm and endoderm layers. Connective tissue cells are not attached directly to each other, and there are changing amounts of extracellular matrix in between them. The most common connective tissue cells are fibroblasts, adipocytes, mast cells, macrophages, and lymphocytes.

Lymphocytes are involved in the immune responses and participate in inflammation. Fibroblasts secrete the main material of the extracellular matrix; the collagen and adipocytes store the fat. The matrix of the connective tissue provides the strength to these specific tissues and is composed of the ground substance and fibers. The ground substance is mainly a water-retaining component, and this is achieved by the polysaccharide-based molecules such as glycosaminoglycans, proteoglycans, and glycoproteins. In a hard and load bearing tissue like bone, the strength of the ground substance is caused by the minerals included (mainly calcium compounds such as various calcium phosphates). The other main component of the connective tissue matrix, the fiber, is mainly composed of collagen, which is the most abundant protein in the body and provides the strength of the matrix. On the other hand, the elastic fibers confer the resiliency.

Connective tissue is found in various easily identified forms such as fibrous and adipose tissues but also as highly specialized tissues like the elastic tissue, lymphoid tissue, blood, cartilage, and bone. In the various connective tissue types, the components and their proportions vary. For example, in a fibrous connective tissue such as the dermis layer of the skin, the predominant entity is the collagen fibers. In the fatty tissue, adipocytes are the most abundant, while in the lymphoid tissue, it is the lymphocytes. In some other connective tissues, some components are completely missing. In blood, there are no fibers; the form of the ground substance is fluid- or gel-like. In bone, the ground substance is strengthened by inorganic crystals.

The connective tissues vary greatly but have in common the presence of a cellular and an extracellular component made by the cells of the tissues. Connective tissues may have loose, dense irregular, or dense regular structures. Cartilage, bone, adipose tissue, and blood are more specialized types of connective tissues. Cartilage is found in the noses and ears. The embryos and the newborn have cartilage in place of some of the bones. Cartilage is also found in the structure of the bones. Both the cartilage and the bone have collagen in their extracellular matrix; bone has calcium phosphate-based compounds in the collagenous matrix.

Adipose tissue stores the neutral lipids, is found under the skin, and provides protection as well as serving as an energy source. In adipose tissue cells, the fat droplets fill most of the cell where the organelles are located in the space between the cytoplasm and the nucleus.

Blood is a more complex connective tissue than the previous ones as it is a fluid carrying several types of cells, biomolecules and water. The red blood cells are one of the most important cell types in the blood where they carry hemoglobin which is an important molecule carrying the oxygen to the tissues. Hemoglobin also carries the carbon dioxide produced as a result of the metabolic activities. Other important cell types are white blood cells (leukocytes) found in various forms including the

lymphocytes, fighting infections and disease, platelets (thrombocytes) involved in blood clotting.

9.3.3 Muscle Tissues

There are three major types of muscles: skeletal (striated), cardiac, and smooth. The skeletal muscle is under the voluntary control and is the major “effector” organ of our bodies. The commands of the nervous and endocrine systems are carried out by the skeletal muscles. They are attached to the skeleton through tendons. The cardiac muscle is involved in maintaining the heartbeats. The smooth muscle on the other hand is the muscle that keep the other internal organs like those of the digestive tract functioning. These muscle tissues are identified by their organization. The skeletal muscle tissue is striated (has stripes) that indicate each muscle cell. These cells extend along the length of the whole muscle.

Smooth muscles have a uniform appearance. Their cells are not elongated as those of the skeletal muscle because the motion created by these muscles is much lesser in extent than the striated muscles. Only the smooth muscles have individual cells, but they also contain the contractile proteins actin and myosin. Smooth muscles line the blood vessels and all the internal organs. They are not under voluntary control.

Cardiac muscle tissue also has striations similar to that of the skeletal muscles where the cells are smaller. They have a distinct branched structure highly suited to the task of contraction and expansion while pumping blood. Cardiac muscle resembles skeletal muscle, however it is not under voluntary control like the skeletal muscle.

Skeletal muscle cells (myocytes) are made up of **myofibrils** and fibrous structures and have several nuclei scattered along the length of a single cell. **Skeletal muscle fibers** are made when myoblasts fuse together. The presence of multinucleated cells leads to rapid dissemination of information along the cell. Cardiac and smooth muscle cells have the traditional single nucleus, where the nuclei of the smooth cells are somewhat elongated like the cells themselves.

Most nutrients are provided to the muscles through the bloodstream, and each muscle cell stores a small amount of fat and glucose as a source of energy to be able to function any time.

9.3.4 Nervous Tissues

The nervous system in vertebrates is composed of neurons which are cells that conduct the impulse originating at the sensory organs or the nervous system. A variety of cells support the activity of the neurons and is called collectively the neuroglia. Neurons have a cell body that encompasses the nucleus and then there are the extensions called dendrites and axons that are involved in signal transmission. The axons are the longest of the cell extensions in mammals and can be several feet long,

extending between the spinal column to the toes and fingertips. The axons being so long need to be supported by an inner structure, and these are the microtubules which are also present in other cells but much shorter. The nervous system involves reception of signals from the sensory organs, processing of the information and transmission of response to appropriate organs and tissues. There are as many kinds of neurons based on the location and function within this cycle. These are sensory, integrative, and motor neurons.

The nervous system is divided into two: central nervous system (CNS) and the peripheral nervous system (PNS). The peripheral nervous system consists of the nerves and ganglia outside of the brain and spinal cord. Its main function is to connect the central nervous system, brain and the spinal cord, to the limbs and organs. The CNS is protected by the skull and the vertebral column, while PNS has no such physical protection.

The peripheral nervous system is divided into (a) somatic nervous system, and (b) autonomic nervous system. The somatic nervous system coordinates the body movements and receives external stimuli. It does these under conscious control. The autonomic nervous system, on the other hand, is categorized under the sympathetic and parasympathetic divisions; the sympathetic nervous system responds to impending danger by increasing rate of heartbeat and blood pressure, or it is responsible of the “fight-or-flight” responses, and the parasympathetic nervous system is responsible for the constriction of the pupil, the decrease of the heartbeat rate, the dilation of the blood vessels, and the stimulation of the digestive and genitourinary systems, or in other words, it is responsible of “rest and digest” responses.

In the sympathetic system, the preganglionic neurons are shorter, and neurons originate from the spinal cord and travel to a ganglion where they synapse with a long, postganglionic neuron which extends across the body. At the junction points or the synapses within the ganglia, the signal transmission is through release of acetylcholine, a neurotransmitter, by preganglionic neurons that activate the acetylcholine receptors on postganglionic neurons. Thus, it is a chemical that transmits the information at the cell-to-cell contact in the sympathetic system. At the next contact point with the tissues, the postganglionic neurons release another chemical, norepinephrine, to transmit the information to the tissues.

9.4 Systems

Organs are organized in a higher hierarchy, as systems. The major organ systems of the body functions are:

1. The skin, or the integumentary system which serves to protect the organs, help in excretion, and receive of external stimuli.
2. The muscular system is for movement of the body and to maintain posture.
3. The skeletal system is for support, attachment of muscles, red blood cell production, and storage of calcium and phosphate ions needed for the skeleton.

4. The endocrine system includes all the glands and the hormones secreted by them. Its function is to produce hormones that support the nervous system in the control of body functions. This hormonal coordination is achieved through secretion of biochemicals that are then used by appropriate specialized cell types.
5. The cardiovascular (circulatory) system consists of the heart and the blood vessels (arteries, capillaries, and veins) and delivers biomolecules and gases to and removes metabolic wastes from the cells in the organism. It is also involved in nutrition, waste removal, immunity, cellular communication, and thermoregulation. It is a “closed” system in humans and works in close conjunction with the respiratory system in addition to others such the endocrine system.
6. Lymphatic system is complementary to the circulatory system and serves to remove the excess fluid (lymph) released by the circulatory system into the tissues. Unlike the circulatory system, it is an “open” system.
7. The respiratory system has some main parts such as the airways, the lungs and related blood vessels, and the muscles that enable breathing. This system serves to exchange oxygen and carbon dioxide for the energy needs of the body.
8. The digestive system is involved in the metabolism and intake of the nutrients to be used in the production of energy and as the building blocks for the body. It consists of the hollow organs that make up the gastrointestinal (GI) tract, mouth, esophagus, stomach, small intestine, and large intestine. The liver, pancreas, and gallbladder are the other organs of the digestive system.
9. The excretory system is involved in the maintenance of a constant internal environment (osmotic pressure, pH, ion balance, and extracellular fluid volume). It is also responsible of secretion of metabolic wastes such as urea, uric acid and ammonia. The system is constituted of the skin, the kidneys, the ureter, urethra, bladder, skin, and the lungs for the gaseous metabolites.
10. The reproductive system is the only system that is different in women and men. It is involved in the production of gametes (the eggs by women and the sperm by men) and to deliver them to the respective sites for fertilization. The female reproductive system consists of the ovaries, fallopian tubes, uterus, vagina, vulva, mammary glands, and breasts. The male reproductive system involves the scrotum, testes, spermatic ducts, sex glands, and penis.

9.5 Conclusion

In summary, humans are highly complex, hierarchical organisms. Together, the body's components function in harmony. Biomaterials scientists are tasked with developing remedies and, in the present case, substitutes or support materials and devices, in order to increase humankind's quality of life.

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10.1 General Properties

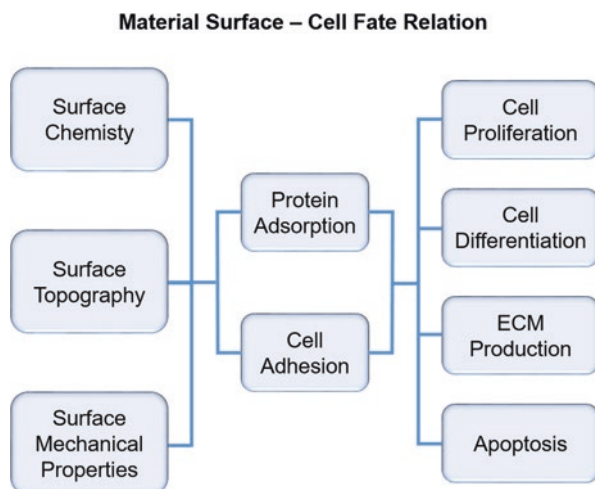
When a foreign material is implanted in the body, tissue responds to the material by showing allergic, toxic, or carcinogenic responses. Meanwhile, the tissue exerts various effects on the material such as corrosion, degradation, or deterioration. The tissue-material interface is critical for a proper implant performance because this is where the two systems meet and the success of the implant is decided. In order to understand the response of the tissues and the implant to each other, the properties of a typical biomaterial surface has to be known.

10.2 Interaction Between the Biomaterial Surface and the Tissue

The critical parameters of a surface are the chemistry (i.e., nature of chemical groups and their reactions with surrounding tissue), topography (the roughness or design), and the physical properties (i.e., porosity, stiffness). Fig. 10.1 shows the phases of biomaterial-cell interactions and their influence on cell fate. Surface chemistry, topography, and mechanical properties are the main properties of an implant that determine its biocompatibility. The first reactions involve the proteins and cells. They adhere or get adsorbed on the surface if the topography, chemistry, and the mechanical properties such as stiffness are suitable for these events to take place. Once this happens, the cells of the neighboring tissue start increasing in number, differentiate if they are stem cells or progenitor cells, and produce their extracellular matrix. If the interaction is unfavorable, the cells either do not attach or they may undergo apoptosis.

Interaction of cells and tissues is more complex than what is presented in Fig. 10.1. Before the material comes into contact with the tissue, it first has to be implanted into the body in most cases, and the first step involves the surgery which might be a minimally invasive process such as introduction of an intraocular lens or

Fig. 10.1 Interaction between material surface and biological system defines the fate of the implant



a major one such as a knee implant. During this initial damage the tissue, the blood vessels and the nerves are cut initiating the repair process starting with blood clotting and inflammation. In addition to this initial damage inflicted on the patient to introduce the device into the body, the later stages require the involvement of a number of systems such as immune systems, cardiovascular system, nervous system, and others due their role in the healing process. Besides the body, the implant will show its response against the foreign environment. In this chapter both the damage and the repair process will be covered in addition to the effect of the properties of the material that have a significant impact on the tissue and the healing process, and the effect of the tissue on the material.

10.2.1 The Polymeric Materials

Polymers present a range of hydrophilicities due to their chemistry, ranging from the very hydrophobic (Teflon, polyethylene, polystyrene) to the very hydrophilic (polyacrylic acid, gelatin, hyaluronic acid, chondroitin sulfate).

The hydrophilicity is a result of the polarity and the charges carried by the molecule which favors interactions with the omnipresent water. The molecules that do not carry any polarity or charge are hydrophobic. The charges carried by the molecules vary with the pH of the medium, and the physiological conditions present a variety of pH to the molecules, such as pH 1 for the gastric juice, pH 5 for the lysosomes, pH 6 for the small intestine, pH 7.4 for the extracellular fluid, and pH 8 for the large intestine. The main groups responsible for the charges are the carboxylic acid ($-\text{COOH}$) groups which dissociate at higher pH values to yield a negative charge and amines ($-\text{NH}_2$) which get protonated at lower pH's and carry positive charges. The presence or absence of charge is an important property that attracts or repels biological molecules and as a result determines the level of interaction. For example,

chitosan, when protonated, attracts negatively charged molecules and cells with its positive charge, and this is sometimes referred to as a cause of toxicity or incompatibility. Meanwhile, the negative charge on polyacrylic acid (at high pHs) repels the cells and the other negatively charged molecules, and as a result it has lesser interactions with the generally negatively charged blood elements and cells.

Polar groups like -C=O , -SH , -OH , and -Cl also attract water and help increase the interaction of the molecule with ionic and other polar molecules. In the absence of charges or polar groups on the biomaterial, the hydrophobic molecules in the tissues, in the serum, etc. are attracted to the implant material and are adsorbed on them while the hydrophilic molecules are repulsed.

In the case of blends, the polymer surface could be homogenous if the polymers are completely miscible, but if they are not, then a phase separation might be observed over the surface. Similar observation can be made when the polymer is a copolymer like those of polyurethanes which might be constituted of hard and soft segments, and there might be regions where one or the other is predominantly observed. In the case of a semicrystalline polymer, again, certain regions on the surface might become more attractive for binding the others.

Topography of the surface is also very important in tissue-material interactions. Polymers can be processed into various 2D and 3D forms like porous, nonporous, fibrillar, granular, lamellar, rough, smooth, etc. (Fig. 10.2), and these features are seen on the surface of the material as well as in the bulk. As a result of these, the biological compounds and cells are allowed to penetrate into the polymer bulk or be restricted to the surface, and they are provided physical surface features to attach to. The cells are known to use the surface features as guides or signals for their attachment, orientation, and other metabolic activities. Since it is known that the biological system tends to isolate the foreign materials like implants, these topographic features could lead to full encapsulation of the implant or penetration of cells and ECM into the bulk leading to different biological processes.

In addition to topography, biodegradability which is a result of chemistry plays a major role in the interaction between the polymers and the tissue. If the biomedical polymer is biodegradable, then in addition to the size, the form and the chemistry of degradation products also play an important role. For example, PVC degrades to produce an unsaturated bond on the polymer chain and releases HCl into the medium; the newly formed double bond is susceptible to further interactions, while the acidity of the released HCl may cause cellular necrosis. The particulate

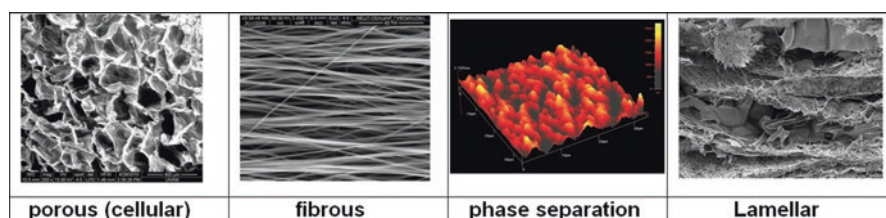


Fig. 10.2 Bulk and surface topographies that biomaterials can present to the tissues [1–4]

degradation products initiate tissue response (inflammation) and may even be taken up by the neighboring cells (endocytosis). Meanwhile, the increased roughness would increase the contact area and adhesion of molecules and cells.

10.2.2 The Surface of the Metallic Materials

Metals have crystalline structures which rarely are made up of single crystals. There generally are a few crystals which could consist of single type or a few types of crystals with different organization and composition. Even if there is only one type of crystal, there will be many grains which would contribute to the chemistry of surface of the metallic materials. The grain boundaries would have high energy due to insufficient bonding by the atoms at the edges of the crystals which would increase the reactivity of the metal. Therefore, reactivity at the grain boundaries, such as oxidation and reduction reactions, would be higher (Fig. 10.3).

Metals can be processed into 2D and 3D porous, rough, fibrillar, granular, or sheetlike structures, and as in polymers these forms define their interaction with the biological system. Similar to polymer degradation, metals can undergo reactions with dissolved air and water in the environment, and the resultant oxidation (corrosion) could lead to particulate degradation products, increased surface energy, and reactivity. It was reported that signs of high rates of corrosion were detected in almost half of the stainless steel implants removed from the patients [6]. They noted that the grain boundaries were the most common corrosion sites. Even though the cases of interfacial corrosion were rarely clinically significant, in the cases of severe intergranular corrosion and pitting corrosion, the result was pain and implant removal.

In addition to material properties, the environment of the implant also has an important effect on its behavior. A typical example is the crevice corrosion which occurs in narrow spaces (crevices) between metal-metal and metal-nonmetal

Fig. 10.3 Crystals and grain boundaries [5]

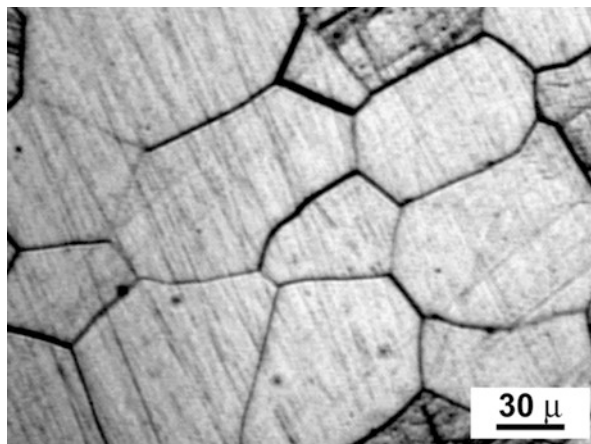
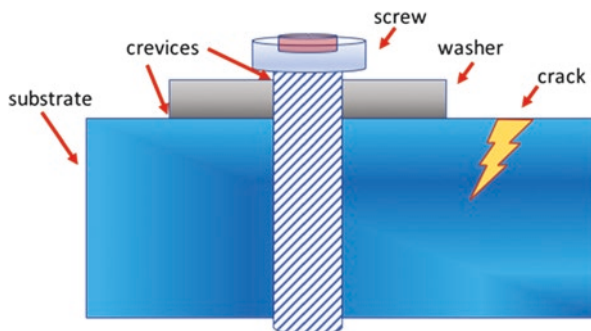


Fig. 10.4 Crevice corrosion at the contact sites where low oxygen availability converts the crevice into an anode



the crevices and the tips of cracks act as anodes because of the low oxygen content in these regions

surfaces. An electrochemical cell forms in the gap due to low levels of oxygen which creates an anodic region, and this leads to corrosion in applications where there are threaded joints, screws, nails, and cracks. The oxidized (ionized) metal is solubilized and leaves the surface as ions and/or as particles (Fig. 10.4).

$M \rightarrow M^+ + e^-$ The crevice serves as the anode

$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ The environment acts as the cathode

The reactions at which the metals undergo could also lead to reaction products such as oxides which are preserved on the surface and stop further oxidation and lead to passivation of the surface preventing loss of any particulate or soluble corrosion products. Nitinol (NiTi) is an equiatomic titanium nickel metal, the surface of which can be passivated to become a predominantly titanium oxide layer (TiO) similar to that on Ti alloys. This oxide layer is biocompatible, protects the bulk NiTi implant from further oxidation and corrosion, thus decreasing the release of Ni ions into the medium which are known to be cytotoxic [7].

The many different types of molecules that contact metallic implants have a tendency to be adsorbed onto them; the extent of adsorption is pH dependent, and the multi- or monolayer adsorption changes with the protein properties, and these adsorptions could lead to increased ion release indicating that biocompatibility of the implant is very dependent on the microenvironment [8].

10.2.3 The Surface of the Ceramic Materials

Even though ceramics are crystalline, they are different than metals in that their bonding type is not metallic but ionic. These bonds make them very rigid and brittle, and plastic deformations are not observed. The strict localization of the electrons between the atoms prevents any charge transfer on their surfaces which make them inert and very good insulators. To obtain a preferred form, they are either molded or their particles are sintered and their processing conditions do not leave much room for flexibility. They possess grains and grain boundaries just as the metals do. Their surface geometry is similar to that of metals, but due to its ionic nature, their

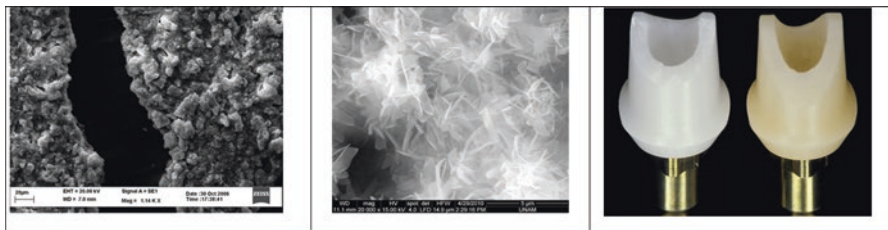


Fig. 10.5 Hydroxyapatite (HAP) granules [9] and nanocrystals produced in situ [10], and zirconia and alumina tooth implants [11]

interaction with tissues is probably higher than polymers and metals. If, however, the ceramics are stable and are not modified by the biological microenvironment, then their surface chemistry does not lead to any response.

Some ceramics are inert due to the highly fused structure formed by application of high temperature during processing. Meanwhile, some ceramics such as biological apatites demonstrate interaction with the environment can adsorb large amounts of proteins, and they can dissolve easily. Depending on the requirements, ceramics are used to coat surfaces of implants such as metals to prevent their direct contact with the biological environment and the physiological fluid within the tissue. One other type of application is to improve the surface properties of implants as in the coating of hydroxyapatite to improve cell adhesion capability and therefore integration of metal implants (Fig. 10.5).

10.3 Effect of the Biological Medium on Biomaterials

Biomaterials are changed in the biological medium in accordance with their chemistry and topography. Chemistry determines whether the biomaterial is degraded or not and the rate at which this happens. The rate is critical because the ability of the biological system to handle the emerging chemicals and particulates depends partly on how rapidly these are produced and how rapidly body deals with it (degrades, removes, encapsulates, or initiates an inflammatory response).

10.3.1 Polymers

Polymers can be hydrolyzed, eroded (not degraded but solubilized), engulfed, or encapsulated in the biological medium. If the polymer is of biological origin (e.g., collagen, hyaluronic acid) or made with biological building blocks (e.g., synthetic polypeptides like poly(L-lysine) (Fig. 10.6)), then it can be hydrolyzed by various enzymes to its smallest constituent which could be amino acids or mono- or disaccharides. For example, starch can be hydrolyzed by amyloglucosidase, alpha- and beta-amylase, glucoamylase, and pullulanase. Collagen is hydrolyzed by collagenase type I, and type II, pepsin, and papain, while hyaluronic acid is hydrolyzed by β -glucuronidase and β -N-acetyl-hexosaminidase. The bacterial polyester

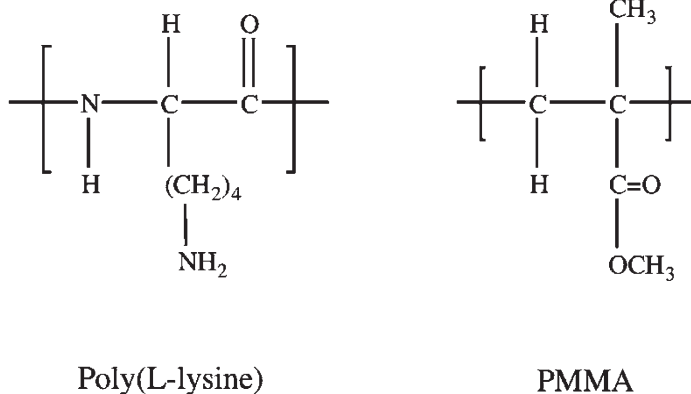


Fig. 10.6 Poly(L-lysine) (PLL) and poly(methyl methacrylate) (PMMA)

polyhydroxybutyrate (PHB) can be hydrolyzed by a variety of PHB-depolymerases isolated from a large number of bacteria.

If the polymers are synthetic with no biological resemblance, then simple hydrolysis is the method of degradation and is effective on polycondensation polymers (such as polyesters) but not on addition polymers (such as vinyls like polymethyl methacrylate (Fig. 10.6) or polyvinylchloride). Meanwhile, there are some oxidative processes such as water-mediated reactions which break the bonds and create free radicals. These radicals are active and may lead to further interactions with the molecules present in the neighborhood. If the polymer is not degradable, that is if its backbone is not hydrolyzed, it could still be removed by dissolution.

For example, hydrophobic polymer molecules could be dissolved away from the surface of the implant material, and depending on the chain dimensions, they could be excreted through the kidneys if the Mw is around 30,000 Da or less. It can be accumulated in the reticuloendothelial system (RES, mainly liver, kidney, and spleen), as observed in surface-eroding systems if Mw is higher (Fig. 10.7). If the polymeric materials or the products of degradation are particles small enough to be engulfed by the macrophages, then they are removed from the application site. This is most often observed with micro- and nanoparticulate systems as in controlled drug delivery and in degradable systems which produce particulate degradation products such as the degradable sutures or the polyethylene acetabular component of total hip prostheses. Free radical processes are also active on this type of materials.

If the polymeric material is too large to be engulfed by the macrophages and its surface or bulk does not change with time, then the biological system tries to exclude it and prevent its contact with its environment by surrounding it with a thick sheath of collagen. A typical example of this is the poly(methyl methacrylate) (PMMA) implants. Polymers which are not degraded or coated can absorb water, swell, and change dimension. Such a change is observed in cross-linked polymers like hydrogels. When a polymer absorbs water, the chains are plasticized due to the newly attached water molecules which makes the chains mobile but also very weak under shear forces especially if an application at the joints are considered.

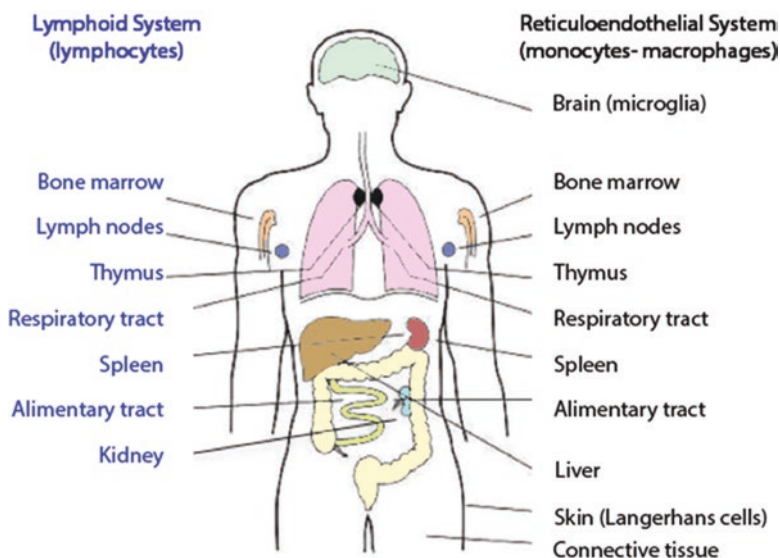


Fig. 10.7 Major organs in the lymphoid and reticuloendothelial systems

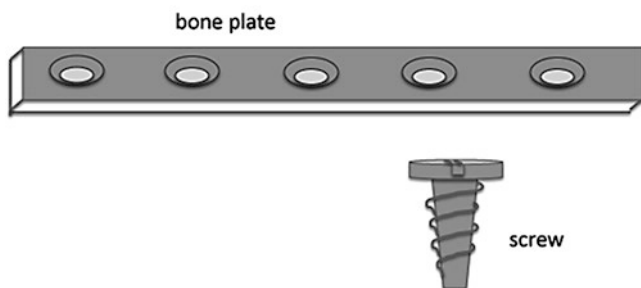


Fig. 10.8 Bone plate and screw

10.3.2 Metals

Metallic implants are used especially in orthopedic and dental applications (Fig. 10.8). A metallic material can be either oxidized to create rust-like, soluble oxidation products which leave the surface creating a fresh surface to be oxidized. However, in the case of titanium or aluminum, the surface layer of oxidation is so tough that it passivates the surface and prevents further oxidation [12]. Under these conditions the body does not anymore react with it except by coating with the collagen sheath. In the former case when degradation proceeds, the degradation products are treated by the body as was done with the polymeric particulate degradation products that is engulfed or covered by a collagen sheath.

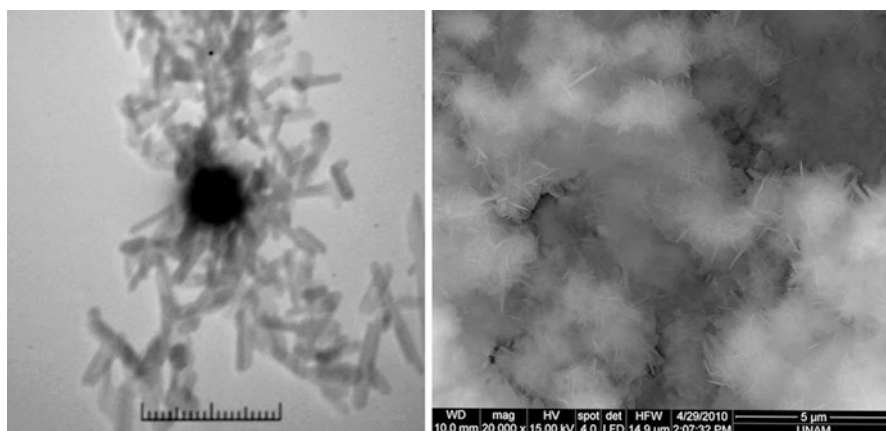


Fig. 10.9 HAp crystals [1, 9]

10.3.3 Ceramics

Ceramic materials are inert in the biological system. Molecules from the biological medium can get adsorbed onto their surfaces, but these do not change the basic properties. If, however, the ceramic biomaterial is in the form of nano- or microparticulates, then the response of the biological system is similar to that observed with the other biomaterials. In other words, they will be phagocytosed and accumulated in the RES. When bioactive ceramics or bioglasses are used then one cannot expect them to behave as typical inert ceramics would. They are a group of glass materials considered to be ceramic biomaterials in the composition of which there are SiO_2 , CaO , Na_2O , and P_2O_5 . In the physiological environment they exchange ions, some Si-O-Si bonds are broken forming silanol groups, and the glass network is disrupted. This changing and receding surface allows the neighboring bone tissue to grow into it and make bonds. The most commonly used ceramic is hydroxyapatite (HAp) which is a bioactive material and stimulates bone regeneration (Fig. 10.9).

10.4 Effect of Biomaterials on Cells

The biocompatibility of biomaterials is studied at various levels of complexity. Initially the extracts of materials are obtained and chemically analyzed to investigate the presence of leachables, compounds which can come out of the material when in contact with the body. This step is called the in situ testing phase. Then the extracts and the materials or samples processed in the exact same way as the finished product are brought in contact with the cells (in vitro) and with the animals (in vivo) before moving to testing on humans (clinical trials) if successful.

10.4.1 Integrity

Depending on the surface properties of the material, cell integrity could be harmed. For example, a highly positive charge such as that on chitosan is generally used to complex with negatively charged species for a number of uses but could be cytotoxic due to excessive interaction and restriction of the cell membrane mobility leading to membrane damage and pores that may cause leakage of intracellular content.

10.4.2 Conformation

The conformation of the cells are dictated by their phenotype; however on surfaces with distinct chemical or physical properties, the cells respond by adapting their conformation to those dictated by the surfaces. It is reported that these changes lead to alterations in various properties of the cells including fate.

10.4.3 Attachment

The attachment of the cells in the body is modulated by what functionalities that the ECM possesses and offers to the cells. Actually, in repair processes the cells themselves create their own microenvironment. However, on new and artificial surfaces, cell attachment is dependent on what the new surface offers. On surfaces that do not provide any attachment cues equivalent to ECM, cells attach to a minimal level until they secrete their own ECM. On highly hydrated surfaces, cells cannot make any stable contact with the surfaces due to the aqueous film layers formed and are therefore compelled to minimize their surfaces for energetic reasons.

10.4.4 Metabolic Activity and Proliferation

The anchorage-dependent cells show changes in their metabolic activities due to their inability to properly adhere or excessively adhere to surfaces. Among these are some important activities such as proliferation or as in case of alkaline phosphatase production by the osteoblasts enzymatic activities are decreased or delayed.

10.4.5 Differentiation

A final effect on cells is especially critical for the stem cells. The surface properties, in addition to absence of certain bioactive agents in the growth media, influence the adhesion, the metabolic activities, and conformation and eventually lead to fate of stem cells. For example, mesenchymal stem cells (MSC) are differentiated into bone cells when they can adhere to the surface but become chondrocytes when the surface is excessively hydrophilic and nonadhesive.

As a result, properties that might not be effective when the cells are in a tissue become very critical when they are on a 2D surface.

10.5 Effect of Biomaterials on the Biological Tissues

The biological system is affected by the implanted materials in various forms. If the biomaterial undergoes a reaction before solidifying while in contact with the tissues, such as bone cements and tissue adhesives, then there is risk to the tissues. These reactions are generally exothermic, and the local temperature measured under in situ conditions can go as high as 100 °C which denatures all the surrounding biological structures including cell membranes. These will have a necrotic effect on the neighboring tissue. The solidification process involves low molecular weight, highly reactive, and highly hydrophobic chemicals with significant solvation power forming large molecules called polymers. These compounds interact with the neighboring tissue and damage the local structure including the cells by the solvation capability, by the heat produced or simply by toxicity. These effects are acute.

There might be slow progressing degradation reactions which would continuously produce degradation products (particles or low molecular weight molecules like lactic acid or HCl) which could cause chronic damage (lowering the local pH as stated earlier) for the cells and reactive functional molecules in the environment. Another category of damage could be observed on the soluble elements of the blood, such as platelets and factors involved in the coagulation cascades. The damage in these would interfere with the metabolism in the body.

The unpolymerized low molecular weight ingredients or monomers are very mobile and might leach out and be transported through the circulation. Bone cements have two main components where one is a monomer and the other is a polymer powder (Fig. 10.10). A producer of bone cements and biomedical products warns that “the premature insertion of bone cement may lead to a drop in blood pressure, which has been linked to the availability of methyl methacrylate at the surface of the product, although this has not been proven. This drop in blood pressure, on top of hypotension induced either accidentally or intentionally, can lead to cardiac arrhythmias

Fig. 10.10 PMMA-based bone cement



or to an ischemic myocardium.” This indicates the seriousness of the risks run by the use of bone cements arising from unpolymerized leachables. This warning does not include the necrotic effect caused by the heat produced during setting of the bone cement. In addition, the surface topography and the physical form could be damaging mechanically if especially located at a moveable site such as joints.

10.6 Responses of the Body to Implantation

There are basic responses of the body to implantation even if the operation is sham because the incision made to introduce the biomaterial into the body causes a series of damages. The main damages are inflicted on the vasculature, on the extracellular matrix, and on the nerves. These trigger a series of events which can be summarized as the inflammation process and wound healing.

10.6.1 Inflammation

Inflammation is the response of the body to injury whether it is caused by accidental tissue damage, trauma, surgery, or infection. The main indications are redness (due to increased blood flow), warmth (increased metabolic activity), swelling (exudate accumulation due to leaked extracellular fluid and blood), and pain (nerve end stimulation).

When there is a tissue damage, neutrophils are attracted to the site of injury, along with the mononuclear cells or macrophages (Fig. 10.11). The microorganisms causing the infection and the damaged tissue debris are attacked by these cells and endocytosed (internalized) and digested if possible. Endocytosis is achieved through phagocytosis, pinocytosis, or receptor-mediated endocytosis where the foreign material uses the membrane bound receptors to be transported into the cell (Fig. 10.12). In some cases the phagocytosed particles cannot be digested, and if cytotoxic they may even lead to death of the macrophages; otherwise they are removed for storage in the reticuloendothelial system.

The sequence of events that follow the initial stage of inflammation are completed within 4–5 days. During this period the vascularization of the damaged area, reconstruction by the arrival of fibroblasts, fibrosis followed by reorganization of the fibrotic site is achieved. This is the normal sequence of events if there is no persistent cause for damage. In the case of implantation, however, the process of inflammation is either acute or chronic (prolonged) depending on the biomaterial in question.

After the tissue is damaged, histamines are released, and an increase of blood vessel diameter (vasodilation) is observed which leads to increased blood flow (redness symptom), and with that local heat is increased. The leakage of plasma and its proteins from the damaged blood vessels lead to swelling. During this time the leukocytes and phagocytes migrate out of the blood vessels and go to the site of injury. Phagocytes internalize the bacteria (if any), the dead cells, and cellular and other debris. Clotting factors are also released from the damaged vasculature into

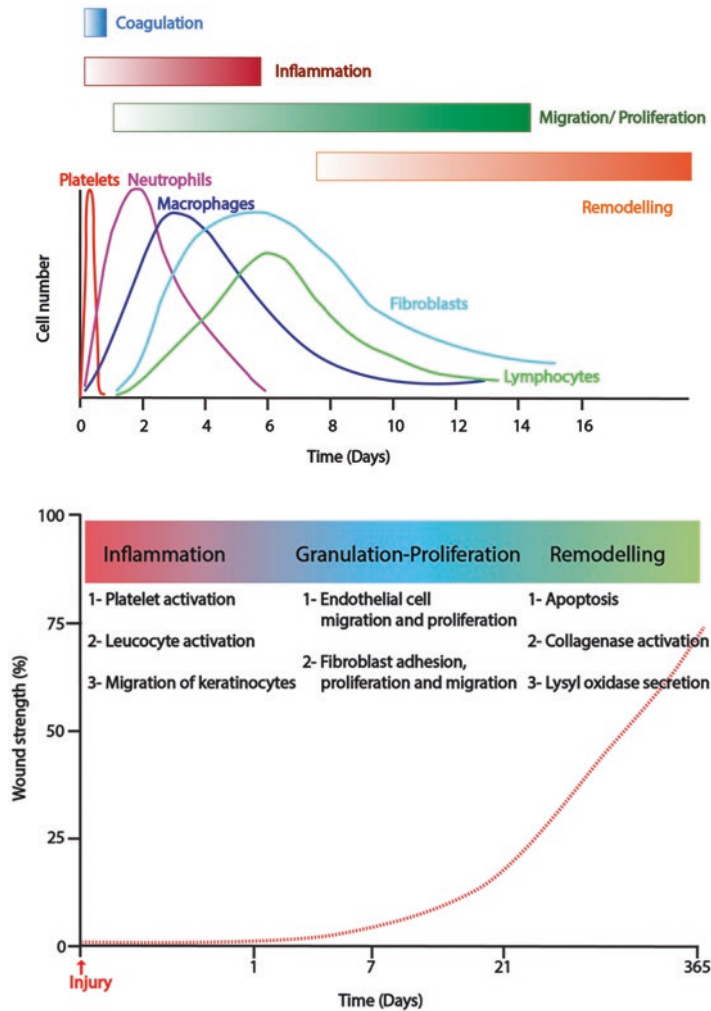


Fig. 10.11 Time frame of inflammation and cell involvement

the wound site. Blood coagulation occurs as a result of the reaction between the blood elements and damage site. The coagulation system which blocks the damage site with the fibrin mesh deposited is later removed by fibrinolysis and helps remodeling of the injury site.

If an irritant is present for an extended period, then chronic inflammation is observed. Highly crystalline degradable polymers, such as those of highly crystalline PLLA, continuously produce nano- and microparticulates and lead to chronic inflammation due to long degradation duration, and, therefore, inflammation continues until the stimulus is removed.

In order for the tissue to fully heal, the inflammation stage must be terminated by removal of the inflammatory agents (microbes, particulates, etc.).

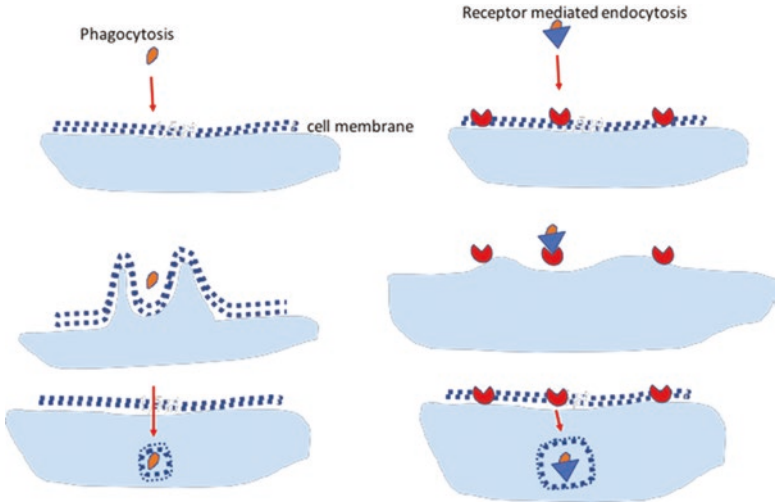


Fig. 10.12 Internalization of a foreign material through phagocytosis and receptor-mediated endocytosis

Table 10.1 Wound healing phases and major events

Phase	Time	Events
1. Inflammation	Early	• Vasoconstriction • Platelet aggregation • Clotting • Inflammation • Vasodilatation • Phagocytosis
2. Proliferation	3 days onward	• Fibroblasts deposit collagen • Contraction of the wound • Epithelialization
3. Remodeling	Weeks to years	• New collagen deposition • Wound strength is increased

10.6.2 Remodeling

The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. Basically, the sequence starts with inflammation as a result of injury, then proliferative phase takes over when tissue starts healing and scar tissue forming, and finally remodeling takes place to complete the healing process (Table 10.1).

After the initial inflammation phase, the proliferative and then maturation phases start. The former takes 3 days to 3 weeks and the latter from 3 weeks to years (Fig. 10.13).

The main events during this step are fibroblasts laying a bed of collagen and associated extracellular matrix components to fill the defect, and neovascularization

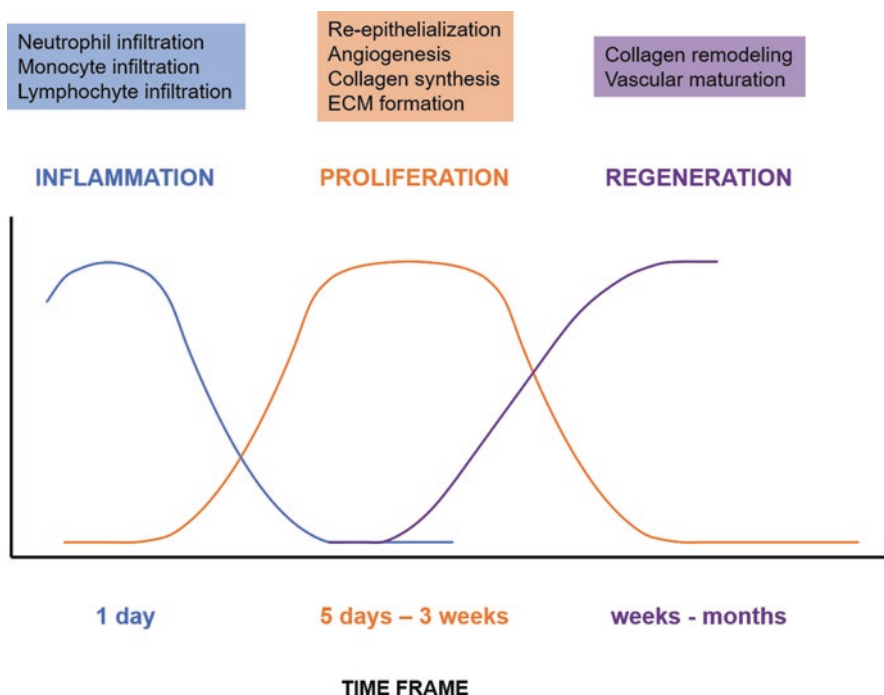


Fig. 10.13 Repair process

to provide nutrients to the damage site through new capillaries. Contraction starts where the wound edges are pulled together to reduce defect size. These are followed by epithelialization and remodeling or maturation. During this phase new collagen is laid to increase mechanical properties of the wound.

10.6.3 Responses to Biomaterials During and After the Healing

The biomaterials elicit so many different types of responses once introduced in addition to the initial damage inflicted by the implantation procedure. These damages are short and long term depending on a number of properties of the biomaterials involved.

10.6.3.1 Response Time Frame

The responses shown by the body to materials can be classified according to the rapidity and duration of the response as acute and chronic. Acute responses are those which have short duration responses. When the bone cement sets, the increase in the temperature is short term but extremely damaging for the cells and the tissue. Once the temperature decreases to normal, there will be no more response due to temperature changes. Similarly, when a tissue adhesive of cyanoacrylate type is used to glue tissue, again the damage to cells is excessive, but once the glue sets,

then there will be no more strong response toward the remaining set glue. However, if a sharp implant is introduced, the mechanical damage will be a prolonged effect and so will be the response. When a biodegradable material is introduced, the body will respond to it by attempting to remove the particulate matter, but since it will continuously be produced, the response will be long term. This will be observed with implants with erodible surfaces and with those that crumble or degrade to produce particulate debris. In the erodible case, the implant will be unsuitable for endocytosis and coating will be attempted, but since the surface of the implant continually recedes, it is not possible to coat it.

10.6.3.2 Response to Implant Shape and Dimension

The tissue responds to shape of implants from the point of removal or isolation. The first attempt is removal, and for this, macrophages are the tools used by the body. In order to achieve this, the size of the implant should be suitable for this process of endocytosis followed by transfer into the lysosomes and hydrolysis. If the implant size is around a few microns, then this is possible. However, if the implant is too large to engulf, then the other approach is used: isolation through coating with a collagen, ECM components, and conformal fibrotic tissue sheath.

10.6.3.3 Response to Production (Setting) Phase

As mentioned above acrylic glues and bone cement involve an initial phase of organic reaction of non-biocompatible molecules and/or heat. In the case of bone cement at the time when the biomaterial is introduced, a temperature rise of up to 80–90 °C is possible. Besides there are unpolymerized acrylic-based monomers which act as solvents of the cell membranes and other cellular components and cause undesirable side reactions. In the case of cyanoacrylate glues, there is no heat involvement, but solvent effect (the dissolution of the lipid organelles) is still sufficiently destructive for the neighboring tissue. Studies are continuing to decrease the setting temperature to more tolerable levels.

10.7 Conclusion

In this chapter, the reactions that take place between host tissue and biomaterials such as polymers, metals, and ceramics, which are introduced into the body were summarized. The effect of the biological medium on biomaterials and the effect of biomaterials on cells were both considered. Biomaterials need to be bio- and hemo-compatible; they should not elicit negative responses in the body. Their responses to the rather hostile and difficult environment within the body should not affect their performance as a device. With the multitude of materials and bioactive agents being introduced as biomedical materials, we can generalize the properties of a suitable biomaterial only very broadly. The development of new production technologies, coating processes, and new composites will allow for new ways to create materials suitable for use in the biomedical field.

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11.1 General Introduction

One of the first definitions of a biomaterial was that of the Clemson University Advisory Board for Biomaterials [1] which was “a systemically and pharmacologically inert substance designed for implantation within or incorporation with living systems.” Biocompatibility was defined by Williams (2008) as “the ability of a material to perform with an appropriate host response in a specific application” [2]. According to ASTM on the other hand, it is defined as a “comparison of the tissue response produced through the close association of the implanted candidate material to its implant site within the host animal to that tissue response recognized and established as suitable with control materials.” Another definition of biocompatibility provided was “The condition of being compatible with living tissue by virtue of a lack of toxicity or ability to cause immunological rejection” [3].

As can be seen from these definitions, initially the kind of response required of a biomaterial was that it had to be systemically and pharmacologically inert, but in time in the new definitions of biocompatibility, a biomaterial was allowed to give an appropriate response for a specific application. Another important change in the approach to biocompatibility was that in the earlier definitions, biosafety or absence of any deleterious effect of the biomaterial on the tissue was required, whereas in the newer definitions, instead of inertness an appropriate response needs to be evoked. So, for example, if a blood-contacting device is being designed, the response evoked by the final product has to differ depending on whether cell or protein adsorption is desired or to be avoided.

The stages of a device coming to the market from a design in the lab follow the path given in Fig. 11.1.

According to Anderson [5] the evaluation of biological responses to a medical device is carried out to determine that the medical device performs as intended without presenting any significant harm to the patient. The goal of evaluation of biological responses or biocompatibility testing is, therefore, to make sure that the biomaterial or the medical device presents no harm to the patient when in clinical use.

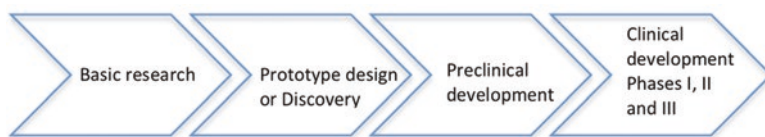


Fig. 11.1 The stages of a device coming to the market from a design in the lab [4]

How can a biomedical device pose a threat to the patient? The device in its manufactured physical form can cause physical or mechanical damages just due to its presence within the tissue by interacting with the cells, organelles, or molecules. The device's chemical composition might initiate adverse responses, molecules contained in the device might be released through leaching after solubilization or after creation by the degradation or hydrolysis of the device components or by the combination of all these. Thus, there are many ways that a biomedical device can pose a risk to a patient. In order to ensure that the device performs without any risk, the device itself and its contents must be examined by various tests with increasing complexity and finally on volunteer patients.

The tests needed for this process involve a series of steps: (1) in situ testing of the material in simple solutions and after being extracted in buffers or oils for anything that can be released by the device. This also serves as a screening test for eliminating very risky materials. (2) The second step involves testing with cells; these are the in vitro tests where cell lines are used to determine the damage caused by the material or its extract on well characterized simple biological systems. (3) The next step is the use of animals (e.g. rats, rabbits) which can stimulate the responses of a series of physiological systems instead of a simple cell. Here, a multiplicity of systems and their collective responses such as irritation or sensitization or responses to an implant in contact with the tissue for long durations are tested. If the device necessitates a higher level complexity, animal models with physiology closer to the humans such as sheep, pigs, dogs, or even primates can be used. This is the highest or most complex level before initiating the clinical testing.

According to ISO 10993-1 [6], the biological safety of a new device can be investigated by following the guidelines presented below:

1. Identification of the biological hazards that might originate from the test sample after taking into consideration the type of exposure and the duration of contact (a polymer has the potential to degrade, so leachables has to be tested; metallic implants can corrode, so mechanical properties might deteriorate in time and in the meantime particulates and toxic ions are released).
2. Selection of tests appropriate for revealing the extent of risk by these potential biological hazards (is the contact duration long enough to release degradation products or to cause irritation or sensitization?).
3. Conductance of the tests after deciding the sample form (film, sheet, powder, solution) and test conditions (in tensile testing the rate of extension and in extraction the duration and temperature pair).

Table 11.1 10993 Biocompatibility testing guide

Part 1. Evaluation and testing
Part 2. Animal welfare requirements
Part 3. Tests of genotoxicity and reproductive toxicity
Part 4. Selection of tests for interactions with blood
Part 5. Tests for cytotoxicity: in vitro methods
Part 6. Tests for local effects after implantation
Part 7. Ethylene oxide sterilization residuals
Part 8. Selection and qualification of reference materials for biological tests
Part 9. Framework for identification and quantification of potential degradation products
Part 10. Tests for irritation and sensitization
Part 11. Tests for systemic toxicity
Part 12. Sample preparation and reference materials
Part 13. Identification and quantification of degradation products from polymeric medical devices
Part 14. Identification and quantification of degradation products from ceramics
Part 15. Identification and quantification of degradation products from metals and alloys
Part 16. Toxicokinetic study design for degradation products and leachables
Part 17. Characterization of materials
Part 18. Chemical characterization of materials
Part 19. Physicochemical, mechanical, morphological, and topographical characterization of materials
Part 20. Principles and methods for immunotoxicological testing of medical devices

11.2 International Standard 10993

Once steps 1 and 2 are carried out, then the procedures mentioned in step 3 are followed. These procedures are defined in the international standard ISO 10993 which consists of various “biocompatibility tests” as presented in Table 11.1.

Test selection procedure is very critical because if inappropriate tests are selected, then the evaluation would be incomplete and the test outcome unreliable. And if the number of tests deemed necessary is too high, then the process takes too long and becomes extremely expensive. Therefore, a well-chosen set of limited type and number of tests need to be applied. The selection algorithm is presented in Fig. 11.2 (adapted from ISO 10993-1). The tests are typically classified according to their type and duration of their contact with the body.

According to the guide, the need for biological evaluation has to be assessed according to the flow chart presented in Fig. 11.2. Once it is determined that a medical device or material needs to be tested, then the ISO 10993-1 Evaluation and Testing Charts are consulted (Table 11.2).

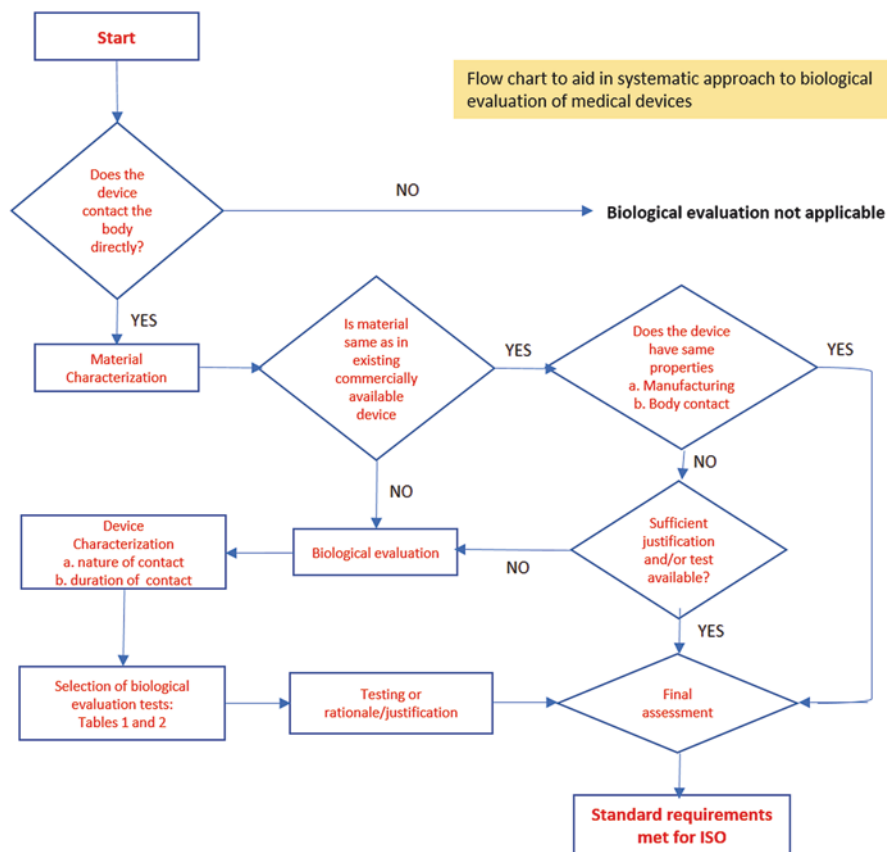


Fig. 11.2 Flow chart to aid in systematic approach to biological evaluation of medical devices

Table 11.2 guides the evaluation process according to:

1. *The body contact type*: surface (skin), external communicating devices (transcutaneous catheters), and implant (heart valves). Here there are also subcategories such as the site of contact whether it is tissue or blood, and tissue state (compromised vs. undamaged).
2. *Duration*: limited (less than 24 h), prolonged (up to 1 month), and permanent (longer than 1 month).

Based on these categories, making a selection from a battery of tests is recommended. These tests are cytotoxicity, sensitization, irritation, systemic toxicity, chronic toxicity, genotoxicity, implantation, hemocompatibility, and reproductive and developmental tests. They are carried out directly or indirectly through the use of aqueous or oily extracts of the finished product or a sample made of the same material that was treated in an identical way as the finished product.

Table 11.2 Categories of evaluation tests [7]

Medical device categorization by			Biological effect												
Nature of body contact		Contact duration	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Acute systemic toxicity	Material-mediated pyrogenicity	Subacute/subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic toxicity	Carcinogenicity	Reproductive/developmental toxicity	Degradation
Category Surface device	Contact	A—limited (≤24 h)	X	X	X										
		B—prolonged (>24 h to 30 days)	X	X	X										
		C—permanent (>30 days)	X	X	X										
	Mucosal membrane	A	X	X	X										
		B	X	X	X	O	O	O	X	O		O			
		C	X	X	X	O	O	O	X	O					
	Breached or compromised surface	A	X	X	X	O	O	O							
		B	X	X	X	O	O	O							
		C	X	X	X	O	O	O	X	O			O	O	
External communicating device	Blood path, indirect	A	X	X	X	X	O				X				
		B	X	X	X	X	O	O			X				
		C	X	X	O	X	O	X	X	O	O	X	O	O	
	Tissue*/bone/dentin	A	X	X	X	O	O								
		B	X	X	X	X	O	X	X	X	X				
		C	X	X	X	X	O	X	X	X	X		O	O	
	Circulating blood	A	X	X	X	X	O		O		X				
		B	X	X	X	X	O	X	X	X	X	X			
		C	X	X	X	X	O	X	X	X	X	X	O	O	

(continued)

Table 11.2 (continued)

Medical device categorization by			Biological effect													
Nature of body contact		Contact duration														
Category	Contact	A—limited (≤24 h) B—prolonged (>24 h to 30 days) C—permanent (>30 days)	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Acute systemic toxicity	Material-mediated pyrogenicity	Subacute/subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic toxicity	Carcinogenicity	Reproductive/developmental toxicity	Degradation	
Implant device	Tissue*/bone	A	X	X	X	X	O	O	X	X	X					
		B	X	X	X	X	O	X	X	X						
		C	X	X	X	X	O	X	X	X		O	O			
	Blood	A	X	X	X	X	X	O	O	X	X					
		B	X	X	X	X	X	O	X	X	X					
		C	X	X	X	X	X	O	X	X	X	X	O	O		

These are only the initial guidelines. Each test has to be planned in more detail based on the application area and the type of the material.

11.2.1 Test Example

It might be better to choose an example such as a polymeric tube that is designed to serve as a catheter for delivering serum to explain this point. In the above evaluation guide, this product is in the category of a transcutaneous device that will be in contact with the body for less than 24 h, and it will not have direct contact with blood. This product is in the external communicating device category, contacting the tissue only for a short period. The table recommends that it has to undergo only the cytotoxicity test (ISO 10993-5). So, it appears as one of the simplest products to test in terms of the number of tests. But, in what form will it have to be tested? In standard cytotoxicity test methods, cell monolayers are grown to near confluence in flasks and are then either directly exposed to test or control articles or indirectly by means of exposure to extracts of the articles. In this example then, the polymeric tube has to be extracted or directly used as is. In the elution test method, which is widely used, extracts are obtained by placing the test and control materials in separate cell culture media under standard conditions (e.g., 3 cm² or 0.2 g/mL of culture medium for 24 h at 37 °C). Each extract obtained is then applied to a cultured-cell monolayer after removal of the culture medium. In this way, the cells are supplied with a nutrient medium containing the extractables, the molecules extracted from the test article (or the control). The cultures are then returned to the 37 °C incubator and periodically removed for microscopic examination at predetermined time points for as long as 3 days. Cells are observed for visible signs of toxicity (such as a change in the size or appearance of cellular components or a disruption in their configuration), in other words morphological changes and cell damage. Other categories of testing, such as cell growth and cellular metabolism, would be more quantitative in response to the test and control materials.

If the tests are conducted on extracts, then they will either be aqueous or oil extracts of the finished product, or a sample made of the same material and treated as the finished product. Aqueous media could be culture medium with serum or without serum, isotonic saline (0.9% NaCl in distilled water), or water.

The study needs to involve positive and negative controls, too. In general a negative control is a material which does not produce any cytotoxic responses. Among the frequently used control materials are silica-free polydimethyl siloxane (PDMS) and high-density polyethylene (HDPE) for polymers and aluminum oxide for the ceramics. Positive controls are materials which consistently produce cytotoxic responses. For polymers these can be organotin-stabilized polyvinyl chloride (PVC) and dilute phenol solution.

Extraction conditions could be >24 h at 37 °C, to mimic the body conditions or accelerated treatment using higher temperatures but shorter durations such as 72 h at 50 °C, 24 h at 70 °C, or 1 h at 121 °C (sterilization conditions). In our catheter example, the conditions of extraction would be with isotonic saline or serum to be

similar to the fluid it will transport and the temperature 37 °C and exposure to extract at least for 72 h.

Of course since dose is important sample dimensions are also important. These are expected to be around 10 mm × 50 mm for polymers, and the ratio of surface area to volume of extraction is in the 0.5–6 cm²/mL range. The extracts must be tested fresh to avoid spoiling due to oxidation or microbial contamination.

In the cytotoxicity test the other variable is the cell type. The cells are commercial cell lines obtained from recognized suppliers such as American Type Culture Collection (ATCC). They are fibroblasts from healthy tissues of mouse connective tissue cells CCL1 (NCTC clone 929), mouse embryo CCL 163 (Balb/3T3 clone A31), human lung CCL 171 (MRC-5) and human lung CCL 75 (WI-38), green monkey kidney CCL 81 (Vero), hamster kidney CCL10 [BHK-21(c-13)], and hamster lung V-79 379A.

Thus, the stated cell lines are used for the changes in their morphology and growth, and the results are compared with those of the positive and negative controls.

11.3 Hemocompatibility

If the biomaterial in question is to contact blood, then blood-material interactions and the hemocompatibility of the material has to be studied according to ISO 10993-4.

The devices contacting blood can be non-contact devices, external communicating devices, or devices for implantation depending on the way of contact with blood.

Non-contact devices are devices that do not contact the whole blood in the circulation, but as in the glucose sensor, they contact the blood removed from the body. External communicating devices are transcutaneous devices which contact blood while serving as a conduit into the vascular system. These can be cannula, tubing, hemodialysis equipment, pacemaker electrodes, extracorporeal membrane oxygenators, or percutaneous circulatory support systems. These generally are short-term applications. Implant devices are generally long-term devices placed in the body and are in intense contact with the vascular system. For example, natural or synthetic heart valves, artificial hearts, vascular grafts, arteriovenous (AV) shunts, drug delivery devices, catheters, intra-aortic balloon pumps, and stents are all in this category (Fig. 11.3). The tests for hemocompatibility need to take into account the geometry and the conditions of contact between the device and blood during clinical application.

11.3.1 In Vitro Testing

The tests conducted in vitro include gravimetric analysis (thrombus weight) and light microscopy (morphology of adhered platelets, leukocytes, erythrocytes, and aggregates). They also include examination counts of platelets, leukocytes, and erythrocytes. Blood coagulation tests such as partial thromboplastin time (PTT) and

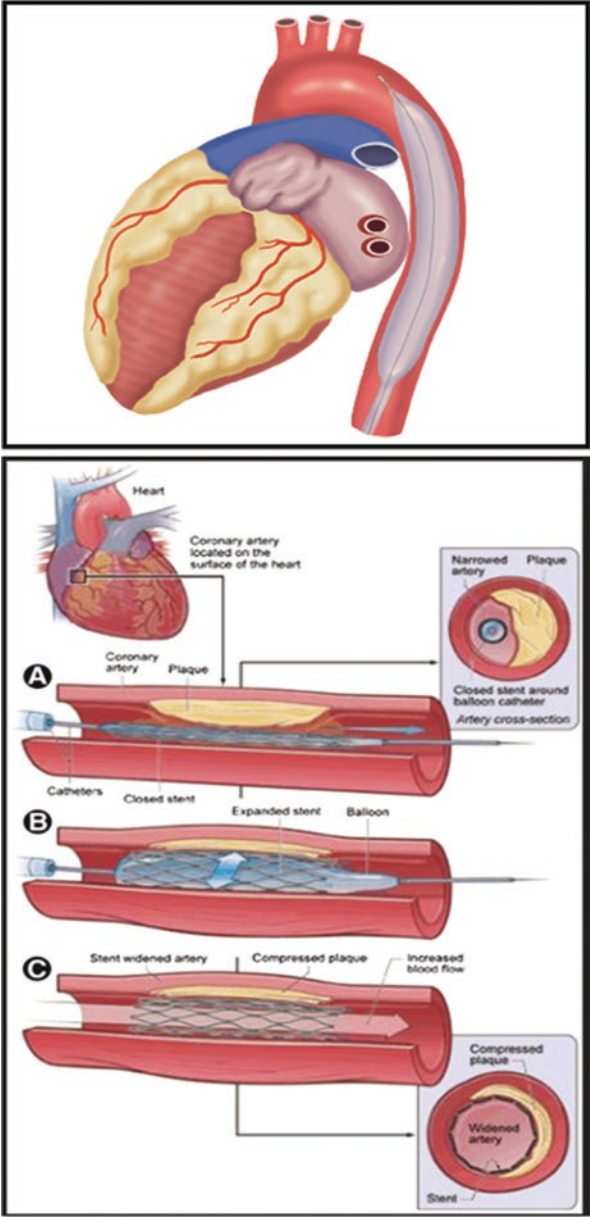


Fig. 11.3 Scheme of an intra-aortic balloon [8] and a stent [9]

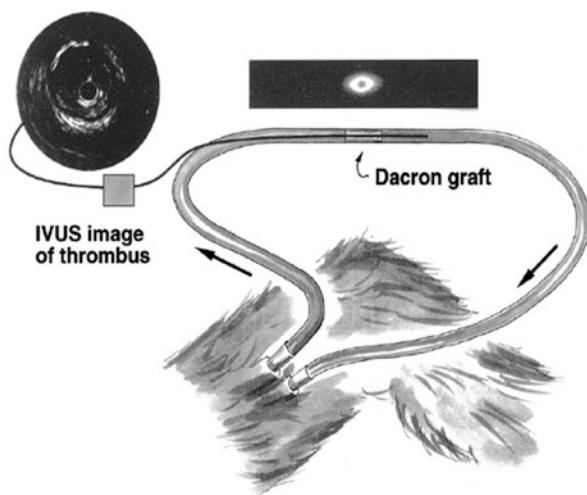


Fig. 11.4 Ex vivo shunt used in testing medical devices [10]

prothrombin time are kinetic tests. In these tests the blood is exposed to the test materials either under static conditions or in a closed loop system where the inner surface of the tubing or the whole tubing is the test material.

11.3.2 Ex Vivo Tests

Ex vivo are the tests where blood of the test animal flowing outside the body (in a shunt, or a loop) is in contact with the test sample (Fig. 11.4). Its advantage is that it mimics the natural flow conditions so it is a test closer to natural conditions.

11.3.3 In Vivo Tests

In vivo tests involve implanting the material or device in animals for significantly longer periods than in vitro or ex vivo testing. In the in vivo tests, adsorption of plasma proteins and lipids, platelets, leucocytes, and erythrocytes (resulting in reduced flow) in the interior of the device; formation of a pseudo-intima or tissue capsule; changes in the mechanical, chemical, and physical properties of the device; any activation of blood elements; formation of thrombi; and damage to circulating blood cells (e.g., hemolysis) are all tested.

11.4 Clinical Trials

A “clinical trial” is defined in Article 2 of Directive 2001/20/EC.1. [11] According to this article “all clinical trials conducted within the European Community (EC), must comply with the requirements of Directive 2001/20/EC of the European

Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of *good clinical practice* in the conduct of clinical trials on medicinal products for human use. Clinical trials, conducted outside the European Community, which relate to medicinal products intended to be used in the European Community, shall be designed, implemented and reported on the basis of principles of good clinical practice and ethical principles, which are equivalent to the provisions of Directive 2001/20/EC. They shall be carried out in accordance with the ethical principles that are reflected, for example, in the Declaration of Helsinki.”

As can be seen from the above quote, running clinical trials require following very strict rules.

EU states that it is not economically feasible and not justifiable to subject all medical devices to the most rigorous conformity assessment procedures [12]. A graduated system of control is more appropriate. In such a system, the level of control corresponds to the level of potential hazard inherent in the type of device concerned. A medical device classification system is therefore needed, in order to apply to medical devices an appropriate conformity assessment procedure. The current classification of medical devices is a system based on the level of risk the human body is exposed to when the device is used. Duration of contact with the body, degree of invasiveness, and local and systemic effects are the main parameters used in the estimation of the level of risk.

11.4.1 The Main Criteria for a Medical Device

Some main criteria that a medical device must fulfill can be summarized as follows [13]:

- The devices must be produced in such a way that they should not harm the patient or aggravate their situation.
- The devices must perform as planned.
- The properties and the performance of the device must be preserved throughout the duration of service.
- The properties of the devices should not deteriorate during transportation or storage on the shelf.
- Any side effect should be at an acceptable level.
- The material choice should be very careful and should take into account its risks due to toxicity, flammability, etc.
- The device must be biocompatible under the conditions and site of use.
- The devices must not pose a risk due to content to the people involved in its production, transportation, and use.
- Devices intended to administer medicinal products must be compatible with these products.
- The products must not create a risk of infection to the patient, user, and third parties.

- Tissues of animal origin must be from animals bred and treated under appropriate veterinary surveillance.
- Use of tissues, cells, and materials of animal origin must be performed under optimal security, avoiding transfer of viruses and other transferable agents.
- Devices must be sterilized using appropriate, validated methods.

The procedures to be applied during clinical trials need to be approved by the local Ethical Committees and performed by physicians involved in such treatments.

11.4.2 The Categories of the Devices According to the Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA, USA)

The categorization is based on the risk associated with the device in question.

11.4.2.1 Class I

Class I devices are the lowest-risk category (e.g. surgical instruments, elastic bandages) and are subject to general controls, which are published standards pertaining to labelling, manufacturing, post-market surveillance, and reporting. Devices are placed into Class I when there is reasonable assurance that general controls alone are adequate to assure safety and effectiveness. Formal FDA review is not required for most Class I devices before their market introduction.

11.4.2.2 Class II

Class II devices are higher-risk devices (e.g. infusion pumps, surgical drapes), and general controls alone are not considered sufficient. Special controls are applied such as performance standards, design controls, and post-market surveillance programs. Most Class II devices require FDA clearance of a premarket notification application (PMA or 510(k)) before the device may be marketed. In the 510(k) application, the medical device manufacturer must provide data to demonstrate that the new device is “substantially equivalent” to a legally marketed device.

11.4.2.3 Class III

Class III devices are the highest-risk implants (e.g. heart valves, pacemakers, implantable cardioverter-defibrillators, and coronary stents). They are of substantial importance in preventing impairment of human health, or present a high risk of illness or injury. Most Class III devices require FDA approval of a PMA before they can be legally marketed. Approval of the PMA generally requires clinical data demonstrating reasonable assurance that the device is safe and effective in the target population.

In the European Union Notified Bodies (NBs), independent commercial organizations that implement regulatory control over medical devices bear the main load [14]. NBs can issue the CE mark, the official EU mark for medical devices. NBs are

controlled by national authorities, and most of the FDA/CDRH functions are performed by NBs.

11.4.3 Clinical Trial Phases

Clinical trials are conducted in a series of steps, called phases. There are four phases as presented below:

Phase I: Testing of a new device, drug, or treatment in a small group of people for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.

In Phase I, the study is a pilot study where a small patient population and limited number of sites are used. Viability is the main concern checked.

Phase II: The drug or treatment is given to a larger group of people to see if it is effective and to further evaluate its safety.

In Phase II the patient population and the numbers of sites are significant. Safety and efficacy are the main concerns.

Phase III: The drug or treatment is given to large groups of people to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.

In Phase III the patient number and sites are more numerous, and the expectation is the verification of the Phase II results in this much larger test medium.

Phase IV: Studies are done after the drug or treatment has been marketed to gather information on their effect in various populations and any side effects associated with long-term use.

Phase IV is the study of the results after the product is already on the market.

11.5 Conclusion

Biocompatibility testing is essential before a product can be marketed. Selecting the correct tests requires experience and guidance, since, otherwise, costs could skyrocket. On the other hand, the costs of implant failure are also very high to the manufacturer and especially to the patient, so great care must be taken to avoid unnecessary cost-cutting. The labs where tests are performed is another issue that must be carefully considered. As the ingredients of biomedical materials and devices include more bioactive agents, the testing process becomes more complicated, especially for growth factor and cell-containing devices. Biocompatibility testing is a matter of life and death and must be properly performed under all circumstances, regardless of time and cost.

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12.1 General Information

Hemocompatibility is a specific and advanced state of biocompatibility which is especially important for blood interfacing biomaterials. It is important due to its systemic consequences, mainly a blood clot traveling to distant sites and causing unforeseen problems. Any biomaterial which is shown to be biocompatible may not necessarily be hemocompatible, but a hemocompatible material has to be biocompatible. This is because the components in the blood and the processes that take place in it are so different than the rest of those in other tissues that this issue deserves a separate treatment. In order to understand hemocompatibility, we should first look at the circulatory system and the elements of the circulatory system.

12.2 Circulatory System

Blood is enclosed in a system called the circulatory system, and hemocompatibility is related with the maintenance of blood properties and composition in the event of introduction of a new material to interface with the blood (Fig. 12.1).

In the body, the cells require nutrients (glucose, proteins, amino acids, electrolytes) and oxygen to properly function, and upon processing them, the waste products (gases like CO₂ and O₂, toxic metabolites such as urea, and creatinine) that form need to be removed. An efficient system is needed to distribute the blood throughout the body using a tubular structure ranging from the very large blood vessels like arteries and veins to the very fine vasculature called capillaries. The diameter and wall thickness of the aorta are 25 and 2 mm, for a typical artery about 4 and 1 mm and for arteriole 30 and 6 μm . For the vena cava, they are 30 and 1.5 mm, for typical veins 5 and 0.5 mm and for the venules 20 and 1 μm . The capillaries connect the two vessel lines, and their dimensions are 8 and 0.5 μm [1]. In the biomaterials sector, blood vessels narrower than 6 mm are classified as “small-diameter vascular grafts” [2] and are not successfully produced.

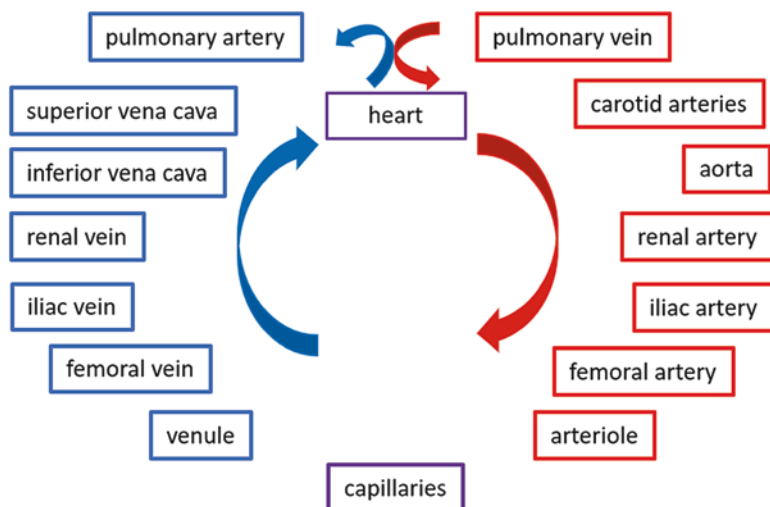


Fig. 12.1 Main elements of the circulatory system

In addition to serving as a transportation network, the circulatory system regulates the internal body temperature. Besides, the blood vessels protect the fragile blood elements from loss and damage, and maintain the internal pressure needed for the blood to flow. Blood also carries immune cells needed in case of infection, and contains damage control elements such as platelets to plug defects in the vasculature [3].

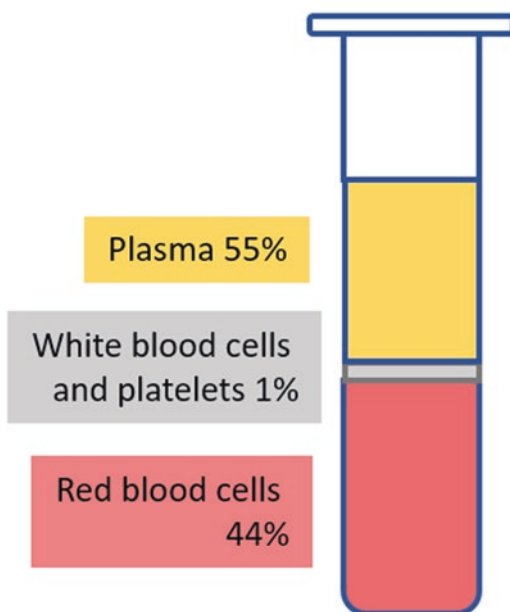
12.2.1 The Elements of the Circulatory System

The circulatory system consists of the heart, blood vessels, and blood, where the first two constitute the *cardiovascular* system. The heart is the organ that pumps the blood through the body, and the blood vessels are a network of hollow biological tubes through which the blood is transported. The main reasons why hemocompatibility is an issue are that blood elements are very fragile and can be easily damaged, and a variety of interactions involving them lead to blood clotting.

12.2.1.1 Blood and Cells

The amount of blood of a 70 kg human is around 4.5 L. It has a fluid portion called the plasma which consists of water and the dissolved gases, the nutrients (proteins, sugars, vitamins, minerals, etc.), and the waste products. The cellular portion of the blood consists of cells, mainly red blood cells, white blood cells, and thrombocytes (or platelets) (Fig. 12.2). These are mainly produced in the bone marrow. In the human adult, the bone marrow produces all of the red blood cells, 60–70% of the white blood cells (i.e., the granulocytes), and all of the platelets. The lymphatic tissues produce the lymphocytes, and the spleen, liver, lymph nodes, and other organs produce the monocytes [4]. Each of the liquid and cellular fractions constitutes approximately half of the blood volume.

Fig. 12.2 Three main components of blood can be separated using a centrifuge. When the blood is settled, it briefly separates into layers of plasma, white blood cells and platelets, and at the bottom erythrocytes



The straw colored supernatant after centrifugation of blood is devoid of cells (erythrocytes, leukocytes, and thrombocytes) and is called the plasma. Plasma, therefore, consists of proteins (albumin, antibodies, enzymes, and hormones), sugars (glucose), and fat particles.

The main cell types present in the blood are shown in Fig. 12.3. Erythrocytes or, as they are commonly known, red blood cells (RBC) are very high in number; there are $4\text{--}6 \times 10^6$ cells/mL blood and have a lifetime of about 120 days. The RBCs lack a nucleus and are simply a shell with hemoglobin in it. RBCs are biconcave in shape and about $7.5\text{--}8\text{ }\mu\text{m}$ in diameter. They are one of the two main elements of clot formation.

Leukocytes are white blood cells (WBC), also called the white corpuscles, a cellular component of the blood; unlike erythrocytes they have a nucleus and defend the body against infection and disease by ingesting foreign materials and debris, and destroying infectious agents and cancer cells. Their concentration in blood is in the range $4,000\text{--}11,000$ per μL . These cells are motile. Most leukocytes are found in the tissues where they are ready to fight infections. These cells are highly differentiated and are grouped into three major classes—lymphocytes, granulocytes, and monocytes—and each of these classes have multiple subclasses with a variety of defense functions.

Platelets are among the most crucial elements of blood clotting. They are irregularly shaped fragments of megakaryocytes that originate from stem cells in the bone marrow, have a lifetime of about 12 days, and circulate in the blood until they are either activated to form a blood clot or removed by the spleen. Their concentration in a 70 kg person is in the range $30,000\text{--}60,000$ per μL .

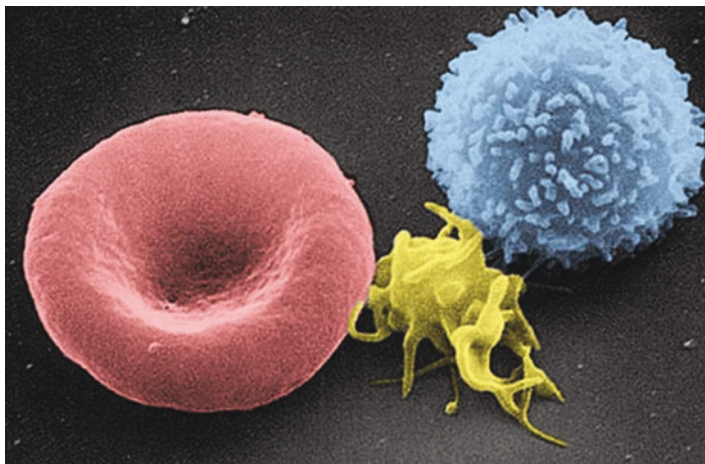


Fig. 12.3 Blood cells: erythrocyte (red blood cell), platelet (thrombocyte), and leukocyte (white blood cell) (from left to right) [5]

Platelets circulate for about 9 days. If they encounter damaged blood vessel walls, or artificial surfaces which do not fit the natural form and chemistry of the blood vessels, they are activated to form a blood clot. This is the basis of thrombosis and the reason for the failure of blood interfacing biomedical devices, and this is why hemocompatibility of new medical devices are strictly monitored.

12.3 Blood Coagulation and Clotting Factors

Coagulation is basically the process of conversion of soluble blood protein fibrinogen to insoluble fibrin. This process takes place with a complex mechanism which involves a number of molecules called protein factors.

Under normal circumstances, coagulation is activated by the interaction of tissue factor (TF) and Factor VII, which activates Factor VII. This, in turn, activates Factor X (Fig. 12.4). Activated Factor X promotes the production of thrombin and the formation of fibrin. Thrombin (Factor IIa), which is produced by cleavage from prothrombin, stimulates the formation of fibrin, activates the coagulation cascade, and stimulates the aggregation of platelets; however, the initial attachment of platelets to each other, once stimulated, is mediated by fibrinogen, which serves as a divalent platelet-platelet bridge.

There are two independent mechanisms for initiating blood coagulation and for activating Factor X:

1. Negatively charged surfaces initiate blood clotting through the intrinsic pathway (Factors XII, XI, IX, and VIII)
2. Tissue factor (TF) on cells outside the blood participates in the extrinsic pathway (Factor VII)

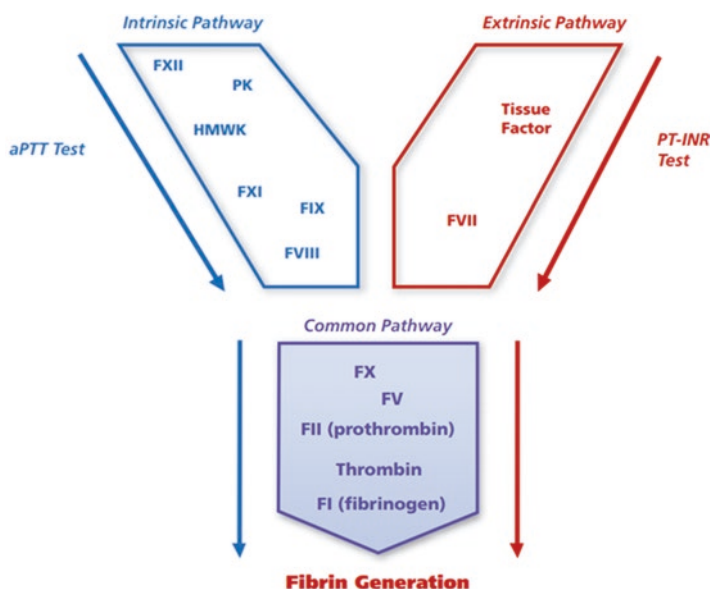


Fig. 12.4 Coagulation mechanism [6]

The common pathway (Factor X, Factor V, prothrombin, and fibrinogen) is shared by both systems. The physiologically important pathway of blood coagulation is the extrinsic pathway initiated by tissue factor.

When a blood vessel is damaged, molecules that are normally not in direct contact with the blood flow, such as collagen and von Willebrand factor, cause the platelets to adhere to the damaged vessel surface. Once the platelets adhere to the surface, they release chemicals that attract more platelets to the damage site and lead to a platelet aggregate. The protein-based system involving the fibrinogen conversion into fibrin (the coagulation cascade) serves to further stabilize the clot that has formed. All of the factors that are involved in the cascade have one inactive and one active form. Once activated, these factors serve to activate the next one down the sequence until fibrin is formed.

Fibrinogen is a large, complex glycoprotein composed of three pairs of polypeptides linked together by 29 disulfide bonds. The final clotting process involves thrombin, an enzyme which catalyzes the lysis of a few peptide bonds of fibrinogen, and two fibrinopeptides with molecular weights of 1900 and 2400 Da are obtained. Fibrin molecules link together by polymerizing to form staggered oligomers to build protofibrils, which continue to lengthen to make long fibers that wind around one another to make multi-stranded, thick bundles, and 3-dimensional networks or the fibrin clot.

12.4 Factors Influencing Hemocompatibility

Materials used in construction of blood-contacting devices have to be hemocompatible. In other words they should not lead to blood clotting, not harm or affect the amount of blood constituents such as decreasing their number by damaging or absorption. For that purpose these devices are treated in a number of ways to gain these properties. Surface chemistry and topography have great importance in succeeding in this difficult task, and researchers study and try to control these properties.

12.4.1 Surface Chemistry

12.4.1.1 Polarity and Hydrogen Bonding

Polarity generally yields a hemocompatible surface. If it is essential, the surface of any material can be modified without affecting the bulk properties. In one study, the surfaces of PLGA nanocapsules were functionalized with thiol groups, and thiolation was observed to be an effective strategy in reducing the protein adsorption, complement activation, platelet activation (as shown by decreased P-selectin amounts), and hemolytic activity [7].

Polar nanoparticles with cetyl alcohol-polysorbate in their structure were found to be harmless toward the integrity of the membrane of the red blood cells. Similarly, heparin-carrying polyethersulfone particles were also non-hemolytic (<5% hemolytic activity).

Attachment of polar molecules such as polyethylene glycol (PEG) or polyethylene oxide (PEO), glycidyl methacrylate (GMA), or N-vinylpyrrolidone is reported to be an effective means to improve the hemocompatibility of hydrophobic polyolefins [8].

In a study, carbon nitride (CN) was deposited as a coat on the multiwalled carbon nanotubes (MWCNTs) using chemical vapor deposition (CVD) to improve surface hemocompatibility. The results revealed that the higher the N/C ratio, the more prolonged was the kinetic blood-clotting and recalcification, implying improved hemocompatibility [9].

In situ testing with whole human blood was carried out with self-assembled monolayers (SAMs) of alkanethiols with various terminating groups such as hydroxide, methyl, and carboxylic ($-\text{OH}$, $-\text{CH}_3$, $-\text{COOH}$) and their binary mixtures, and the results showed that the adhesion of leukocytes was diminished on a methylated (hydrophobic) surface and enhanced with hydroxylated ($-\text{OH}$) groups. An extremely important point for hemocompatibility is prevention of platelet adhesion, and a hydrophilic surface was found to be better [10].

12.4.1.2 Charge

Phospholipids are integral components of cell membranes which have a zwitterionic functionality that mimic the outer, blood-contacting surface of the lipid bilayer structure of normal cell membranes and those of blood corpuscles,

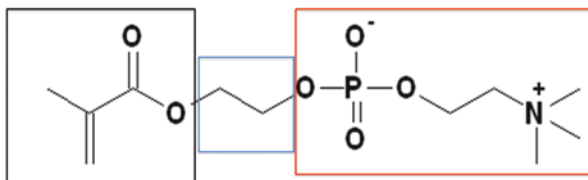


Fig. 12.5 Methacryloyloxyethyl phosphorylcholine

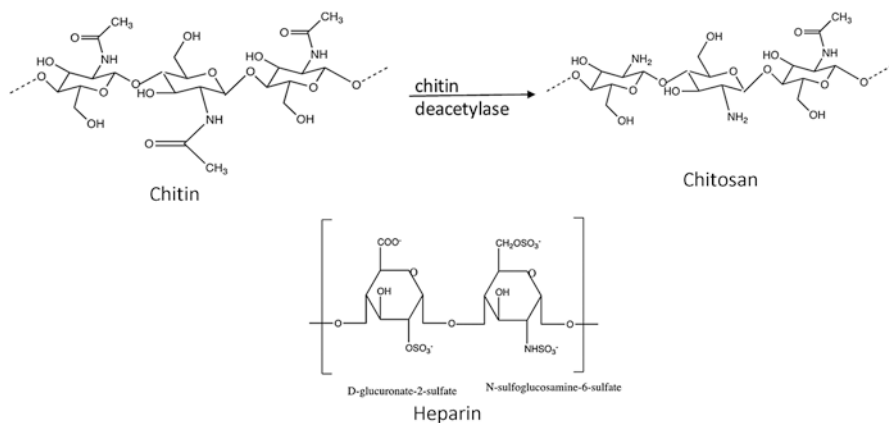


Fig. 12.6 The formulae of chitin, chitosan, heparin

and are expected to be hemocompatible. Methacrylates containing phospholipid side chains such as 2-methacryloyloxyethyl phosphorylcholine (MPC) (Fig. 12.5), and polymers containing them in their backbones as copolymers with various hydrophobic methacrylates and styrene were shown to support this expectation. Poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) exhibits excellent hemocompatibility as shown by the reduction of platelet adhesion and aggregation and suppression of protein adsorption due to the presence of the negative charge of the phosphate and the positive charge of the quaternary amine [11, 12].

It was shown that molecules that impart a negative charge to a biomaterial make them hydrophilic and also more hemocompatible. Same cannot be stated for the positively charged molecules. As examples of negative charged hemocompatible molecules, polysaccharide-based natural molecules such as heparin, polyalginic acid, hyaluronic acid, chondroitin sulfate, all of which are organic acids and have a net negative charge at physiological pH, can be stated. Among the synthetic molecules, poly(methacrylic acid) is one important example.

Meanwhile the positively charged and, therefore, hydrophilic cationic molecules such as chitosan and poly(L-lysine) have a tendency of being cell attractive since the cell surface is anionic and is cytotoxic and also non-hemocompatible (Fig. 12.6).

These are all related with the charge on the cellular elements being negative and, therefore, repulsed by negatively charged species, while positively charged species attract them and lead to accumulation and aggregation. Heparin is also a molecule which has a negative charge due to being a highly sulfated glycosaminoglycan. Its anticoagulant effect is not just due to the charge and hydrophilicity, but also its interference with the coagulation mechanism by binding to the enzyme inhibitor [antithrombin](#) III (AT) causing its activation which inactivates [thrombin](#) and other proteases involved in blood clotting.

12.5 Protein Adsorption

It is stated that interaction of blood with foreign surfaces start with plasma protein adsorption, and this influences the blood-material interactions that follow. Adsorption of fibrinogen from plasma plays a central role in coagulation, and especially fibrinogen was reported to be the main protein involved in platelet adhesion. Hydrophobic surfaces were shown to adsorb more protein, and the affinity of fibrinogen was higher for methyl (hydrophobic) rather than hydroxyl (hydrophilic)-carrying surfaces implying that hydrophilic surfaces are more compatible with blood [13].

Adsorption of proteins gives an indication as to whether the surface is hemocompatible or not. Studies show that not only the amount of adhered proteins but also the structure and the type of the proteins that adhere determine the subsequent platelet reaction and thus hemocompatibility. Albumin is considered to be nonadhesive to platelets as it lacks any known amino acid sequences for binding platelet receptors. It has been considered to be unable to support platelet adhesion and hence is widely used for blocking nonspecific platelet-surface interactions in platelet adhesion studies, and also as a hemocompatible coating for biomaterial surfaces to create passivating surfaces. Studies demonstrated that in addition to the amount and type, the conformation of the proteins plays an important role in the interaction with platelets. Thus conformation changes observed after adhesion on the surface appear to be also very important in platelet adhesion. Fibrinogen adsorption can be given as an example since its conformation changes after adsorption in order to bind and activate platelets.

The adsorption of proteins does not produce a layer stable over time. Depending on the type of the protein, they can desorb soon after adsorption and be replaced by other proteins. Vroman effect states that at first the proteins with highest mobility arrive and get adsorbed, and then they are replaced by proteins slower but with a higher affinity for the surface.

A major indicator used in detecting whether a surface is hemocompatible or not is the preference for the adsorption of albumin and fibrinogen. In situ testing of adsorption of these two proteins give a good indication: when albumin is preferentially adsorbed onto a surface, then the surface is more likely to be hemocompatible, and when fibrinogen is adsorbed more, then the surface is likely to be non-hemocompatible.

Pyrolytic carbon, titanium alloy Ti-6Al-4V, tissue culture polystyrene (TCPS), and polystyrene sterilized by gamma irradiation were tested for their hemocompatibility [14]. Among the materials studied, pyrolytic carbon induced the lowest level of protein adsorption, platelet adhesion and activation, and titanium induced the most. Pyrolytic carbon showed high levels of fibrinogen adsorption, but this did not induce platelet adhesion. The study also found a correlation between decreased protein adhesiveness and increased hydrophilicity. Interestingly, no significant differences were reported in terms of platelet activation and protein adsorption caused by surface roughness differences.

In addition, surface chemistry (charge, polarity, hydrophilicity) is also important in hemocompatibility. In the study reported above about thiolated surfaces, protein adsorption was decreased upon treatment. Even though fibrinogen adsorption was more than that of albumin on treated and untreated surfaces upon thiolation, both these surfaces were harmless against blood cells.

When bovine serum albumin (BSA) was immobilized onto polypropylene using polyacrylic acid as a linking molecule, the water contact angles revealed that hydrophilicity was improved, protein adsorption and platelet adhesion significantly decreased, and the whole blood clotting time was significantly enhanced, all indicating improvement in hemocompatibility [15].

12.6 Surface Topography

Surface topography plays a significant role in hemocompatibility. In the design of the blood vessels, surface roughness is not desired because any protrusions or depressions disturb the flow of the blood and its elements, leads to their accumulation, and eventually might trigger coagulation. Obviously smoothness by itself is not satisfactory for hemocompatibility. In cases where there are interstices, as in blood vessels, they might attract blood proteins to accumulate in the pores and fill them to create very smooth and compatible surfaces.

In addition to surface chemical modifications, surface topography is also an important parameter in hemocompatibility of materials. In a study with HUVEC (human vascular endothelial cells) and with whole blood, nano level cues were formed on the surface of titanium stents, and tests revealed negligible hemolysis upon contact with human blood under constant shear and static conditions. Meanwhile surface roughness induced no changes in the normal clotting times and in thrombus formation and showed only minimal inflammatory reaction. The nanotextured Ti surface promoted endothelialization, and with all the positive responses against blood, it was accepted to be antithrombogenic [16]. Nanotopography was also reported to influence fibrinogen adsorption. Fibrinogen adsorbed on rough titanium oxide surfaces bound platelets significantly faster compared to flat substrates [13].

It can be concluded that hemocompatibility is a complex issue and a single parameter cannot by itself determine the response of a surface.

12.7 Testing for Hemocompatibility

Hemocompatibility testing involves the evaluation of critical interactions of foreign materials with blood to explore possible adverse effects on the patient arising from the exposure of the materials to blood cells and components. Such adverse effects are varied and complex and generally very risky for the well-being of the recipient of the material. Approval of blood-interfacing implants and other biomaterials is tightly regulated.

The devices exposed to blood for short or long durations involve vascular grafts, shunts, heart valves, stents, pacemakers, hemodialysis systems, heart-lung machines, left and right ventricle assist devices, and total artificial hearts. The testing guidelines are given in the International Standards as ISO 10993-4.

In general, the *in situ* aggregation tests use treated blood or certain fractions of it to study the influence of the foreign materials or their extracts on activating elements of the coagulation or clotting mechanism. Hemolytic activity tests are performed to determine the adverse effects of the implant or its extracts on the erythrocyte integrity. The *in vitro* assays are designed to assess the level at which the biomaterial adversely affects the cellular components of the blood. When the test article itself is used, it is called the direct approach, and when an extract in oil or aqueous solution is used, it is called the indirect approach. *In vivo* experimentation is also essential because a living animal with all its systems is tested for its reaction toward the foreign material.

The tests include:

1. *In situ* tests involving protein adsorption
2. *In situ* tests on the elements involved in clotting
 - (a) Complement activation
 - (b) The prothrombin time assay (PT)
 - (c) The unactivated partial thromboplastin time assay (uPTT)
 - (d) The activated partial thromboplastin time assay (aPTT)
 - (e) Lee-White clotting time
 - (f) Platelet aggregation
3. Hemolytic activity tests
4. *In vitro* hemocompatibility assay
5. *In vivo* thrombogenicity (in dogs)

12.7.1 Protein Adsorption Tests

As stated above, hydrophobic surfaces preferentially adsorb fibrinogen, and fibrinogen leads to coagulation. Thus, hemocompatibility of material surfaces is sometimes tested by assessing the preference of the surfaces for fibrinogen or albumin (Fig. 12.7). Correlations were found between the amount of adhering blood platelets and the fibrinogen/human serum albumin (Fg/HSA) preference ratio and also the absolute amount of Fg adsorbed in competition with HSA.

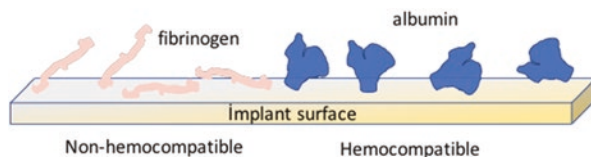


Fig. 12.7 Implant surfaces that adsorb fibrinogen are generally not hemocompatible, but those that adsorb albumin are

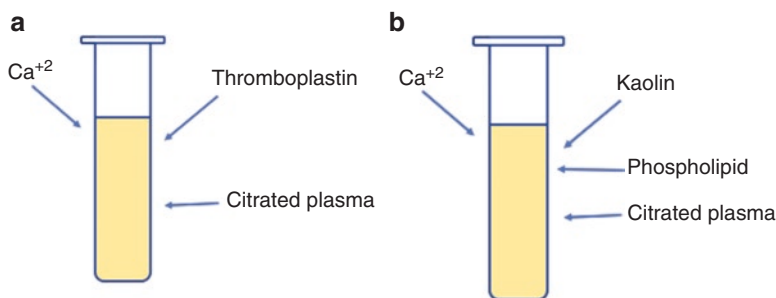


Fig. 12.8 Blood-clotting tests. (a) Partial thromboplastin time (PT) test, (b) activated partial thromboplastin time (aPTT) test

12.7.2 Blood Clotting Tests

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are by far the most common screening tests for blood clotting (Fig. 12.8). In the PT assay, the amount of tissue factor (TF) used to trigger *in vitro* clotting is much more than under *in vivo* conditions. It is reported that the *in vivo* coagulation mechanism cannot be mimicked fully by PT tests because repeated bleedings might occur in hemophilia even when the PT values are normal.

In performing the test, a sample of blood is decalcified by treating with oxalate or citrate ions to prevent the clotting process prematurely. The blood cells are separated by centrifugation and removed. The test is performed by adding this plasma to some source of Tissue Factor such as thromboplastin that converts prothrombin to thrombin and keeping the mixture in a warm water bath at 37 °C for 1–2 min. Then calcium chloride is added to neutralize the effect of citrate ions and initiate clotting. The prothrombin time is the period between the addition of the calcium chloride and the clot formation as shown by a glass rod becoming immobile in the test tube due to the clot.

The PT test measures how long it takes for blood to clot. The normal time for clotting to occur is between 25 and 35 s. If the implant interferes with clotting by interacting with blood elements, it generally decreases the time for clotting. The PT test uses blood which is decalcified to prevent clotting before the test begins.

The aPTT test also uses blood which is decalcified to prevent clotting before the test begins. The plasma is separated by centrifugation. Calcium ions and activating

substances such as kaolin (hydrated aluminum silicate) and cephalin are added to the plasma to start the intrinsic pathway of the coagulation cascade. Kaolin serves to activate the contact-dependent Factor XII, and cephalin substitutes for platelet phospholipids. The partial thromboplastin time is the time it takes for a clot to form, measured in seconds. Normally, the sample will clot in 35 s.

12.7.3 Hemolytic Activity Tests

Hemolytic activity tests are used to evaluate damage to erythrocytes, in the form of membrane damage and thus hemolysis, caused by physical and chemical interactions with the environment. The tests can be carried out indirectly using extracts of test samples. Erythrocytes are separated from plasma by centrifugation, diluted (1:9) in physiological phosphate buffer solution (PBS). Next, 1 volume of erythrocyte suspension is mixed with equal volume of extract or brought into contact with the test device. As blank, blood and PBS are mixed (1:1). For complete hemolysis, erythrocyte suspension is mixed with ninefold higher volume of distilled water. After incubation, the absorbance of the released hemoglobin is measured with a UV Vis spectrometer at 405 nm.

12.7.3.1 In Vivo Tests

In vivo tests involve implantation of the material or the device in animals. Vascular grafts, prosthetic rings, heart valves, and “right and left ventricular assist devices” are among the devices tested. The extent of occlusion and the mass of the thrombus formed are determined by gross and microscopic examination of the organs downstream from the device and the device itself. Histopathological evaluation of the neighboring tissue and organs is also necessary. Such evaluations need the termination of the test animal, but there are also other methods where terminating the animals is not necessary.

For example, imaging of vessel blockage using radiopaque agents or scintigraphy and detection of accumulation of platelets by pre-labelling the platelets with radioactive chromium can be used to monitor the extent and the kinetics of platelet deposition.

12.7.3.2 Ex Vivo Tests

Ex vivo tests are performed when the intended use of the device is ex vivo, such as an external communicating device or when monitoring of blood-material interactions is more convenient with this approach. An example of that could be the case of a vascular graft. Such an approach allows the retrieval of the test sample during testing and analyzing it without having to terminate the test animal.

These observations could be for vasculature occlusion, platelet adhesion, protein deposition, analysis of cell types adhering on the graft including platelet consumption, and activation. Similarly, changes in the blood flow rates can be measured to

deduce the level and development of thrombus deposition. A major advantage of ex vivo tests over in vitro tests is that dynamic blood flow is used mimicking the physiological conditions whereas the test fluid in the in vitro tests requires treated blood use or use of blood components.

12.7.4 Some Commercial Vascular Grafts in the Market

The vascular grafts are produced by two main methods: knitting and weaving. The method applied influences the interstices, permeability, extensibility, and adsorption of blood elements. The main materials used are various Dacron (polyethylene terephthalate), PTFE and ePTFE (polytetrafluoroethylene), and polyurethanes. The chemistry and the functionalization treatments of the basic structures are complex. The following are a list of commercially available vascular graft types:

Dacron (PET, polyethylene terephthalate):

- Knitted polyester (requires pre-clotting)
- Knitted velour polyester
- Knitted, collagen coated
- Woven polyester

ePTFE (extended polytetrafluoroethylene):

- Woven polyester
- Goretex and Impra

Polyurethane:

- Knitted

It is very complicated to simply categorize vascular grafts as the properties of the main product, and those of the modified versions are extremely influential on the final properties of the graft, including the most essential property, hemocompatibility.

12.8 Conclusion

In this chapter, hemocompatibility, the factors affecting thrombotic properties of biomaterials and testing for hemocompatibility in order to gain familiarity with blood interfacing biomaterials were discussed. Compatibility of the biomaterials with blood and the factors influencing this phenomenon are especially important in cardiovascular applications.

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13.1 General Information

Sterilization is a necessary and an important part of the manufacture of any biomaterial because infection is the last thing a patient who has undergone an implant placement surgery needs. This would hamper the healing process, probably make the implant unsuccessful and make the patient incapable of dealing with this additional health problem, while the actual surgery might have been a very serious one such as a cardiovascular implantation. Sterilization is needed because the implants could be carrying harmful microorganisms due the implant production environment being not sufficiently sterile, the operating theater may not be microorganism-free, or the surgical instruments could be contaminated. The solution for the last problem is proper sterilization of the implant. Of course in the operating theatre, all the instruments used during the surgery should also be sterilized. The selection of an appropriate sterilization method is an important issue. The goal of sterilization is to reduce the amount of microorganisms found in the surgical environment and on the devices to an internationally acceptable level. However, the methods used to achieve this could have a significant effect on the physical, chemical, and toxicological properties of a biomaterial and as a result on its performance; therefore care should be exercised in the selection of the correct method of sterilization.

Sterilization is defined as a condition of complete destruction or removal of all forms of life on an instrument to be used or on a device to be implanted. In practice, however, the acceptable level of microbial contamination of a sterilized object is 10^6 or less.

13.2 Methods of Sterilization

Methods of sterilization are either physical (ionizing radiation) or chemical (reactive gases and liquids) (Table 13.1). Most liquids used are disinfectants and not sterilants; thus, they do not kill spores. Liquids are also not appropriate for use with

Table 13.1 Biomaterial sterilization methods

Methods	Conditions	Comments
Dry heat	170 °C, 60 min	Used for glass, metal, silicon, nylons, PTFE. Longer times above 170 °C can effectively inactivate endotoxins
Steam under pressure	121 °C, 15 min 126 °C, 10 min 134 °C, 3 min	Generally suitable for metals and ceramics. Softening or deformation may occur with polymers
Ionizing radiation	2.5 Mrad (25 kGy)	Crosslinking or degradation may occur with polymers
Ethylene oxide	Relative humidity 40%, 55 °C	Very suitable for most biomaterials. Requires degassing especially of porous materials

microporous objects or devices with complex geometry (where trapped air can protect microorganisms by acting as a barrier preventing contact of the liquid with the targeted microorganisms).

13.2.1 Dry Heat Sterilization

This method is not considered suitable for most polymeric biomaterials because temperatures in excess of 140 °C are required for long periods of time (60 min or more). Most polymeric products soften, deform, or are oxidized under these conditions. The advantage of dry heat application is that endotoxins (pyrogen) are removed if performed at 180 °C for 4 h.

The disadvantages of the method are that diffusion of heat is slow in materials with low thermal conductivity, and it requires long sterilization times and high temperatures. Many materials are damaged by heat or oxidation, and also heat zones tend to develop.

13.2.2 Steam Under Pressure (Autoclaving)

This method is at least seven times more effective at killing spores than dry heat at the same temperature because of its latent heat of vaporization. According to USP 1229, steam sterilization is carried out as heating with steam-saturated air in an autoclave (Fig. 13.1) under pressure (ca 10 atm) for at least 15 min at a minimum temperature of 121 °C. Shorter times with higher temperatures can also be used.

13.2.3 Ethylene Oxide (EtO) Gas Sterilization

Gas sterilization can be extremely effective if certain conditions are met. Ethylene oxide (EtO) and formaldehyde are considered to be the sterilants of choice. Highly porous implants carry the risk of entrapping the gas in their structure.

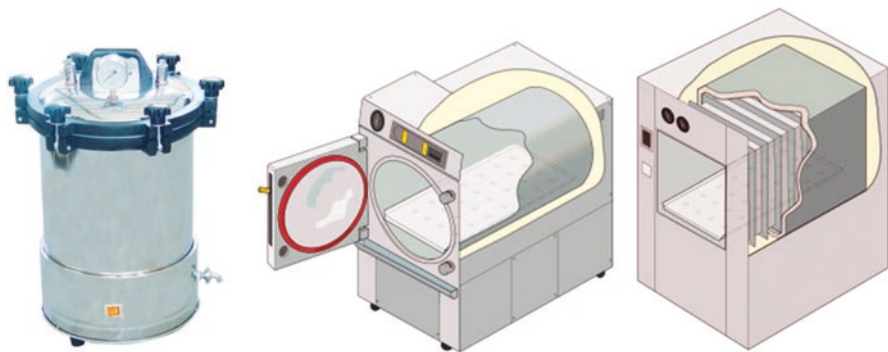


Fig. 13.1 Typical autoclaves: cylindrical and rectangular designs [1]

Ethylene oxide is highly explosive in air in its pure form, but addition of carbon dioxide or Freon reduces the risk. It is most effective when relative humidity is 40% and the temperature is in the range 50–60 °C. The maximum safe level acceptable in an implanted device is 250 ppm EtO after the sterilization. Its disadvantages are that it is more expensive than heat, more monitoring is required in each cycle, it does not kill spores in crystals, and it is necessary to determine the residual EtO or its products in the biomaterial.

13.2.4 Vaporized Hydrogen Peroxide

One of the newest gas sterilization methods uses vaporized hydrogen peroxide (H_2O_2) for terminal sterilization of clean and dry reusable metal and nonmetal medical devices used in healthcare facilities since 2011 [2]. A similar gas-based system uses low-temperature H_2O_2 gas plasma technology at 47–56 °C and utilizes 59% liquid hydrogen peroxide for terminal sterilization of heat and moisture-sensitive medical instruments and devices.

13.2.5 Ionizing Radiation

Ionizing radiation types related with sterilization of medical devices mainly consists of gamma radiation (γ -radiation), ultraviolet radiation (UV), and electron beam (e-beam). Their cost, widespread acceptance, and effectiveness define the sterilized device categories. γ -Radiation and UV are non-particulate, electromagnetic radiation (EMR) without any energy carriers except the photons, while in e-beam the energy utilized in the sterilization process is transmitted by highly energetic electrons. In the following section, the advantages, disadvantages, and uses of these methods are being discussed.

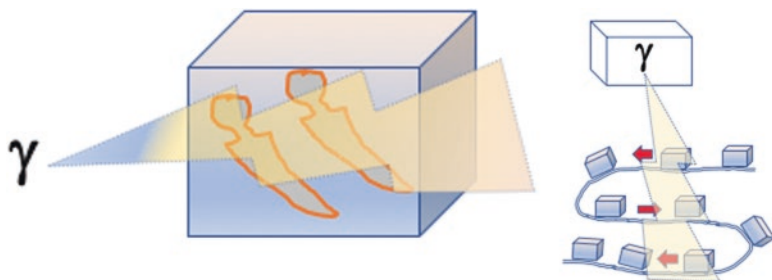


Fig. 13.2 Sterilization of metallic implants with gamma radiation: radiation penetrating a boxed device container and conveyor belt system to irradiate from every angle

13.2.5.1 Gamma Irradiation

Sterilization of single-use medical devices by gamma radiation using a cobalt 60 (^{60}Co) source has been an accepted practice for the last 40 years (Fig. 13.2). During the early years of gamma radiation, sterilization with 25 kGy (kilo Gray or 2.5 Mrad) was the standard used in the sterilization of medical devices. Current practice, however, is to use lower levels of radiation that assure sterility in tests to the desired sterility assurance level (SAL). The commonly accepted SAL for invasive medical devices such as implants is reduction of the viable microorganisms on any device by one million times. This is referred to as “SAL of 10^{-6} .” Still, in Europe an item is allowed to be labelled sterile only after receiving 25 kGy, regardless of the bioburden. In Fig. 13.2, the advantage of using gamma sterilization is presented. A product completely sealed and boxed is paraded in front of the gamma source, where the height and direction of the conveyor belt changes to allow for complete irradiation.

With gamma irradiation fewer controls than EtO is required, and continuous processing can be achieved at substantially lower running costs. However, most polymeric materials are affected by the electromagnetic radiation. This effect is either in the form of crosslinking and leads to embrittlement of the material, or degradation through chain scission and leads to lower mechanical properties.

13.2.5.2 UV

Ultraviolet (UV) radiation is generally used for disinfection in the labs because it does not produce any known toxic residuals or by-products. However, despite its wide use, there is a lack of information available on the efficacy of UV radiation for disinfecting or sterilizing surgical tools.

The UV spectrum is commonly subdivided into three categories: UVA (400–315 nm), UVB (315–280 nm), and UVC (280–200 nm).

UVC radiation has the highest energy due to the wavelength range and thus is more effective. In addition it can denature the DNA of microorganisms because these molecules have a high absorbance at 254 nm. Denaturation is caused by the formation of pyrimidine dimers, resulting in the inactivation of the bacterium by blocking DNA replication [3]. Although the entire UV spectrum has been known to kill or inactivate many microorganisms, some researchers suggest that a wavelength

of 254 nm (UVC) is most effective. Thus, it is suggested that monochromatic UVC radiation should be the industry standard. The type of radiation varies not only with the wavelength of the UV light source but also with the quantity of the energy transmitted. The dosages required for inactivation of microorganisms and spores vary and sterilization conditions must be calibrated to achieve desired level of microorganism reduction.

13.2.5.3 E-Beam Sterilization

Sterilization by electron beam is cost-effective and fast, and especially important for processing polymer-based medical devices in large numbers. Electrons of the e-beam kill the microorganisms by damaging the DNA, and creating free radicals and other active species which also react further with the microorganisms.

It is reported to have the following advantages:

- Penetrates all types of product packaging,
- Allows control of temperature during irradiation.
- Dose range can be controlled well.
- Works quickly (minutes) and is therefore efficient.

Polymeric devices are susceptible to oxidative degradation as a result of irradiation. Recent reports indicate that rapid delivery of a dose decreases the degradation and embrittlement of a polymer caused by excessive crosslinking.

Currently, surgical dressings, wound care products, IV kits, dialyzers, cardiac catheters, and stents are sterilized by this method. The electron beam sterilizer operates by passing packages on carriers in front of the e-beam, and the sterilized packages can be safely handled as soon as they exit the chamber unlike some other treatment methods.

Side effects of exposure to electron beam are typical of any irradiation; crosslinking which makes the products brittle and chain scission which decreases the mechanical properties. These are dependent on the dose and the chemical composition of the polymeric material. In some, crosslinking predominates and in others, chain scission. Besides, the presence or absence of gases during sterilization can modify the surface properties of the products.

13.2.6 Chemical Sterilization

A number of chemicals are used in sterilization of devices using their solvating power or by oxidizing the organic compounds of the microorganisms.

- [Ethylene oxide](#)
- Hydrogen peroxide
- Ozone
- Chlorinated agents (hypochlorous acid, hypochlorite)
- Glutaraldehyde

- Formaldehyde
- Detergents
- Isopropyl alcohol
- Ethyl alcohol

For example, EtO (ethylene oxide) or hydrogen peroxide rapidly turns to active form and attacks the biological molecules starting with those of the membrane or wall, and finally the DNA. Molecules like glutaraldehyde ($\text{OHC-CH}_2\text{-CH}_2\text{-CH}_2\text{-CHO}$) and formaldehyde (HCHO) crosslink the proteins of the microorganisms making them nonfunctional or killing them. Hypochlorous acid (HClO) and hypochlorite (ClO^-) oxidize and damage the biological molecules and lead to death of the microorganisms. Detergents or solvents like ethanol and isopropyl alcohol ($\text{CH}_3\text{-CHOH-CH}_3$) dissolve the membranes of the microorganisms to eliminate them.

13.3 The Influence of Sterilization Methods on Biomaterials

13.3.1 Polymers

Polymers are the substances that are affected most by the applied sterilization technique. Three standard sterilization methods, gamma irradiation (35 kGy, for 10 h, in air, at room temperature), ethylene oxide (EtO) (716 mg/L, at 45 °C, for 5 h), and steam sterilization (121 °C, for 10 min, at 15 psi), were tested on poly(glycerol sebacate) (PGS), and neither of the applied methods induced any adverse change in the surface properties [4]. The sterilized samples preserved their original thermal and mechanical properties. Thus, PGS was found to be sterilizable with the above methods without property change.

The initial clinical application of polyethyletherketone (PEEK) was in the Brantigan lumbar intervertebral body fusion cage (DePuy spine). Pristine PEEK or its carbon-reinforced forms are extensively used in a range of load-bearing implants such as a hip stem in total joint replacement, in disc arthroplasty bearing surfaces as Lumbar intradisc and Cervical disc arthroplasties, and as internal fracture fixation plates [5]. PEEK was exposed to gamma irradiation at a dose range of 25–40 kGy as recommended for sterilization of biomedical devices. The aromatic stable structure of PEEK was expected to withstand a gamma irradiation dose level well over 10^4 kGy without significant degradation of properties, and this was observed to be true when the fatigue properties were tested. This exceptional resistance to radiation was explained by the short life of the free radicals created during exposure to gamma. However, this resistance was not shown by most other polymers.

Poly(D,L-lactide-co-glycolide) (50:50) of two different molecular weights was sterilized by exposure to 25–40 kGy gamma radiation [6]. This exposure did not change the glass transition temperature (T_g) or the polydispersity indices of the polymers; however, the molecular weights were decreased to their half or lower.

The irradiation significantly increased the ultimate tensile strength of the high molecular weight (MW) sample whereas this effect was slight in the lower MW samples. The Young's modulus value, on the other hand, was not changed for the high MW sample but was significantly increased for the low MW sample. The surface chemistry was apparently changed because the contact angles of the irradiated samples were very low with respect to unirradiated counterparts. Finally, the mass loss in solution was substantially higher in the sterilized ones coupled with an increase in the diameter of fibers implying scission of chains and increase in hydrophilicity. These indicate chain scission and crosslinking caused by the irradiation led to the increase in the stiffness showing that gamma irradiation significantly changes the mechanical properties of the polylactides. These changes also caused the increases in hydrophilicity and mass loss.

In a review article, sterilization of the nanoparticles of polymeric origin was covered. Of course the concerns about the suitability of various methods to sterilization of polymers are valid, and there is the additional problem of the small size of the object [7]. One of the sterilization methods, filtration, is applicable to nanoparticulate products and does not cause any alterations in the properties of the particles, but they are suitable only when the particles are much smaller than the filter pore size of 0.2 μm . For example, nanoparticles (NP) designed for use in the targeted drug delivery through the EPR (enhanced permeability and retention) effect, where particles around 50 nm diameters are preferred, is a good example where this technique is suitable. However, higher sized nano and microparticles cannot be sterilized by this method because they would be left on the filter along with the undesirable biological entities. The authors recommended a contaminant-free synthesis process if removal of endotoxins from NPs is essential.

The researchers compared the effects of different sterilization techniques on the properties of *Bombyx mori* silk fibroin thin films prepared for use in corneal tissue engineering [8]. The transparency, tensile properties, corneal epithelial cell attachment, and degradation of these natural protein films were used to study the suitability of the method of sterilization with gamma irradiation 15 kGy from a ^{60}Co Gammacell 220 facility which had a dose rate of 1.4 kGy/h (in air or nitrogen) and steam (134 °C and 216 kPa for 12 min) and aqueous ethanol (70% v/v, at room temperature, for 1 h) treatment. It was found that gamma irradiation, in air or under nitrogen, even though it caused changes was not so significant. Gamma and ethanol treatment decreased the modulus of elasticity, the ultimate strength, and increased elongation at break. Steam increased the modulus and ultimate strength and decreased the elongation at break. The films sterilized by gamma irradiation or with aqueous ethanol had a transparency greater than 98% and tensile properties comparable to human cornea or to amniotic membrane, a biological tissue used in the reconstruction of the ocular surface. Steam sterilization made the films stronger, stiffer, and less transparent, and also decreased cell attachment due to changed, wrinkled topography. Table 13.2 summarizes some sterilization methods suggested for synthetic and natural polymers.

Table 13.2 Sterilization methods suggested for synthetic and natural polymers

Polymer	Ethylene oxide	Radiation	Autoclave
Acrylonitrile-butadiene-styrene (ABS) rubber	+	+	—
Butyl rubber	+	—	+
Cellulose acetate	+	—	—
Cellulose, regenerated	+	—	+
Epoxides	+	+	+
Natural rubber	+	—	+
Polyamides, aliphatic	+	—	—
Polyamides, aromatic	+	+	+
Polycarbonate	+	+	+
Polyester	+	+	+
Polyethylene (PE)	+	+	—
Poly(2-hydroxyethyl methacrylate) (PHEMA)	+	—	+
Polymethyl methacrylate (PMMA)	+	—	—
Polypropylene (PP)	+	—	+
Polystyrene (PS)	+	+	—
Polytetrafluoroethylene (PTFE) (Teflon)	+	—	+
Polyurethanes	+	+	+
Polyvinyl chloride (PVC)	+	—	—
Silicones	+	+	+

13.3.2 Metals

Metals have crystalline structures and are bonded with metallic bonds which give them both the crystal properties like melting at a fixed temperature and highly organized structure and also the malleability due to bond type. Their melting temperatures are much higher than those of polymers. Surgical instruments made of metals for reusability, strength, and sharpness need to be sterilized after use as all other medical devices. On the other hand, there are metallic implants which are especially preferred due to their mechanical and electrical properties. These metallic implants and instruments have advantages and disadvantages over the sterilization of polymers and ceramics.

Advantages:

- Metals are heat-resistant and therefore can be sterilized at temperatures much higher than those of the polymers.
- Metals can be sterilized with ionizing radiation even when completely boxed due to the penetration of the radiation, and little or no harm is inflicted on the device by the radiation.
- Metals are chemical-resistant (except water in some cases) and can be scrubbed, ultrasonicated, immersed in detergent solutions, and then washed.
- Unless porous by design metals do not absorb liquids or contaminants, the contaminants; can only be adsorbed on the surfaces and are easier to clean than absorbed contaminants.

Disadvantages:

- They have risk of corrosion in aqueous media (even stainless steel), and therefore cannot be left moist [9].
- They are not radiotransparent and do not allow ionizing radiation (e-beam, gamma rays, UV) to pass through, so they have to be exposed to radiation from various angles or sides.

The metallic implants can be boxed and then irradiated by gamma or E-beam as these rays or beams can penetrate the packaging materials. The radiopacity problem behind the metal (radiation cannot eradicate microorganisms present behind the implant) is solved by exposing the implant from different directions and heights through the use of conveyor belts.

13.3.3 Ceramics

Al_2O_3 and ZrO_2 ceramics are used as implants and endoprostheses. Nanostructured hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, are used as bioactive surfaces for bone tissue treatments and oral surgery. Especially the hydroxyapatite nanoparticles on implant surfaces facilitate new bone tissue formation and implant fixation inside the body. Ceramics are used as surgical implant devices because of their biocompatibility, mechanical strength, and very low thermal and electrical conductivity. Ceramics are especially used in areas of friction and bony structures of the skeleton and dentistry. However, they have the disadvantages of low ductility and brittleness.

The heat resistance, inertness against fluids and chemical reactions allow ceramic implants to be sterilized with almost all approaches applicable to metals. According to one source, ceramic implants can be sterilized only by ionizing radiation, and all other sterilization processes are inadmissible due to regulation [10]. This might be due to the difficulty in sterilizing porous ceramic structures without leaving traces of the sterilizing agents. However, EtO, irradiation, chemical vapors or liquids, and detergents are all applicable to ceramics for sterilization purposes basically because of the inertness of ceramics against these approaches. As an example, in a study, zirconia-alumina composite ceramics were developed for total hip arthroplasty [11]. The implants were contaminated with *Staphylococcus* (CNS) and *Escherichia coli* (*E. coli*). It was found that 50 kGy of gamma irradiation effectively sterilizes both the surfaces and interiors of these materials while the commonly used ethylene oxide (3 h at 55 °C), and 25 kGy gamma irradiation (the normally recommended dose) were not satisfactory deep inside the femoral head. As expected, no changes in the chemical or mechanical properties of the composites were observed.

13.3.4 Natural Tissues

Depending on the individual case, the doctors might elect to use natural tissue of human origin including that of the patient (autograft) or another person (allograft) and also from another species (xenograft) such as porcine. Natural tissues are pre-treated even before the sterilization stage to remove all the allergenic materials including the cells (decellularization). Sometimes the decellularized tissue is reconstituted (dissolved in appropriate solvent and then reshaped like that is done with collagen or hyaluronic acid), or the tissue is decellularized, but the process is done with care to preserve the tissue architecture especially when this processed tissue is planned to be used in place of the same kind of tissue of the patient. Decellularized vasculature can be given as an example. The biological tissue in question could be bone tissue when the architecture of the bone and its mineral content is needed for the application.

The method of sterilization to be used for allografts is a serious concern because as with other implants, the allografts are prone to modification of their chemical and mechanical properties, but there is also the serious risk of disease transmission, which is more probable than with manufactured implants. Recently, supercritical carbon dioxide (scCO₂) treatment was shown to be capable of terminally sterilizing a range of bacteria and viruses, while preserving the static mechanical properties of the tissues. Supercritical carbon dioxide is the most commonly used SCF (supercritical fluid) due to its mild operating conditions (T_c : 31.1 °C, P_c : 73.4 bar), which allows it to be used to treat thermally labile biological tissues without deleterious effects on proteins and enzymes. In one study, the effect of scCO₂ treatment was compared with two doses of gamma irradiation. Whole rabbit humerus was dissected and sterilized with scCO₂ (with or without sporicidal and bactericidal addition) and with gamma irradiation at 10 or 25 kGy. It was found that scCO₂ treatment with or without additive chemicals did not alter static or dynamic mechanical properties. Gamma irradiation, however, had a deleterious effect, leading to reduction in all static mechanical parameters when exposed to 25 kGy. The effect was more prominent in fatigue tests in both the 10 and 25 kGy dose groups revealing that high-energy radiation is highly damaging and scCO₂ was more appropriate for this bone tissue [12].

In a review article of the standard sterilization methods used for the allo- or xenogenic grafts, it was reported that in tutobone (a xenograft of bovine origin), a purification sequence of different chemical solutions was applied, followed by “gentle” solvent dehydration and sterilization by gamma irradiation [13]. In Puros, an allograft, the biological sample was degreased, and osmotic therapy, oxidation process, solution drying, and low-dose gamma irradiation were performed. For OsteoBiol, xenograft from Italian farm animals, sterilization by gamma irradiation was the main method.

As can be seen in any adverse biological response, namely, contamination or risk of infection from allografts or xenografts were prevented by a variety of solvent treatments followed almost always by gamma irradiation regardless of the reported decrease in mechanical properties.

13.3.5 Other

In addition to the materials discussed above, there are biomaterials which are in the gray area, because they do not belong to any of the above.

13.3.5.1 Liposomes

Liposomes have been frequently used as a model cell to study the influence of membrane components on physicochemical properties of the cell membrane and also as a delivery system of cosmetics and drugs. There have been many types of liposomes and different manufacturing techniques used to prepare and to preserve the service life of liposomes since they are very labile. The most common method is ultrasonication which may be followed by extrusion to narrow the size distribution. Due to the fragile nature of the liposomes which makes them easy to damage or degrade, sterilization using the common methods remains very harsh. Currently, filtration is recommended under aseptic conditions for the preparation of sterile liposomal products designed to be used by routes other than transdermal. Novel, aseptic manufacturing techniques such as dense gas techniques have been devised to eliminate the need for terminal sterilization [14]. Filtration with a 0.2 micron filter can be used, but then larger liposomes such as MLV (multilayer vesicles) cannot pass through the filter. If they are gamma irradiated, then they do undergo peroxidation, and this changes the properties such as stability, membrane rigidity, and release rate from the liposomes. UV sterilization is just a milder version of gamma irradiation, and the effects are similar, and maybe there is the risk of ineffectiveness of the UV due to limitations in its transference across containers. Steam or dry heat sterilizations lead to degradation and fusion of liposomes because the temperature used is above the critical temperature for the liposomes. Ethylene oxide, on the other hand, is difficult to apply to a solution unless it is bubbled through the liposome suspension or applied after the liposome is lyophilized and turned into a powder which then has the risk of association with the phospholipids of the liposome. Use of CO₂ under supercritical fluid conditions is recommended as a sterilization method with potential because it is cytotoxic. But this might not be as effective as a sterilization method where the use of organic solvents is damaging to the cell and liposome membranes. So it seems that production and filtration under aseptic conditions is the only remaining method.

13.4 Conclusion

In this chapter, the methods used in the sterilization of biomaterials and the effect of sterilization on the properties of these materials were discussed. Sterilization does not have a standard procedure; a series of guidelines must be followed using a case-by-case approach because there are no distinct rules of thumb to dictate which method is best for each biomaterial. The changes caused by one method of sterilization may be acceptable for one case, but not for another. In spite of the complexity of choosing the right method, the choice and the process of choosing are both critical and, therefore, well worth the time and effort spent.

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Biomaterials and Devices in Soft Tissue Augmentation

14

14.1 General Information

Soft tissues constitute a significant portion of the human body, and the ECM of the soft tissues are made up of mainly collagen, as the strong material, hyaluronic acid and chondroitin sulfate as the water and form retaining materials, and elastin as the elasticity component. Biomaterials are designed and used to support their performance and to substitute them when there is a need. Sutures, tissue adhesives, wound dressings, temporary fillers are some examples.

14.2 Sutures

A suture is a fibrous, natural or synthetic material, used to stitch together wounds and soft tissues including the skin and blood vessels. It serves to hold tissues together that are separated by surgery or trauma. During this function, it closes the gaps, preventing fluid and blood loss and invasion of microorganisms, provides strength to the sewn tissues until the natural connection between the separated tissues is healed, and helps speed up healing by allowing tissue contact leading to minimal scar formation. Similar function can be performed on harder tissues by staples and wires, on superficial damages with polymeric tapes, and with a variety of natural and synthetic adhesives.

14.2.1 Characteristics of Suture Materials

The ideal suture should be inert and not cause any adverse tissue reaction. Its handling and making knots should be easy enough for the surgeon, and the suture should maintain the knot under wet, damaged tissue conditions. A list of requirements that should be fulfilled by the sutures and their degradation products (if any) is presented below:

- Not toxic, allergic, or carcinogenic
- No immune response
- Ease of handling
- Inertness
- Not support bacterial growth
- Appropriate tensile strength
- Ease of sterilization
- Appropriate service life

A suture could be resorbable and, therefore, dissolve away in time or be stable and stay within the tissue until physically removed by the surgeon. Each has its own benefits and disadvantages.

14.2.2 Classification of Sutures

Sutures are, in general, categorized according to the type of material (natural or synthetic), the lifetime of the material in the body (absorbable or nonabsorbable), and the form in which they were made (braided, twisted, and monofilament). They have well-defined thicknesses depending on the application area (Table 14.1). Suture production comes under the control of appropriate regulatory bodies in every country. In the USA, it is the Food and Drug Administration (FDA).

Manufacturing and testing guidelines for the US medical industry is provided by the United States Pharmacopeia (USP). The USP system was established in 1937 for standardization and comparison of suture materials, corresponding to metric measures. Sutures are designed to meet many different needs. Sutures for deep tissue wounds are different from the ophthalmic applications such as corneal treatments. Designers of a new suture have to take into account many factors such as the material used, stability of the sutures, number of filaments in the fiber, and the coat present (if any) as given in Table 14.2.

14.2.2.1 Materials Used in Suture Production

Sutures are from either of natural or synthetic sources and are macromolecules processed and converted into fibers. Natural materials are of animal origin with examples like catgut, reconstituted collagen; or from insects such as silk; or from plant sources such as cotton; also bacterial polyesters such as polyhydroxyalkanoates. Their common property is that the backbone bonds of the macromolecule consist of heteroatoms and the polymers are produced by natural mechanisms.

Synthetic sutures on the other hand are made of petroleum-based polymeric compounds such as polypropylene (PP), poly(ethyleneglycol terephthalate) (PET), polyamides (PA), nylons, and polylactides. Their backbones could be only carbon or heteroatoms like the natural polymers. Most of the biological sutures are biodegradable, even though the plant and insect originated sutures might not be or are very slowly degradable due to the absence of appropriate enzymes in the human body. Silk fibers are actually made from silk fibroin, which is a natural protein that

Table 14.1 Suture size and application areas

Suture size (USP)	Metric dimension (mm)	Application area
10-0	0.02	Typically used in the most delicate surgeries
9-0	0.03	Common in ophthalmic (eye) surgery
8-0	0.04	For repairing small damaged nerves often due to lacerations in the hand
7-0	0.05	Used for repairing small vessels and arteries or for delicate facial plastic surgery
6-0	0.07	Common for use in vascular graft sewing
5-0	0.1	Used for larger vessel repair such as an abdominal aortic aneurysm
4-0	0.15	Skin closure
3-0	0.2	Skin closure when there is a lot of tension on the tissue, closure of muscle layers
2-0	0.3	Repair of bowel in general surgery
1-0	0.35	
0	0.35	For closing of the fascia layer in abdominal surgery, the joint capsule in the knee
1	0.4	
2	0.5	For repair of tendons
3	0.6	Repair of high tension structures
4	0.6	
5	0.7	Repair of large orthopedic surgeries
6	0.8	
7	0.9	

Table 14.2 Suture classification

Category	Type	Material chemistry/form
Material	Synthetic	Polyester
		PTFE
	Natural	Collagen (CatGut)
		Silk
Stability	Absorbable	Collagen, PLGA, PGA, PCL, PLA-PCL Polydioxanone
	Nonabsorbable	PTFE, silk, polyamide 6,6 (nylon 6,6), polyester, polypropylene
Filament no	Multifilament	Braided
		Twisted
	Monofilament	Straight
Coat presence	Coated	Wax, silicone, PTFE, PGA
	Uncoated	

forms the fibrous substance of the cocoon threads of silk worm (*Bombyx mori*). Similarly, most of the synthetic sutures are nondegradable because of the absence of enzymes or their bonds being not susceptible to hydrolysis. Details of suture properties and specific sutures are provided below.

14.2.2.2 Stability

Suture materials can be chosen as biodegradable (or absorbable, resorbable) or durable (nondegradable, stable) depending on the type of application. For example, if the suture removal could be a problem due to the location of the wound site, it would be best to use a biodegradable one, whereas if the suture is expected to maintain its strength for a long period, then it can be of a durable or stable type. The following are the materials used in these two major categories.

Absorbable Sutures

The most important advantage of absorbable sutures is their removal in the human body after a certain time depending on their properties such as chemistry and composition. In this way they do not induce any collagen sheath formation, and there is no need for their removal after tissue healing.

Catgut is an acronym derived from cattle gut or in general the intestines of cattle, sheep, or goat. Collagen isolated from the intestines is purified and twisted or braided to form long protein fiber threads. Catgut sutures are available as plain catgut (untreated) and chromic catgut because of the cross-linkage achieved by chromium trioxide treatment used in increasing the stability of catgut which if untreated is resorbed very rapidly and loses most of its strength within a week through enzymatic and aqueous hydrolysis. Upon chromic salt treatment, this period is extended two-fold. Catgut sutures are stored in alcohol (such as ethanol or isopropanol) to maintain their flexibility and stability.

Synthetic absorbable polymers are mostly of the polylactide family polyesters (Fig. 14.1). PGA was the first in the 1970s, of a series of homo- and copolymers produced from cyclic lactones such as dilactides. Polyesters degrade by hydrolysis, and the rate of degradation is determined by many factors such as crystallinity, chemical composition, and ingredients (Table 14.1). This hydrolytic degradation process leads to short and long polymer molecules. Since most of these molecules are polyesters, the degradation products are acidic molecules, and this leads to a local pH drop, and this can end up causing sterile sepsis. For example, with polyesters as the length of the aliphatic chain increases, the rate of degradation decreases [1].

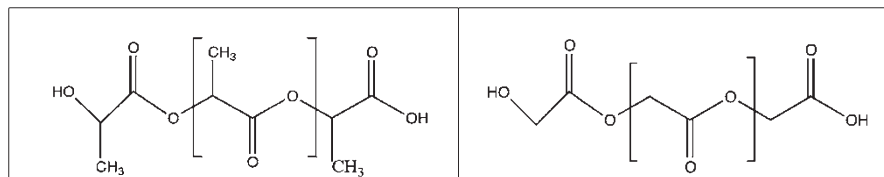


Fig. 14.1 Chemical structure of polylactide and polyglycolide

There are many polyesters available under the commercial names of Dexon (polyglycolic acid homopolymer); Vicryl (polyglactin 910) is a copolymer of glycolic acid and lactic acid with the molar ratio of 90:10; and Monocryl (polyglycaprone) is a poly(ϵ -caprolactone-co-glycolic acid) copolymer. A modified version of polyglactin called Vicryl Rapide[®] is an irradiated version of Vicryl which has an increased rate of degradation due to chain scission caused by exposure to gamma radiation.

Panacryl is another suture from the PLGA family but with composition opposite to Vicryl; it is a poly(glycolic acid-co-lactic acid) with molar ratio of 10:90. It has a longer degradation time (more than 6 months) than Vicryl. Biosyn[®] is a monofilament suture based on a terpolymer (composed of three monomer types) of p-dioxanone, trimethylene carbonate (TMC), and glycolic acid (GA). There are also other biodegradable sutures with different chemistries (Table 14.3).

BSR (breaking strength retention) plot (Fig. 14.2) shows the need by the wound site for the support by the suture. The sutures should not fail in strength until the wound site has sufficient strength to resist the load applied. The wound site undergoes a repair process as was explained in wound healing section, and the strength of the wound site increases gradually. In the meantime, the load experienced by the wound site increases more rapidly. As a result some mechanical support is needed until the wound strength matches the load applied onto the wound, and this is provided by the suture especially when the suture is degradable.

Table 14.3 Some absorbable polyester-based sutures

Trade name	Type	Composition
Vicryl (polyglactin 910) Vicryl rapid Panacryl	Polyester	Poly(lactic acid-co-glycolic acid)
Dexon	Polyester	Poly(glycolic acid)
Monocryl	Polyester	Poly(lactic acid-co- ϵ -caprolactone)
Biosyn	Polyester	Trimethylcarbonate, p-dioxanone, glycolic acid

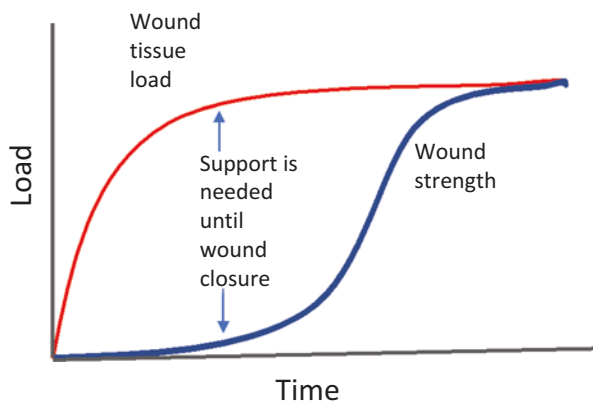


Fig. 14.2 Wound healing: load versus time

Nonabsorbable Sutures

Almost all nonabsorbable sutures are polymeric petroleum-based materials except silk which is the only natural, nonabsorbable suture material in common use today. Silk sutures are actually made of fibroin, a protein obtained from the cocoon of the silk worm, *Bombyx mori*. It is therefore quite inexpensive. Silk due to the protein nature which is not natural to human system induces intense inflammatory response. This inflammatory reaction can be suppressed by coating the suture with other inert materials such as silicone. Coating is a procedure also applied to other suture materials to change their properties, such as to increase smoothness. Even though it is stated to be nondegradable, silk still loses most of its tensile strength in 12 months and physically deteriorates in about 2 years [2]. It is stated that it is possible to control the degradation rate of silk fibroin by changing its crystallinity, pore size, porosity, and molecular weight distribution.

Nylon is another polyamide like silk fibroin but is really nonabsorbable because it is not a protein despite the presence of amide group in its structure. It is produced by using a diamine and a dicarboxylic acid molecule, hexamethylenediamine, and adipic acid, respectively (Fig. 14.3), rather than by molecules carrying both carboxylic acid and amine ends as in amino acids which form peptide bonds as a result of condensation. Since the succession of these groups does not resemble that of a protein, degradation of nylon is not by the enzymatic route but instead by simple hydrolysis.

Polypropylene is a real nonabsorbable synthetic suture material that has no heteroatoms in the polymer backbone (Fig. 14.3) and therefore is not hydrolyzed and does not induce any tissue response due to hydrolysis products. It is also one of the least thrombogenic sutures and is, therefore, ideal for vascular surgery applications.

Stainless steel is biologically inert and the strongest of the suture materials. It is commonly used in applications where load bearing is needed and orthopedics is one such field. Stainless steel suture has some disadvantages such as a tendency to cut through tissue and difficulty in manipulation conforming to the defect contours. For soft tissues, stainless steel sutures are not a material of choice, but stainless steel staples are more appropriate due to the ease of application and high

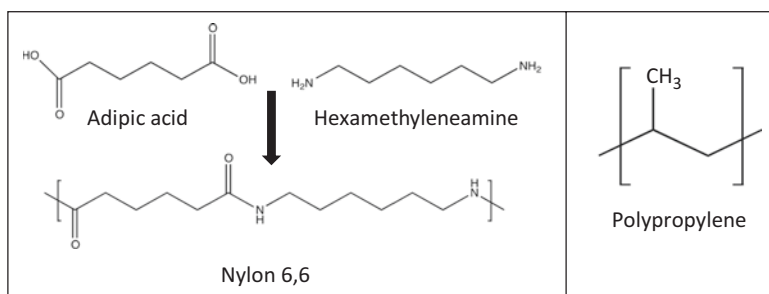


Fig. 14.3 Production of Nylon 6,6 from adipic acid and hexamethylene amine and the chemical structure of polypropylene

Table 14.4 Nonabsorbable sutures

Trade name	Material	Composition
Ethilon® Nylon 6 Nylon 66 Nurolon® Surgilon™ Dermalon™	Polyamide	Hexamethylenediamine and 1,6-dicarboxylhexane
Prolene® Surgilene® Surgipro™	Polyhydrocarbon	Polyethylene, polypropylene
Novafil™ Vascufil™	Polyester	Polybutylester

speed compared to suturing. Different types of nonabsorbable sutures are given in Table 14.4.

14.2.2.3 Number of Filaments

The sutures can consist of a single filament or multifilaments. In the multifilaments several filaments or strands are braided or twisted together. The use of multifilaments improve the strength, have better handling and knot holding ability due to their flexibility, but have a rough surface with crevices. In contrast, the monofilament sutures have a smooth surface and cause no trauma in the tissue during suturing, but their handling is difficult; they have a memory (from the original packaging), and the knot is not as stable as that in the multifilaments (Fig. 14.4).

14.2.2.4 Suture Coating

Since the multifilament sutures pass less easily through tissue than the smooth monofilament sutures and have crevices into which microorganism accumulation risk is high, these sutures are coated with appropriate polymers. Coatings fill the crevices between the filaments to prevent them from harboring bacteria, to make the suturing process smooth and untraumatic, and also to give color for ease of detection at the wound site. In the recent years, antimicrobial coatings carrying antibiotics for slow release are being developed to prevent surgical site infections occurring sometime after suturing. The source of the microorganisms could be endogenous or exogenous bacteria (from the personnel, tools, the environment, or the bacterial colonization of the suture).

Among the traditional coating materials used are natural materials like bees wax and paraffin wax and synthetic materials such as silicone and Teflon (poly(tetrafluoroethylene), PTFE). For proper adherence of the coat onto the suture, the coat is generally selected to be chemically similar to the suture. The coating material is also chosen based on the degradability of the suture.

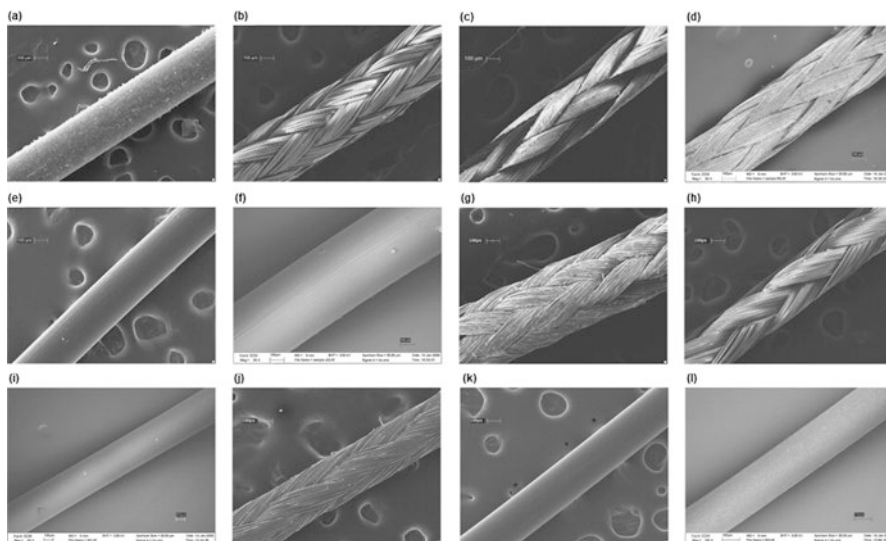


Fig. 14.4 Scanning electron microscopy images of some commercial sutures with multi and monofilaments (a) Chromic catgut, (b) Dexon, (c) Vicryl, (d) Vicryl Plus, (e) PDSII, (f) MonoPlus, (g) Silk, (h) Mersilene, (i) Novafil, (j) Nurolon, (k) Ethilon, and (l) Dermalon. [3]

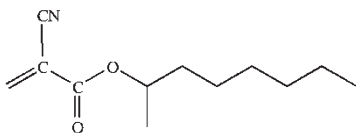
14.3 Tissue Adhesives

Tissue adhesives are advantageous over other fixation systems in that they are quicker, avoid leaving dead space, prevent fluid leakage, and do not leave behind scars due to application. They do not need a dressing to prevent infiltration of micro-organisms. They come from two sources, synthetic and biological.

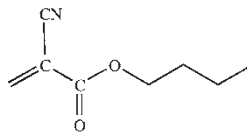
14.3.1 Synthetic Tissue Adhesives

Synthetic tissue adhesives are similar to the commonly used adhesives, but they have to be biocompatible. They are expected to set in wet environments and stay stable during tissue healing and are expected to be biodegradable. They are quite suitable for use in the repair of delicate tissues where not much strength is needed. Synthetic adhesives are generally obtained by the reaction of liquid adhesive components, mostly monomers, which set very rapidly upon mixing. Important examples of this category include cyanoacrylate monomers such as 2-butylycyanoacrylate or 2-octylcyanoacrylate (Fig. 14.5). Their polymerization can be initiated by moisture (especially in the presence of hydroxide ions) or molecules like proteins that are present on the skin. The reaction is generally very fast and completed in a matter of seconds sealing the wound edges and making them water tight due to the hydrophobicity of the polymer formed. They are degradable at rates dependent on their molecular weight and form [4] and are removed along with the keratinized

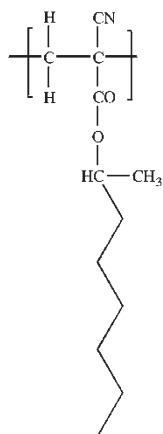
2-octylcyanoacrylate



butylcyanoacrylate



Poly(2-octylcyanoacrylate)



poly(butylcyanoacrylate)

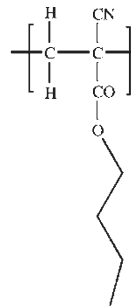


Fig. 14.5 Chemical formulae of monomers and polymers of two cyanoacrylate adhesives

epithelium. They might carry remnants of additives such as stabilizers and coloring agents. The most commonly used biological or synthetic tissue adhesives are summarized in Table 14.5.

14.3.2 Biological Adhesives

The natural adhesives either benefit from standard biological reactions that lead to a coagulated mass that adheres to the sides of a wound, or biological molecules are treated to become insoluble in aqueous media through cross-linking using aldehyde molecules. Biomimetic adhesives mimic the chemistry and the reactions of biological molecules that serve to attach biological species such as mussels to the objects in the environment such as boats, rocks, and wood.

14.3.2.1 Fibrin Glue

Fibrin is coagulated fibrinogen-based matrix. Fibrinogen consists of three pairs of polypeptide chains linked by disulfide bridges and form a dimeric structure. The central domain is cleaved during conversion of fibrinogen into fibrin by the protease thrombin which forms an insoluble fibrin network during the blood coagulation process. By learning from nature, researchers have developed what is called a “fibrin

Table 14.5 Biological and synthetic tissue adhesives

Main group	Material chemistry	Trade name	Company
<i>Biological</i>			
Protein based	BSA-glutaraldehyde	BioGlue	CryoLife, USA
Collagen based	Bovine collagen	Floseal Proceed Co-stasis VitaGel	Baxter Ethicon US surgical Stryker
Polysaccharide based	Chitosan		
	Chondroitin sulfate with methacrylate groups		
Mussel adhesive	Biomimetic, DOPA	Nerites	Nerites, USA
Fibrin sealant	Human fibrinogen, human thrombin	Tisseel Tissucol, Co-stasis Crosseal/ Evicel TachoSil	Baxter Healthcare Baxter, Austria US Surgical Ethicon Nycomed
<i>Synthetic</i>			
Polyethylene glycol	PEG	Coseal	Baxter Healthcare
Cyanoacrylate based (ethyl, octyl)		Dermabond	Ethicon
Methacrylate based	Methacrylate with colloidal silica	Clearfil SE	Kuraray, Japan
		Excite	Ivoclar Vivadent

glue,” which actually is a fibrinogen and thrombin-based product to seal especially soft and fragile tissue defects, to adhere tissues together and control bleeding. A typical two component preparation consists of two solutions: (a) fibrinogen solubilized in CaCl_2 , and (b) thrombin in CaCl_2 . These two solutions are mixed to form a gel (Fig. 14.6). The strength has been reported to be quite suitable for tissue repair: 9 kPa tensile strength and 1.1 kPa–23.4 MPa shear strength [5].

Fibrin glue is used in the repair of Achilles tendon ruptures, to seal nerves, to repair osteochondral fractures, to adhere periosteal grafts onto medial chondyles, to fuse herniated disks, in skin grafting, burns and ulcers, mammary reduction, control bleeding in operative field, seal vascular grafts, seal fetal membranes, and bladder perforations.

14.3.3 Other Adhesives

There are also other biomimetic adhesive systems:

1. Proteins such as bovine collagen cross-linked with glutaraldehyde (Fig. 14.7)

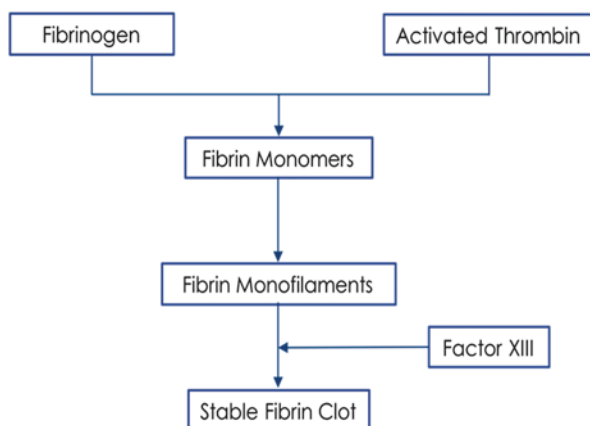
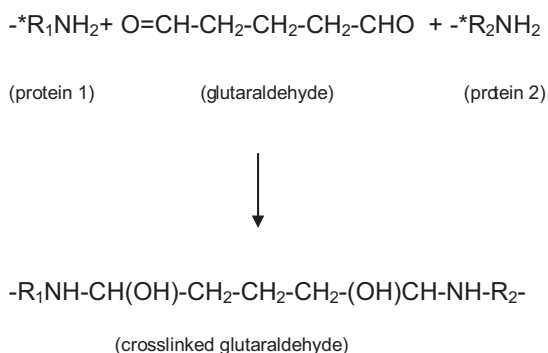


Fig. 14.6 Fibrin clot formation in the fibrin glue



* RNH₂ could be a protein or a molecule carrying a primary amine such as chitosan

Fig. 14.7 Cross-linking of proteins by glutaraldehyde

2. Polysaccharides such as chitosan and chondroitin sulfate modified with methacrylate groups
3. DOPA-based adhesives mimicking that of the mussel

Of these adhesives, especially the DOPA-based adhesive that mimics the mussel is important because the high adhesive strength and stability, and not swelling under wet conditions are properties that cannot be matched by the polysaccharide or protein-based adhesives (Fig. 14.8). An interfacial bonding strength of 15.85 ± 3.23 MPa is close to the tensile strength of acrylic bone cement (28.9 MPa) and much higher than that of fibrin glue [6].



Fig. 14.8 Mussel attaching to a surface and the main molecule involved in mussel attachment, DOPA or dihydroxy phenylalanine [7]

14.4 Burn Dressings

Burn injuries constitute a major worldwide public health problem and cause more severe physiological stress than other traumas. Superficial burns usually heal with minimal scarring, but treatments for second and third degree burn injuries remain far from optimal. Burn-induced full thickness skin injuries result in rapid and dangerous levels of water loss and expose the body to microbial attack, and mechanical protection is no longer available.

In partial-thickness wounds (second-degree burns), the external sheaths of hair follicles and sweat gland ducts are partially preserved and could serve as a source of reepithelization of the damage sites. In deep wounds (third-degree burns), repair needs to be made by filling the defect with something similar to connective tissue structures which mainly consists of collagen. The healing process results in scar tissue formation. Burn injury induces the formation of cellular granulation tissue which is gradually converted into a collagen-rich structure. Then the collagen in the scar is cross-linked covalently, and the scar tissue contracts.

The objective of the use of a burn dressing material is to accelerate wound healing by preventing fluid loss and bacterial infiltration. Therefore, a successful burn wound covering should control the absorption of exudates, prevent infection, and allow exchange of gases across its structure. Therefore, antimicrobial agents can be integrated with the wound dressing materials. Burn wounds are most commonly covered with a gauze impregnated with paraffin (to prevent the dressing from sticking to the wound bed) and an absorbent (generally cotton wool). Silver sulfadiazine (SSD) is commonly used as the bioactive agent to prevent infection.

The wound dressings come in various forms; these include films, felts, hydrocolloids and hydrogels, sponges, gauzes, and biological dressings. Almost any polymer can be used in the construction of wound dressings in the physical form of fibers, films, membranes, sponges (foams).

Gauze can be used on wounds where other fabrics might stick. Polymers or antibiotic ointments can also be added on the gauze to make it more nonstick. It is

especially suitable when the wounds are draining. They are readily available in many sizes and forms and other chemicals can be added to improve the properties, but it needs to be changed frequently if wound becomes dry.

The hydrocolloid dressings contain a gel forming agent, such as gelatin, and create an absorbent, self-adhesive, and waterproof seal of the wound site. They are especially suitable for low or moderately draining wounds, under compression wraps or as stockings, and are available in numerous sizes, shapes, and thicknesses. It is easy to apply them, help reduce the pain from the wound, and provide thermal insulation.

Hydrogels are designed to create a moist environment during the healing of the wound. Their weight is consisted of 80–90% water, and this water helps keep the wound tissue moist. They are advantageous because topical drugs can be added, and can be removed without disturbing the wound area. Hydrogels are somewhat similar to the natural extracellular matrix. In addition to their utility as sponges, hydrogels can also deliver bioactive agents (cytokines and growth factors, antibiotics, and even cells) to help achieve better and faster wound healing. Sun et al. [8] report to have tailored the properties of dextran hydrogels to achieve rapid and functional neovascularization in vivo.

Silver dressings contain silver that is released to create microorganism (especially *Staphylococcus*)-free environment and can be found in many forms such as transparent films, foams, hydrofibers, etc.

Collagen films, membranes, or sponges have been widely used as burn dressings because they are quite pliable and soft in moist environments like that of the wound bed. In order to increase its mechanical strength collagen is cross-linked. These cross-linked structures do not adhere well, but they are more stable at the wound site and disintegrate at a much slower rate. The membrane also serves as a barrier in preventing bacterial contamination. Membranous collagen-based dressings are transparent and allow visual inspection of the dressed wound. Sponges made of other polymers, mainly of polyurethane (PU) or poly (vinyl alcohol) (PVA), have been studied and are commercially available.

14.5 Artificial Skin

Skin is the largest organ of the body (Fig. 14.9). When there is significant loss of skin, then the cover of the tissue is removed, and this results in fluid and heat loss and creates a risk of infection. Traditionally deep burns are covered with an autograft that is healthy skin harvested from another part of the patient's body. When the damage area is extensive, donation of such amounts becomes difficult because there might not be enough skin to harvest. The other source is allograft, skin donated by another human being. The main limitation of the grafts is that it is difficult to find right amount of donated skin. Skin substitutes have been developed to overcome this problem.

One of the first artificial skins was a bilayer, composite construct composed of a collagen-chondroitin-6-sulfate dermal layer, and a 100 μm thick medical grade

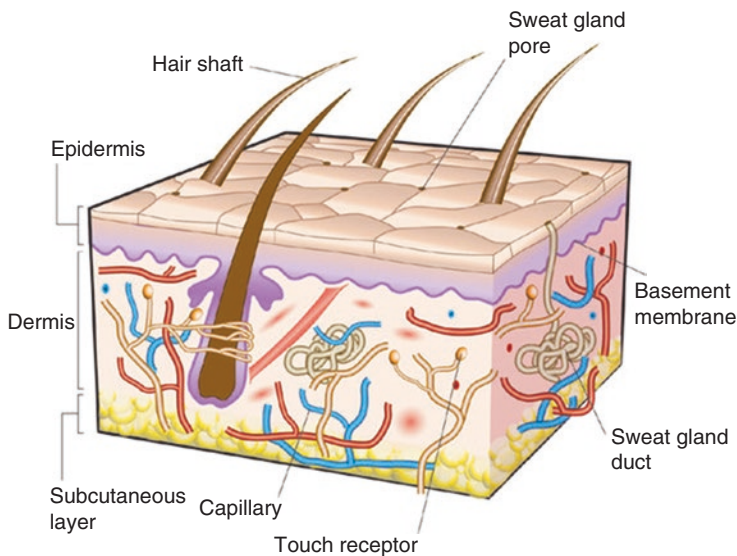


Fig. 14.9 Scheme of human skin [9]

silicone cover serving as the epidermis [10]. For the dermal layer, collagen and chondroitin-6-sulfate were converted into a sponge by lyophilization and cross-linked by dehydrothermal treatment and by using glutaraldehyde. Some of the commercially available artificial skins are given below.

14.5.1 Integra

Integra™ Bilayer Matrix Wound Dressing is very similar to that proposed by Yannas and Burke in the early 1980s designed to cover deep wounds in full- or partial-thickness burns when there is an insufficiency of donor material [10]. It is an acellular porous matrix of cross-linked bovine tendon collagen and glycosaminoglycan (chondroitin-6-sulfate) and a semipermeable polysiloxane (silicone layer) which controls water vapor loss and provides a flexible protective layer that increases the tear strength. Collagen-glycosaminoglycan matrix is biodegradable and allows cellular invasion and capillary growth. The collagen is reabsorbed and structured into a new matrix within 3–6 weeks.

14.5.2 Apligraf

Another is Apligraf® produced by Organogenesis and tissue engineering is involved in its design. It is a bilayered skin substitute and was the first engineered skin that the USFDA has approved for the healing of ulcers that have failed standard wound care [11]. This construct consists of bovine type I collagen matrix seeded

with neonatal fibroblasts isolated from human foreskin, and on top of this layer, neonatal epidermal keratinocytes derived from human foreskin are added to achieve stratification. This structure provides both a template for the new tissue to form, and the right types of cells required for the healing skin.

14.5.3 Biobrane

There are also other artificial skins commercially available. One of these is Biobrane[®] which is produced by Smith-Kline and Nephew, and it is reported to be a temporary skin dressing used especially on superficial and partial-thickness wounds and donor sites. It is a multilayer construct; a silicone membrane is bonded to a nylon mesh which is bonded to peptides from porcine dermal collagen resulting in a composite dressing.

14.5.4 Epicel

Epicel[™] is a cell therapy product which basically is an epidermal autograft grown from a biopsy from the patient's own skin for use in the treatment of deep dermal or full-thickness burns comprising areas as large as total body surface area [12]. The cell type used is keratinocytes, and they are cultured aseptically for several weeks to increase the cell population. The cell processing is done by Genzyme. The keratinocytes are grown together with mouse cells and contain residual mouse cells, and therefore, FDA considers it a xenotransplantation product. The grafts are two-to-eight cell layers thick.

14.5.5 OrCel

OrCel[™] is defined as a bilayered cellular matrix produced by Ortec Int. Inc. and contains live human cells, epidermal keratinocytes and human dermal fibroblasts which are cultivated in two separate layers of type I bovine collagen [13]. The fibroblasts from the donor are cultivated on the internal side of the bovine collagen matrix, and keratinocytes from the same donor are cultivated on the exterior. According to the manufacturer, approximately 2 or 3 weeks after the application, there should be no traces of allogeneic DNA in the wound.

14.5.6 TransCyte

TransCyte[™] is a human fibroblast-derived temporary skin substitute by Smith-Nephew and is another cell therapy product which appears to be result of tissue engineering as a temporary burn wound covering made of human cells and a silicone mesh-like material. It is recommended for use in the treatment of second-degree

burns that are expected to heal on their own. This temporary wound covering is specifically for surgically excised full-thickness and partial-thickness burns. TransCyte consists of a polymer membrane and newborn human fibroblast cells cultured under aseptic conditions in vitro on a nylon mesh [14]. Prior to cell growth, this nylon mesh is coated with porcine dermal collagen and bonded to a silicone membrane. Silicone membrane serves as a transparent synthetic epidermis, and as the fibroblasts proliferate within the nylon mesh during the manufacturing process, they secrete human dermal collagen, matrix proteins, and growth factors. Following freezing, no cellular metabolic activity remains; however, the tissue matrix and bound growth factors are left intact. Following drying the covering peels off.

14.6 Tissue Augmentation and Cosmetic Application

Injectable cosmetic wrinkle fillers are soft tissue fillers approved in the category of medical devices by the American Food and Drug Administration (FDA) [15]. A nonabsorbable, FDA-approved, injectable cosmetic wrinkle filler is designed to correct facial tissue around the mouth. These are solutions injected into the skin to fill in facial wrinkles to restore the original smoother appearance. Depending on the substances used, these wrinkle fillers could be temporary because they are eventually resorbed. The effect from the biodegradable materials lasts for about 6 months. The materials used in these injections can be permanent or temporary.

- Temporary (bioresorbable) filler.
Collagen: collagen is obtained from cows or humans after high levels of purification.
Hyaluronic acid is a natural molecule of the human body located in tissues which have forms (ears, nose) and elasticity is needed. It readily forms a gel with water and serves as a protective lubricating gel.
Poly(L-lactic acid) is a synthetic material, which is biodegradable and biocompatible and has been widely used for many years in various biomedical and specifically biomaterial applications.
- Permanent fillers: One of the permanent (nonabsorbable) fillers is polymethyl methacrylate (PMMA). It is a synthetic acrylate-based polymer which is biocompatible but not biodegradable. When used in wrinkle filling, it is in microsphere or nanosphere form or as beads; they are smooth, spherical polymeric particles (Fig. 14.10).

Facial folds and wrinkles are a serious concern for aging women. In a typical solution for this problem, Artecoll®, a product consisting of PMMA microspheres (diameter 30–40 μm) (Fig. 14.10a), is suspended in a solution of collagen and injected at the wrinkle area in order to fill the wrinkle region. The viscous product is injected subdermally using a tunneling technique (Fig. 14.10b). The collagen gradually degrades in 1–3 months, and the microspheres become totally surrounded by a fine fibrous capsule produced by the patient's body as an immune response.

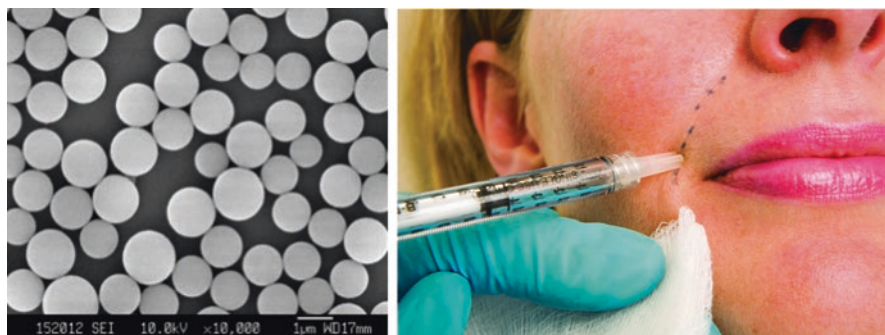


Fig. 14.10 PMMA microspheres used as wrinkle filler. (a) The microspheres [16], (b) scheme of application [17]

The results are expected to be long term because the PMMA is not biodegradable. Hydroxyapatite (HAp) does not dissolve rapidly so can retain its form for long periods and therefore it can be used as a filler like PMMA.

14.7 Soft Dental Tissues

Soft tissue grafting is recommended when there is gum recession that has left the root of a tooth exposed or the person is at risk of root exposure due to recession. Soft tissue grafting is a common procedure that is intended to recreate the gumline and prevent further deterioration of the gums. Normally, the tissue needed for a grafting procedure is taken from another part of the patient's mouth, through an additional surgery. In cases when this cannot be done, then other sources are used. For example, certain allografts may be used without need for additional surgery.

In a "free gingival graft," a small strip of tissue is removed from the palate. The tissue is then stitched to the gum tissue in the area being treated. In a "connective tissue graft," a flap is cut in the palate; some tissue from under the flap (subepithelial connective tissue) is removed and slipped under the gum tissue that surrounds an exposed root. A "pedicle graft" uses a flap of tissue from the gum of a tooth next to the one with recession. The flap, a pedicle, is not completely removed and has one edge still attached. It is slid sideways to cover the exposed root and stitched into place. A pedicle graft usually heals quickly because at least some of the blood vessels are intact.

14.8 Breast Reconstruction Strategies

Women who undergo mastectomy either wear an external prosthesis or have prosthetic devices implanted (Fig. 14.11). There are two basic types of prosthetic devices available: fixed-volume breast implants and tissue expanders. These implants and expanders are manufactured in a broad range of sizes and shapes. Their design is significantly different.

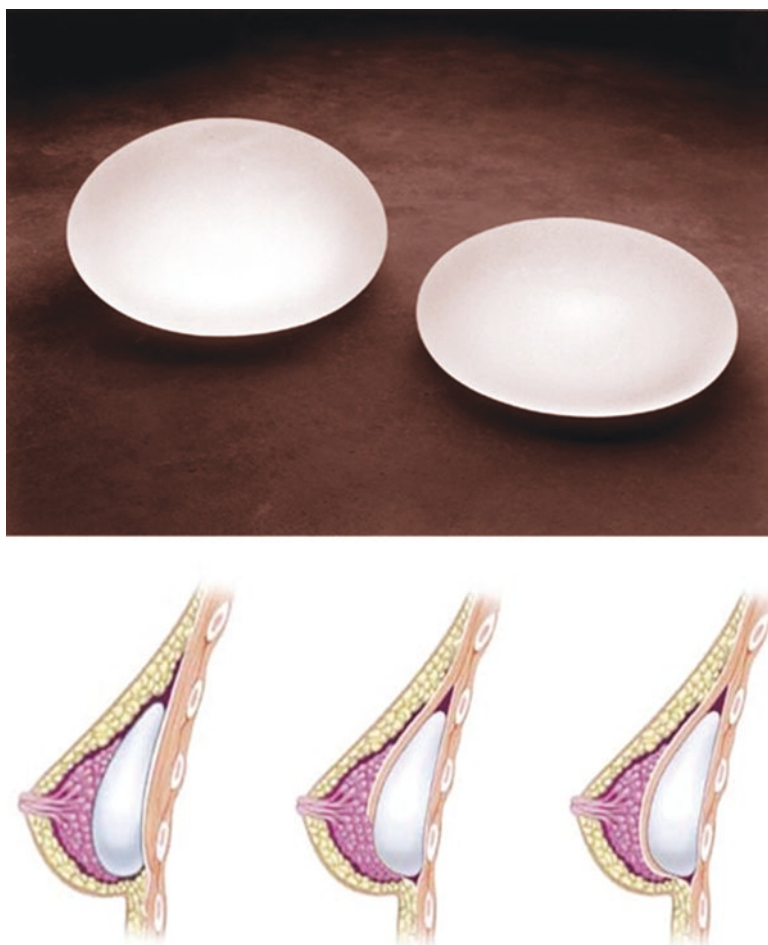


Fig. 14.11 Breast implant applications. (a) Silicone bags filled with saline or silicone oil. (b) Different locations a breast implant is placed with respect to muscle: subglandular, dual plane, and submuscular (from left to right) [18]

Fixed volume implants have an outer or skin layer of vulcanized silicone (cross-linked to make it strong) and are usually filled with a saline solution [19]. Implants can also be filled with silicone gel but are less in comparison to saline-filled ones [20, 21]. There still is a controversy about the health issues reportedly arising from these implants. Siloxanes, platinum or saline, have been reported to leak out from the implants, but the health issues were never proven one way or the other [22]. Recent events about the use of industrial silicone which was also thinner than it should be caused a great furor about the certain implants, and in that case, too, a definite health risk associated with the use of these particular implants could not be assessed.

Tissue expanders are implants the volume of which can be altered after implantation. An inflatable silicone tissue expander is placed submuscularly and is gradually inflated by the injection of physiological saline over a period of weeks to months. When expansion is complete, the tissue expander is removed and is replaced with a permanent breast saline- or silicone-filled implant to provide the volume necessary for breast mound restoration.

Both implants and expanders are available with a smooth or textured silicone surface. Textured surfaces result in a lower incidence of capsular contracture following implantation than smooth surface implants. Textured surfaces also serve to stabilize the implant and facilitate tissue expansion. One drawback of a textured envelope, however, is that these implants possess a slightly thicker vulcanized silicone shell that may be more visible, exhibiting a rippled appearance through the skin in some patients who possess a thin skin envelope. The thicker shell may also contribute to the implant folding and potentially deflating following insertion [19].

14.9 Conclusion

In this chapter, materials used to replace, augment, or treat soft tissues. Among these materials are those used as sutures and tissue adhesives in soft tissue reconstruction, artificial skin, and cosmetic and dental applications were discussed. Since the human body has a very large variety of complex multilayer tissues, the spectrum of materials used is also varied. When working with soft tissues, materials which have low density, low mechanical strength and stiffness, and high flexibility are chosen. For this reason, most of the materials used are either polymers (synthetic or natural) or natural tissues from donors.

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Biomaterials and Devices in Hard Tissue Augmentation

15

15.1 Introduction

Bone loss or damage can result from illnesses such as cancer and osteoporosis or by trauma sustained in an accident. The lost bone has to be substituted using alternative materials or methods such as tissue engineering or additive manufacturing, while damages like cracks or breaks are repaired by use of implants such as fracture fixation devices in the form of screws, wires, pins, staples, or more complex load-bearing devices until healing is achieved. There are five types of bone in the human body. These are the long bones, femur (upper leg), tibia (lower leg), and humerus (upper arm); short bones, carpals (wrist) and tarsals (ankle); flat bones, scapula (shoulder bone), sternum (chest bone), cranium (skull), pelvis, and ribs; irregular bones, vertebrae, sacrum (lower end of the vertebra), and mandible (lower jaw); and sesamoid bones, bones in tendons (patella and pisiform). The places of the bones in the skeleton are shown in Fig. 15.1. [1, 2].

Each type of the bone has an outer layer of dense, smooth and compact bone (i.e., cortical bone) and an inner porous part with growing porous core having cellular, cancellous, and spongy nature called the trabecular bone which carries in it the bone marrow. The mechanical properties of the cortical bone are higher than the trabecular bone and depend on the anatomic site it is located because of the load-bearing needs and the stress applied by the muscles, ligaments, and joint capsules attached are different. Cylindrical long bones, such as the femur bear of the highest loads, are subjected to strong muscular forces and have a thick and hard cortical bone, while exterior of the short and/or irregular bones have a thinner outer layer.

For an implant to be successful, it is expected to have appropriate mechanical properties that are the properties similar to that of the natural bone. Otherwise, the implant may carry more load than the natural bone and lead to stress shielding in the natural bone, which is a decrease in the bone density due to insufficient load bearing. The parameter to match under these circumstances is the modulus of elasticity (or the Young's modulus, E). The other parameter to study is the geometry or the design of the implant. Since the bones are the load-bearing elements of the body, the

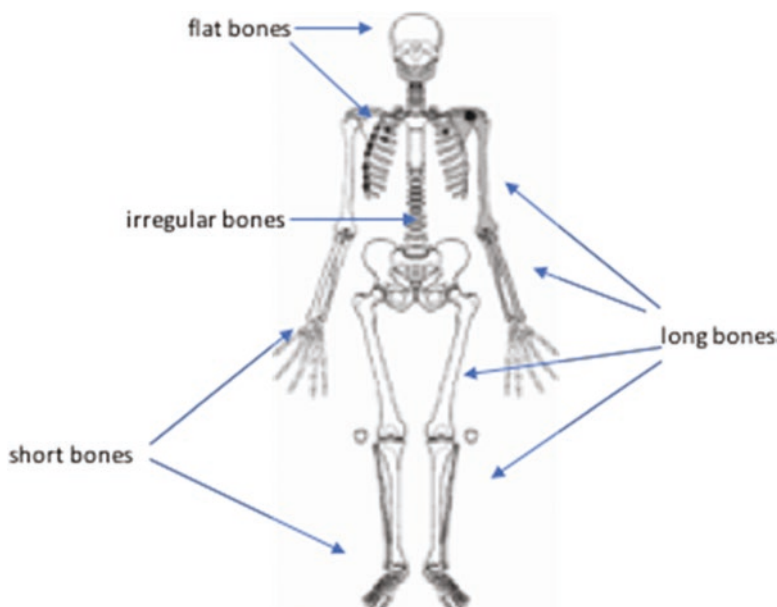


Fig. 15.1 Main types of bones in human body [2]

implants designed to substitute them for short or long periods have to have appropriate strength and are generally selected among metals or ceramics as polymers are weaker and softer and are susceptible to deform under load. Among the metals used are cobalt-chromium alloys, titanium and its alloys like NiTi (nitinol, the shape memory alloy), stainless steel, and bioerodible metals such as magnesium. Other properties needed in an implant are non-corrosiveness, biocompatibility, and ability to integrate with the tissue. In order to gain biological compatibility, a metallic implant is generally coated with a synthetic polymer (PLLA, PGA) or biopolymer (heparin, collagen, hyaluronic acid), or a ceramic (hydroxyapatite, alumina).

Metals are generally used in the treatment of hard tissue defects due to their higher mechanical strength compared to polymers, and therefore, orthopedic and dental applications (as internal fixation materials such as plates, screws, pins, rods, or hip and dental implants) are the common areas for metals. Also, their conductivity makes them suitable for use in the production of pacemakers for cardiovascular treatments.

15.2 Internal Fixation Materials for Fractures

Sherman vanadium steel was the first metallic alloy designed specifically for biomedical applications, as plates and screws to join fractured bones together.

15.2.1 Bone Plates

Bone plates are surgical tools used to assist in the healing of broken and fractured bones. The breaks are first set and then held in place using bone plates in situations where casts cannot be applied to the injured area. Bone plates are often applied to fractures occurring on facial areas such as the nose, jaw, or eye sockets. Repairs like this fall into an area of medicine known as osteosynthesis. Currently osteotomy equipment is made primarily of titanium and stainless steel. The broken bones are first surgically reset into their proper position. Then a plate is screwed onto the broken bones to hold them in place, while the bone heals. This method has been proven both successful and useful in treating all manner of breaks; however there are still some drawbacks. After initially placing the plate on the osteotomy defects, the bones are compressed together and held under some slight pressure, which helps to speed up the healing process of the bone. Unfortunately, after only a couple of days, the tension provided by the steel plate is lost, and the break or fracture is no longer under compression, slowing the healing process.

Forearm fractures account for 41.1% of all fractures in children. Most of these are fractures of the distal radius or ulna [2]. The most common reason of injury is a fall onto an outstretched hand. The treatment of non-union fractures of the humeral diaphysis is complicated because on the one hand, there are surgeons who prefer closed treatment and recommend the use of intramedullary nailing or external fixators and thus to reduce the risk of sepsis, whereas others prefer open treatment with screws and plates because they wish to correct the existing anatomic deformity and to obtain absolute stability with a powerful stimulus of osteogenesis essential to achieve strong bony union. In adults, open reduction and plating are the optimal treatment for diaphyseal forearm fractures. In some studies, the open reduction approach with stable internal fixation using a compression plate performs much better [3]. In most studies researchers used metallic materials such as flexible titanium nail or K-wire and Rush rods (Fig. 15.2).

Since the bones have anisotropic properties and are exposed to complex loading conditions during the daily activities, fiber-reinforced glass fiber-biodegradable polymer composite bone plates were developed both to meet the mechanical requirements and provide adequate stiffness (Young's modulus for human cortical bone is 18.6 GPa [5]) but also appropriate flexibility to avoid stress shielding. In a study, composite multilayer bone plates of phosphate glass fiber (PGF) and polylactic acid (PLA) were used [6]. They prepared a number of plates with graded components and as a result a variety of mechanical properties with Young's modulus around 20 GPa (3.80 GPa for PLA and 51.5 GPa for PGF) and bending stiffness in the range 1.7–2.2 Nm/degree were obtained. The sample with lowest bending stiffness presented the best healing performance compared to stainless steel indicating an inverse relation between healing and bending stiffness.

In another study [7], various stainless steel, plain weave carbon/epoxy fabric composite (WSN3k), and plain weave glass/polypropylene composite (Twintex) plates were tested. In this study also, the flexible composite bone plates provided the most beneficial effects on callus generation and development (Table 15.1).



Fig. 15.2 Metallic bone plates for radius and craniofacial applications [4]

Table 15.1 Mechanical properties of some bone plates [8] and natural materials

Material	Young’s modulus (GPa)*	Poisson’s ratio*
Stainless steel	193.0	0.300
WSN3k [0]18 carbon/epoxy	$E_r = 10.0$	$\nu_{r\theta} = 0.019$
	$E_\theta = 70.0$	$\nu_{rz} = 0.019$
	$E_z = 70.0$	$\nu_{\theta z} = 0.130$
Twintex [0]18 glass/polypropylene	$E_r = 5.3$	$\nu_{r\theta} = 0.021$
	$E_\theta = 20.0$	$\nu_{rz} = 0.095$
	$E_z = 20.0$	$\nu_{\theta z} = 0.780$
Cortical bone	$E_r = 8.5$ (radial)	$\nu_{r\theta} = 0.141$
	$E_\theta = 7.0$ (transverse)	$\nu_{rz} = 0.065$
	$E_z = 18.4$ (longitudinal)	$\nu_{\theta z} = 0.099$
Trabecular bone	1.1	0.260

* r radial, θ transverse, z longitudinal

Epoxy resin (EPOMIK R-140) was used to introduce toughness and decrease the brittleness of PAN (polyacrylonitrile)-based carbon fiber yarns (HTA 6K: Toho Rayon) which were braided at various angles (15–25°) [7]. It was observed that the bending strength of composite plates changed with the braiding angle: for 15° braided yarn, it was 622 MPa, almost 30% higher than that of 25° specimen. Similarly, the highest bending modulus was seen in the same 15° sample (the more straight braided sample) indicating that not just inclusion of braided yarns but also their braiding angles influence the mechanical properties of the products.

Another composite bone plate consisting of poly(L-lactic acid) (PLLA) and braided bioactive glass fibers that had mechanical properties close to that of the cortical bone was tested [9]. Braiding was achieved at an angle of 15°. The bone plate was prepared by molding and hot pressing bioglass (BG): PLLA (45%, v/v) at 130 °C under 150 MPa for 40 min. Under tension the average longitudinal strength was 194 MPa and elastic modulus 22.1 GPa, values near those of the cortical bone. The ultimate tensile strength (UTS) of the samples decreased to 35% of its original in 4 weeks in PBS at 37 °C.

A bioresorbable poly(D,L-lactide) (PLA 5:95) plate/screw system (volar PLA95) was used in four patients for intraosseous fixation after open reduction of distally displaced radius fracture in four patients with the controls being metal locking plates Volar T (Synthes, USA), and they were followed for up to 11 months. At the final assessment, it was concluded that all of the distal radius fracture sites were united and the results were acceptable showing that the bioresorbable PLA95 copolymer plate/screw system implants were suitable for the repair of distal radius fractures.

It is reported that currently in Japan, resorbable materials are being used in the repair of facial fractures. Although titanium materials being resistant to corrosion due to the surface oxide film, and are nontoxic, the reason for not using titanium is the necessity of removal of the implant under certain conditions. Especially in young patients, growth of the patient might require implants of larger sizes. In addition, the absence of sufficient information about the long-term effects of titanium and the risk of adverse effects due to retained metallic devices such as osteopenia of cortical bone induced by protection from stress and corrosion make removal an important consideration. Besides, it is stated that in cold and hot climates, metallic implants are not comfortable due to their conductivity, and their removal is recommended.

In 1997, a bioresorbable polymeric device made from poly(L-lactide) (PLLA) started to be used in Japan. The device called FIXSORB MX is reported to be absorbed in 3–4 years. It however is not without problems; complications such as foreign body reactions and late degradation tissue responses were reported. Another bioresorbable device, based on the same polymer, was designed as an osteosynthetic device OSTEOTRANS MX which was a composite of unsintered hydroxyapatite particles with PLLA (u-HA/PLLA) [10]. In a study with this product, 17 patients with 86 fracture sites were treated using 1.0 mm plates and 5 or 7 mm screws; the patients were observed for 6–60 months postoperatively. Fracture site healing was achieved in all of the patients, and the plate was almost fully absorbed after 60 months. Thus, the problem of implant remaining in the body or the need for removal with a second surgery was avoided. Another advantage of this implant was its thermoplasticity (deformation upon heating). During surgery, after reducing the fracture, the surgeons were able to adapt the bone contour with a template, shaping the miniplate according to this template. To deform the plate, all they had to do was to warm the device with hot water (65–68 °C for 10–60 s). This is a major advantage because a similar adaptation of form with a titanium implant would not have been carried out as easily.

Fig. 15.3 Bioresorbable plating system [11]



In another resorbable craniofacial implant application study, 13 randomly selected patients underwent treatment for zygomatic–complex fractures (two site fractures) and mandibular fractures using 1.5/2/2.5-mm INION CPS biodegradable plates and screws [12] (Fig. 15.3). The biodegradable system was found to need further refinement in material quality and handling to match the stability achieved with metal system, but they were an ideal system for pediatric fractures with favorable outcome.

A similar 2.5-mm resorbable plating system from Inion CPS was used in the fixation of 50 mandibular fractures on 34 patients. The plates were secured with resorbable screws, and the patients were followed for a period of 6 weeks–10 months. Primary bone healing was reported in all the patients when assessed according to the incidence of soft tissue infection, osteomyelitis, plate fracture, non-union, and malunion among others. It was concluded that the 2.5 mm resorbable plating system was a viable option for the treatment of mandible fractures [13].

15.2.2 Screws, Pins, Rods, and Wires

Bone screws are used for internal fixation more often than any other type of implant. Although the bone screw is a simple device, there are several designs based on how the screw will be used. The screws can be used alone to hold a fracture, as well as with plates, rods, or nails. The design may have some variations for a specific type of fracture and for the producer. Screws may be left in place or removed after the bone heals (Fig. 15.4).

Pins hold pieces of bone together. They are usually used in cases when the fragments of the bone are too small for fixation with screws. The pins are usually removed after a certain amount of time but also may be left in permanently for some fractures.

In some fractures of the long bones, the best way to align the bone ends is by inserting a rod or nail through the hollow center of the bone that normally contains some marrow. These nails or rods held in place by screws until the fracture heals. They may be removed or left in the bone after healing is complete.



Fig. 15.4 Metallic bone screws [12], bone pins [14], intramedullary rod [15], metallic K-wires used in fracture fixation [16]

Wires are often used as sutures or threads to “sew” the bones back together. They can be used in conjunction with other forms of internal fixation to hold bones together. They can be used alone to treat fractures of small bones, such as those found in the hand or foot. Metallic devices can easily be detected and observed in the X-ray and MR analyses.

15.3 Total Hip Implant

Hip implants are medical devices used to restore mobility, eliminate damage, and relieve pain of the patients. The deformations of the hip joints are usually associated with arthritis, hip diseases, or injuries. Although the traditional metal and polyethylene implants have been in use since the 1960s, the technological advances since then made novel materials, ceramics, and titanium alloys more preferable.

The hip implants have some advantages as well as some risks, as every implant. This depends on the design, shape, size, and the materials used in the production. Different outcomes also depend on the health, age, sex, and activity level of the patients.

Metals used in the production of femoral stem, femoral head, and acetabular cup can be produced from metals and metal alloys. In general, the femoral stem is made from titanium, titanium-cobalt, stainless steel, and cobalt-chromium alloys, where the head and acetabular parts can be made of either similar metal alloys, or plastics or ceramics. In some cases, combination of all materials is used.

The extensive use in modern times of metallic alloys is related to the availability and success at the beginning of the twentieth century of several different alloys made of the noble metals. Implants made from iron, cobalt, chromium, titanium, and tantalum are commonly used. Clinical studies have demonstrated that alloys made from these metals can be used safely and effectively in the manufacturing of orthopedic implants that are left in place for extended periods. The mechanical, biologic, and physical properties of these materials play significant roles in the longevity of these implants.

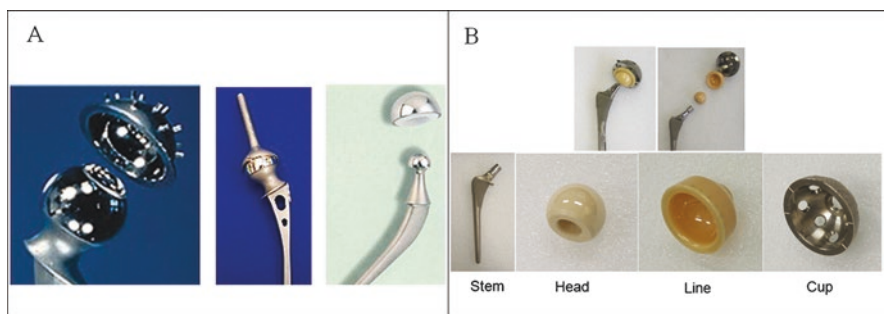


Fig. 15.5 Total hip implants. (a) Completely metallic hip implant (metal cup, metal ball). (b) Composite hip implant made of ceramic (head and cup), polymer (line), and metal (stem) components [17]

The most common cause of hip joint deterioration is arthritis. Other causes include congenital hip disorders, osteonecrosis (avascular necrosis), and trauma due to sports and accidents. When the standard treatments (weight loss, physiotherapy, pain relief agents, use of glucocorticoid injection into the joint) fail and the pain is not relieved, then surgery is performed. In a total hip replacement (total hip arthroplasty), the damaged femoral head and the acetabulum are removed and replaced with a complex system consisting of a metal or ceramic ball and a metallic stem on the femoral side and the acetabular cup of mostly of UHMWPE (ultrahigh molecular weight polyethylene), locked in a ceramic cup. The metal stem is placed into the hollow center of the femur where the bone marrow is found (Fig. 15.5).

The combinations of the ball and the cup can be metal-metal, metal-polymer, ceramic-polymer, ceramic-ceramic, ceramic-metal, and metal-ceramic-polymer. The main criterion is that the ball and the cup do not damage each other by causing fragmentation, cracks or fractures, particulate material, and erosion. These would shorten the service life of the implant and also cause pain even if they do not completely fail. The stem is introduced in the intramedullary cavity of the femur, and fixation is achieved either by cementing with a polymeric paste called the bone cement, or it is press fit into the bone without the use of any adhesive. In the absence of the cement, the implant stem has to have pores or depressions on its surface to increase the surface area for more attachment and to provide sites to be ingrown by the newly developing bone tissue during healing. In order to avoid infections such as *Staphylococcus aureus*, the bone cement can be impregnated with antibiotics (mostly gentamycin).

15.4 Bone Cement

Polymethyl methacrylate (PMMA)-based bone cement is the oldest, the most common, and successful method used in anchoring orthopedic implants to the bone. Still, the cement might undergo mechanical failure and cause premature failure of

the implant. Researchers are therefore continuing their search for a better bone cement using particulate or fibrous fillers to enhance the mechanical properties and lower the unacceptable levels of heat release during the setting of the cement [18].

The improvement attempts have not been translated to clinical practice and are still in experimental stage due to problems in the strength of attachment between the cement materials and the fillers, mainly due to problems relating to interfacial particle/matrix adhesion and high cement stiffness.

In one such attempt, mesoporous silica nanoparticles (average diameter 200 nm) were used as fillers within a commercially available acrylic bone cement (Palacos R + G) at a level of 0.5, 2, and 5% (w/w). In fatigue testing, the higher levels of silica nanoparticle substantially decreased the number of cycles to failure. The flexural modulus and compressive strength and modulus increased as the silica particle content is increased, but the flexural strength, fracture toughness, and work to fracture all significantly decreased. The incorporation of the highest amount of silica (5%) significantly increased the hydration of the cement. The results were interpreted as either the interfacial adhesion strength between the nanoparticles and the polymer was poor, or proper dispersion of the nanoparticles could not be achieved.

In another study a polymeric material was used as a filler instead of ceramic nanoparticles [19]. The biodegradable biopolymeric fillers were poly(3-hydroxybutyrate) (PHB) and its copolymer with 3-hydroxyvalerate (PHBV), well-known polyesters produced by bacteria. It was shown that the incorporation of PHBV made the cement more resistant, similar to PMMA bone cement. Osteoblastic compatibility of the samples with polymer using human bone marrow stem cells was much better than that of PMMA alone. The addition of a biodegradable polymer significantly decreased the maximum temperature reached when compared with the formulation based on PMMA. With the cements impregnated with the polymeric fillers, the peak temperature was less than 90 °C, the highest value accepted by the ISO 5833 standard for acrylic resin cements (Fig. 15.6). One adverse observation was that the setting time of about 5 min with the original PMMA bone cement was extended to around 10 min upon addition of the polymers.

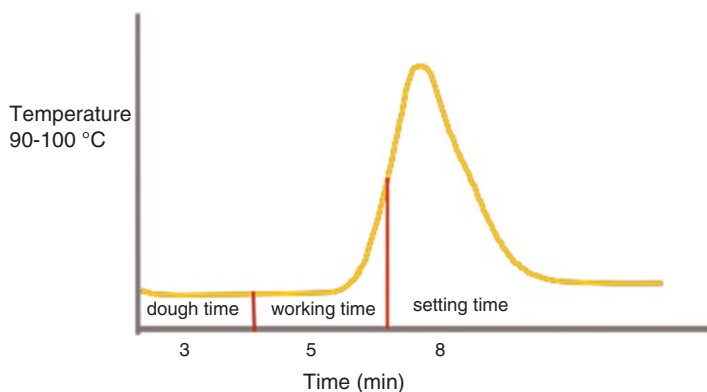


Fig. 15.6 Thermal behavior of acrylic bone cement

Adhesives are very important in the case fixation of bone plates. Their use in fracture fixation in order to stabilize the defect site is, however, more limited due to the need for higher bond strength. Their use in craniofacial surgeries was recently proposed as an alternative to the use of plates and screws [20]. The obvious advantages of adhesive use include the ease of application and avoidance of primary and secondary surgery involving drilling holes, pain, and risk of infection if the plates are of metallic type. Metallic plates also have the disadvantage of extrusion, migration, and growth restriction. If bioresorbable materials are used, then the number of surgeries is decreased to one, but the rest of the problems still exist.

In one study of four adhesives, octyl cyanoacrylate, N-butyl cyanoacrylate, a novel methyl methacrylate, and a novel cyanoacrylate-derived adhesive were compared with Lactosorb resorbable plates and screws [20]. The lowest tensile strength was obtained with the octylcyanoacrylate. Bond shear strength of the N-butyl cyanoacrylate was significantly greater than that of plate and screws, while the others were comparable with the plate and screw system. As such, N-butyl cyanoacrylate had the greatest potential for fixation of fractured bone in craniofacial surgical applications.

Titanium appears to be the most widely used permanent material for reconstruction of orbital floor fractures, but there seems to be a problem with adherence. A limitation of these implants is the risk of late enophthalmos (sunken eye ball, which is sometimes related with dehydration) due reportedly to the lack of adequate support in defects larger than 2.5 cm². In order to avoid this problem, a resorbable PLLA-PGA mesh plate (LactoSorb) was coat with BIOPEX (Hoya Co.) bone cement and then applied to the patient's orbital floor with biodegradable screws [21]. BIOPEX consists of a powder and a liquid which contains various phosphates like calcium phosphates, hydroxyapatite, and chondroitin sulfate as the main ingredients [22]. Only problem the authors observed in this approach was the increased economic cost of combining both implants.

15.5 Dental Implants

Dental implants are developed for augmentation of jaw bone strength for dental root implantation purposes, for stems of implants, or for crowns of dental fixtures. Depending on the site of application, the material type and therefore properties and processing conditions change.

15.5.1 Bone Augmentation

Bone augmentation is needed when a patient requires an implant to substitute for a missing bone, but the site of implantation is very poor in terms of the quality and amount of bony material due to the extent of damage. These defect sites could be devoid of the bone and called cavitations. These cavitations can be caused by physical trauma, dental treatments, infection and diseases, and other toxic materials that

a patient comes in contact during the daily activities. Under these conditions, the material used is generally something that can bind with the new bone, is in powder form, and preferably osseoconductive if not osseoinductive. The approach necessitates the application of the powder at the defect site and induces bone formation by the use of bioactive agents such as bone-inducing growth factors (e.g., bone morphogenetic protein BMP2). The source of the filler could be biological such as allografts or shells from crustaceans. Synthetic materials generally include HAp or β -tricalcium phosphate (β -TCP), both calcium phosphate compounds with different calcium-to-phosphate ratios and different properties (e.g., TCP erodes faster than HAp). Both of these are very similar to the mineral component of the bone. Their advantage is that they are initially hard so they provide strength at the implantation site, but in time they can erode and fuse with the regenerating new bone. Other minerals can be added, but they generally do not integrate with the natural tissue. In order to accelerate the healing process, the powder is mixed with either growth factors or with patient's own blood containing a variety of osseoinductive compounds.

15.5.2 Implants

Implants are needed when the tooth cannot be saved with treatment. For proper chewing and also for cosmetic reasons, the tooth is replaced with a metallic root which then is capped with a material that resists the harsh conditions of the human mouth created by the things eaten that have different pH and T and require different mechanical stress during mastication (Fig. 15.7). The implant is generally made of metals due to the brittleness of ceramics, the only other type of material that can withstand high compressive forces. The main material used in these roots is based on titanium (Ti) due to its inertness and mechanical properties. Also, people tend to have allergies and other immune or toxicity problems with many other metallic implants including nickel. Recently Ti-Zr (titanium-zirconium) alloys are also produced for certain application conditions. However, zirconium oxide alone is not recommended for long-term use [24].

15.5.3 Crowns

Crowns are critical because in addition to an array of properties that they have to have, they need to be esthetically pleasing. Zirconia-based crowns appear to be gaining wider acceptance as an alternative to conventional metal-ceramic materials used in restorations. Zirconia-reinforced lithium silicate and lithium disilicate are apparently being used as crown material. A number of complex CAD/CAM machinable resin-ceramic materials are also available to be formed into patient-specific implant crowns (Fig. 15.8).

Fig. 15.7 A tooth implant with the abutment and crown components [23]

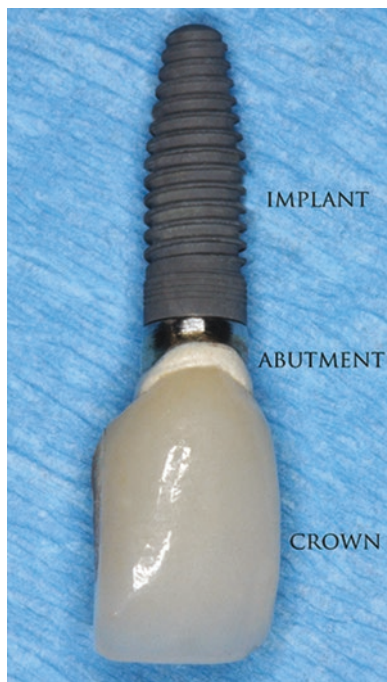
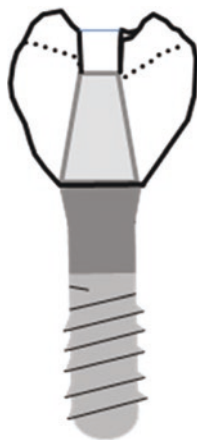


Fig. 15.8 Crown in position with the abutment and the implant [25]



15.6 Conclusion

In this chapter, examples of metal, ceramic, and polymeric materials used for hard tissue augmentations were presented. These materials have applications in orthopedics and dentistry as plates, screws, pins, and wires, as well as bone cements. Metals and ceramics, or their composites prepared by combination with polymers, have a history of successful use in dental and orthopedic applications to treat and augment damaged regions of tissue.

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16.1 Blood Interfacing Implants and Hemocompatibility

Blood is a type of connective tissue and is the most difficult one to bring an implant in contact with because in addition to being biocompatible, a material has to be hemocompatible to serve as a successful implant. Hemocompatibility is defined as the property of a material not to elicit thrombosis and blood coagulation, loss or damage to platelets and other blood elements, or in short, a biomaterial's property should not to initiate any adverse effects on blood constituents or functions. A broad series of tests are conducted in situ, in vitro, and in vivo before a material is tested on humans for its hemocompatibility. The main types of adverse effects are thrombosis (blood clotting), damage to blood cells (e.g., hemolysis of erythrocytes), adherence and decrease of blood elements such as platelets, and immune responses initiated through the various complement activation pathways.

Hemocompatibility is a very important issue because during the circulation through the vasculature in and outside the organs and tissues, the blood contacts many different tissues throughout the body and organs like the heart, kidneys, and the vasculature which frequently have serious problems requiring grafting or some type of treatment (e.g., stents for clogged arteries, dialysis case of kidney failure, replacement of a heart valve, complete heart transplantation). Similarly, downstream effects may take place, and a clot formed on an implant surface may leave and block capillaries resulting in death in a worst-case scenario. Under such circumstances, the properties of the material like chemistry, hemodynamics, dimensions, design, porosity, roughness, and elasticity become very critical in the prevention of incompatibility. The parameters to be taken into consideration are different for each application, and the general guide for these tests and considerations is found in the international standards (ISO 10993-4: Hemocompatibility).

16.2 Vascular Grafts

Blood vessels carry the blood between the heart and the tissues and organs. They form a branched system of vasculature varying in composition, organization, size, and mechanical properties. The molecular network of the extracellular matrices consists primarily of collagen (types I and III), elastin fibers, glycoproteins, and proteoglycans (e.g., hyaluronan). Collagen provides the mechanical strength, elastin gives the elasticity of the vessels, and the proteoglycans contribute to the hydration and compressibility; as a whole they are the reason of the elasticity of the vasculature [1].

Autografts are the golden standards for the grafting or replacement of a dysfunctional blood vessel with a blood vessel obtained from another tissue of the same patient. However, such vessels are not readily available especially from a patient who has a disease related with the vasculature. Under such conditions, allografts (from other people), xenografts (from animals), and synthetic grafts (man-made) are used. These natural grafts are generally used as decellularized structures which preserve the internal architecture but are devoid of the biological markers and cells which if implanted in another person than the donor could lead to immune responses or even rejection.

Small-diameter (less than 6 mm) artificial blood vessels are generally made of non-woven materials using mainly poly(tetrafluoroethylene) (PTFE, Teflon) and polyurethane [2]. Large-diameter artificial blood vessels on the other hand are generally made of woven or knitted materials. Woven materials have better stability, while knitted materials have higher elasticity. The materials used are polyesters, PTFE, and natural silk.

The large-diameter vascular grafts are well accepted in the medical market and by the clinicians, but this is not true for the small-diameter grafts. The reason of this is the frequency and ease of thrombus formation and thickening of the neointima which lead to blockages and graft failure.

As an example of synthetic grafts, PTFE used as large-diameter (≥ 6 mm) conduits can be given [3]. Polyurethanes are also among the most commonly used prosthetic vascular grafts.

Allografts from cadavers and human umbilical vein have also been used as vascular tissue substitutes. In situations where small-diameter vessels are required, the synthetic grafts have limited applicability because their patency is low; they lead to blood coagulation. Their patency is less than 25% in 3 years in comparison to the more than 70% with autologous grafts. Therefore, small-diameter blood vessels such as saphenous vein, internal mammary or radial artery cannot be replaced by the synthetic grafts. Besides, there is a need for continuous antithrombotic drug therapy in order to prevent blood clotting. Other problems associated with synthetic grafts are infection, calcification, and formation of aneurysm (formation of a balloon-like swelling or widening of the transplanted vasculature caused by weakening or inherent weakness of the implant) [4]. Even though ePTFE (expanded Teflon) and Dacron (polyethylene terephthalate, PET) are successfully used in arteries larger than 6 mm, they have not been successful as substitutes for small-diameter arteries [1].

There are two main ways of producing synthetic grafts: weaving and knitting. As a result of the technique used, the woven grafts have smaller pores and thus lesser leakage of fluid and blood compared to knitted ones. It is stated that immediately after implantation, a thin layer of fibrin, erythrocytes, white blood cells, and platelets are deposited on the surface and slowly start thickening until it reaches equilibrium [5]. In humans this is observed after 18 months of implantation. In the depth of the graft, fibrin instantly fills the narrow interstices of the graft wall and the space between the graft and the surrounding tissue. Knitted grafts are treated by preclotting and immersion in fluids to decrease the leakage. In order to modify the surface properties, researchers treated the inner surfaces and coated them with proteins (e.g., collagen, albumin) or polysaccharides (e.g., heparin) mainly to reduce adsorption of blood elements that would lead to changes in the rate of clotting. The surfaces were also treated to release antibiotics against infections, especially critical in the early stages of implantation.

16.2.1 Important Parameters in Vascular Graft Design

16.2.1.1 Mechanical Properties

The mechanical properties of the vascular grafts are very critical in the design of prosthetic grafts because the properties of the synthetic grafts have to be matched with the mechanical properties of the natural tissues. The mechanical properties of some human blood vessels are presented in Table 16.1.

16.2.1.2 Porosity

Porosity is especially important for the formation of a smooth layer of proteins due to absorption of albumin or fibrin filling the interstices and formation of an endothelial layer. In the fibrillar grafts like blood vessels, porosity can be varied by changing the fiber thickness, winding angle, and the method (knitting, weaving, electrospinning, or winding). When fiber thicknesses used are in the range 1–30 μm , then pore sizes are 10–60 μm .

It was reported that complete healing of small-diameter (2 mm O.D.) ePTFE grafts with 30 and 60 μm diameter pores yielded better results with the higher porosity graft, due to ingrowth of perigraft capillaries yielding endothelial cells when tested on the rabbit common carotid artery [6]. In another *in vivo* study with dogs, 30–40-mm-long ePTFE graft with internodal gaps of about 60 μm and i.d. of

Table 16.1 The mechanical properties of human femoral artery and veins

Sample	Ultimate tensile strength (MPa)		Ultimate elongation (%)	
	Longitudinal	Transverse	Longitudinal	Transverse
Femoral artery	1.67	1.37	87	69
Femoral vein	2.16	3.04	82	70
Popliteal artery ^a	1.37	1.18	105	70

^aArtery located at the knee, branches off from the femoral artery

6 mm was implanted into the canine abdominal aorta, and no deformation or anastomotic aneurysm (ballooning at the sutured region) was observed. There was no significant deterioration in the mechanical properties such as suture retention strength, and radial or longitudinal tensile strength for up to 80 weeks [7].

16.2.1.3 Surface Chemistry

Surface chemistry of grafts is very important for adhesion of cells, blood components and for clotting, and therefore, surface chemistries of the prosthetic grafts have always been modified to make them more hemocompatible. In order to improve endothelial cell adhesion, researchers have attached cell adhesion peptide sequences, such as the P15 peptide found in type I collagen. Other peptides carrying the three amino acid adhesion sequence of arginine-glycine-aspartic acid, RGD, found in collagen and in extracellular matrices (ECM) were also used. In order to increase endothelial cell adhesion to ePTFE, RGD was used to mimic cell-ECM binding through integrins [3]. Endothelial cell attachment could also be significantly improved on surfaces grafted with fibronectin. Delivery of growth factors such as VEGF (vascular endothelial growth factor) from polymer surfaces to attract endothelial cells to the graft also led to an increase in the rate of in situ endothelialization [8].

In addition to growth factors, heparin, a polysaccharide with anticoagulant properties, was among the surface modification chemicals tested. ePTFE grafts are impregnated with fibrin glue containing FGF-1 (fibroblastic growth factor 1). Heparin increased endothelialization and proliferation of smooth muscle cells (SMC) in a dog model. Solvent cast and electrospun PVDF-HFP (poly(vinylidene fluoride-co-hexafluoropropylene)) samples were highly hydrophobic, and minimal bone marrow-derived endothelial cell (BMEC) growth was observed on them. When fibrinogen was adsorbed onto solvent cast films, the contact angle decreased to 77°, but this did not improve cell binding or proliferation. However, on electrospun meshes which presented a contact angle of 114° (indicating hydrophobicity), cell adhesion properties were improved. As these results show, hydrophobicity did not deter cells from adhering to the PVDF surfaces; the surface topography is, however, another important factor [9, 10].

It is stated that platelets are negatively charged and adhere to opposite charged surfaces on implant surfaces [11]. They also preferentially attach to hydrophobic surfaces. It is recommended that synthetic graft development should focus more on hydrophilic materials and especially those with negative charges in order to prevent blood cell coagulation. In another study, a hybrid small-diameter vascular graft of poly(ϵ -caprolactone) and chitosan was prepared by co-electrospinning, and heparin was immobilized on it by ionic interaction. Heparin was observed to be released from this conduit for more than a month. This functionalization with heparin improved the hemocompatibility as was shown by reduced platelet adhesion and prolonged blood coagulation time. This was also confirmed with an arteriovenous (AV) shunt test. Implantation in rat abdominal aorta showed reduced thrombus formation and increased the patency of the implant.

In order to modify surface charge and chemistry, cold plasma with air or oxygen is also used. A polycaprolactone vascular graft treated this manner was tested

in vitro with smooth muscle cells (SMC), and also implanted in vivo in the rat model for 3 weeks, subcutaneously (s.c.) and as an aortic replacement. The plasma treatment significantly increased the hydrophilicity of the scaffold (complete wetting after 60 s), and in vitro the SMC spread on the surface, while the s.c. implantation revealed a very low foreign body reaction [12].

The current trend is to use negatively charged hydrophilic molecules and biological anticoagulants as graft coating materials to decrease thrombosis.

16.2.1.4 Materials

Nowadays, most artificial vascular prostheses are made of polymers like expanded polytetrafluoroethylene, polyethylene terephthalate, or polyurethane. These materials have excellent mechanical and physical properties and were originally designed for use in other industrial applications. Only in the last decades they found their way to the biomedical field. As stated above these polymers have been successfully used in vascular grafts in large-diameter graft applications [13]. ePTFE vascular grafts are highly thromboresistant and biostable, and in vivo results have demonstrated that these grafts can maintain their structures and functions for up to 6.5 years after implantation. More importantly, no major tissue inflammatory responses have been found. Both PET grafts and ePTFE grafts have been implanted in femoropopliteal bypass with no significant differences in graft patency in 5 years. Polyester urethanes (PEU) and polycarbonate urethanes (PCU) are widely applied as biomaterials for applications in artificial vascular grafts; however, it is reported that the hydrolytic instability of PEU appears to be a disadvantage, and PCU is better in this respect than PEU [13, 14].

16.3 Tissue-Engineered Vascular Grafts

Tissue engineering has emerged in the last 2 decades as an alternative to nondegradable, nonliving implants to solve a variety of implant-related problems, including those related with the vasculature. Tissue engineering has three components: (1) cells, preferably patient's own, (2) a cell carrier or a scaffold to seed the cells on, and (3) growth factors or similar bioactive agents to guide the stem cells toward the desired phenotype. The scaffolds are designed to degrade in an appropriate duration sufficient for the damaged tissue regenerates and gains sufficient strength to perform its function in synchrony with degradation. Initially, these artificial tissues are not as strong or elastic as the natural tissue or the synthetic substitutes. However, they are less prone to cause thrombogenicity, and they grow and are transformed into natural tissue as the scaffold on which the cells are seeded is simultaneously eroded and covered with the ECM produced by the native cells of the environment and cells of the patient seeded. A tissue-engineered vasculature needs to mature in several weeks or more so that the graft has the required level of mechanical properties at the time of implantation in order not to fail (rupture, burst, leak, create an aneurysm, etc.).

These 3D scaffolds have more strict requirements such as efficient material transport to and from the cells (oxygen, nutrient, and waste transfer) in order to achieve cell viability throughout the thickness of the scaffold. These are only possible with appropriate surface and bulk chemistry, porosity, pore interconnectivity, and surface area. Pore size is critical in controlling both tissue ingrowth and the internal surface area available for cell attachment. The organization of the yarn used in the production of the vascular graft is also important. It was reported that when fibrous PLLA-PCL scaffolds were seeded with human coronary artery endothelial cells, the cells on the scaffolds were aligned parallel to each other and along the fibers and stretched [15]. When the fibers were not parallel with each other, then the cells grew randomly without getting oriented. This organization is critical for proper endothelialization of the lumen of the graft. Figure 16.1 shows a nanopatterned collagen conduit. The vascular smooth muscle cells (VSMC) are aligned orthogonal to the conduit axis and parallel to the pattern on the exterior of the tube.

SMC (smooth muscle cells) were observed to adhere and migrate along the axis of fibers woven parallel to each other and presented a spindle-like appearance. Their cytoskeleton was also organized parallel to the orientation of the fibers [16]. In an extreme case, SMC cells were co-spun with the scaffold materials, poly(ester urethane) urea [17]. SMC were found to be mechanically robust and were integrated with the elastomeric, nanofibrous scaffolds. They spread well, were healthy, uniformly distributed throughout the whole thickness of the vascular scaffold. It thus appears that the application of a high electric potential (around 20 kV) to achieve electrospinning did not adversely affect the viability and proliferation of the cells.

In 2001 Shin'oka et al. reported the first clinical application of a tissue-engineered blood vessel [18]. In this approach, cells from ca. 2 cm segment of peripheral vein were isolated and expanded. A tube of PCL-PLA copolymer (1:1, w/w) reinforced with woven polyglycolic was designed to degrade within 8 weeks. Ten days after seeding, the graft was transplanted in place of the occluded pulmonary artery with

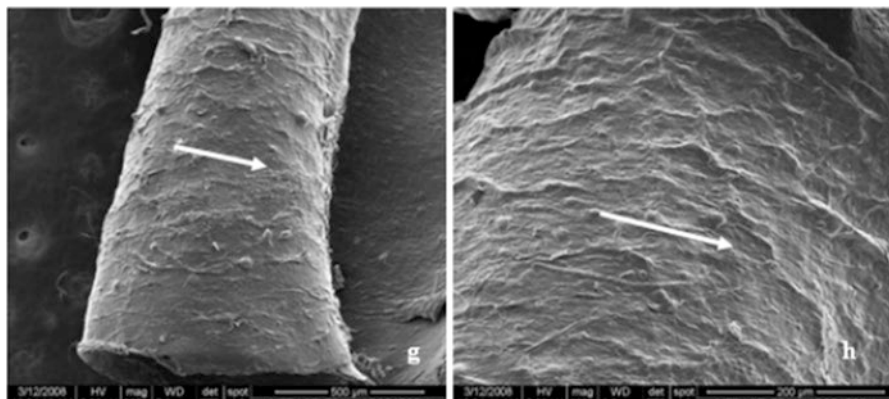


Fig. 16.1 Small-diameter nanopatterned collagen conduit seeded with VSMC at its exterior. Arrows show the directionality of the patterns and the cells that are aligned with it

no postoperative complications, but was found to be not suitable for high-pressure arterial implantation. They were successfully implanted into many patients as venous conduits for cardiovascular applications with >95% patency at 1 year without evidence of stenosis, thrombogenic complications, or aneurysm formation [13].

Cytograft (Novato, USA) performed a pilot clinical study using engineered blood vessels grafting them as arteriovenous (AV) shunts. The carrier was seeded with autologous dermal fibroblasts and endothelial cells. The vessels were allowed to mature in vivo before use. In the construct design, they used cell sheet engineered fibroblasts which were wrapped over a mandrel, matured, and then seeded with endothelial cells. For up to 5 months after implantation, no failures were reported with the first three patients.

16.4 Heart Valves

The function of the heart valve is to assure unidirectional, forward, smooth (nonturbulent) blood flow through the heart chambers and the main vessels during circulation (Fig. 16.2). Anatomy of the valve is complex and the function is vital for the humans. The mitral and tricuspid atrioventricular (AV) valves have leaflets and control the flow from the atria and from the ventricles, and the aortic and pulmonary semilunar (SL) valves have cusps and control the flow toward the rest of the body and the lungs, respectively. The AV valves are large and asymmetric leaflets attached to ring-shaped annuli on the static/attached end, and they are attached to the

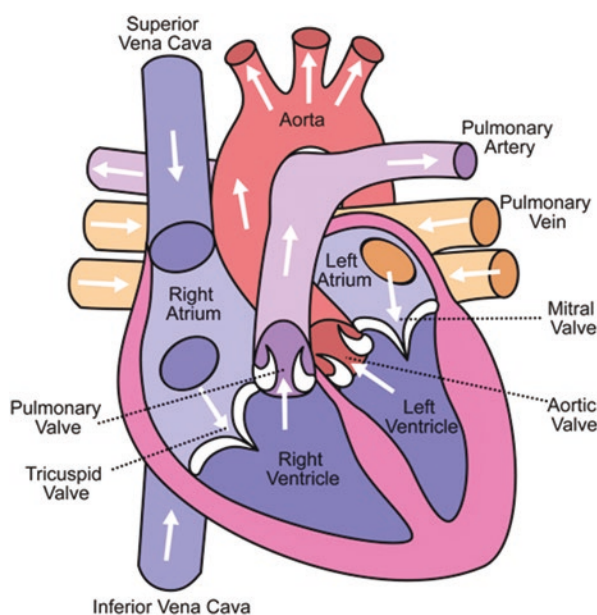


Fig. 16.2 The heart valve locations and blood flow (white arrows) in the heart [19]

ventricles by a complex bundle of fibers and muscles at the mobile end [20]. This function of the heart valves can be affected by heart diseases that could lead to blockage of the blood flow (stenosis) or backward leakage of the blood (regurgitation), or both. Even though conventional therapy might be successful, in severe cases, heart valve replacement is the procedure applied.

16.4.1 Heart Valve Replacement

Heart valves can be replaced with valves from two main sources: biological (bioprosthetic), such as allo- or xenografts, and synthetic heart valves made as composite materials using biomaterials. Biological origin heart valves are scarce, and there are the risks of infection and rejection. However, they have the correct geometry and inner architecture, and therefore, they are also frequently used. The main problems of synthetic heart valves are thrombosis and damage to the blood elements and cells. With time the hemocompatibility, design, and mechanical properties of the artificial heart valves are improving making them a competitive alternative to bio-based valves.

16.4.2 Bioprosthetic Heart Valves (Allografts and Xenografts)

Bioprosthetic heart valves are generally xenografts such as porcine aortic valves or constructed from bovine pericardial membranes that are stretched across metallic frames to mimic the valvular architecture. They can also be left unmounted (stentless) (Fig. 16.3). Xenografts are a potential cause for immune rejection and susceptible to tissue degeneration due to absence of viable vasculature. For this reason, bovine or porcine tissues are cross-linked using glutaraldehyde, a well-known cross-linker, which in addition to strengthening the tissue, it also almost completely prevents tissue antigenicity and thus prevents rejection. Glutaraldehyde kills by fixing all the cells on the material by cross-linking the proteins (including the enzymes), and this prevents degradation by host enzymes. Finally, it serves to sterilize the graft. Tissue-derived heart valves are less thrombogenic than their mechanical counterparts due to better compatibility of their chemistry and design, and as a result they do not require long-term anticoagulation treatments as the prosthetic heart valves need. These heart valves function quite effectively for many years (10–15 years).

16.4.3 Prosthetic Heart Valves

Heart valves (HV) are among the most widely needed and used cardiovascular devices. Recent advances in metals, ceramics, polymers, nanotechnology, and surface modification techniques together have resulted in materials with improved biocompatibility and hemocompatibility properties. HV replacement therapy dates

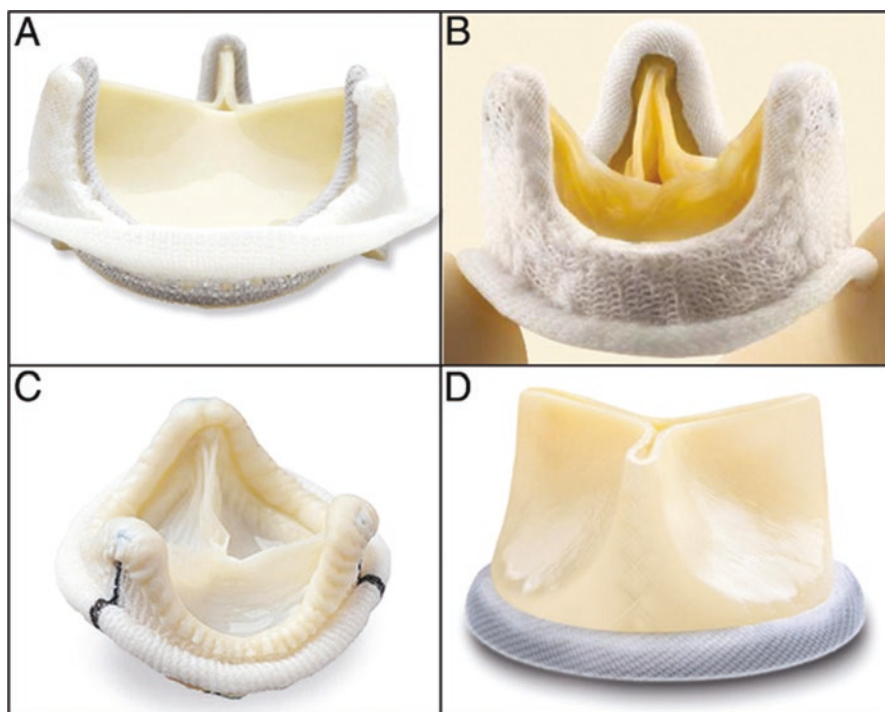


Fig. 16.3 Bio-based heart valves with (a–c) and without (d) frames [21]

back to 1960s, when the first prostheses were implanted into humans. Although clinical outcomes of these initial prototypes were not satisfactory, they evolved into the currently widely used mechanical and bioprosthetic valve prostheses. Even though they are saving lives, still there are problems unsolved. The main complication is thrombogenicity for the prosthetic HV and durability for the allo- and xenografts in long-term clinical application. Consequently, the search for better alternatives is intensely pursued.

16.4.3.1 Material Selection

The choice of material is a crucial part of developing prosthetic HVs. In the current heart valves, there are metallic rings to provide stability for the shape and sufficient mechanical strength [22]. There is also need for a textile material to cover this ring and keep it in place. Then there is the valve material which currently is made of polymers, pyrolytic carbon (as a material or a coat), ceramic materials, or composites. Thus, it has to be a complex list of materials with different requirements. Obviously, they all, individually and as the completed product, need to be hemocompatible.

HV replacements were introduced in the 1960s, using several synthetic polymers including polymeric materials such as silicone and polyolefin rubber as leaflet materials [23, 24], but these were not mechanically satisfactory and did not have the

required durability. Polytetrafluoroethylene (PTFE) also failed for the same reasons and caused a high level of thrombosis and calcification [25]. Polyurethanes are among the most hemocompatible materials known for biomedical application and has been studied since the 1950s. However, their main disadvantage is their low biostability or in other words their degradability. PU degradation is caused by oxidation, and acid or enzymatic hydrolysis. Degradation leads to a decrease in the chain lengths followed by loss of mechanical properties and eventually to tears or cracks on the leaflets. The other major drawback of PUs is their calcification, a problem that was also observed with PTFE and PHEMA which leads to increased rigidity of the leaflets. Modification of the chemical structure with polydimethylsiloxane (PDMS) improved the stability against oxidation. For example, long-term stability was improved when a blend of PU:PDMS = 20:80 (%) was used [26].

A polyolefin (polystyrene-polyisobutylene-polystyrene “Quatromer™” [27]) was developed in the late 1990s, and its mechanical properties were improved by reinforcing with polypropylene (PP) fibers to mimic the collagen fibers in the native valve leaflets. Quatromer was shown to have suitable hemocompatibility and mechanical durability for use in polymer trileaflet heart valves (Fig. 16.4), and fiber reinforcement was considered to be useful in tailoring the mechanical properties of valves [29].

A nanocomposite with excellent bio- and hemocompatibility and biostability was developed based on polyhedral oligomeric silsesquioxane (POSS) nanoparticles (an organosilicate compound) and poly(carbonate-urea) urethane [30]. This material had improved mechanical properties and elasticity and was resistant to calcification. Moreover, peripheral blood stem cells were attracted to this valve material and were able to proliferate and differentiate into endothelial cells on the valve [31].

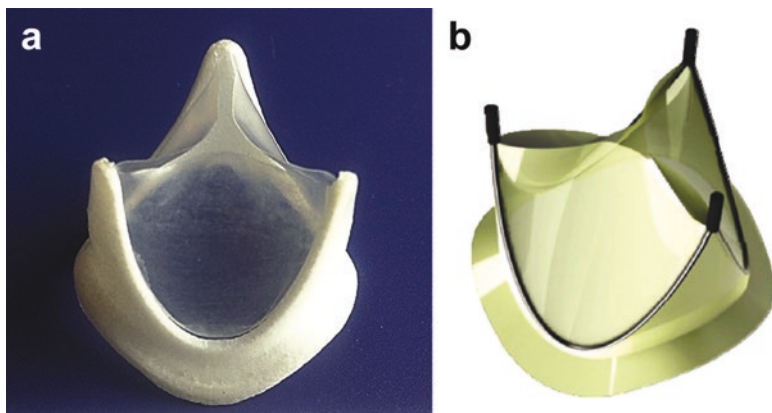


Fig. 16.4 Trileaflet polymer-based heart valves (a) surgical valve on a nylon stent, (b) semi-stented aortic valve [28]

16.4.3.2 Design Parameters

Considering the complex anatomy and the mechanical properties of natural valves, it is impossible to mimic it completely. Unlike bioprosthetic counterparts, the synthetic leaflet valves can be designed in any shape the mechanical and biological properties dictate. The following are some of the requirements to be satisfied by a synthetic valve:

- The valve must fit well into the site of the natural valve it replaces.
- The valve leaflets should create minimum resistance to forward flow and ensure complete sealing to minimize backward flow.
- There should be minimal damage to blood cells.
- There should be no thrombus formation.
- The maximal stress in the valve components should be as low as possible during the entire cardiac cycle.
- The mechanical properties should be satisfactory and preserved during the service life.
- The materials used should be hemo- and biocompatible.

Figure 16.4 illustrates different trileaflet valves with asymmetric (a) and symmetric (b) leaflet valve designs. The design and geometry of the leaflets is directly related to the function of the valve. These characteristics can be optimized by the use of advanced designing tools such as finite element analysis, computational fluid dynamics codes, and fluid-structure interaction analysis.

Figure 16.5 presents the disk-type Björk-Shiley heart valve that was used extensively in the early 1980s. The figure shows the detail of a heart valve with its sewing ring and fabric, the pyrolytic carbon occluder disk, and the restraining struts.

The products in Fig. 16.6 are heart valves manufactured by different companies with different designs and materials representing the developments in these

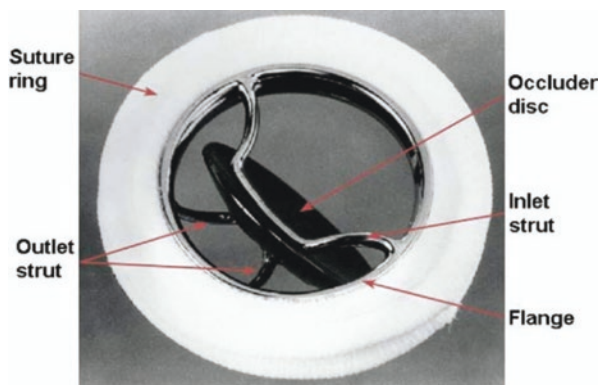


Fig. 16.5 The Björk-Shiley (BSc) mechanical heart valve that was extensively used during the 1979–1986 period [32]

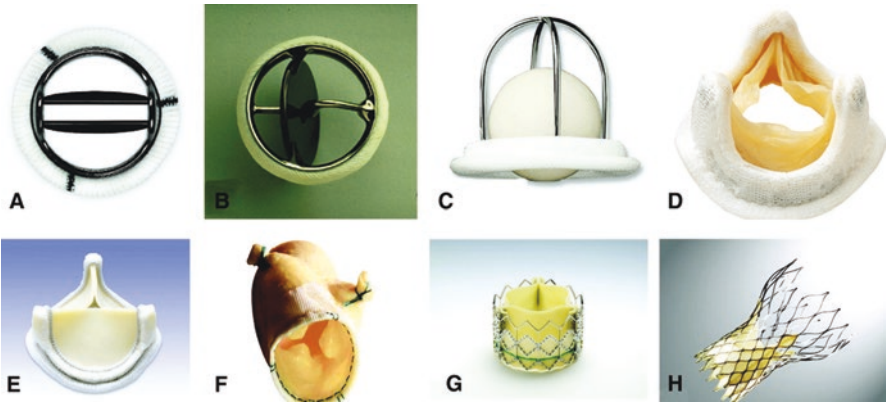


Fig. 16.6 Various types of prosthetic valves produced by different companies [33]. (a) Bi-leaflet mechanical valve (St Jude); (b) mono-leaflet mechanical valve (Medtronic-Hall); (c) caged ball valve (Starr-Edwards); (d) stented porcine bioprosthesis (Medtronic Mosaic); (e) stented pericardial bioprosthesis (Carpentier-Edwards Magna); (f) stentless porcine bioprosthesis (Medtronic Freestyle); (g) percutaneous bioprosthesis expanded over a balloon (Edwards Sapien); (h) self-expandable percutaneous bioprosthesis (CoreValve)

Table 16.2 Biomaterials used in various components of prosthetic heart valves (adapted from Mohammadi and Mequanint [22])

Component	Material
Cage, housing, and hinge	Ti alloys, cobalt-based alloys, pyrolytic carbon (low temperature isotropin (LTI) carbon)
Leaflet, disk, and ball	Pyrolytic carbon, silicone rubber, polyacetate (Derlin), polyolefins (UHMWPE)
Sewing ring	Polypropylene, Teflon, Dacron

categories over the years. Even the same company has several products, some bio based and some fully synthetic.

In Table 16.2 a number of main components of the heart valves made from different materials are presented. The type of component varies with the design because the mechanical and biological responses vary with different models. It is observed that cage and similar stiff components are made from metal alloys or pyrolytic carbon, while the sewing ring is from softer textiles of synthetic polymers.

16.5 Artificial Heart

Research on artificial heart dates back to 1949, when, for the first time, Sewell and Glenn implanted an artificial heart in a dog’s body. Then in December 1967, Dr. Christiaan Barnard made the first implantation of a cardiac allograft [34]. First implant in the heart of a human was a left ventricular assist device that was developed by Domingo Liotta and Michael DeBakey at Baylor University College of

Medicine in 1961, and this was transplanted successfully in 1966 after some unsuccessful attempts. In 1969 the first total artificial heart was implanted into a patient for a period of 64 h and was then replaced with an allograft. The patient died of acute pulmonary infection 32 h after implantation. In this heart, the expandable membrane of the heart was constructed of Dacron with a reticular fabric surface that was embedded in Silastic. Because of their wide orifice and freedom of flow, Wada-Cutter hingeless valves were used in the heart (Cooley). These valves had a regurgitation problem which served to discourage thrombus formation. The problems initially observed were hemolysis and renal failure.

First successful heart substitution with a mechanical device was performed in 1982. Kolff, working with Jarvik and DeVries, designed a compact implantable pneumatically powered total artificial heart with which, in 1982, they made the first attempt at permanently replacing the failed biologic heart. Barney Clark died from multiple organ failure, as did several others who followed, after 112 days with what was called the Jarvik-7 implant. At present, FDA-accredited artificial hearts are CardioWest (in use since 2004, implanted in 900 patients) and AbioCor (in use since 2006, implanted in 14 patients) [33].

Total artificial heart (TAH) of The SynCardia CardioWest is a biventricular orthotopic pneumatic pulsatile pump with two separate artificial ventricles that take the place of the native ventricles (Fig. 16.7). The two artificial ventricles have basically the same construction. Each has a rigid outer “housing” that has a blood containing diaphragm, two intermediate diaphragms, and an air diaphragm, all made of

Fig. 16.7 SynCardia CardioWest total artificial heart [35]

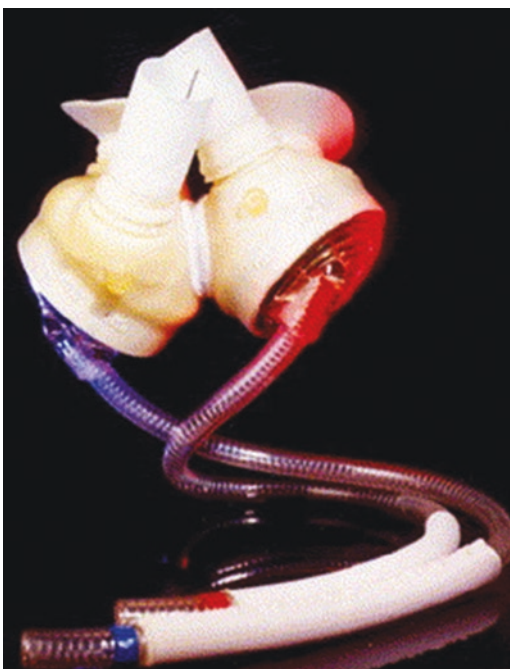
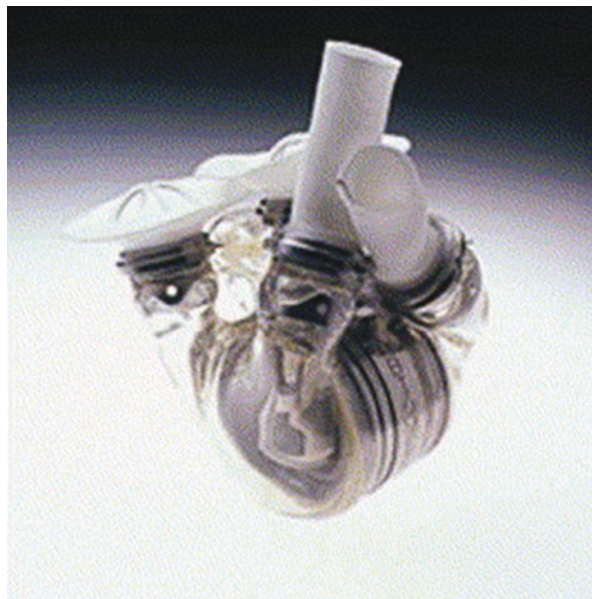


Fig. 16.8 AbioCor (Abiomed Inc.), completely self contained artificial heart [33, 35]



segmented polyurethane, separated by thin coatings of graphite. It has Medtronic-Hall valves for the inflow and outflow.

The AbioCor™ (Abiomed, Inc.) implantable replacement heart was the first completely self-contained total artificial heart (Fig. 16.8). Its design allows it to be totally implanted within the body. On July 2, 2001, surgeons at Jewish Hospital in Louisville, Kentucky, performed the first implantation of the AbioCor in a male patient.

The AbioCor system consists of internal thoracic unit, rechargeable lithium battery, miniaturized electronics package and external lithium battery pack, handheld alarm monitor, and computer console. The thoracic unit of the AbioCor includes two artificial ventricles with artificial valves and a motor-driven hydraulic pumping system.

The system controls the pumping speed of the heart based on the physiologic need of the patient. The internal battery is recharged from the external battery pack using an energy transfer device which transmits power across the skin, without any transcutaneous connection. Moving parts such as the valves and ventricular membranes are manufactured from flexible polymers.

The main difference between AbioCor and the other artificial hearts is that the AbioCor has the energy transfer across the skin, miniaturized microprocessors, and high-capacity batteries, and these eliminate the need for the patient to be permanently immobilized by the tubes or wires that they are connected to the auxiliary devices.

CARMAT bioprosthetic artificial heart is a half cow and half mechanical device and was invented by Carpentier and Lagardère. A 75-year-old Frenchman received this implant after a 10 h long operation, and the patient died after about 3 months

due to complications. In this device, there are two chambers each of which is divided by a membrane that holds the fluid on one side. An electrohydraulic motorized pump moves the hydraulic silicone fluid in and out of the chamber to cause the membrane to move and push the blood. The blood interfacing side of the membrane is bovine pericardial tissue, processed with glutaraldehyde for strength and sterility, and ePTFE. The pericardium increases the hemocompatibility of the device [36]. The power supply of the prosthetic heart is provided by lithium-ion batteries carried by the patient and connected to the device through cables to an external control console and to a plug positioned on the skull behind the ear of the patient. As such the AbioCor is more advanced as there are no transcutaneous wires.

16.6 Stents and Assist Devices

A stent is a device placed into a clogged blood vessel to open it and keep it open. In order to open an artery blocked with a plaque, angioplasty is carried out by inserting a tube carrying a balloon at its tip (catheter) into the artery, moved to the site of blockage; the balloon is inflated to compress the plaque to expand the blocked site. When the balloon is inflated, the stent expands and locks in this form and maintains this shape when the balloon is removed after deflating. The device design, material, and chemistry are very important in the success of a stent. Stents are generally made up of metal alloys such as nitinol (NiTi), Ni-Co steel, and stainless steel.

Stainless steel which is an alloy of iron is the material most commonly used in the balloon-expandable stents. The shape-memory alloy NiTi, on the other hand, is a natural choice for use in the self-expanding stents. Some of the main design factors to consider in both cases are corrosion resistance, biocompatibility, strength, radiopacity, and the material to coat the stent with to control cell adhesion and corrosion. Table 16.3 presents the list of some commonly used stents.

Tissue proliferation on the stent material is another issue to be dealt with as it leads to restenosis, or re-clogging of the artery, at the target site. For example, stainless steel was reported to lead to a tissue proliferation of up to 2 mm thickness around some orthopedic implants, while titanium had a minimal effect [37].

A different approach to stent design was tried by making use of biocorrosion [38]. A coronary stent from an Al-Mg alloy that gradually corrodes and degrades was designed. In a porcine model, a diameter decrease of 40% was shown initially due to an intense inflammatory reaction, but later a remodeling occurred re-enlarging the lumen and leaving behind a patent vessel.

16.6.1 Restenosis and Drug-Eluting Stents (DES)

Restenosis can be seen as the response of the arterial wall to the mechanical injury inflicted by the stent involving smooth muscle cell migration/proliferation and extracellular matrix deposition followed by vessel remodeling. These include platelet aggregation, inflammatory cell infiltration, release of growth factors, smooth

Table 16.3 Some commonly used vascular stents

Stent	Material
Viabahn	ePTFE/Nitinol
Wallgraft	Polyester/Ni-Co-Ti steel
Wallstent	Ni-Co-Ti steel
Jostent	ePTFE/stainless steel
Luminex	Nitinol
Express	Stainless steel
Palmaz	Stainless steel
Symphony	Nitinol

muscle cell (SMC) proliferation, and proteoglycan deposition. All these events eventually lead to the blockage of the artery again. In order to prevent this from happening, an approach is to coat the stents with natural and synthetic polymers carrying inhibitors for cell adhesion and proliferation. In a study, a significant reduction in neointimal hyperplasia (increase in SMC proliferation and ECM secretion) was observed with patients treated with a coronary stent that released the antibiotic rapamycin [39]. Release of anticancer agent paclitaxel also gave promising results. For example, when paclitaxel-coated stents in the coronary arteries were compared with pristine, uncoated stents, a significant decrease in the development of intimal hyperplasia was observed [40]. The SIROCCO I and II trials were designed to compare the effectiveness of sirolimus-releasing nitinol stents with uncoated nitinol stents placed in the superficial femoral artery; no statistically significant differences were observed. This implied that application of stents to peripheral vasculature might yield different results than when used in a coronary application indicating that the site at which the stent is applied matters [41].

Drug-eluting stents (DES) on the whole appear to have dramatically reduced the rates of restenosis through revascularization. The newer DES systems with everolimus, zotarolimus, and biolimus A9 as the bioactive agents perform better than the earlier stents releasing paclitaxel. [42, 43]

DES efficacy was studied with 8,150,763 applications and it was observed that there was a rapid and widespread use of DES (ca. 89%). However, a steep drop to 66% was recorded in 2007 followed by a modest rise to 73% in 2011 [44].

16.7 Membrane Oxygenators

Oxygenators are used to artificially support the life of an adult by providing oxygen to ca. 5 L of blood per minute and to remove the carbon dioxide (CO₂) in exchange. While doing so, the heat exchange with the environment should be minimum, and the priming volume should be small. The choice of the material used for the membrane is very crucial as it must be hemocompatible, highly permeable to O₂ and CO₂, and strong and thin (Fig. 16.9).

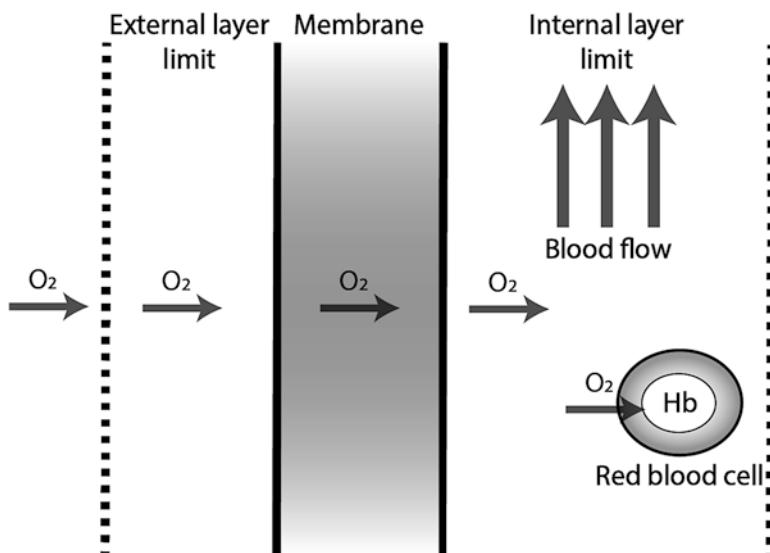


Fig. 16.9 Oxygen diffusion through a porous membrane for oxygenation of blood

The diffusion of CO_2 is easier because it is ca. 20 times more soluble than O_2 and may be exchanged through any type of membrane by only the pressure difference.

During the process blood enters through an inlet port and flows either along the exterior of a bundle of hollow fibers or on the outside of a membrane and leaves the system via an outlet port. The gas could be pure oxygen or a mixture of oxygen, and air enters the oxygenator through a gas inlet port flows through the inside of the hollow fibers or on the other side of the silicone membrane. During the transport within the fibers the blood flows through a heat exchanger, a kind of constant temperature bath, to preserve its temperature at physiological temperature. Another aspect is the removal of the bubbles, introduced during the transport over the many junctions, before the oxygenated blood returns to the body.

A blood oxygenator is often characterized by its gas exchange capacity. “Rated flow” is defined as the flow rate through the oxygenator at which the inlet blood saturation of 70% is increased to 95% at the outlet.

Silicone membrane oxygenators are often used because plasma leakage is not like the case with the hollow fiber oxygenators. These oxygenators contain two silicone sheets sealed at the edges, which are wound around a rigid polymeric, generally polycarbonate, core. Gas flows between the sheets, while the blood flows on the outside in a countercurrent fashion. Diffusion of gases across a nonporous silicone sheet is difficult, and therefore, the thickness of these sheets has to be low (ca. 100–200 μm). Even then, the gas exchange efficiency of hollow fiber oxygenators is higher. In addition, the bubble removal step of the sheet oxygenators is generally more difficult [45].

16.7.1 Comparison with the Lungs in Terms of Rate of Transference, Surface Area, and Oxygenation Efficiency

The natural lung is a very efficient organ for gas exchange, and the development of an artificial lung that has a gas exchange capability similar to that of the natural lung is extremely difficult. The alveoli of the lung are tiny sacs at the end of all the branching airways of the lung, and they achieve close contact between the inhaled air and the blood flowing through the capillaries in the lung. As Fick’s second law of diffusion states, the exchange rate is proportional to the area of the alveoli of the lungs, the concentration gradient across the barrier material, and the solubility of the gas (oxygen and carbon dioxide) in the barrier material (which is mainly bilayers of phospholipids) and inversely proportional to the wall thickness between the alveoli and the capillaries. Thus, to mimic the natural lungs, the exchange area has to be very large, the membrane very thin, and the permeability of the membrane for the relevant gases very high [46].

The natural lung has an alveolar capillary area of 100–150 m², and the gas exchange rate is ca. 200–250 mL min^{−1} for both O₂ and CO₂ while in resting stage, and it is increased by about 10- to 20-fold under exercise conditions [47].

Unfortunately, the current hollow fiber blood oxygenators have membrane areas in the range 1–4 m² and a surface area-to-blood volume ratio 10 times lower. The effective distance that gas diffuses between blood and gas flow pathways in artificial lungs is approximately 10–30 μm whereas it is 1 μm in the natural lung. Thus, the gas exchange duration is the only parameter that can be modified; prolonged to increase the gas exchange. Obviously that also has a limit (Table 16.4).

16.7.2 Hollow Fiber Oxygenators

Hollow fiber oxygenators have microporous polypropylene membranes, constituted of hollow capillary fibers positioned in parallel bundles, and are the most common type in use (Fig. 16.10). The blood circulation can be on the inside of the capillary or the outside, with the oxygen gas flowing in a countercurrent manner on the opposite side. Some examples are Univox[®] hollow fiber oxygenator (Bentley Laboratories), the Monolyth C[®] hollow fiber oxygenator (Sorin Biomedica), the Affinity[®] hollow fiber oxygenator (Avecor), the hilite[®] series hollow fiber oxygenators (Xenios AG), and the CAPIOX[®] series hollow fiber oxygenators (Terumo CVS).

Table 16.4 Comparison of lung and hollow fiber oxygenator performance [48]

Properties	Natural	Oxygenator
Surface area (m ²)	140	2.5
Gas exchange rate (mL min ^{−1})	210–3200	200–400
Wall thickness (μm)	1	10–30
Surface area/blood volume ratio (cm ^{−1})	290	28

Fig. 16.10 Scheme of a hollow fiber oxygenator

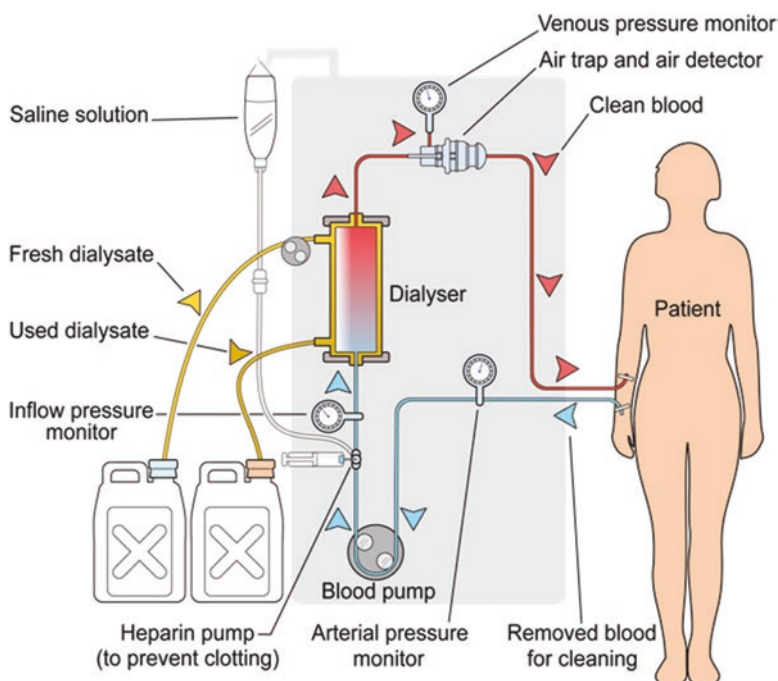
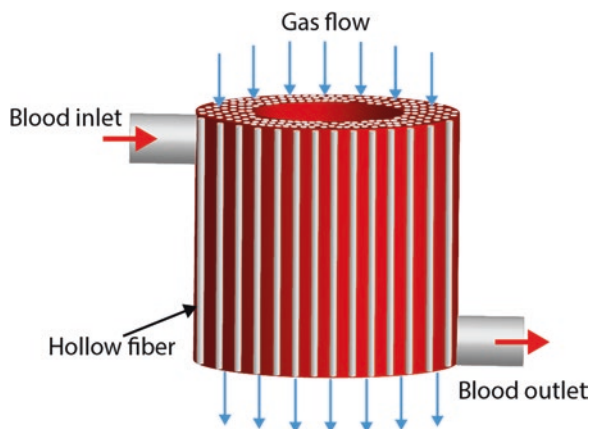


Fig. 16.11 Dialysis system for removal of wastes from the blood [50]

16.8 Dialysis Systems

Hemodialysis is the removal of the wastes and extra fluid from the blood when the kidneys cannot perform this function (Fig. 16.11). Chronic renal failure (CRF) is defined as a progressive decline in kidney function associated with a reduced glomerular filtration rate (GFR) and is measured clinically by the rate of creatinine clearance.

The most common causes of these symptoms are diabetes mellitus, glomerulonephritis, chronic hypertension, and failure of the kidney. In older individuals, the most commonly diagnosed causes of CRF are renovascular disease and diabetes mellitus, although other causes include polycystic kidney disease and pyelonephritis. The treatment of CRF includes dietary changes, correction of systemic complications, and dialysis or renal graft procedures. Dietary and fluid restrictions may be required to handle the reduced excretory capacity of the kidneys. Acidosis and increased levels of potassium can be treated by reducing dietary intake of potassium-rich foods, such as bananas, and sodium restriction can aid the control of hypertension. It is sometimes necessary to reduce protein consumption to minimize nitrogenous waste products. Despite these, most patients progress to end-stage renal failure (ESRF), requiring dialysis or transplantation.

A microporous, semipermeable membrane allows small molecules to pass through while not allowing larger molecules. In the dialysis systems the same principles apply: they allow the transport of small molecules and ions but keep larger molecules such as proteins and blood cells in the blood (Fig. 16.11). Blood introduced into a dialysis system flows on one side of a microporous membrane, while a special salt solution called the dialysate flows on the opposite side. The wastes and toxic products are originally only in the blood and not in the dialysate; the concentration gradient forces them to go to the dialysate and be eventually discarded. It, therefore, is very similar to oxygenator in its working principle.

Chronic renal failure is the final common pathway of a number of kidney diseases. The choices for a patient who reaches the point where renal function is insufficient to sustain life are (1) chronic dialysis treatments (either hemodialysis or peritoneal dialysis) and (2) renal transplantation. With renal failure of any cause, there are many physiologic possibilities. With this renal failure problem, homeostasis of water and minerals (sodium, potassium, chloride, calcium, phosphorus, magnesium, sulfate) and excretion of the daily metabolic load of fixed hydrogen ions are no longer possible. Toxic end products of nitrogen metabolism (urea, creatinine, uric acid, among others) accumulate in the blood and tissue. Finally, the kidneys are no longer able to function as endocrine organs in the production of erythropoietin and 1,25-dihydroxycholecalciferol (calcitriol).

In dialysis, instead of adding more drugs, therapy involves physical diffusion and ultrafiltration to remove the chemicals as well as water from the blood. The most important points are temperature and pressure. The dialysate must be brought up to body temperature and drawn into a dialyzer by negative pressure (Fig. 16.12). The dissolved gases introduced in the dialysate should be removed by heating and negative pressure. Bubbles inside the dialyzer lead to pressure build up and decrease the effective exchange area. Therefore, the concentration of dissolved gases in dialysate should be kept low for efficient osmotic exchange with blood [51]. In the last decade, compact and wearable dialysis systems which the patients can easily handle by themselves without the need to visit a healthcare center are being developed (Fig. 16.13).

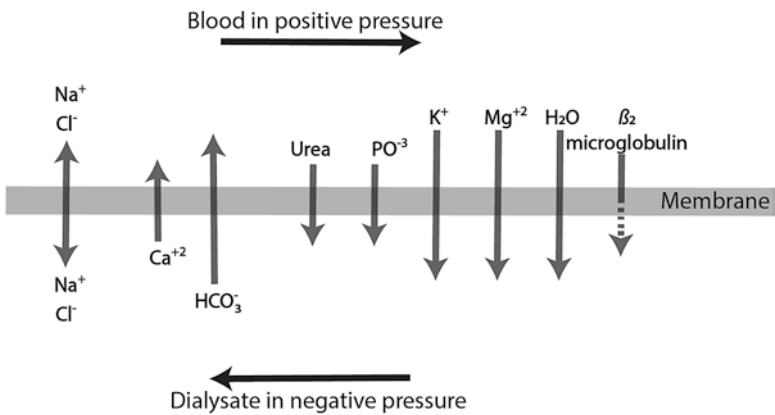


Fig. 16.12 Hemodialysis scheme



Fig. 16.13 Portable dialysis system [52]

Table 16.5 Improvements in hemodialysis membranes

Type	Improvements
Traditional membranes	Biocompatibility: new materials and chemical modifications of side chains
	Refinement of pore dimensions to enhance permeability and middle molecule clearance
Innovative membranes	Microfluidics and membraneless systems
	Nanofabrication with synthetic channels
	Nanofabricated silicone membranes with slit pores
Living membranes	Endothelial cell-lined membranes for hemocompatibility
	Tubule cell-lined membranes for active transport and metabolic activities

Cellulose-based materials have been used for this purpose since the first human hemodialysis treatment with collodion tube membranes. Later, with the original development of hollow fiber dialyzers in the 1960s, regenerated cellulose was utilized as the principal membrane material. Table 16.5 gives information about the improvements in hemodialysis membranes.

16.9 Conclusion

In this chapter, devices such as vascular grafts and heart valves (allografts and xenografts), total artificial heart implants, stents, oxygenators, and dialysis systems were discussed. These devices can be made of metals, ceramics, polymers, or their composites. Since they come in contact with blood, either in or outside the body, these devices require unique parameters in their materials selection and design. The materials used in these devices should be highly hemocompatible in addition to being biocompatible.

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17.1 General Information

Drugs are bioactive agents used to treat or prevent diseases and illnesses through chemical action in the body. In order for a drug to be effective, it has to be at the site of cause of the illness, whether it is an infection, a blockage of an artery, pain, or some other malfunction of the bodily organs or tissues due to genetic causes or a trauma or aging. In order for the drug to reach this target site, it has to be introduced to the body (administration), cross barriers (distribution), get modified by the enzymes within the body (metabolism), and be removed from the body (elimination or excretion). All these processes affect the rate, dose at the target, efficacy, and the fate of the drug. In order for the drug to have prolonged or predetermined period of presence and sufficiently high concentration and to be localized specifically at the target tissue, “controlled release systems” are designed. All the above mentioned topics have to be discussed before a controlled release system can be designed.

17.2 The Journey of a Drug Molecule in the Body

Drugs can be introduced to the body by many different routes and have a number of barriers to cross before they can reach their targets.

17.2.1 Administration

Bioactive agents, or in other words drugs, are administered through a variety of routes such as oral, intravenous (i.v.), intramuscular (i.m.), intrathecal (i.t.), intra-peritoneal (i.p.), subcutaneous (s.c.), and topical or transdermal. A drug administered in the form of tablets, creams, ointments, or injectables has to pass through a large number of barriers to reach its targeted site of action. In order to achieve this, the drug is transported within the body through the vascular system. Thus, the drug

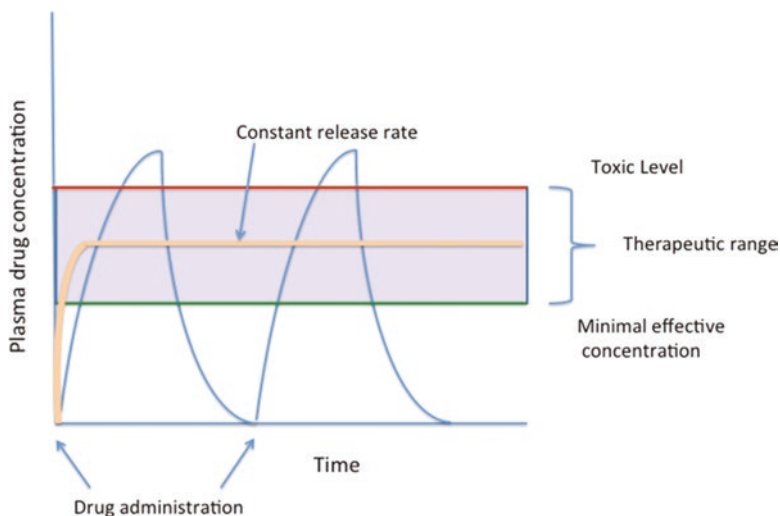


Fig. 17.1 The pharmacokinetics of drugs depending on the administration and formulation type

molecule has to be introduced to the body using one of the administration routes, transported within the body and reach the plasma. The concentration of the drug molecule is decreased by distribution to the tissues, metabolism in certain organs (e.g., liver), and excretion through a variety of routes (e.g., skin, kidney, lungs). In order for the appropriate amount of drug to be available at the target site it has to be administered as the kinetics dictate. As a result, the plasma level of the drug increases and decreases by roughly following the first-order kinetics (Fig. 17.1). On the other hand, controlled release systems lead to a constant release rate.

In the body, most reactions and processes happen via the first-order kinetics; in other words, concentration changes in time depending on the concentration in plasma according to the following equation:

$$-dA / dt = k \cdot A$$

where A is the concentration of the drug, t is time and k is the rate constant.

The integrated version of this equation is:

$$A = A_0 \cdot e^{-kt}$$

where t is time, A_0 is the drug concentration at time zero (in the plasma), and A is the concentration at time t .

It can be seen that the amount released decreases exponentially as the drug concentration decreases. So, soon after administration the concentration of the drug in plasma starts increasing, reaches a peak but after a while when there is no more administration, and the metabolic and elimination processes take effect, the concentration starts to drop. When the concentration falls below the MEC (minimum effective concentration), it is no longer effective. In order for the drug to be effective, its

concentration has to be brought up to the effective level, and this requires another administration by injection or by taking another pill. Excess of the plasma concentration is another problem; it causes toxicity. If the drug administration is too frequent, then its concentration in the plasma could reach toxic levels, but if it is too infrequent, then it falls below the concentration needed for the treatment. Thus, the concentration must be maintained between the ineffective and the toxic levels. In other words, the plasma concentration of the drug should be maintained in the therapeutic range.

When the administration is through injection, it is uncomfortable and painful. If it is an oral tablet or pill, then this administration approach increases the path length. Painful or not, patient compliance, patient's following the drug administration times and doses, is another issue. If the patient is not compliant, then avoiding to take the drug leads to lower plasma doses, making the therapy ineffective. Also, in some cases the rise and the fall of the plasma drug levels could cause discomfort as in the case glaucoma treatment. Pilocarpine used for this purpose might cause blurred vision, stinging, or itching of the eye, which is uncomfortable. In some cases, the patient might be noncompliant because they are unable (unconscious, in too much pain, etc.) to administer the drug. In order to circumvent all these problems, controlled and targeted drug delivery systems were developed.

17.2.2 Distribution

The distribution of drugs in the tissues is important in the efficacy of the drug and also in the elimination kinetics. Sometimes the drug is bound to plasma proteins, and this could make them too large to cross membranes or change their surface charge, thus modifying the distribution pattern. Distribution basically depends on the physicochemical properties of the drug such as the hydrophilicity/hydrophobicity, charge, size, pK_a , etc. These determine the ability of the drug to cross the cell membranes and the other physiological barriers (such as the blood-brain barrier, BBB), their ability to accumulate in the aqueous compartments.

17.2.3 Metabolism

Drug molecules are unavoidably metabolized because they are generally mimics of biological compounds, and there are enzymes to metabolize the original molecules and also the drugs. The main goals of metabolism are to modify the drug to be more easily eliminated (by conjugating with molecules like glucuronic acid derivatives) and make them highly hydrophilic, to activate (if prodrugs are used) or inactivate them. The liver is the main site where metabolic activity is the highest in the body. Unfortunately when the drugs are orally administered, they go through the stomach in the gastrointestinal (GI) tract which is a vat of enzymes of pH 1 in aqueous medium. Then the intestines are negotiated, and eventually the drug is absorbed by the vasculature to be transported to the liver, which is the main eliminator or

detoxifier in the body that is loaded with different types of enzymes. Thus, elimination becomes a significant issue when the drug is taken orally. The kinetics of enzymatic processes are generally first order with respect to drug concentration.

17.2.4 Elimination and Excretion

The drug, is metabolized sometimes even before showing its effect, and eliminated through the kidneys by filtration, through the large intestines by excretion, by exhalation from the lungs, or by diffusion through the skin. The route of elimination depends on the physicochemical properties (form, size, charge, etc.) of the drug.

As a result of these four processes, the drug concentration in the body continuously changes after the administration, and this is not well accepted by the patients due to the problems associated with the “sawtooth” variation of the plasma drug concentration due to repeated administrations as presented in Fig. 17.1.

As a result of these, researchers have developed the controlled release systems which deliver drugs into the medium at a constant rate, without any fluctuation in time until the very end, when the system is depleted of the bioactive agent. In this way the sawtooth appearance of plasma drug concentration is replaced by a plateau between the minimum effective and toxic levels, thus maintaining a safe and effective dose in the plasma.

17.3 Advantages of Controlled Drug Delivery

Controlled drug delivery has certain inherent advantages over classical drug formulations:

- Patient compliance problems are minimized (more effective treatment).
- Less drug is used (both healthier and more economical).
- Side effects are decreased (less damage to the patient through use of large doses).
- Drug accumulation upon chronic use of drugs is decreased.
- Discomfort due to frequent administration is avoided.
- The required amount of drug is provided for the duration of the illness.

Pilocarpine, the drug used in the treatment of glaucoma, was mentioned above for the disadvantages when applied periodically as droplets. In the case of the drug delivery system called Ocusert, the drug pilocarpine is released at a constant rate and absorbed through the cornea at a very low level (1% of applied), and this helps maintain a constant level of drug in the target tissues for extended periods because first some of the drug penetrates, and then some more of the drug accumulating outside would penetrate the corneal epithelium. Since the release from the system is constant, it will be like many injections in a very short time periods. There will of course be loss of the drug through drainage from the eye and also by absorption into other tissues, but these are taken into account when designing the drug release rate

from the CRS. In one study, patients were treated with the Ocusert pilocarpine system, and it was effective, provided continuous release, was convenient for the patient, especially those who had to rely on others for treatment (e.g., children and the elderly), was judged to have definite advantages, and was a highly desirable method of therapy in selected cases of glaucoma [1, 2].

17.4 Methods to Achieve Prolonged or Sustained Drug Delivery

Prolonged or sustained delivery approaches aim to extend the bioavailability of the drug in the body by modification of the drug or the carrier of the drug. This does not have to be zero order, constant for a long period, but rather decreasing in time very gradually so that it does not need the frequent administration.

17.4.1 Approaches

There are a variety of approaches to achieve sustained delivery. Some of them are provided below:

- Simplest is when the drug in powder form is mixed with particles of an inert (and preferably bioresorbable) carrier and compressed to form a pill or a film. The carrier creates a barrier through which the agent has to diffuse out and thus the availability of the drug is delayed (Fig. 17.2), or the carrier could erode gradually and the drug is released when the aqueous medium interacts and solubilizes the drug. A combination of these routes can also be employed. These systems are called the monolithic systems (Fig. 17.3).

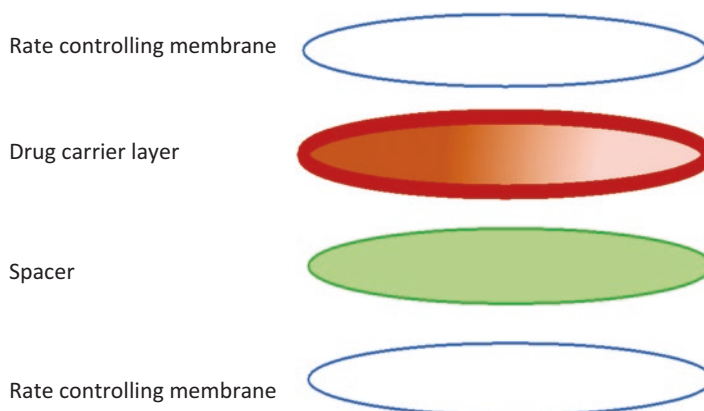


Fig. 17.2 Reservoir-type drug delivery system (DDS)

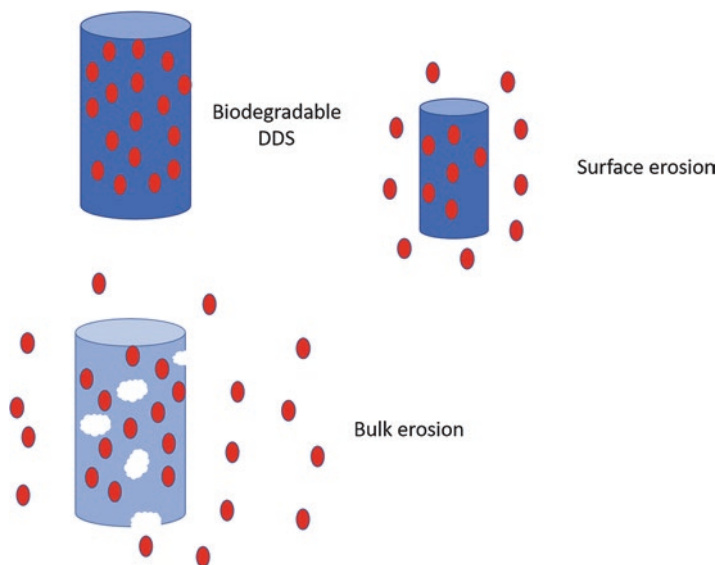


Fig. 17.3 Different types of biodegradation used in delivery systems

- The drug can be entrapped within a gel or used in the form of a solution or a powder and is loaded into a gap between two membranes. The drug's release is then controlled by the diffusion of the water in between the membranes and the drug's diffusion out through the capsule membranes. In the case of Ocusert®, the core had alginic acid-pilocarpine gel, and the rate-controlling membrane was ethylene-vinyl acetate polymer (EVAc) similar to that in Fig. 17.2.
- A solution of the drug can be placed in an impermeable balloon, and then osmotic pressure is used to squeeze this balloon and force the drug to be released through a gate with a valve.
- A drug molecule is chemically bonded to a polymeric carrier, and as the bond is hydrolyzed, the drug is released at a rate depending on that of hydrolysis.

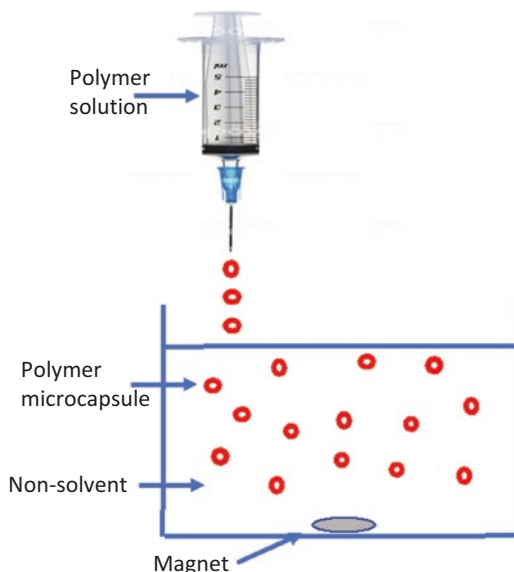
17.4.2 The Processes

Drug delivery systems are designed in a variety of forms in order to satisfy the need for controlling the release rate, conforming to the shape of the application site, suitability to the route of administration, and length of application. As a result, capsules or spheres (nano or micro size), films, membranes (porous and nonporous), films, or fibers are loaded with one or more bioactive agents.

17.4.2.1 Encapsulation in Micro- and Nanospheres or Capsules

Microencapsulation can be used to encase particles, liquids, solids, or gases, and several approaches can be employed to encapsulate materials. Normally drug

Fig. 17.4 Microcapsule production



delivery involves hydrophilic and hydrophobic drugs to be entrapped, and depending on the nature of the drug, the solvents need to be changed (Fig. 17.4). In the case of a hydrophilic drug to be entrapped in a capsular form, water-in-oil-in-water (w/o/w) system is employed. The drug is dissolved in an aqueous solution and then added dropwise into a much larger volume of organic, low boiling point solvent that has the hydrophobic polymer dissolved in it. After proper mixing and sonication, this suspension is added into a larger volume of aqueous solution carrying surfactants to avoid fusion of the particles. After stirring for a sufficient time to evaporate the organic solvent and thus harden the polymer in the form of a capsule, the drug-carrying capsules are removed. The release of the drug from these capsules will be controlled by the diffusion through the hydrophobic polymeric membrane and might therefore be zero order or, in other words, constant in time.

When a drug molecule is to be entrapped in a sphere, then the drug is either dissolved or dispersed in the polymer solution, depending on the solubility of the drug in the solvent of the polymer. This then is introduced dropwise in a non-solvent for the polymer and stirred until the solvent is evaporated leaving behind spheres of the drug-carrying polymer. In this case, the release of the drug would be by diffusion through the polymeric matrix, and the rate will decrease gradually with time.

17.4.2.2 Entrapment in Micro- and Nanofibers by Electrospinning

Electrospinning is a method transferred from the textile industry to make fibers of micron and nanometer width. The main approach is to extrude or inject a polymer solution, while a potential is applied to the injector needle and a metallic collector (Fig. 17.5). Under the influence of the potential, the polymer is extruded in the

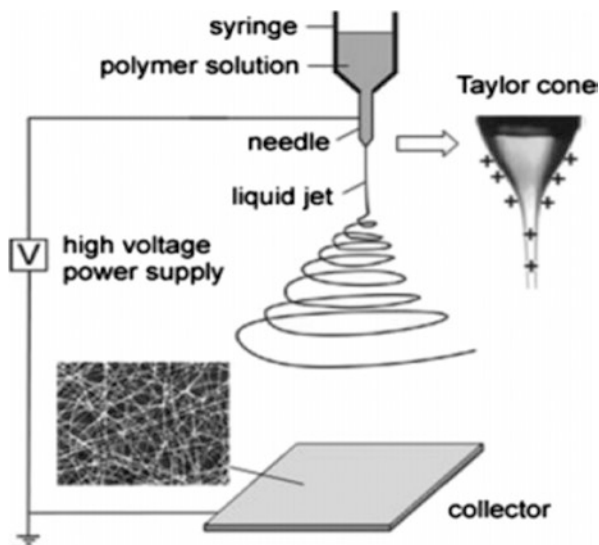


Fig. 17.5 Electrospinning to produce drug delivery from fibrous materials [6]

form of thin fibers which dry during the trajectory toward the target or soon after reaching there. Depending on the polymer concentration, solvent type, distance between the injector needle tip, the collector (a rotating mandrel, a metallic sheet or parallel bars), and the potential applied, the fiber thickness, beading (formation of bead-like formations along the length of the fiber), and the level of sticking of the fibers to each other are determined. The fibers in a well-prepared fiber mat could be made to align parallel or stay disorganized depending on the need. The fibers could be made to be in core-shell form with the core and shell chemistries being different from each other, or a number of fibers could be co-extruded to create a multitude of fibers with different compositions or contents in a single mat. The rotating mandrel-type collector is used to prepare tubular structures such as vascular grafts, or they can be cut after preparation to create parallel fiber mats. It is reported that non-woven, nanofibrous mats were prepared by electrospinning from regenerated silk fibroin solution (e.g., in formic acid) with a large surface area for attachment and high porosity, too. In the case of treatment with methanol, the solubility of silk fibroin in aqueous media is diminished, and this increased the mechanical properties [3]. Researchers have used the electrospun fibers to prepare tissue-engineered vasculature, bone, neural guides, tendon/ligament [4], and cardiac patch [5] and also in drug delivery.

17.4.2.3 Entrapment in Membranes

In order to prepare drug-carrying membranes, the chemical properties of the drug and the membrane material are important. If they have the same type of chemistry, hydrophobicity or hydrophilicity, the drug and the polymer can be dissolved in the same solvent, and the solution is allowed to dry; this is what is called solvent casting. If they have opposite chemistries, then the approach involves suspending the

drug particles in the polymer solution and again allowing to dry gradually, leaving behind drug crystals embedded in the membrane or the film. The process could be melt pressing the polymer-drug mixture at a temperature higher than the T_g of the polymer and below the T_m of the drug. This leads to smooth-surfaced films with embedded drug crystals. The main limitation of this approach is the thermal denaturation of the drug especially if it is a macromolecule. This process could be modified to have a sandwich of the drug crystals in between two polymeric membranes and heating as above. In this approach, the drug crystals are distributed in the middle of the bilayer structure which would lead to a more controlled release than the others because of the membranous barrier between the drug particles and the medium.

17.4.2.4 Entrapment in Sponges

Sponges are highly porous thin membranes or thick structures generally with interconnected micro- or macropores. There are a variety of ways to incorporate the drug molecules in the structure of a sponge.

1. The drug could be adsorbed into the pore walls if the solvent of the drug is not a “good” solvent for the sponge material. If the solvent of the drug is a poor solvent of the polymer, there would be some swelling of the polymer which would lead to deep penetration of the drug with possible attachment onto the walls (adsorption).
2. In addition to the “ready membrane” approach, the spongy membranes could be made by lyophilization of the polymer solution in which the drug could be dissolved or suspended. The only difference would be the form of the drug; if suspended the drug would be particulate; if dissolved then it would be dissolved in the membrane material forming a solid solution.
3. One final approach would be formation of the sponge by salt leaching. In this case, the salt is generally a water-soluble small molecule, and therefore the drug and the polymer are dissolved in a hydrophobic solvent and then dried, followed by leaching of the salt by immersion of the membrane in aqueous solvent to remove the salt and leave behind a porous structure with the polymer finely integrated in the bulk.
4. Drugs can be physically entrapped within polymer shells and matrices, or they can be covalently attached to the polymer backbone [7]. Covalent attachment requires the presence or creation of functional groups on the polymeric sponge to form a covalently bonded drug which is not easily leached if the linkage is not labile. This is also somewhat risky because of the possibility that the drug might lose its activity during the binding process especially if the drug is a macromolecular one such as an enzyme or nucleic acid.

17.5 Parameters Important in Achieving Controlled Release

There are various parameters depending on the system and the type of the drug which affect the delivery of the drug to the target site with a desired dose. Some are described below.

17.5.1 The Properties of the Drug

The physicochemical properties of the drug are very influential on the drug release rate and are generally modified to control the release. These are:

- Aqueous solubility (hydrophilicity)
- pK_a (acid dissociation constant)
- Partition coefficient (ratio in oil to that in water, K_d)
- Molecular weight (Mw)

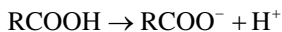
17.5.1.1 Aqueous Solubility (Hydrophilicity)

Aqueous solubility is important in the transport of the drug simply because to reach the target site the drug has to be in an aqueous environment which is determined by the route of administration. For example, intravenous (i.v.) applications introduce a drug molecule directly into the blood, and unless it is soluble in this medium, the drug would precipitate out causing problems related with the blockage of the capillaries. If the application is oral, then the drug molecule will be carried in aqueous media of different pH values and then absorbed. The drug will therefore need some water solubility for the transport. When, however, the drug molecule comes across the biological barriers which it has to cross to be distributed in the body, then the nature of the barrier, which is almost always hydrophobic, gains importance. For a hydrophilic molecule to cross a hydrophobic membrane through diffusion is very difficult unless it is transported inside using a special mechanism like active transport, carrier-mediated transport, or endocytosis. Once in the cell it has to cross another barrier to go out, to the systemic circulation.

17.5.1.2 pK_a (Acid Dissociation Constant)

Molecules like amino acids have carboxylic acid groups and amine groups (Fig. 17.6). These are generally shown as zwitterions, where both groups are ionized.

In the figure the simplest amino acid glycine is presented in its zwitterionic form. These ionizations are not always simultaneous and depend on the pH of the medium and on the acid dissociation constants of the carboxylic acid group and the ammonium group. Acid dissociation is shown below:



which is represented as:

$$K_a = \frac{[RCOO^-][H^+]}{[RCOOH]}$$

With rearrangement, the equation becomes:

Fig. 17.6 A typical amino acid molecule. R defines the nature of the amino acid

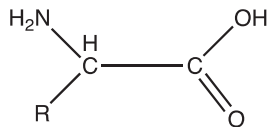
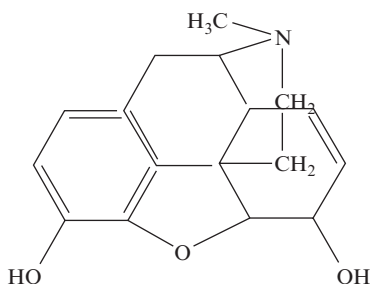


Fig. 17.7 Relation between the medium pH and the charge of the drug molecule morphine. The nitrogen is protonated at lower pHs and not protonated above indicating that its solubility would be higher when the local pH is decreased

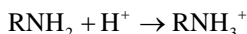


Morphine p Ka 7.9

$$pK_a = pH - \log\left(\frac{[RCOO^-]}{[RCOOH]}\right)$$

When pH and pKa are equal, the amounts of $[RCOO^-]$ and $[RCOOH]$ are also equal.

A similar equation can be written for the amine group



Glycine has a pKa of 2.34 and another of 9.60 and an isoelectric point of 5.97. This means that at pHs lower than 2.34 the molecule is positively charged, at pHs higher than 9.60 it is negatively charged, and at around pH 6 it will be neutral. For a molecule to dissolve in aqueous media such as the extracellular fluid, blood, or cytoplasm, it should be hydrophilic, and being ionic is an important contributor to this property. In the example below aspirin is mainly in ionic form at pHs above 3.5, meaning in the extracellular fluid it is highly soluble and easily be transported but it will have difficulty crossing barriers (Fig. 17.7).

17.5.1.3 Partition Coefficient

Partition coefficient actually completes the picture about water solubility and transference across barriers and transport within the body. Partition coefficient (K_d) is expressed as:

$$K_d = [C_o] / [C_w]$$

where $[C_o]$ is the concentration of the drug in oil (or organic solvents or in cell membrane) and $[C_w]$ is the concentration in an aqueous medium (like blood). For a drug both lipid solubility and water solubility are needed: the first one for crossing lipid barriers and the second for transportation throughout the body. As already shown above, the pH of the medium in which the drug is present determines its being charged or not, and this leads to an increase or decrease in the K_d ; a high K_d is preferred for easy crossing lipid barriers and a low K_d for movement within an aqueous environment or compartment.

17.5.1.4 Molecular Weight

Molecular weight or, maybe to be more accurate, molecular size is important in the transport and elimination of the drug through kidneys.

Elimination through the kidneys is simple to understand because the kidneys mainly act as a filter through which molecules with sizes smaller than that of the glomerular filter (cut off molecular weight is around 30 kDa) are filtered out. Therefore, any drug administration should avoid to send the drug to the kidneys before it has time to be systemically distributed.

For the transport the relation between size and transportation is somewhat more complicated.

Rate of transfer (v) across a membrane is presented as:

$$v = D \cdot K \cdot A \cdot [C_2 - C_1] / l$$

where D is diffusion coefficient, K is partition coefficient, and $[C_2 - C_1]$ is the concentration gradient (difference) across a thickness of l . D , the diffusion coefficient, is presented as:

$$D = \frac{kT}{6\pi\eta a}$$

where k is the Boltzmann constant, T is the temperature, η is the medium viscosity, and a is the diameter of the molecule.

Thus, as the molecular weight becomes higher (and therefore, the size becomes larger), the diffusion coefficient becomes smaller, and when D is smaller, the rate of transport across a barrier (or across a distance within which these differences are valid) is decreased. It can be seen that molecular weight and size have a significant influence on drug transport. This would reflect on the rate of release in different ways depending of the device designed. For a membrane-coated CRS device, the drug molecule has to cross a barrier, and all the equations stated above are effective. The stability of the coat (degradable or nondegradable) is another parameter because if stable, l is constant and if not it will vary. If there is no coat, then diffusion in the matrix becomes important, and these equations are also valid, but this time there is no fixed thickness membrane but a polymeric material to cross, and the distance of the drug to the exterior of the device will be important.

17.6 The Properties of the Drug Carrier

The properties of the drug carrier are also important in the design of a CRS system. Among these parameters the carrier's form, state, and type of drug-carrier interaction are important.

Carrier's form can be fibrous, particulate, or sheet type. These are discussed in another section in this chapter so state and drug-carrier interaction type will be discussed here.

The state of the biomaterial can be solid or hydrogel type. Solid drug carriers function as the membrane material or the matrix material through which the drug has to diffuse. If the solid material is hydrophobic, then solid drug hydrophobic materials will be dispersed in them as solid solution, and the release rate will depend

on the aqueous solubility of the drug, the extent the carrier can imbibe water, and the carrier's degradability or resorption. When the water molecules hydrate the matrix and dissolve the drug, the molecules need to diffuse through the polymeric chains or move through the pores available in the system. In the case when the system is not porous and there are no pore formers which dissolve to create interconnecting pores, then the release of the drug will be quite slow.

In cases where the matrix is resorbable, the drug molecules contact with water by resorption of the matrix, and release might be controlled by the rate with which the resorption takes place.

If the carrier is a hydrogel, then the carrier's bulk will be highly hydrated, and drugs with low molecular weight or high water solubility are not suitable for this kind of an application because they would be very rapidly released. If the drug molecules are of high molecular weight, then they will wiggle through the polymer chains of the hydrogel causing a delay in the release. The release rate would then depend on the molecular weight of the drug and the polymer, the cross-link density of the polymer (if it is cross-linked), its hydrophilicity/hydrophobicity and its swellability.

17.7 The Pharmacokinetics of Drug Bioavailability

Generally a constancy of the drug concentration at the target site is needed. This means the drug release rate does not change with time; in other words the rate is zero order. This is mathematically expressed as:

$$v = -dA / dt = k$$

The drug transference from inside a carrier to the outside requires diffusion of the drug molecules through membranes in the case of encapsulation between membranes or diffusion between solid particles which might be stable or eroding.

Fick's First Law states that flux of drug or its rate of release is proportional to a diffusion coefficient and the concentration difference between the present position and the "other" side. This is expressed as:

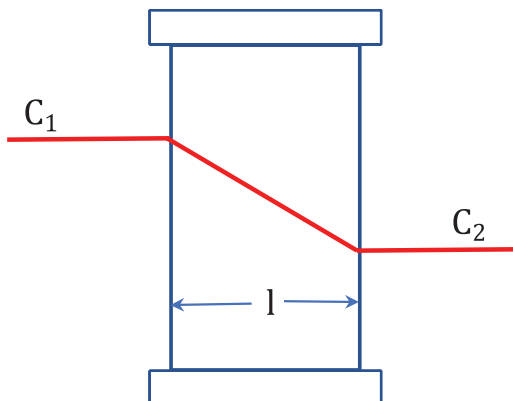
$$J = -D \cdot (dc / dx)$$

where J is the flux of drug (amount permeated per area per unit time), D is the diffusion coefficient, and dc/dx is the concentration gradient (the concentration difference between two sides of a membrane or in two neighboring chambers where dx is the separation between these two sides or the thickness of the membrane). The diffusion coefficient is expressed as:

$$D = kT / 6\Pi\eta\rho$$

where k is the Boltzmann constant, T is the temperature, η is the intrinsic viscosity, and ρ is the radius of the molecule. These give the parameters that allow a molecule to diffuse through other molecules in a solution.

Fig. 17.8 Concentration gradient across a membrane



Diffusion of molecules across membranes can be rewritten as:

$$dM / dt = A \cdot D \cdot K \cdot \Delta C / l$$

where dM/dt is the rate of permeation, A is the area through which permeation takes place, D is the diffusion coefficient, K is the partition coefficient (the ratio of the drug solubility in an oil phase to that in the water phase), ΔC is the concentration difference between the two sides or points, and l is the thickness of the membrane (Fig. 17.8).

This equation states that as the concentration of the ΔC is kept constant, then the release rate is constant because A , D , K , and l do not change with time. If through a first-order reaction the concentration of the drug leaves the CRS and the difference in its concentration between the inside of the CRS and the outside gets smaller ($C_1 - C_2$ gets smaller), then the rate of release gets lower. Thus, the main thing to achieve if one needs a zero-order release or controlled release is that the ΔC must be kept constant. This can be achieved if the high concentration side has constant concentration, and this is normally achieved by a saturated solution.

For a drug with a very short half-life, the rate of release should be quite high. On the other hand, controlled release systems are not suitable for drugs with long biological half-lives. These drugs are available in the plasma for long periods, and their concentration gradient (ΔC) will get smaller, so they will be effective for a longer duration than planned, and they will practically achieve an undesirable prolonged or sustained release.

17.7.1 Higuchi Equation

Higuchi equation is developed because the explanations above are very simplistic because the release from matrices depends on the tortuosity of the route, and the carrier might be eroding. There are also assumptions such as that the steady state is accomplished during release, there is excess solute drug that is present ($A \gg C_s$), the

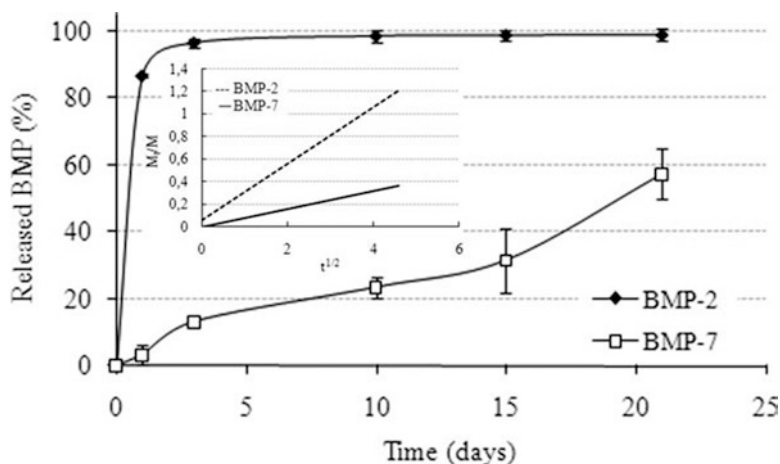


Fig. 17.9 Bone morphogenetic protein (BMP) release from polymeric nanocapsules. BMP-2 from PLGA nanocapsules and BMP-7 from PHBV (inset graph presents the kinetic analysis of BMP release according to Higuchi diffusion model). BMP-7 release fits the Higuchi model best [8]

drug particles are smaller than those of the matrix, the diffusion coefficient stays constant, and no interaction takes place between the drug and the matrix. In order to consider all these parameters, a practical equation has been proposed by Higuchi which is reported to be valid during the release of the initial 40% of the drug in the carrier (Fig. 17.9). For practical purposes it is used as $M_t/M_o = kt^{1/2}$.

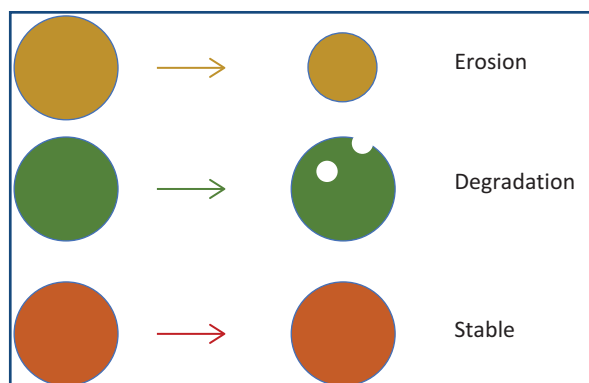
17.8 Classification of CRS Systems

Controlled release systems can be classified in a number of ways such as stability, shape, and responsiveness. These are parameters that affect the release kinetics significantly and can therefore be used to achieve the desired level of control and dose at the target site.

17.8.1 Stability Related Classification: Erodible and Nonerodible Systems

Erodibility is about the disappearance of the CRS in time. This could be achieved by two main routes: chemical change of the drug carrier by a route such as hydrolysis of the polymer backbone or its dissolution. Hydrolysis of a backbone is also called degradation because the molecule is changed irreversibly into a shorter or a different molecule (the side chains could be modified). Dissolution happens by the polymer carrier dissolving in the medium and disappearing like a hard candy in the mouth without being modified. In this case, no chemical change takes place; it is

Fig. 17.10 Difference between erosion and surface degradation versus bulk degradation



only a form change (Fig. 17.10). If the dissolution rate is high, then a CRS cannot be formed; therefore, the dissolution is slowed down by either modifying the polymer chemically or by modifying its molecular weight (e.g. increasing). If the system is erodible, then it complicates the release kinetics of the systems which would normally release according to Higuchi or zero order.

There is another category of low stability systems where none of the above mentioned instabilities is valid. This is observed in liposomal drug delivery systems where the vesicular carrier is held together by self-assembly of phospholipids which have polar heads and nonpolar tails. These carriers are advantageous for several reasons, but their instability is a major problem against their widespread use. Local temperature and the medium in which they are placed lead to their disintegration with time. In addition, they can be made to disintegrate on demand or by the environmental conditions before their normal time by incorporation of pH or light- or heat-responsive molecules into the liposomal structure. For example, they can be made pH responsive by incorporation of molecules that can protonate at the pH of the lysosomes, or by local application of heat to raise the temperature above their critical temperature. This would lead to the release of their content at sites where the temperature is locally high.

17.8.2 Shape-Related Classification

This group releases its content depending on their shape. The release system could be spherical (capsule or sphere), sheet, or fibrous. These forms and their sizes are determinants of the release.

17.8.2.1 Spherical Devices (Capsules and Spheres)

The main difference between microspheres and microcapsules is that the capsules have a coat around a hollow or solid core. The spheres have a matrix material such as an inert polymer that serves to obstruct the release of the drug which is evenly distributed throughout the sphere. As the drug on the surface is depleted, the drug

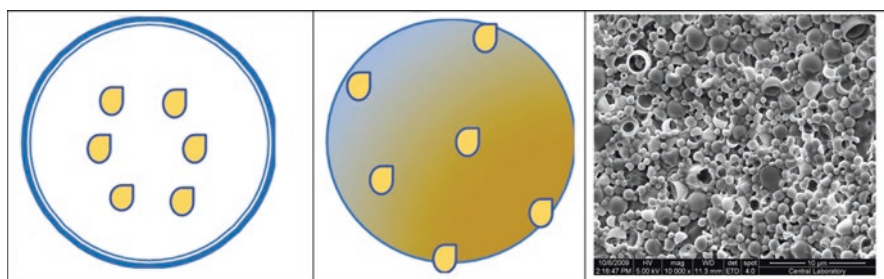


Fig. 17.11 Spherical drug delivery systems. (a) Micro- or nanocapsule with drug particles in the core, (b) micro- or nanosphere with drug particles in the bulk, (c) SEM of polymeric microcapsules [9]

molecules buried deeper in the structure are released, but since the distance to the surface gets longer, the route is more tortuous and the surface area of the inner drug-laden region is smaller, and the rate of release decreases with time.

In the case of the microcapsules, the drug can be in the core or on the membrane that forms the surface (Fig. 17.11). As long as there is a low-solubility drug in the core and the core is saturated with the drug, the inner concentration stays the same, while on the outside, the drug which has been released will be washed away by the body fluids so the outside concentration will be low. Thus, a concentration difference and the rate will be maintained and so will the rate of release.

17.8.2.2 Sheet Devices

These devices are basically laminates either carrying the drug evenly distributed in their internal structure, and there are layers above and below to protect the core, to control the rate, and maybe to help adhere to the tissues such as the skin (Fig. 17.12). For example, transdermal drug release systems are built like this. As explained earlier, if there are protective/rate-controlling membranes, then the rate of release is zero order for most of the release period. Meanwhile, the single-layer CRS will release by simple diffusion based on Higuchi kinetics and provide sustained release (gradually decreasing) rather than zero-order (constant in time) release.

17.8.2.3 Fibrous Devices

Fibrous devices are less frequently used. Still, antibiotic releasing sutures, drug releasing dental fibers, growth factor releasing vascular grafts, and nerve guides are among the few to be discussed. They are produced like any textile material, as is done recently, by electrospinning, using classical materials such as synthetic polymers (e.g., nylon) or using materials obtained from natural resources such as silk fibroin which is isolated and purified from silk worm *Bombyx mori* cocoons and then processed into fibers. The drug can be introduced to the fibers during or after processing. For example, electrospinning of fibers could achieve core-shell-type products where the drug is in the core and the carrier polymer forms the coat preventing the instant solubilization of the drug. When the drug is blended with the

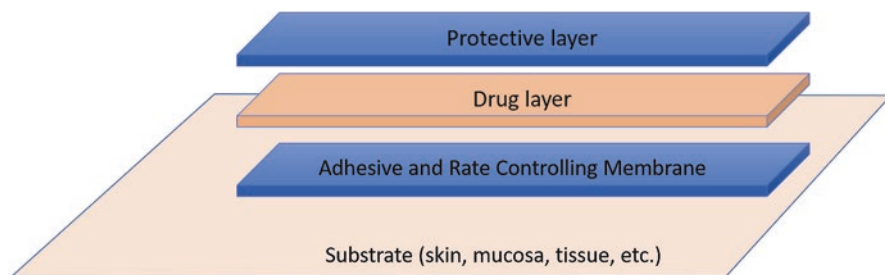


Fig. 17.12 Drug delivery system in multilayer sheet form

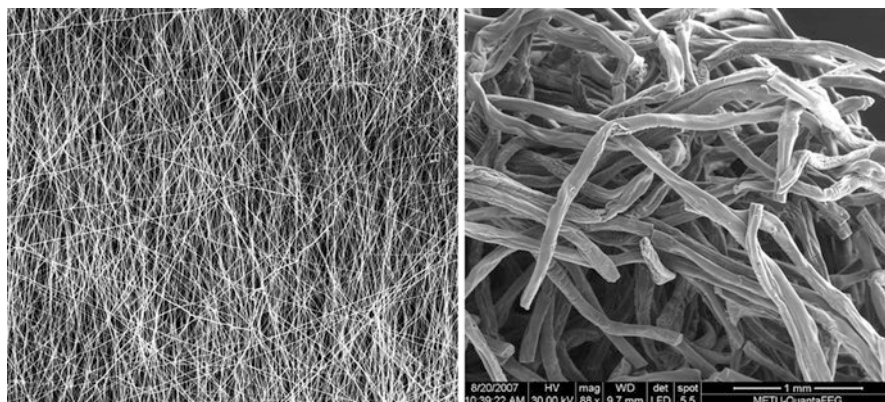


Fig. 17.13 Electrospun and wet spun fibrillar biomaterials

polymer during the making of the fiber, the drug release is more like by diffusion through the polymeric matrix via the Higuchi mechanism. In a third approach, the drug could be absorbed by the fiber after production. This leads to higher rates of release of the drug with release rate mimicking that of absorption (Fig. 17.13).

17.9 Responsiveness Related Classification

All the release systems mentioned above are expected to deliver their contents continuously and mostly at a decreasing rate. However, the need for medicine might not always be continuous, and then we might wish to deliver them on demand, for example, introduction of insulin for the diabetes patients after a meal when the blood glucose level is higher than appropriate for the patient. The researchers might also wish to reduce the side effects of the systemically introduced drugs to healthy cells away from the targeted and diseased tissue. Then the patient might be given the opportunity to use it in on-demand fashion. The best known examples are the pH, temperature, and photoresponsive systems also called the intelligent systems.

17.9.1 pH-Responsive Systems

These systems release their contents at sites where the pH protonates or deprotonates the carrier, and it becomes more permeable at this target site. For example, a carboxylic acid carrying drug carrier loses its hydrogen at high pHs and becomes ionized. The ionized form is more hydrophilic, imbibes more water, and releases more. A similar behavior is observed with the amine-containing molecules which get protonated and ionized at low pHs. The pH of the lysosomes is known to be low and this is also true for the environment of solid tumors. So if the carrier has amine groups, then release at these pHs will be higher, or at the normal pH of healthy tissues, the release rate will be much smaller sparing the untargeted, healthy tissue.

In a typical application, doxorubicin was used as the anticancer agent, which is also known for its cardio- and nephrotoxicity. The researchers prepared a folate-bovine serum albumin (BSA)-cis-aconitic anhydride-doxorubicin where folic acid served as the tumor-targeting agent, BSA improved the water solubility, and doxorubicin was attached through cis-aconitic anhydride, a molecule which hydrolyzes at low pHs such as that of lysosomes in the cell cytoplasm. Thus, this folate-targeted system also carried a labile linkage for the anticancer drug to release it when taken by endocytosis into the cells, transferred to the lysosomes where it found the low pH needed for doxorubicin release. They observed that this prodrug (due to doxorubicin being released after hydrolysis) was rapidly released when the pH was 5.5 or lower while it was stable when it was in the neutral or alkaline range.

17.9.2 Temperature-Responsive Systems

Temperature difference to trigger such on demand release is generally initiated externally since temperature differences between the body parts do not exist. This property is especially observed with carriers which have low melting temperatures, somewhat higher than the physiological temperature. The main cause of the observed phenomenon is that below the critical point, the groups in the molecule energetically prefer to make bonds (H bonds) with the solvent. Once the temperature is raised above the critical temperature, the bonds between the solvent and the molecule are broken and instead intra- and intermolecular interactions take place leading to precipitation of the molecule or to the shrinkage of a gel. Thus, a hydrogel swollen at room temperature could shrink when introduced to the body (Fig. 17.14). The systemically applied carriers are distributed throughout the body, but externally warming the target sites makes them release their contents especially at these heated sites because they lose their integrity.

17.9.3 Photoresponsive Systems

Photoresponsiveness is similar to temperature responsiveness, and in these applications, carriers which are photosensitive, those that lose their integrity when exposed

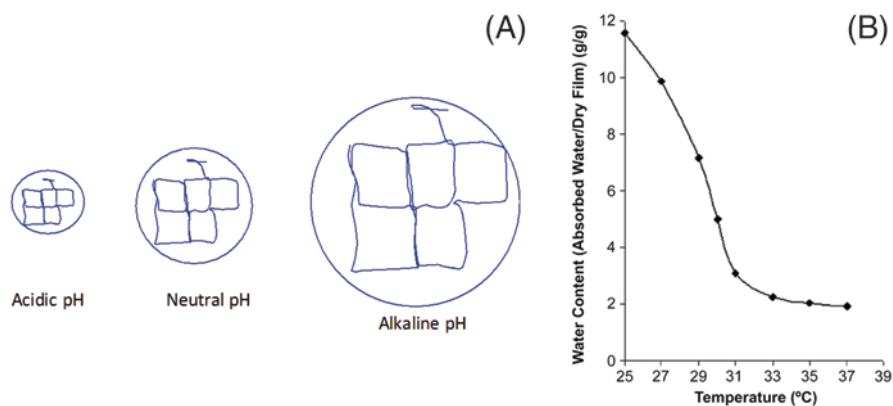


Fig. 17.14 pH- and temperature-responsive systems. (a) pH responsiveness, (b) water content decrease of NIPAM with increase in temperature for the LCST systems

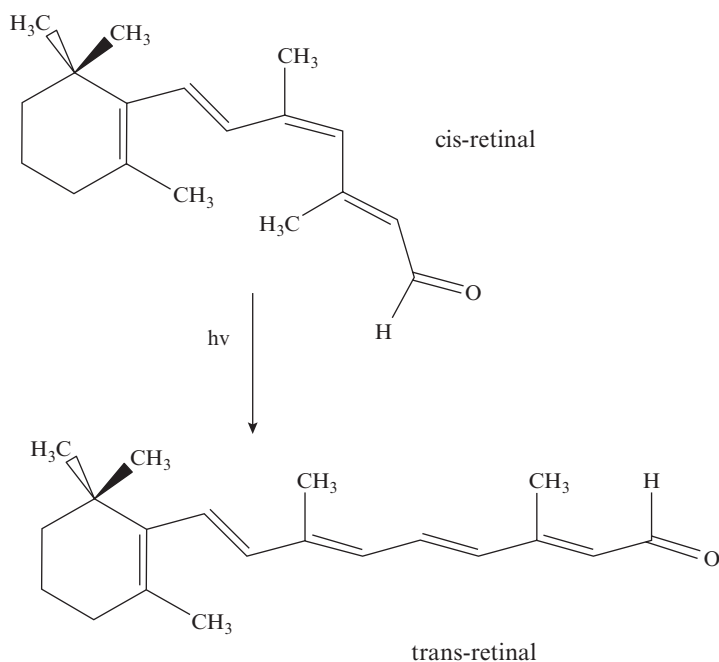


Fig. 17.15 Photoresponsiveness: conversion of cis-retinal to trans-retinal upon exposure to light

to a light of certain intensity and wavelength are flashed onto them, release their contents. The release at the target site due the exposure to light at that site ensures higher drug concentration at the target site thus decreasing the side effects, even though the drug carrier is distributed evenly throughout the body.

As shown in Fig. 17.15, cis-retinal is converted to its trans form upon exposure to light wave in the visible range. When this molecule is entrapped in an easily

deformable carrier like a liposome membrane, then this change of form would destabilize the membrane and increase permeability of the contents such as drugs.

17.10 Targeted Delivery

Almost all the drug administration applications introduce the drug to the body at various rates, but the effect is in most cases systemic, except may be the topical applications, the drug applied onto the skin. Researchers aim to localize the bioactive agent at the site of the disease [11]. Since the drugs are active molecules, their bioactivity could be harmful on the healthy tissues which receive the drug as a result of its systemic distribution. Targeted drug delivery systems are designed to deliver their payload directly to the desired site of action. This is especially important for anticancer drugs which aim to kill the cells into which they are taken up. Targeting can be achieved by attaching the drug to a molecule which is selectively taken up into the cells of the target tissue or by using the properties of the drug carrier, such as chemistry and size.

Figure 17.16 presents a typical drug attached to a polymer and also to targeting moieties. The purpose of using the targeting group is to either achieve attachment to a specific tissue where the cells express certain proteins on their membrane specific to the target cells.

In a liposome-based study to prevent systemic toxicity, an anticancer drug-carrying liposome was conjugated with a RNA aptamer specific to the prostate-specific membrane antigen (PSMA) expressed on the surface of prostate cancer cells [12]. Liposomes carried the anticancer drug doxorubicin (Dox) and were more toxic to the targeted cancer cells than to nontargeted cancer cells. Dox-carrying liposomes administered to xenograft nude mice were selectively retained in the tumor tissue indicating the effectiveness of the cell membrane binding by the conjugated liposomes.

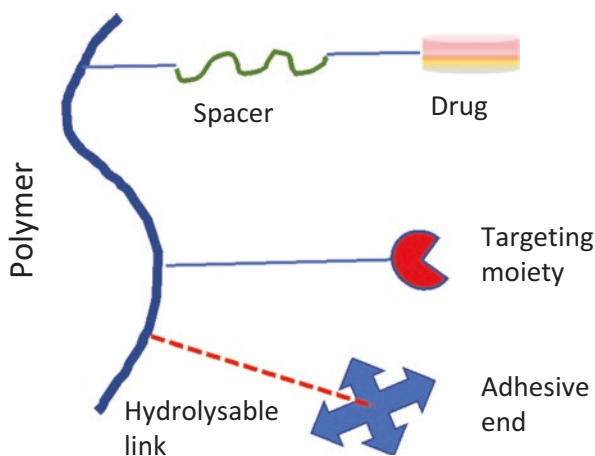


Fig. 17.16 A targeted polymer-drug conjugate

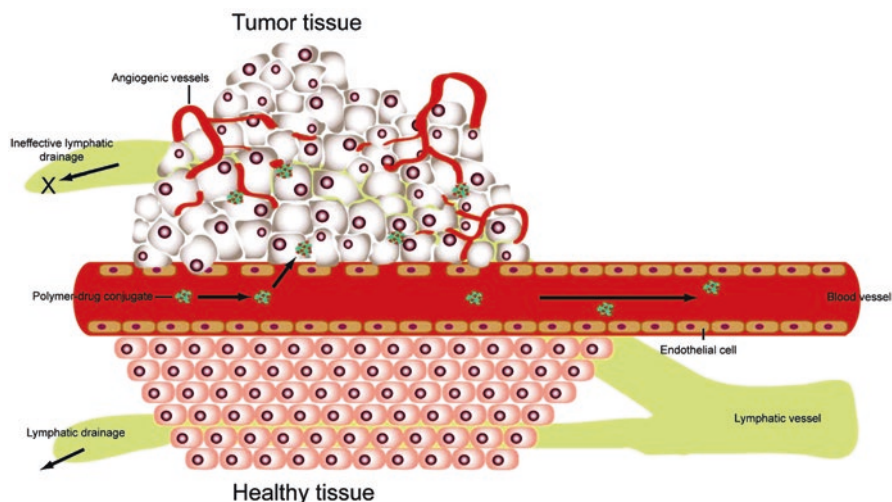


Fig. 17.17 Targeting through EPR effect [14]

Another advantage of using a targeting moiety is that they can be preferentially transported into the cell and even into the nucleus. This can be achieved by encapsulation of the drug in carriers with appropriate surface chemistry. Related with this is the chemistry of the drug molecule which also is important in restricting the regions in which the drug is soluble. For example, a hydrophobic drug spends long periods if injected in the lipid tissue. These molecules can penetrate membranes with ease, but due to their low solubility in the aqueous compartments, they cannot easily diffuse in such media. Niosomes were tested to enhance the *ex vivo* percutaneous penetration of diclofenac sodium, and the permeation of free diclofenac was less than that from niosomal formulations, implying that niosomes acted as enhancers of penetration [13].

Size is effectively used in the recent years for the targeting of drug molecules in cancer tissues. Taking advantage of the EPR (enhanced permeation and retention) effect, drug carriers with low nano size (ca. 100 nm or less) can leak out of the disorganized vasculature at the cancer site (Fig. 17.17). Since the vasculatures at the healthy sites have tight cell-to-cell contact at the capillaries, the drug-carrying nanoparticles are delivered/targeted to the cancer tissue. This is a passive targeting approach in contrast to active targeting above.

17.11 Conclusion

In this chapter, the routes of drug administration, the forms of the drug carriers, and the various parameters that influence drug loading and release were discussed. Delivery can be achieved via oral, intravenous, intramuscular, intrathecal, intraperitoneal, subcutaneous, transdermal routes, and through the use of controlled release systems. Design of targeted delivery systems to specific sites, intelligent carriers, and the kinetics involved in these delivery systems were covered.

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18.1 Important Concepts: Development of Tissue Engineering and Regenerative Medicine

Biomaterials and biomedical devices can be constructed of a variety of materials, and depending on the end use, incorporation of bioactive species such as drugs, enzymes, growth factors, and other molecules is possible. Until the last 15 years, a complete biological entity such as a cell was not incorporated into the biomedical devices. Most of these devices were generally expected and designed to be stable, to have service lives long enough to serve as long as the host lived, except for a few cases such as resorbable sutures and short-duration implants. However, the thought of biodegradable cell-seeded devices that would completely integrate with the biological system during the wound healing process was very appealing because these implants were to be designed to blend with the tissues in the body, and this would be a cure and would not leave behind any traces after a certain implantation period. As a result of these important advantages, this approach became a very appealing solution for many problems arising from the long-term implantation of durable materials. This new field, now called “tissue engineering,” is supported by a number of interdisciplinary fields (Fig. 18.1). The main components of tissue engineering are a scaffold or a cell carrier, mature or stem cells, and bioactive molecules such as growth factors (Fig. 18.2). Meanwhile cell therapies were introduced into the field of novel therapeutic tools where the main difference from tissue engineering was the absence of the scaffold. Over time these two fields together started to be called regenerative medicine.

18.2 Definition of Tissue Engineering

Tissue engineering has been defined in a variety of ways by many authors and international institutions. The pioneers of the field, Robert Langer and Charles Vacanti, have defined it in 1993 as “an interdisciplinary field that aims at the development of

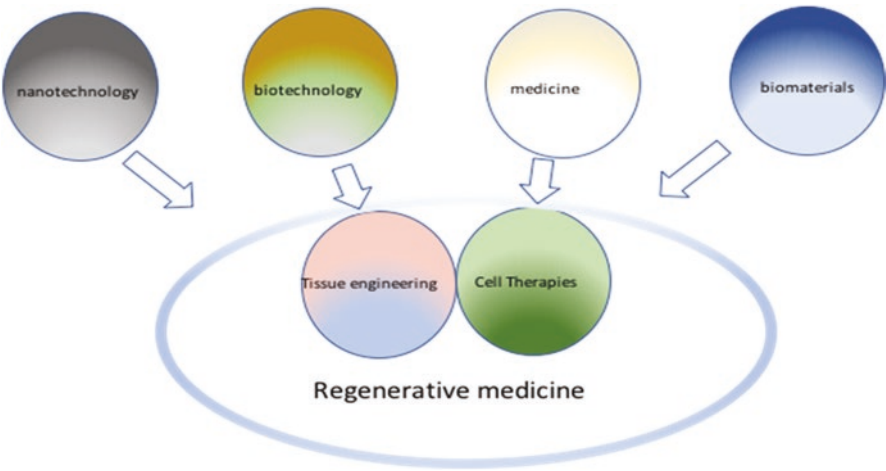


Fig. 18.1 A scheme showing the relation between tissue engineering, regenerative medicine, and the contributing fields

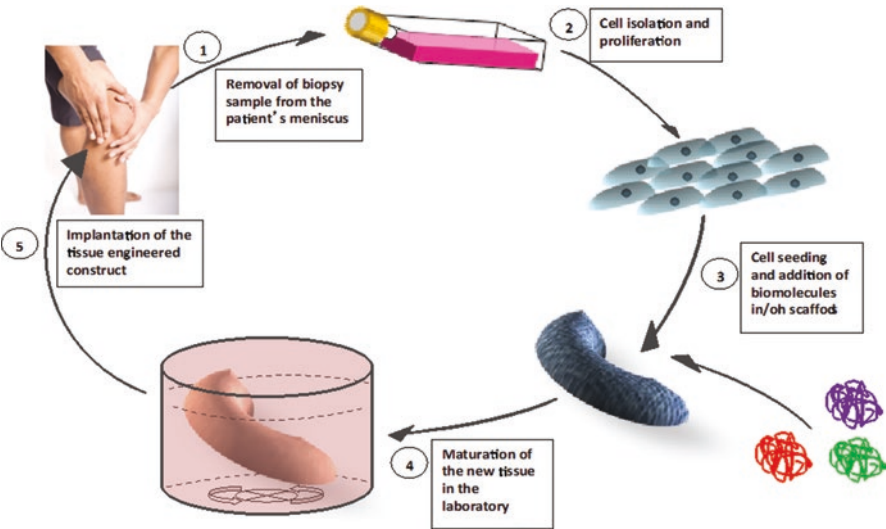


Fig. 18.2 The scheme for tissue engineering of meniscus [1]

biological substitutes that can be used to replace, restore or improve tissue function” [2]. According to a report by the National Science Foundation (NSF, USA), the term “tissue engineering” was coined at an NSF-sponsored meeting in 1987, and at a later NSF-sponsored workshop, tissue engineering was defined as “...the application of principles and methods of engineering and life sciences toward fundamental

understanding and development of biological substitutes to restore, maintain and improve [human] tissue functions.” NSF [3] states that this definition was intended to include procedures where the biological substitutes are cells or combinations of different cells that may be implanted on a scaffold made of natural polymers or synthetic, biocompatible polymers to form a tissue.

ASTM came up with a range of definitions in the field (Designation: F2312–11, Standard Terminology Relating to Tissue Engineered Medical Products) and defined tissue engineering as “the application, in vivo and in vitro, of scientific principles and technologies to form tissue engineered medical products (TEMPs) used for medical treatments and diagnoses as diagnostics”. The tissue-engineered medical product (TEMP) is “a medical product that repairs, modifies or regenerates the recipient’s cells, tissues, and organs or their structure and function, or both.” Their regenerative medicine definition is “a branch of medical science that applies the principles of regenerative biology to specifically restore or recreate the structure and function of human cells, tissues, and organs that do not adequately regenerate.” As can be seen from the definitions, there are differences in the goals: inclusion of diagnostics is one, and fundamental understanding is the other. On the whole, however, the biomaterials referred to in these definitions are different than the classical biomaterials in the sense that they include cells and their goal is to heal the tissue and not to simply replace.

18.3 Components of Tissue Engineering

In order to achieve the goals stated above, a tissue-engineered construct or tissue-engineered medical product can only be prepared if the following three components are available (Fig. 18.3):

1. A scaffold or a cell carrier to house the cells and serve as their microenvironment
2. Appropriate cells to fill the empty scaffold and convert it into the target tissue
3. Certain bioactive compounds (growth factors) to guide the cells in their attachment to the scaffold or during their proliferation and differentiation.

Once this construct is prepared, it is either immediately implanted or allowed to mature in the lab for several days or weeks before implantation into the patient.

18.4 Scaffolds

An ideal tissue repair environment is created by reproducing the intrinsic properties of the natural tissue or the autografts. Scaffolds are needed for the cells to attach and then to serve as their microenvironment, and their form (fibrous, foam, with and without chemical and physical surface decorations) and chemistry (hydrophobic,

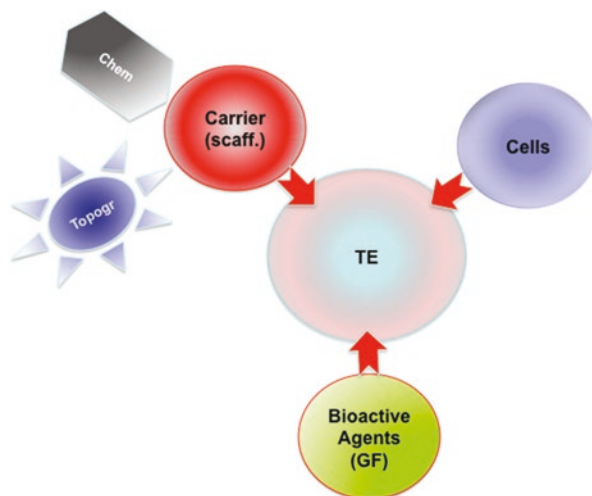


Fig. 18.3 Components of tissue engineering

hydrophilic, carrying certain functional groups) are of utmost importance. A scaffold is expected to possess certain properties:

- The scaffolds are 3D structures; they have to be highly porous to allow penetration of cells, culture medium, growth factors, and other compounds into the scaffold body and the metabolic waste products to move out.
- The pores have to be of an optimum size (generally 200–300 μm wide) and interconnected to allow complete population by the cells.
- They have to be resorbed in a time span paralleling that of healing (Fig. 18.4).
- The scaffold surface chemistry should be suitable for cell adhesion.

They are required to be removed by the time the tissue heals; in other words, the two processes progress simultaneously in harmony as shown in Fig. 18.4. It is essential that the implant properties (mechanical, physical, chemical) are maintained until the healing tissue is capable to fully take over.

18.4.1 Scaffold Forms

The form of the scaffold is one of the most debated issues in the tissue engineering circles. Some researchers argue that the scaffold should not be anything more than a sponge or foam to allow the cells to modify it as they please, while others design the scaffolds meticulously to guide the cells toward forming the target tissue which

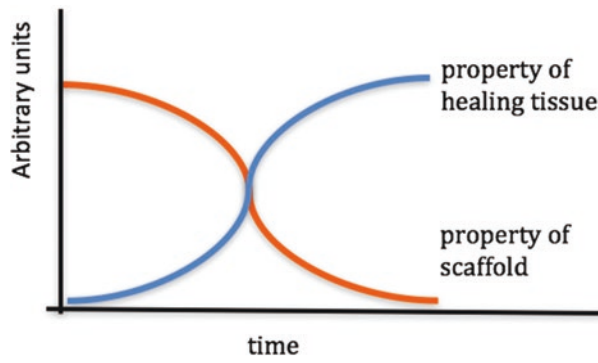


Fig. 18.4 Tissue healing and scaffold resorption relation

(A)	(B)	(C)	(D)	(E)	(F)
Rapid prototyped	Wet spun	Lyophilized sponge	Fibrous	Lamellar	Channel

Fig. 18.5 Various 3D macroporous scaffold types. (a) Rapid prototyped (additive manufactured) [4], (b) wet spun [5], (c) lyophilized sponge [6], (d) fibrous [7], (e) lamellar [8], (f) channel [9]

might have special anisotropic organizations such as muscles, nerves, etc. The main types of forms are:

- Macroporous, foam or sponge
- Fibrous, random or oriented
- Lamellar or filmlike, with or without patterns or designs

Some examples of these scaffold categories are presented in Fig.18.5.

18.4.1.1 Macroporous Foam Scaffolds

These are the simplest to manufacture and require very simple or no equipment to make them. Simply layering a bottom of a Petri dish with water-soluble crystals of compounds like sucrose or sodium chloride, pouring a polymer solution (with an organic solvent) to cover the crystals, drying in air, and washing in water remove the crystals leaving behind a macroporous sponge (Fig. 18.6). The dimensions of the macropores are defined by the crystal dimensions. Foam- or sponge-type scaffolds can also be made by lyophilization or freeze-drying producing a macroporous

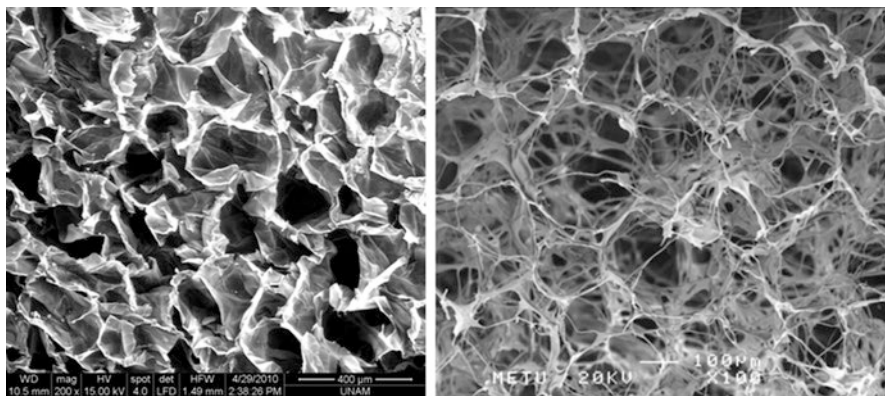


Fig. 18.6 Sponge structure showing interconnected pores with nonuniform but appropriate size for cell penetration [6, 9]

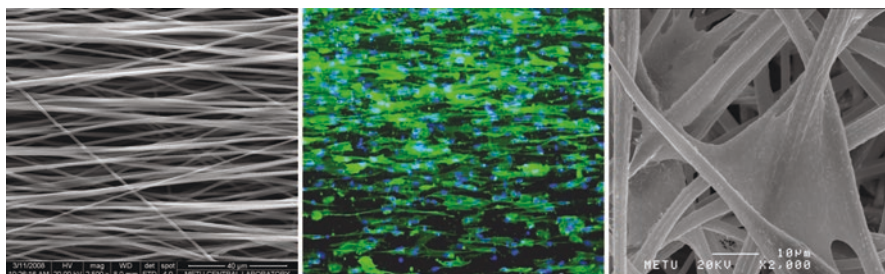


Fig. 18.7 Cells on micro- and nanofibers. (a) SEM showing highly aligned nanofibers, (b) confocal micrograph showing cells adhered and aligned on these fibers, and (c) SEM of cells adhered on random fibers [7, 10]

structure where the pore size distribution is controlled by the rate of freezing (freezing temperature), the solvent used to dissolve the polymer in, and the polymer concentration. They have mostly irregular-shaped pores with a size in the range 100–400 μm that are interconnected. Interconnectivity is needed to allow for cell penetration and population of the scaffold.

Porosity is also essential for the transport of nutrients and metabolic wastes. Thus, a minimum pore size of 10–20 μm and pore interconnectivity are essential for transport purposes. Identical-sized pores can also be made by using special techniques. Sponges are most suitable for producing 3D tissue substitutes such as the skin, bone, cartilage, and meniscus because it is difficult to build sufficiently thick structures with other approaches such as fibers or multilayers.

18.4.1.2 Fibrous Scaffolds

Fibrous scaffolds can be made by a variety of methods with random or precisely controlled thicknesses, orientations, and porosities. These scaffolds are more porous

than the foams (Fig. 18.7). In this case there are no well-defined pores with measurable diameters but rather gaps between fibers. In fibrous scaffolds important parameters and properties that influence the performance of the scaffold are the thickness, the size of the gaps, and orientation of the fibers with respect to each other.

Also important is the relative of orientation of layers with respect to each other if a multilayer structure is controllably made. Fluid flow through these scaffolds is relatively easy, but the fiber thicknesses are critical especially if sizes smaller than that of the cells are being used. When the sizes are at nano levels, then the cells cannot balance themselves on a single fiber, and they attach to a few. If the diameters of the fibers are about the size of the cells, then the curvature of the surface becomes an issue for the cells. When the alignments of cells are required, then fibers with thicknesses much larger than the diameter of the cells are not very effective; fibers similar in dimension to the cells are much better. One major property of the fibers is that if they are at nano level generally the gaps between the individual fibers are so small that penetration of the cells below the surface is generally not possible. This could be advantageous or disadvantageous depending on the aim.

18.4.1.3 Lamellar Scaffolds

Two-dimensional (2D) scaffolds are not preferred for applications with the belief that they cannot mimic the microenvironment of the 3D tissue. They are, however, very useful in carrying out preliminary studies on cell adhesion, proliferation, differentiation, study of parameters, and microscopic investigation and mechanical testing (tensile). They are also very useful when rolled into tubes (if the target tissue is tubular) or stacked into multilayer structures to create a 3D form. This approach allows the individual layers to carry intricate patterns or designs on them. It also enables them to mimic the tissue organization better especially when the orientation of various layers of a tissue (such as the blood vessels or skin) is different and when they house different cells at each layer (Fig. 18.8). In such a case, seeding the individual layers with cells and then combining them in a 3D construct can be very informative even if not applicable in the clinic. It is possible to examine individual layers after the experiment by simply unrolling or unstacking the construct. Thus, they are more convenient for basic studies about cell-material interactions.

18.4.2 The Scaffold Material

As any biomaterial, the scaffold material can be of synthetic or biological origin with the limitation that it should mainly be polymeric because resorbability is essential for a successful tissue engineering application. The synthetic polymers used are generally condensation polymers such as polyesters (polylactides (PLA), polyhydroxyalkanoates (PHA), polyaminoacids, polyamides, and polyurethanes. Biological polymers are also quite frequently used in tissue engineering. Among these are mainly polypeptides (collagen, gelatin, silk fibroin) and polysaccharides (chitosan, cellulose, hyaluronan) (Fig. 18.9). Although quite rarely, the scaffold material could be of ceramic origin (e.g., sintered bioglass powder [12]). The reason

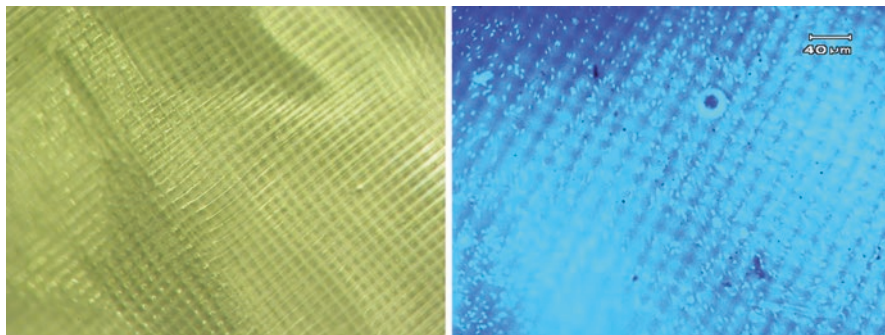


Fig. 18.8 Multilayers of lamellae (a) before and (b) after cell seeding [11]

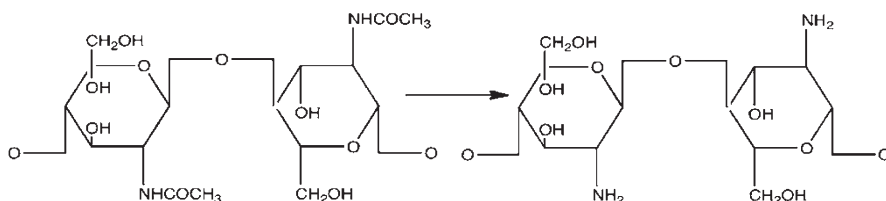


Fig. 18.9 Chitosan, a frequently used scaffold material, is obtained by deacetylation of the natural polymer chitin found in the shells of the crustaceans (e.g., crabs, lobsters, and shrimps)

for the scarcity of ceramics as scaffolds is that tissue engineering in principle prefers full replacement of the construct; however, there is difficulty in the processing of the ceramic materials into a relatively rapidly resorbing material. This still has not prevented researchers from designing ceramic-based tissue engineering scaffolds especially when maintenance of mechanical strength without a gradual decrease and without degradation products is essential. A typical recent application is a trachea substitute which necessitated the use of a strong and stable tubular form that would not collapse. An intermediate solution is the use of composites, mainly those formed with ceramics (e.g., hydroxyapatite (HAp) and tricalcium phosphate (TCP)) with polymers (e.g., PLLA, PCL).

Even though rarely, biodegradable metallic implants are also reported as scaffolds for tissue engineering of hard tissues. The authors report that in vitro cultures show that these scaffolds have good cytocompatibility, allow osteoblastic differentiation and in vivo exhibit acceptable inflammatory responses, and are almost fully degradable [13].

The synthetic polymeric material is either synthesized from its starting compounds (e.g., monomers) to prepare a material suitable to be processed into a scaffold or it could be commercially obtained and used with or without chemical modification. A typical example of the second category is chitosan, a water-soluble biopolymer obtained from the partial hydrolysis of chitin, a material abundantly found in the shells of crustaceans like shellfish and also produced by *Aspergillus niger*.

Biological source of polymers for tissue engineering could be microbial (bacterial polyesters, recombinant collagen), plant (potato starch, wood cellulose), or animal and insect (silk fibroin from silkworms or spiders, collagen from Achilles tendon or rat tail or cow hide, hyaluronic acid and chondroitin sulfate from rooster comb and shark fin) (Fig. 18.10).

Cellulose is an abundantly available polysaccharide which can be used as is or after modification. Silk fibroin is another biological polymer produced mainly by the silkworm *Bombyx mori* (and also by spiders) and constitutes 67% of silk. Fibroin is obtained after removal of a glycoprotein and an integral polypeptide coating the whole structure, sericin, which has a high content of amino acid serine (Fig. 18.10). Fibroin consists of a heavy (H) and a light (L) chain linked together by a disulfide bond.

Polyhydroxyalkanoates (PHA) are polyesters formed as polymers or copolymers of various hydroxyalkanoates that are synthesized by many gram-positive and gram-negative bacteria from many different genera, the major source of the commercially used PHA is *Alcaligenes latus* and *Alcaligenes eutropha*. These polymers are accumulated intracellularly under conditions of nutrient stress, constitute about 90% of the cell dry weight, and serve as a carbon and energy reserve [14].

Another source of scaffolds is the tissues themselves. Tissues are processed to remove cells (decellularized) or certain components such as minerals (HAp or calcium phosphates) or proteins and fats, and the remaining structure is used as scaffolds to seed cells. A good example of that is decellularized bone which retains all the intricate architecture of the bone without the organic components [15]. Similarly, the skin is used in its decellularized form to construct a skin substitute [16]. This approach allows the cells of the donor to populate the biological origin substrate which carries the appropriate architecture and therefore makes a more suitable implant than artificially designed. However, donor scarcity, tissue availability, and cell penetration into the whole structure are some of the main disadvantages.

18.4.3 The Scaffold Chemistry

Scaffold chemistry is another important issue. The parameters to be considered are the presence and kind of electrical charge, hydrophilicity, and the presence of biological moieties.

Charge is important for cell-material interactions. It has been found that cells prefer to adhere to surfaces with a contact angle of about 60°, and this requires the presence of charges or highly polar groups on the material surface. Cell adhesion studies were carried out using human MG63 osteosarcoma cells and silicone surfaces, and optimum cell adhesion was observed at 64° [17]. A similar result was reported with NIH/3T3 fibroblast cells proliferated best on oxygen plasma-treated, low-density polyethylene (LDPE) with a surface contact angle of 60° [18]. They recommended surfaces with a water contact angle of 50–60° for cell adhesion and growth. Obviously a factor which complicates the conclusion of what is best surface contact angle is the surface roughness. Which is reported to decrease the contact angle and increase cell adhesion, but not due to biochemical interactions.

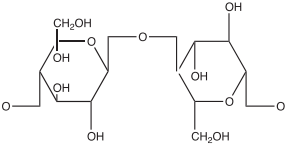
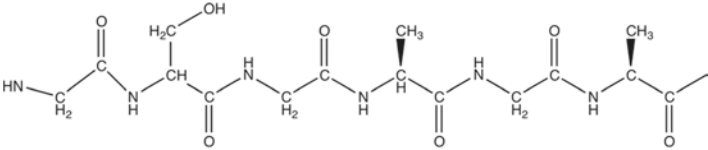
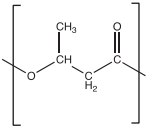
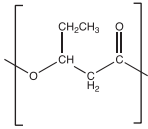
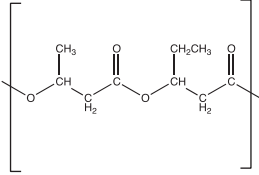
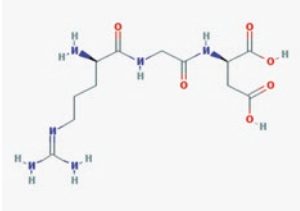
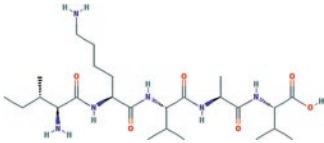
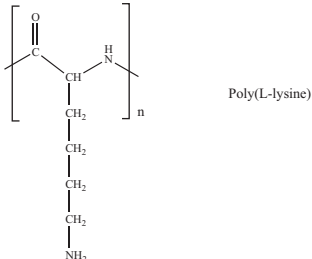
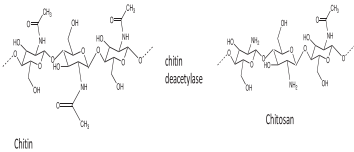
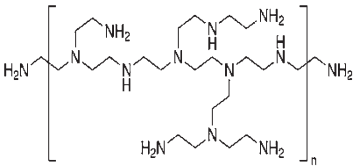
A 	Cellulose
B  Gly Ser Gly Ala Gly Ala	Silk fibroin
C 	P3HB
D 	P3HV
E 	PHBV

Fig. 18.10 Chemical formulae of various biopolymers. (a) cellulose, (b) silk fibroin, (c) poly(3-hydroxybutyrate) (PHB), (d) poly(3-hydroxyvalerate) (PHV), (e) poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)

Table 18.1 Cell adhesive molecules or moieties

Name	Amino acid sequence	Molecular structure	Specificity
Arginyl-glycylaspartic acid	RGD H-Arg-Gly-Asp-OH		RGD sequence can bind to multiple integrin species
IKVAV	IKVAV Ile-Lys-Val-Ala-Val		Cell adhesion, neurite outgrowth
Poly(L-lysine)		 Poly(L-lysine)	Cell adhesion
Chitosan			Cell adhesion due to positive charge
PEI			Cell adhesion due to positive charge

These indicate that fully hydrated surfaces like that of a hydrogel of alginic acid, hyaluronic acid, or chondroitin sulfate are not suitable for cell attachment. Since positive or negative charges like those in the above mentioned molecules or high polarity like in polyethylene glycol (PEG) make materials very hydrophilic, they are not highly recommended in the production of cell adhesive surfaces. Table 18.1 shows some cell adhesive molecules or moieties which can be added on the surfaces of the scaffolds to enhance interaction of the cells with the material.

The type of charge is also critical as it is known that a typical animal cell or nuclear membrane has an overall negative charge under physiological conditions. Thus, if a scaffold is needed to be populated by cells, it is not advisable to make it from a cell-repellent material, a molecule with negative charge. It is, therefore, quite understandable that poly(L-lysine) (PLL), a positively charged molecule under physiological conditions, is very well liked (adhered to) by the cells and therefore used to treat cell culture plates for better adhesion. Chitosan, which also is positively charged under the physiological conditions, is also well adhered by cells; this attraction by chitosan and PLL may be too much that it is reported to have a toxic effect. It is sometimes not the charge presence but instead charges in a given order could be beneficial for cell adhesion. The presence of molecules or groups on the surface of a scaffold is another way to make the scaffolds suitable for cell attachment. The main approach in selecting such groups is to try to mimic the extracellular matrix (ECM) which constitutes the microenvironment that the cells are attached to. This can be achieved by attaching fibronectin, a molecule found in the ECM and binds to cell membrane through its integrins, onto the scaffold material using a variety of transient (adsorption on the surface) or permanent (covalent bonding) linkages. There are also well-known amino acid sequences which have affinity for certain cell types. For example, it is reported that proteins that contain the Arg-Gly-Asp (RGD) attachment amino acid sequence constitute a major recognition site for cell adhesion, and this sequence appears to be recognized by nearly half of the over 20 known integrins, the proteins cells use to attach to their environments. Incorporation of such a molecule onto a scaffold composition definitely makes it attractive for the cells to attach to [19]. Another molecule with similar property is laminin which is a basement membrane glycoprotein. Among its biological activities are promotion of cell adhesion, migration, differentiation, growth, and neurite extension [20]. Synthetic peptides carrying a specific sequence of amino acids from the active region of laminin, the pentapeptide IKVAV (Ile-Lys-Val-Ala-Val), have been shown to produce these increased adhesion effects. Similar to RGD scaffolds that carry this sequence alone or as a part of a synthetic polypeptide are being tested to improve adhesion of nerve cells.

18.4.4 Production of Scaffolds

The scaffolds mentioned above with their different topographies are produced in a number of ways. These are presented in Table 18.2.

The 2D structures can be brought together in multilayers to construct 3D forms. When solvent casting is performed on patterned templates such as those prepared on wafers produced through microelectronic processes, films with finely detailed surfaces can be prepared that can guide cell growth and orientation (Fig. 18.11). Similarly, when electrospinning is coupled with rotating mandrels as the fiber collector, well-aligned fibrous mats could be obtained. Once obtained, these scaffolds can be treated with oxygen or other kinds of plasma or modified by binding cell adhesive molecules or moieties (as given in Table 18.1) through adsorption or covalent bonding onto their surfaces.

Table 18.2 Various scaffold production methods and the forms of the products

Method	Form and dimension of the scaffold
Rapid prototyping	Microfibrinous, 3D
Electrospinning	Nano-microfibrinous, 2D
Wet spinning	Microfibrinous, 3D
Lyophilization	Foam, sponge, 3D
Contact printing	Micro or nano, 2D
Solvent casting	Film, patterned or smooth, 2D
Particulate leaching	Foam, 3D
Gas foaming	Foam, 3D
Phase separation	Film, 2 or 3D
Self-assembly	Film, 2D
Layer-by-layer technology	Film, 2D, 3D

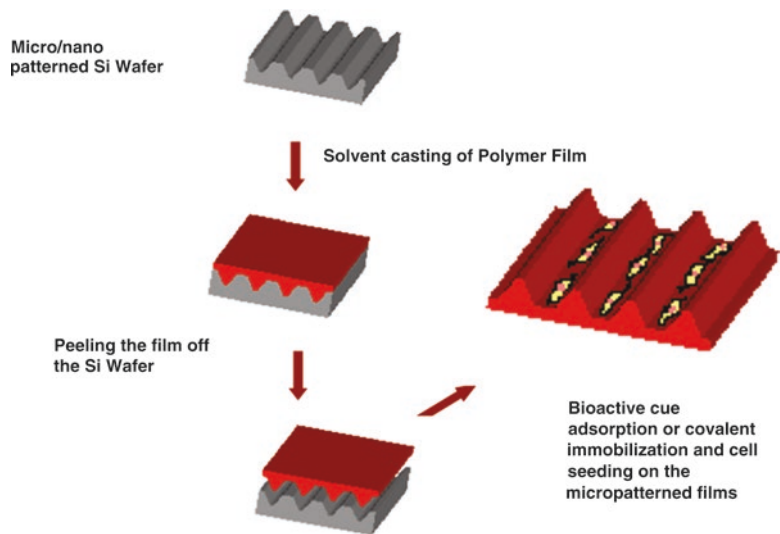


Fig. 18.11 The preparation of 2D and 3D patterned scaffolds through photolithography [21]

Cells respond to the surfaces with natural or artificial surface topographies by adapting their conformation and also metabolic activities. Figure 18.12 shows cell behavior on scaffolds with different surface topographies such as microchannels, sponges, and fibers. It can be observed that the shape and dimensions of the surface features of the topographies lead to these changes.

18.5 Cell Types

The second ingredient of a tissue-engineered product is the cells. Most animal cell types require a surface on which to attach, spread on, and divide. This is anchorage dependence of human cells. The cells used are either primary (fully differentiated)

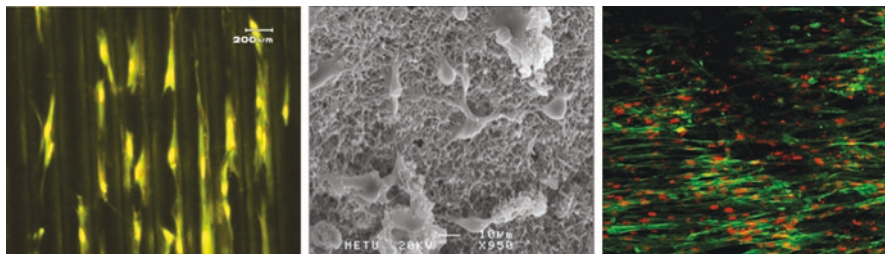


Fig. 18.12 Cell adhesion and conformation on surfaces with different topographies: (a) BMSC on PHBV microchannels, (b) Saos-2 cells on PLLA sponge, (c) Wharton's jelly cells on electrospun PLGA fibers [10]

cells or stem cells that have a significant potential to differentiate into the cells of the target tissue.

18.5.1 Primary Cells

Primary cells, mature, differentiated cells that are obtained from tissues and have not undergone any passaging (expansion in the lab), are used in tissue engineering and can be classified according to their source as follows:

Autologous cells: Autologous cells are isolated from the diseased or damaged tissue of the patient. The extracellular matrix of the biopsy specimen is removed, the cell density is increased, and then they are seeded on a scaffold. Since they originate from the donor, there is no risk of immune response or pathogen transmission.

Their main disadvantage is that harvesting of cells causes a new damage at the donor site. The pain, the risk of infection, and the limited availability of suitable donor sites make autologous cells not a very practical source.

Allogeneic cells: Allogeneic cells are obtained from a donor of the same species and, in this case, from another human being. In using this source, there is a risk of pathogen transmission and immune rejection, but at least donor site morbidity and associated pain are not an issue for the patient in using this cell source.

In a study, 1142 patients were treated with novel cellular therapies, in which 40% of the patients were treated with allogeneic cells and 60% with autologous cells [22]. The problems associated with the transplantations were:

- Graft versus host disease (26%; 11% autologous)
- Musculoskeletal disorders (25%; 93% autologous)
- Cardiovascular disorders (20%; 100% autologous)
- Epithelial disorders (16%; 44% autologous)
- Autoimmune diseases (11%; 55% autologous)
- Neurological disorders (2%; 62% autologous)

As can be seen from the list, the frequency of various problems arising from transplantation of tissues is significant in the use of both autologous and allogeneic cells, and the share of the risks observed with the allogeneic donors is not very different than those originating from autologous donors.

18.5.1.1 Xenogeneic Cells

Xenogeneic cells are obtained from donors from another species; in the case of tissue engineering, this means nonhuman cells. These are especially useful in the preparation of generic, off-the-shelf, engineered tissues. The human use of xenogeneic cells is associated with serious problems such as:

- Risk of transmission of known and unknown pathogens, with the potential risk of introducing new infectious diseases into the general population.
- Risk of immunological rejection.
- Maintaining the survival and functions of the cells in the long term.
- Use of immunosuppressive treatment leads to weakening of the host defense system.
- Careful choice of the donor animal.
- Uncertainty of the transplanted cells to provide the desired function in the long term [23].

When all these risks are considered, the use of xenogeneic cells becomes less appealing than autologous or allogeneic cells, neither of which shows most of these risks.

18.5.2 Stem Cells

Stem cells are not fully differentiated cells which have the capability to differentiate into a variety of tissues or organs. Two key characteristics define stem cells or separate them from adult primary cells: (i) they are unspecialized cells capable of self-renewal through cell division and (ii) potency, the inherent ability to differentiate into specialized cells under certain physiologic or experimental conditions. Thus, depending on their type, they have the potential to develop into a variety of cells in the body and even grow organs or complete living species. Besides, they serve as a type of internal repair and regeneration system. A stem cell can divide to form new stem cells or differentiate into a more specialized cell. Dividing without limit serves to replenish and thus acts as an internal repair system, as long as the person or animal is alive. The bone marrow, serves as a stem cell source, these cells regularly divide to repair damaged tissues. In some other organs, such as the heart, however, stem cells only divide under special conditions. There are three main types of stem cells as given below:

1. *Totipotent cells*: The totipotent cells are the first four cells of an embryo which have the ability to differentiate into any kind of cell in the body. One can even grow a whole organism using these cells.

2. *Pluripotent stem cells*: These cells are capable of differentiating into any cell type in the body except those of the placenta. Embryonic stem cells (ESCs) are stem cells derived from the undifferentiated inner mass cells of a human embryo; they are pluripotent, and therefore, they are able to differentiate into all derivative tissues of the three primary germ layers of the embryo, ectoderm, endoderm, and mesoderm.
3. *Multipotent stem cells*: Multipotent stem cells can only differentiate into a limited number of cell types. Multipotent adult or somatic stem cells are able to self-renew. They can also form a number of differentiated cell types, all within a distinct tissue, organ, or physiological system. They are found in the bone marrow, brain, and liver. For example, mesenchymal stem cells can differentiate into the bone, cartilage, and fat cells. The best studied adult stem cells, the hematopoietic stem cells (HSC), undergo cell division, differentiate into all mature blood elements [24]. HSC, however, are not a cell type that can be used in tissue engineering.

Mesenchymal stem cells (MSCs): They are defined as self-renewing and multipotent cells capable of differentiating into multiple cell types, including osteocytes, chondrocytes, adipocytes, hepatocytes, myocytes, neurons, and cardiomyocytes [25]. MSCs are isolated from the bone marrow stroma and have been also identified in tissues such as lipid tissue, epidermis, and cord blood. They have a significant attachment and expansion capability in culture. MSC plasticity (ability to differentiate into a number of cell types) and the opportunity to use them as an autologous cell source for tissue engineering are very important for researchers and clinicians.

Cell lines: When the primary cells are treated and are made immortal, then they become a cell line, some of which are often available through commercial sources. A cell line is a permanently established [cell culture](#) that will [proliferate](#) indefinitely as long as its culture medium and appropriate space are provided. Some [species](#), particularly [rodents](#), give [rise to lines](#) relatively easily, while the establishment of [cell lines](#) from [human tissue](#) is more [difficult](#). Many researchers consider a cell line [abnormal](#) and believe that they do not sufficiently represent the primary cell. However, especially the expansion ability and its relative ease, the commercial availability and sturdiness of cell lines make them very useful in research for obtaining and comparing results between laboratories (Fig. 18.13).

18.6 Growth Factors

Growth factors are protein molecules that are involved in the regulation of cell division, differentiation, migration, and cell survival. They are growth stimulators (mitogens) and inhibitors, act as chemotactic agents, and are involved in angiogenesis and apoptosis. Growth factors are found in membrane-bound form. Examples of important growth factors are BMP, EGF, FGF, NGF, PDGF, VEGF, IGF, TGF, and erythropoietin.

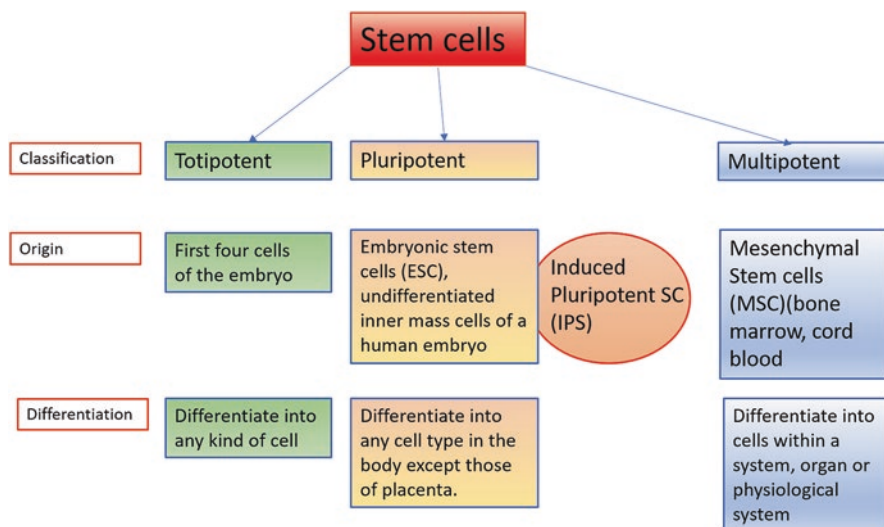


Fig. 18.13 The categories of stem cells and their capabilities

The insulin GF family include insulin, insulin-like growth factor (IGF), and multiplication-stimulating factor (MSF). A second family consists of sarcoma growth factor (SGF), transforming growth factors (TGFs), and epidermal growth factor (EGF). In addition, there are growth factors, such as nerve growth factor (NGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), for which structural homologs have not been identified. The classical growth factor list is presented in Table 18.3.

TGF- β superfamily has more than 35 members and 12 known receptors. Among the members are TGF- β 1, TGF- β 2, and TGF- β 3, 15 bone morphogenetic proteins (BMPs), 6 growth differentiation factors (GDF), 4 glia-derived neurotrophic factors (GDNF), and 4 activins [47]. Major members of the superfamily include TGF- β 's, activins/inhibins, BMPs, growth and differentiation factors (GDFs), and anti-Müllerian hormone (AMH) [48]. Transforming growth factor- β isoforms (TGF- β 1, TGF- β 2, and TGF- β 3), bone morphogenetic proteins (BMP), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) have been reported to affect bone healing [49]. Of the three isoforms, only TGF- β 1 is known to play a major role in bone formation.

Thus, growth factors with not so clear role definitions in the present state are proving to be very important in tissue repair and regeneration, and as such they are an important component of tissue engineering studies.

Table 18.3 Some important growth factors and their functions

Name	Abbreviation	Function
Nerve GF	NGF	NGF promotes survival of cholinergic neurons [26] and promotes the survival and differentiation of sensory and sympathetic neurons [27]
Vascular endothelial GF	VEGF	VEGF induces angiogenesis and endothelial cell proliferation, and plays an important role in regulating vasculogenesis, causes vasodilatation, stimulates cell migration, and inhibits apoptosis [28] It also induces migration, proliferation, and survival of endothelial cells [29]
Platelet derived GF	PDGF	PDGF is a potent mitogen for mesenchymal cells, (smooth muscle cells, glial cells), induces proliferation of undifferentiated mesenchyme and some progenitor populations, is involved in tissue remodeling and cellular differentiation, directs the migration, differentiation, and function of a variety of specialized mesenchymal cells [30]
Insulin and insulin-like GF	IGF	IGFs control cell growth and survival [31], affect osteoblast proliferation and differentiation, function in local regulation of bone formation [32]
Epidermal GF	EGF	Control cell growth by stimulating division [33]
Fibroblast GF	FGF	FGF regulates a broad spectrum of biological functions, including cellular proliferation, survival, migration, and differentiation [34]
Transforming GF beta	TGF-β	TGF-beta induces osteoblast proliferation and differentiation, regulates bone formation [26]

Bone morphogenetic proteins	BMP1	BMP1 influences osteoblast proliferation and differentiation, regulates bone formation [26] BMP1 plays a key role in ECM formation, and in the regulation of TGF-beta family [35, 36]
	BMP2	BMP2 plays important roles in bone repair and regeneration [37]
	BMP3 (osteogenin)	BMP3 is the most abundant BMP in adult bone [38] It stimulates ALP activity and collagen synthesis It stimulates sulfated proteoglycan synthesis [39] It repairs cartilage [32]
	BMP4	BMP4 stimulates the synthesis of sulfated proteoglycans [32] It repairs the cartilage It acts as a hematopoietic growth factor and has a crucial role in hematopoietic stem cell development [40]
	BMP5	Involved in chondrocyte differentiation and growth of long bones [41]
	BMP6	It is involved in chondrocyte differentiation and growth of long bone [34] It is involved in chondrogenesis induction of MSCs [42]
	BMP7	It induces adipogenic differentiation [43] It stimulates osteogenic differentiation [44] It is involved in differentiation and migration of bone-forming cells [23]
	BMP8	BMP8 plays an important role in embryonic development and in generation of the central nervous system [23]
	BMP9	BMP9 increases expression of aggrecan and cartilage oligomeric matrix protein It is important for neurogenesis It promotes chondrogenic differentiation of mesenchymal precursor cells [45]
	TNF- α	TNF-alpha causes necrosis in normal tissues but contributes to liver repair and regeneration [46]
Tumor necrosis factor		

18.7 Conclusion

In this chapter, we presented the basic concepts of tissue engineering and regenerative medicine. The need for engineered tissues and the components of a tissue engineering construct, namely, the materials used in scaffold design, the bioactive agents, and cells, were discussed. Scaffolds can be produced in different forms and are loaded with cells. The type of cells added depends on the needs of the patient and the location of the defect area. Tissue engineering and regenerative medicine are the means through which we can decrease or even completely eliminate the need for tissue donation from the patient, other humans, or even other species.

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19.1 Importance of Nanoness

Richard Feynman (winner of 1965 Nobel Prize in Physics) gave a talk at the American Physical Society meeting on December 29, 1959, titled “[There’s Plenty of Room at the Bottom](#)” at the California Institute of Technology (CalTech) upon which the whole discussion on the topic of nanotechnology started.

Nanotechnology is the science, engineering, and technology conducted at the nanoscale, which is about 1–100 nm as defined by the US National Nanotechnology Initiative [1] (Fig. 19.1). In the biomaterials field, this range of up to 100 nm is used literally; something less than a micrometer in size is considered nano. Nanoparticles, nanofibers, etc. all refer to materials with nanometer-level diameters, widths, or thicknesses, respectively. Then there are nanometer thick coatings or surfaces created with various treatment techniques and nanometer-level thicknesses. Oxygen plasma treatment of any material would lead to the formation of an oxidized layer with several nanometers thick [2].

Importance of nanoness in the biomaterials field is different than that in physics where a sudden, unexpected behavior is observed with the nano-systems. For example, “by confining excitations within their tiny volumes, quantum dots can harvest excess energy that otherwise would be lost as heat, and therefore, greatly increase the efficiency of converting photons into usable free energy” [3]. This happens when the dimensions are around a few nanometers. In the biomaterials field, no unexpected changes such as the above example are present. However, the very small dimensions have important contributions to the functions of the nanobiomaterials.

19.2 Nanoparticles

In medicine nanoparticles are used for diagnosis and in therapeutic applications. Therefore, their transport in the biological media, release of their cargo, and degradation are all important in medical applications.

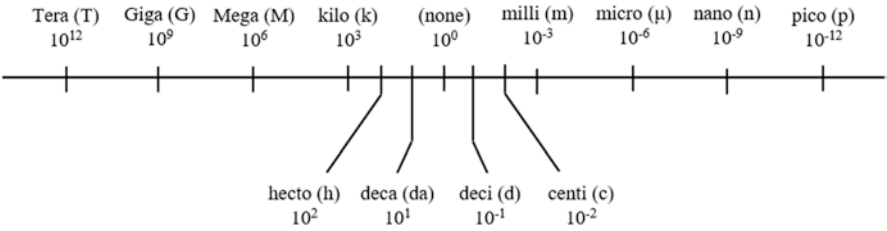
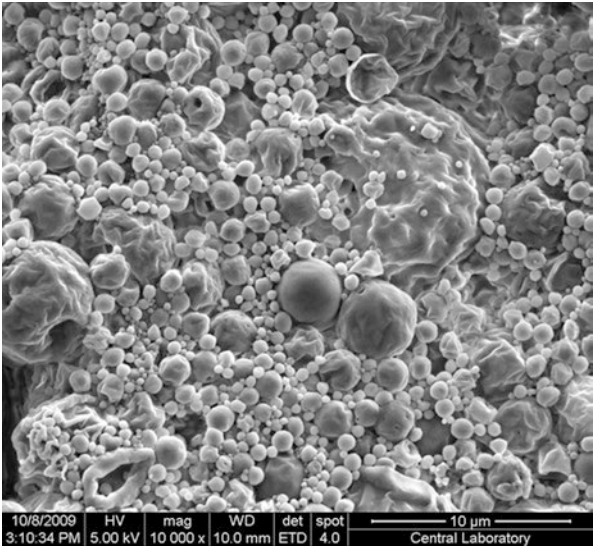


Fig. 19.1 Nanometer-to-meter scale with objects on a scale line

Fig. 19.2 Nanocapsules designed for drug delivery [4]



19.2.1 Transport of Nanoparticles

Nanoparticles (Fig. 19.2) are used in the biomaterials field as carriers of drugs and other bioactive agents. The reason for using a carrier is to protect fragile molecules such as enzymes, growth factors, or nucleic acids during their transfer to their targets in the relatively hostile biological environment. During this journey, the efficiency of delivery to the target site depends on the success rate of passing across a number of biological barriers such as membranes and mucous layers. The smallness of the carrier obviously is an important advantage in crossing these barriers as can be seen from the radius in the following flux equation:

Particle flux is:

$$J = -D \cdot (dc / dx) \text{ where } D = kT / 6 \Pi \eta r,$$

where k is the Boltzmann Constant, T is the temperature, η is the medium viscosity, and r is the radius of the permeant. Thus, all other parameters being the same, the smaller radius particle will penetrate better than a larger radius one.

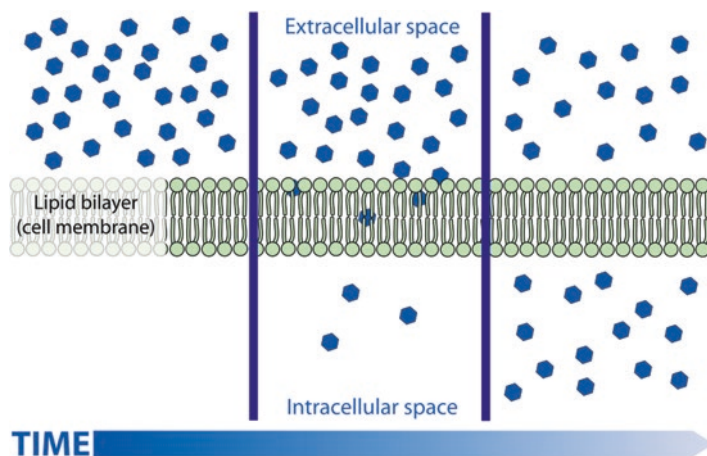


Fig. 19.3 Diffusion of molecules across membranes

19.2.2 Release Rate

Nanoparticles carry their contents in aqueous media, the body fluids of different pHs. Release of a molecule across a nanocapsule membrane is dependent on the ratio of surface area to volume; the higher this ratio is, the more is the rate of transport according to Fick's law. A small nanocapsule has a higher surface area than a larger capsule prepared from the same amount of material, and therefore the rate of release from a nanoparticle would be faster than from a larger one. The rate of diffusion is given as:

$$dn / dt = D \cdot A \cdot K_p \cdot (C_1 - C_2) / l$$

where D is the diffusion coefficient, A is the area of the membrane, K_p is the partition coefficient (between the oil and aqueous phases), l is the membrane thickness, and $(C_1 - C_2)$ is the concentration gradient. Thus, a carrier with a larger surface area (A) will allow a higher rate of diffusion out of its walls (Fig. 19.3).

19.2.3 Degradation

Degradation is also dependent on the surface area that is in contact with the degrading media, and in the body the aqueous medium is the principal hydrolytic agent. Therefore, a smaller particle will get hydrolyzed faster than a larger particle and thus release its contents faster because of its faster degradation. Depending on the intent, this could be advantageous or disadvantageous. If a rapid release is needed, then the particles need to be smaller, and the best are nanoparticles.

19.2.4 A Negative and a Positive Effect of Nanosize

Nanoparticles in the biological media may have various positive and negative effects as described below.

19.2.4.1 Toxicity

The permeation capability of nanoparticles is an advantage for permeation into cells and across barriers, but then again it is a disadvantage if the material is toxic by itself or becomes toxic upon accumulation in certain tissues such as the lungs. Since the particles are very small, the prevention of their entry and therefore their clearance from the tissues is more difficult than it is for the micro- or larger particles. Besides, as small particles, they are known to penetrate into the cells making accumulation of such particles more dangerous. When the particles are not degradable, it means that they pose a risk for the health of the person involved. This becomes especially an issue when nanoparticles are produced in large quantities for commercial use.

19.2.4.2 EPR Effect

Among the current applications of nanoparticles, one important type is the passive targeting of anticancer agents to solid tumors. In the normal tissues, the endothelial cells do not have any gaps between them. This type of tumor tissues are difficult to penetrate, however, have highly permeable vasculature due to formation of leaky vessels due to the high rate of production that leads to improper sealing of the endothelial layers (Fig. 19.4). It is expressed as tumor microvasculature being hyperpermeable to macromolecules. Angiogenesis apparently leads to high vascular density in solid tumors, and large gaps exist between endothelial cells in tumor blood vessels, and tumor tissues show selective extravasation and retention of macromolecular drugs [5]. It was found that N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer, with a molecular size up to 778 kDa and α 2-macroglobulin (α 2-M) (720 kDa), exhibited this permeability, and so did the bacteria larger than 1000 nm, which constitute examples of the EPR effect. This indicates that nanocarriers of any size if applied i.v. could extravasate into the tumor tissue and deliver their content to the extracellular medium of the cancer tissue.

19.3 Nanofibers

Nanofibers are produced to serve as drug carriers, as fibrous mats for use in tissue engineering, and as biomaterials. Their production is mainly by methods such as electrospinning or wet spinning where the fibers are extruded under electric potential or pressure (Fig. 19.5). The fibers can be produced to be aligned or to have a random nature for use as guides for cells, to act as membranes, or to form the biomaterial, but their thinness prevents the preparation of large-sized fibrous meshes as biomaterials (Fig. 19.6).

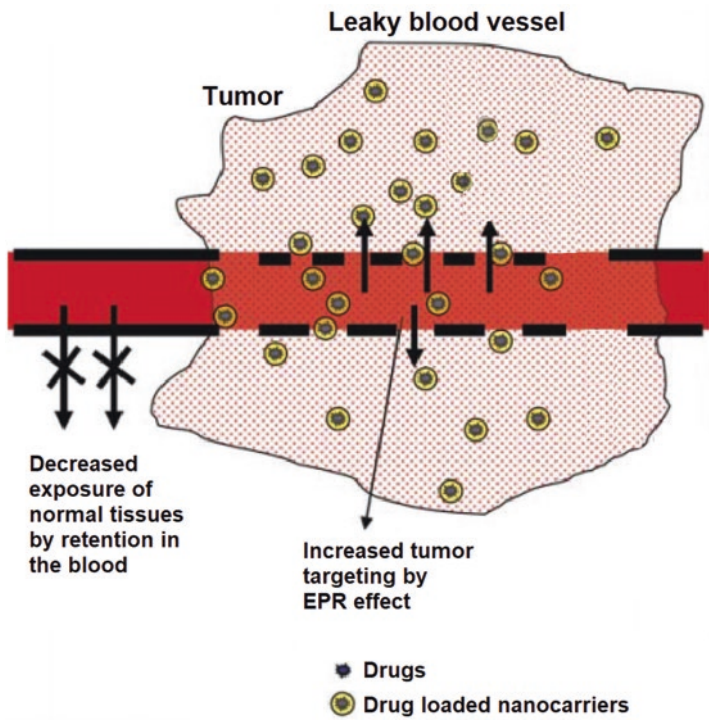
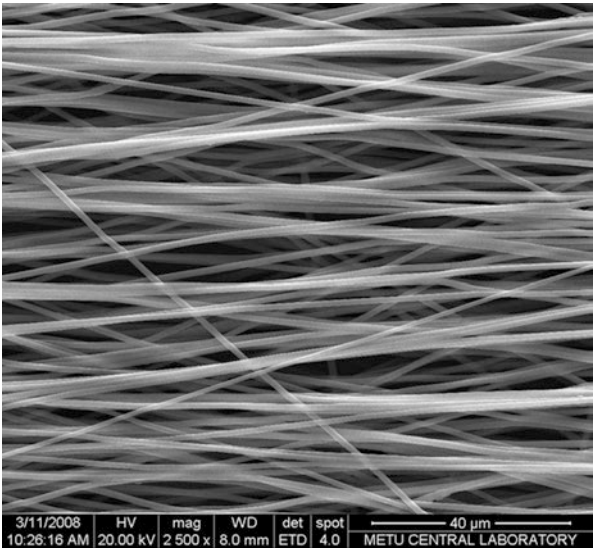


Fig. 19.4 Targeting of drugs to solid tumors through EPR effect [6]

Fig. 19.5 Nanofibers produced through electrospinning [7]



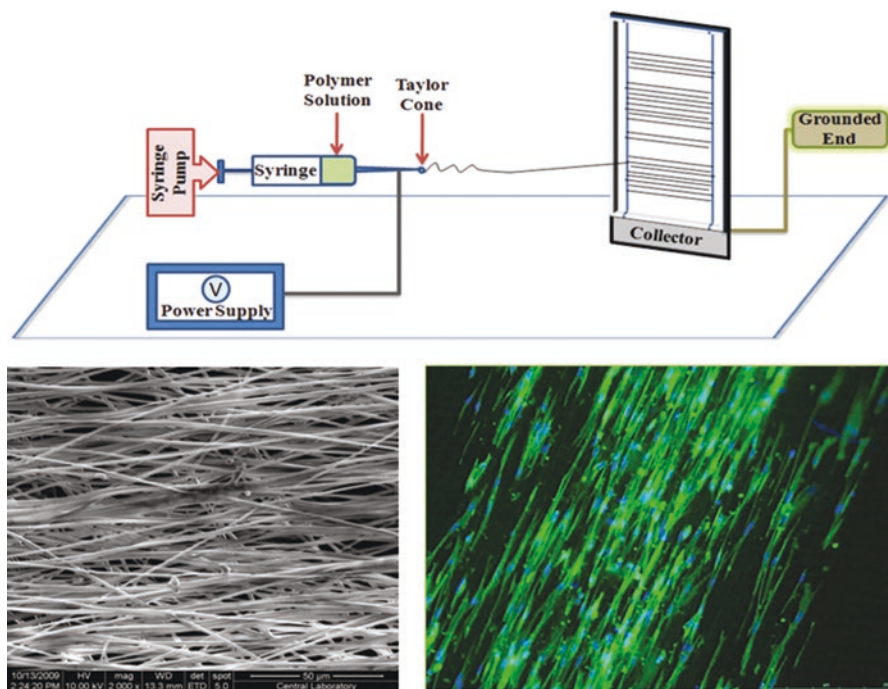


Fig. 19.6 Electrospinning system and cell behavior. Wharton's jelly mesenchymal stem cells on electrospun PHBV-PLA fibers (SEM and confocal laser scanning microscopy) [8]

Drug, or in general bioactive agent, delivery is possible by incorporating the molecule in the core, as part of a composite fiber composed of drug and carrier material in mixed form or the drug coated onto the fibers. The release rates naturally depend on the location of the drug with respect to the fiber, the composition, and the degradability of the carrier polymer. Due to thinness of the fibers and the small distance between the fibers, especially when they are aligned, permeability of the cells is generally very low. The small width of the fibers does not allow cells to be guided by individual fibers but by a number of them. Since the distance between the fibers is small, the cells can attach and spread across a number of them.

19.4 Nanosurfaces and Coats

Surfaces have a very critical role on the performance of biomaterials because they constitute one of the two components of the interface with the body. Thus, its biocompatibility affects the compatibility of the whole implant. For example, the surface roughness might act as an irritant, tissue adhesive, or repellent and change the whole performance of the implant. With the current methodology available today, it is possible to modify the surface of a biomaterial or a device without altering the

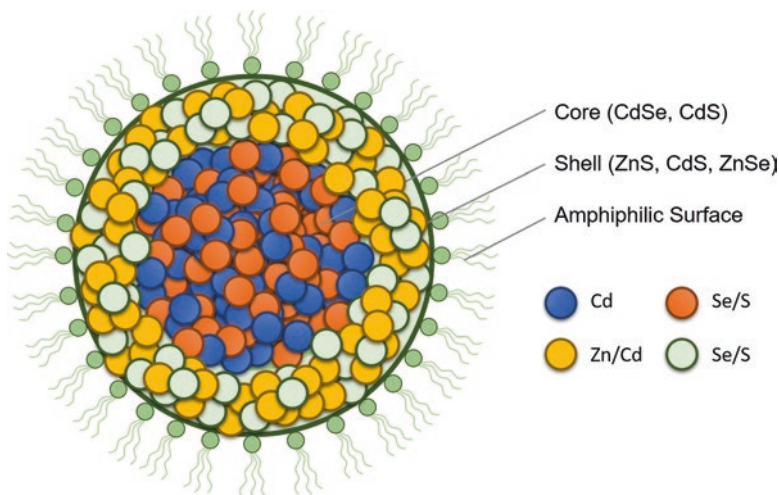


Fig. 19.7 Quantum dots coated for targeting and for biocompatibility

bulk properties, thus obtaining quite a biocompatible material from a toxic material. For example, quantum dots are nano-sized particles which are used in imaging because of their strong and stable fluorescence properties (Fig. 19.7). When they are injected in the i.v. route, they can travel throughout the body and penetrate even the cells due to their small size. They are semiconductors such as CdSe, ZnO, and SnO₂ made from Cd, Zn, Sn and other heavy metals and have the potential to be toxic. Upon administration into the test animals for imaging purposes, they accumulate in cells and tissues such as the spleen and liver because they are not biodegradable or not excreted due to their size [9]. The risk of accumulation might be small due to low frequency of application and the resultant low amount accumulated. When they are coated with synthetic or biological polymers such as polystyrene, graphene, or polypeptides, the toxicity is masked by the coat which acts as a neutralizing interface.

Another kind of coating or surface modification is applied by cold plasma treatment, generally using oxygen (Fig. 19.8). In the treatment of a very hydrophobic implant such as PMMA with oxygen plasma, the side groups and the main chain of the polymer get activated and oxidized, creating a series of oxygen-carrying groups on the surface of the polymer. The increase in oxygen-carrying groups increases the polarity of the molecules at the surface to a depth of several hundred nanometers creating a hydrophilic layer over the hydrophobic surface. The resultant change is generally measured by contact angle measuring devices such as a goniometer, and the low angle between the water droplet and the substrate shows a wettable substance. This new surface is active for a short while during which attachment of any kind of molecule could be achieved on these surfaces. It can be made attractive or repellent for the cells by attaching other molecules which have the capability to bind specifically to their counterparts, thus acting as biosensors.

Fig. 19.8 Oxygen plasma device for surface modification of biomaterials



19.5 Nano- and Micro-features (NMF) and Their Importance in Implant Performance

Implants, because of their location in the body, have to be in contact with the various types of tissues. The first contact could be with biological fluids like blood or extracellular fluids; proteins, lipids, and other molecules can attach onto them. As a result of this intensive interaction, biocompatibility of the implant and therefore its success depends very much on the nature of this contact. Researchers are studying the contact by creating nano- and micro-sized patterns on substrates and try to mimic the environmental conditions mainly *in vitro* using a variety of primary or stem cells or cell lines. In this chapter the creation of these surfaces and the interactions are discussed with examples from the literature.

19.5.1 Biological Macromolecules and Natural NMF

Surfaces with nano- and microstructures are being tested to improve or decrease cell attachment and motility [10, 11], modify or completely avoid foreign body responses

[12], create biosensors and detect analytes [13], and mimic tissue organization to prepare better implants and other devices used in everyday life [14]. These features are pores or depressions, ridges or pillars or posts, grooves or channels, fibers, nodes, and combinations of these. The origin of all these lies in the observation that nano- and micro-sized topographical features are abundant in the tissues and organs of all the living systems and they are very important in their proper functioning. These are found in the form of posts or pillars, channels or crevices, fibers or fibrils, and others which are less geometric than the artificial ones but are at least as effective in influencing cell and tissue behavior. These features serve to form the structure of the tissues and give them strength, resilience, hydrophilicity, or hydrophobicity and provide attachment sites for macromolecules and cells. For example, the nano-sized posts on the water lily make their leaves extremely hydrophobic and, therefore, water repellent. Similar is true for cicada wings (150° contact angle) which are also highly reflective due to the presence of the nano-sized posts [15]. The nano-sized protrusions on the lacuna provide attachment sites for the osteocytes, whereas the canaliculus channels provide a route for the osteocyte extensions (filopodia) to reach out and contact other cells. In this way tissues can perform their complicated tasks and the whole macroorganism is able to survive.

Human tissues are complex structures consisting of many cell types embedded in the extracellular matrix (ECM) which is mostly made up of proteins and glycosaminoglycans. The cells and the ECM are connected with each other through a complex series of components bound together ionically or covalently. These interactions involve macromolecular aggregates such as focal contact points and fibronectin component of the ECM and the transmembrane integrin of the cell binding in a very specific manner. The field of biomaterials and tissue engineering is involved in the activity of creating tissue and organ substitutes that support or replace the damaged tissues to help the function to be recovered.

Inspired by these natural patterns of ECM, nanotopography-guided approaches have been increasingly investigated in the last several decades [16]. It was shown repeatedly that surfaces with micro- and nanoscale features prepared in a well-controlled, “engineered”, manner significantly affect cellular and subcellular topography, function, and fate [17]. These surface features induce spatially and temporally resolved stimuli responses from the cells and control the behavior of biomolecules and cells at the solid-liquid interface [18].

19.5.2 NMF on Biomaterial Surfaces

In recent years, research has concentrated on modifications of biomaterial surfaces aiming to control and direct cell behavior. In an attempt towards this goal, the molecular and physical cues that govern such activities including protein adsorption are being mimicked at micro- and nano-level on biomaterial surfaces. The main aspect of this control is on the interaction of the cells with the surfaces starting with cell adhesion, proliferation, and differentiation.

When the adhesion of proteins on biomaterial surfaces is considered, literature indicates that the driving forces behind this process involve contributions from ionic, van der Waals, electrical double layer, and hydrophobic interactions [19]. In terms of cell behavior, cells in various tissues respond differently according to diverse factors such as physical topography or rigidity [20], cellular adhesion sites [21], electric fields [22], and chemical factors [23]. As a result, different cells may behave oppositely against identical stimuli. For example, on a surface with graded stiffness, while the fibroblasts migrate toward the more rigid region, neurons stretch the neurites toward the softer side.

19.5.3 Physical, and Chemical and Biological NMF

Among the various factors described above, physical cues are of great importance since cells reside in a physical microenvironment with topography and mechanical properties specific for the tissue. Such physical cues are frequently observed in the human body such as in the neuronal network, in the aligned myofibrils of the heart and skeletal muscles, and in the anisotropic organization of the collagen fibers in the skin. Since these structures are involved in specific functions essential for the tissue and organ, pathological conditions that lead to changes in these structures result in the malfunctioning of the tissue or disconnection of signal or force transfer. For example, it is well known that neurons in the human brain have a radially spreading morphology that connects the cerebral cortex and the white matter. In the case of physiological malfunctions such as Alzheimer's or Parkinson's diseases, interconnections between the neurons are severely damaged, thus disrupting the informational pathways [24]. The main goal of regenerative medicine is to restore the damaged physiological structures and the resultant disabilities and minimize the side effects using micro- and nanostructured constructs through guidance of cell migration, proliferation, and even differentiation. To mimic the natural physical features, various materials and fabrication methods have been developed over time.

Nano- and microstructures in the human body can be found to be involved in the functions and physiological environment of four types of tissues (Fig. 19.8): (1) protective tissues (skin), (2) mechanosensitive tissues (bone, ligament, tendon), (3) electroactive tissues (nerve tissue, skeletal muscle, heart), and (4) tissues subjected to shear stress (cardiovascular system components like vasculature). It should be remembered that some tissues are found in several of these categories making choosing a property to mimic difficult. Since these tissues are in significantly different environments and are exposed to different stimuli, an understanding of the microarchitecture and microenvironment of each is required to adequately substitute them through regenerative medicine.

19.6 Patterning Techniques

A number of methods are available for producing micro- and nanopatterned areas on material surfaces (Fig. 19.9). Depending on the pattern type, the approach used changes. When the feature components have 3D dimensions, then they are the

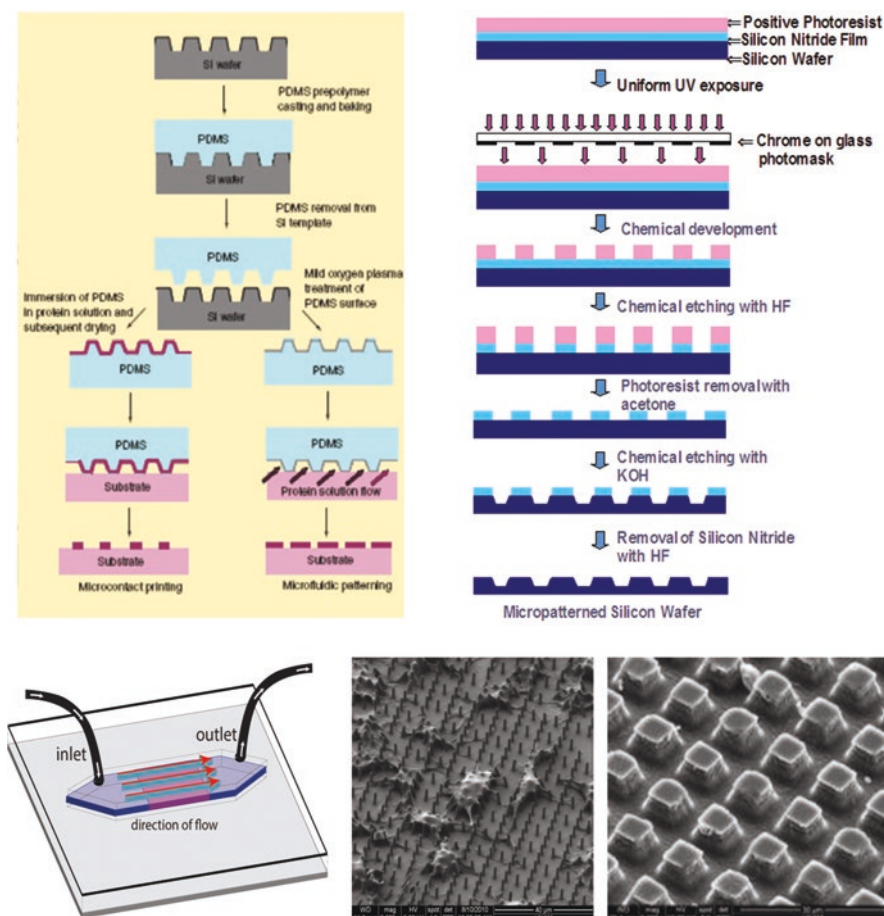


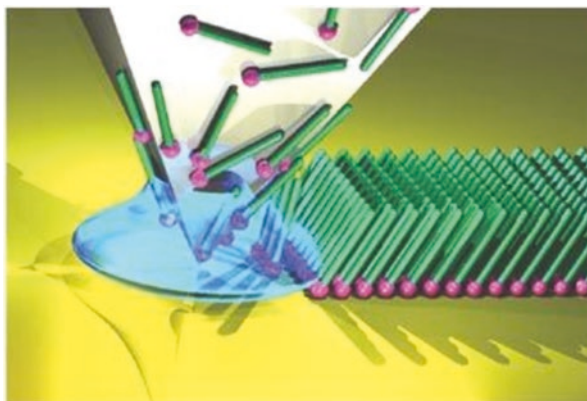
Fig. 19.9 Microcontact printing and photolithography approaches and, microfluidic system and substrate surfaces (with and without cells) obtained [7, 25]

physical patterns, whereas 2D patterns consisting only of molecules and macromolecules are the chemical or biological patterns. The approaches used in making them differ accordingly.

19.6.1 Physical Patterning

These approaches are usually classified as bottom-up and top-down techniques [26]. Top-down methods start from a bulk material, which is transformed into a material with micro- and nanoscale features; this is very similar to carving a sculpture from a block of marble. These methods are based on lithographic techniques such as photolithography, scanning beam lithography (SBL), scanning probe lithography (SPL), or soft lithography (SL) [27].

Fig. 19.10 Dip-pen lithography [34]



Photolithography [28] is based on the exposure of a photosensitive resist to a light of certain wavelength (generally UV) through a mask that has opaque and transparent regions, to transfer the pattern in the mask onto the resist. SBL techniques, such as electron beam lithography (EBL), focused ion beam lithography (FIBL), or X-ray lithography (XRL) [29–31], use resists exposed to a high-energy beam of electrons, ions, or electromagnetic radiation (UV or X-rays). Electron beams or X-rays degrade or cross-link the exposed regions of the resist. Once the uncross-linked (left unstable or unstabilized) regions are washed off, the remainder is the patterned material aimed. Similarly, FIBL could deposit or remove material from the surfaces, too.

SPL techniques [32, 33] (e.g., dip-pen nanolithography (DPNL) or chemical nanolithography (CNL)) are based on the localized modification of a surface by oxidation or by material transfer using a sharp probe that makes a contact with the surface (Fig. 19.10).

SL technique [35] uses soft organic materials to transfer a pattern on the substrate. A well-known method is microcontact printing that uses stamps, usually of polydimethylsiloxane (PDMS) carrying features at the micro- or nanoscale, and transfers the pattern by putting the substrate in contact with the stamp previously inked with the substance (such as proteins, cell adhesive molecules, etc.) to transfer.

Bottom-up methods start out with building blocks, i.e., atoms, molecules, and colloids, that self-assemble to form nanostructures on surfaces. Examples of these methods are colloidal lithography (CL) [36] and block copolymer lithography (BCPL) [37].

BCPL uses different types of block copolymers to form nano-regions. The more soluble block forms a shell around the less soluble block. Using a proper solvent, the more soluble block is removed creating a nanopattern of the second polymer on the surface. CL is based on the ability of the colloid particles to form a two-dimensional monolayer array on a surface. A monolayer of colloidal particles is deposited on the surface and used as a mask for etching or sputtering processes.

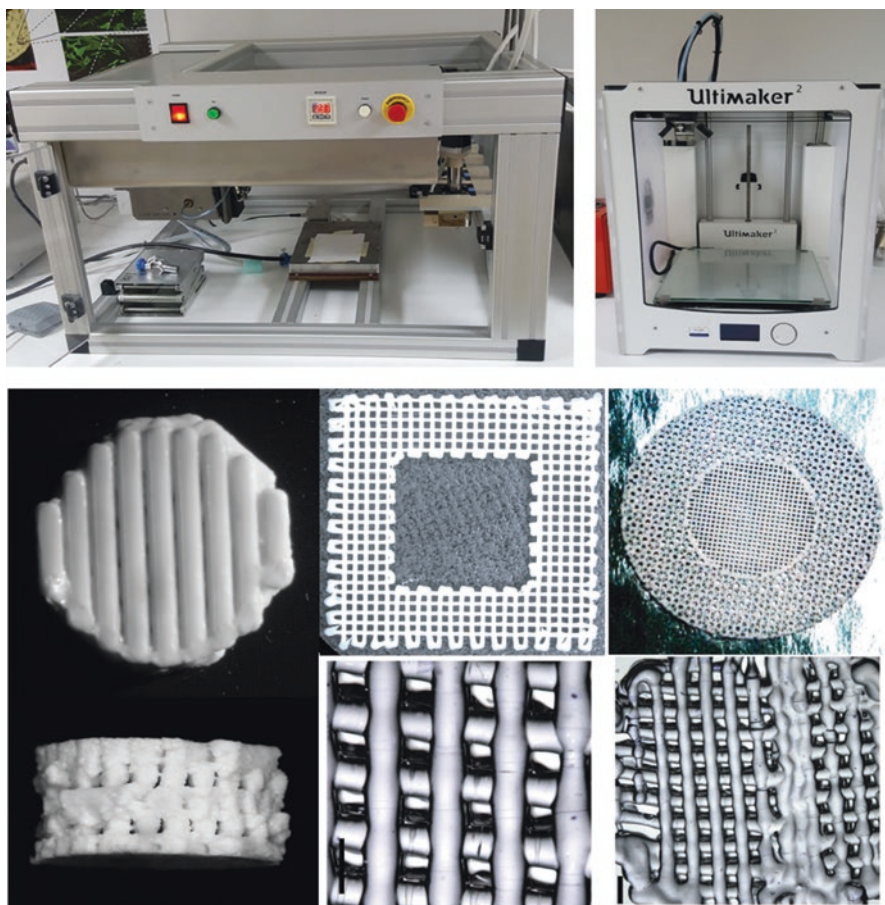


Fig. 19.11 Rapid prototyping equipment and a polymeric mesh produced by fiber deposition modeling (FDM) [38]

When the level is increased to micro, then the range of methods is increased. Rapid prototyping, or additive manufacturing, is one such approach (Fig. 19.11). In the figure samples produced by fused deposition modeling approach of 3D printing are presented along with two devices with this capacity. The top left device can also print viable cells in photocross-linkable gels allowing printing of soft tissues such as the cartilage, muscle, blood vessels, and liver.

Various fabrication methods are employed. In general, they can be classified into two categories: template-assisted and template-free. The template-assisted fabrication methods require the involvement of a mold which can have different levels of (1) transparency depending on the use of radiation, (2) elasticity depending on the pressure requirements during release from the mold, and (3) permeability depending on the use of solvents. Depending on the properties of the polymers and the mold material used, an appropriate method is adopted.

19.6.2 Chemical Patterning

With chemical factors, pre-defined patterns can be transferred onto the target substrate in a way similar to stamping, called microcontact printing (μ CP) [39]. To be used as a stamp, a material has to meet several requirements such as flexibility for appropriate contact, low surface energy between the stamp and the substrate for efficient transfer, and inertness toward the substrate so that degradation of transferred materials, the ink, which actually carries the bioactive species to attach to the surface, is minimal. In order to meet such requirements, μ CP usually utilizes PDMS stamps prepared using various approaches based on molding. Materials such as proteins, self-assembled monolayers (SAMs), DNA, siRNA, cell adhesive molecules, antibodies, gold nanoparticles, and even metals and ceramics can be used as the ink to be transferred through μ CP to the substrate surface. The minimum feature size is determined by the resolution of the patterns on the stamp. When electrical energy is used to accumulate the molecules on the feature surface, a redox reaction takes place at the interface between the substrate that is conductive and the ions in the “ink” solution. Then an electric field is applied, and the ions in the electrochemically reducible solution accumulate on the substrate. The resulting pattern or morphology can be formed by the use of pre-defined features on the substrate or the stamp itself. Although this method can create features with high precision (depending on the resolution of the features on the guide), it is limited to ionic or conductive materials [40].

Microfluidic systems can also be used to create strips of cell adhesive molecules. The microfluid components are generally made of PDMS because of its hydrophobicity (nonstickiness toward most compounds) and convenience of making complex features with relative ease. Photolithography, with UV or e-beam, is the main method used in creating the template. Once the PDMS cover is placed over the substrate, then the ink is introduced through the channels in the template, thus restricting the inked regions.

Wu et al. [41] fabricated surfaces with gradients of methoxy poly(ethylene glycol) (mPEG, poly(ethylene glycol) methyl ether) brushes on plain surfaces and on such surfaces in strips. Vascular smooth muscle cells exhibited preferential orientation and enhanced migration toward the lower methoxy poly(ethylene glycol) density. By introducing mPEG striped patterns in parallel with the substrate surface gradient direction, the extent of cell orientation and directional migration were significantly improved. Due to the synergetic effects of surface mPEG striped patterns and gradient cues, almost all cells were oriented, and 67% of the cells were observed to move unidirectionally. An interesting aspect is that as the mPEG concentration gets higher, the cell adhesion decreases indicating the avoidance of the highly hydrated PEG.

19.6.3 Biological Patterning

Biological patterning can be achieved using the methods employed in chemical patterning, but there are also other approaches specific to biological patterning arising

from the properties of the biological molecules used as the ink. For example, self-assembly is an application mainly for biological patterning due to the spontaneous formation of ordered structures generally observed with macromolecules. This is an important tool of nanotechnology and nanomedicine because it can be used in spatially orienting peptides with nanoscale precision. Self-assembly is a “bottom-up” approach in which molecules that serve as the building blocks associate with each other in a coordinated fashion to form larger, more complex supramolecular structures. The organization of these building blocks into supramolecules is governed by molecular recognition due to non-covalent interactions such as hydrogen bonding, as well as electrostatic and hydrophobic interactions. Methods commonly using peptides include self-assembled monolayers, amphiphatic peptide self-assembly, polymer-assisted templating, and DNA templating [42].

DNA is an important building material to create complex yet predictable nanoarchitectures using the bottom-up approach. DNA stands out because virtually any nanoscale architecture can be constructed with angstrom-scale precision [43]. In addition, dedicated software programs [44] help design the molecular origami devices from scratch, thereby defining the sequences of attachment of the constituent DNA strands, which can, in most cases, be readily obtained from commercial suppliers. Furthermore, DNA strands can be chemically modified and equipped with attachment linkers or fluorophores to expand the range of the DNA nanostructures that could be constructed. Finally, physicochemical knowledge on the attachment of DNA at the nanobiointerfaces is available [45–47]. There, however, are several limitations in the use of DNA for the construction of bio-based patterns. Compared to chemically more robust synthetic polymers, neither the DNA nor the assembled DNA nanostructures can withstand the harsh acidic/alkaline or high-temperature conditions because of the depurination [48] and unzipping of the constituent DNA duplexes, respectively, and also due to loss of conformation through denaturation. In addition, DNA is highly negatively charged, which can initiate electrostatic interactions with positively charged molecules which might not be desired [49, 50]. DNA is a powerful biomaterial for creating rationally designed and functionally enhanced nanostructures. DNA nanoarchitectures created at substrate interfaces can offer unique advantages leading to improved surface properties relevant to biosensing and cell organization.

Molecularly thin and laterally dense films of DNA structures have been constructed to improve the biomolecular recognition at biointerfaces. In addition to homogeneous coatings on the whole surface of substrates, DNA nanoarchitectures can also be created on surfaces to produce patterns at the nano-level. In principle, nanopatterning with DNA can be achieved either in the bottom-up fashion or in combination with top-down approaches. In its simplest form, bottom-up nanopatterns of nucleic acids can be made with isolated bases and form porous networks [51, 52], obtain porous 2D patterns [53] from flexible single-stranded and surface-tethered DNA molecules, or with nano ribbons chemically modified DNA duplexes that self-assemble [54].

Researchers applied cell adhesive protein fibronectin quite often as a molecule to pattern a substrate with cells. Microcontact printing was used to stamp fibronectin

and control molecules poly(D-lysine) and PEG to create strips of C2C12 myoblasts or primary neurons on the surface of a silicon wafer with stripes of 10 μm width and 100 μm spacing [55]. The stamp was made of PDMS and created by photolithography as most stamps are.

Microcontact printed fibronectin was also used in creating a 100 μm line-and-space grating using PDMS stamps created with photolithography. Then primary cardiomyocytes isolated from ventricles of 1–3-day-old neonatal Wistar rats were seeded onto the surface of a multi-electrode array (MEA). After 3 days on the array, cell stripes were formed, allowing guided excitation along each of the cell stripes [56].

Another molecule that was specifically used for nerve cells was laminin. The stamp-making method (photolithography) and stamp material (PDMS) were the same as above [57]. They used planar substrates with patterned ligands that were used to induce astrocyte alignment. This time the surface was covered with astrocyte monolayers, and dorsal root ganglion neurons isolated from rats were plated on top of oriented astrocyte monolayers. The neurons were observed to exhibit directional outgrowth along aligned astrocytes, demonstrating that purely biological cues provided by the oriented astrocytes were sufficient to provide guidance cues.

19.7 Influence of Surface Topography on Cell Response

The ability to manufacture highly controlled topographies enables the researchers to direct cell function, alone or synergistically with the use of chemical and biological cues. The influence of micro- and nanotopography on cellular response has been explored *in vitro* using a variety of surface features and cell types including osteoblasts [58], fibroblasts [59], macrophages [60], neural cells [61], and endothelial cells [62]. Different cells are used because cells differ as the tissues they constitute differ in their environment, and, therefore, their response to artificial media created of various types of materials with different designs is not the same. Hence, the generalization of the effect of topography on the responses they elicit in various cell types is very difficult. Studies have shown that generalizations are difficult because the responses are rather cell type and application specific. Direct comparison of studies on the effect of topography on cell response is complicated by differences in the methods of cell characterization used, the variety of topographic dimensions, and also the difficulty to isolate the influence of chemistry of the surface features from that of the physical and mechanical cues. Despite these, the general conclusion is that micro- and nanoscale surface topographies are capable of modulating cellular responses, both in the short and long term. For example, substrate microtopography was shown to influence cell adhesion and guide orientation of the cells (contact guidance) as these dimensions are comparable with the cellular dimensions (10–30 μm) [63]. Cells have been found not to penetrate grooves which are less than 2 μm in width or 500 nm in depth [64]. However, these dimension limits are found to vary with cell type. Besides it has also been found that the sizes lower than that of the cells are still able to influence cell behavior.

The kinds of responses that can be quantitatively measured are biological responses such as the rates of adhesion, proliferation, and differentiation, preservation of the phenotype, and the physical responses like changes in the area, circumference, alignment along an axis, and aspect ratio (the ratio of the length to the width) for both the cell and the nucleus. Cell adhesion is a dynamic process involving the initial step of cell-protein contact followed by longer-term process involving various molecules which act together to induce conformational and metabolic activity changes.

Once these responses were observed, then researchers found ways to use them. An important application of tailoring the surface of biomedical implants is to enhance or prevent cell adhesion onto implant surfaces. An example of prevention is the antifouling stents against tumor cells. Stents in general are used to open blocked passage ways like blood vessels for freer fluid flow. Tumor stents, however, are used to prevent the collapse of gastrointestinal, pancreatic, and biliary ducts from which tumors have been surgically removed. A persistent problem that interferes with normal stent function, however, is the adhesion and growth of tumor cells on the stent surface. As the stent is blocked, this increases the morbidity and mortality in patients, and there is a need for strategies for preventing the adhesion of the tumor cells onto the stent surface [65, 66]. Similarly, preventing macrophage adhesion onto titanium or other metal implant surfaces is crucial for the success of bone prosthesis [67]. Platelet adhesion and subsequent clogging of cardiovascular stents are another problem that needs antifouling measures [68]. A class of nanostructures that shows promise for reducing mammalian cell adhesion is a surface covered with upright nanoscale cylinders, in the form of nanorods, nanopots, nanowells, or nanoislands [69–71]. Below the responses are grouped for some frequently used cell types.

19.7.1 Osteoblasts

The effect of surface micro- and nanotopography on osteoblast behavior has been widely investigated to gain a more fundamental understanding of bone biology and also make advances in bone tissue engineering and regenerative medicine strategies. In general, surface nanotopography is frequently observed to promote the adhesion and proliferation of osteoblastic cells [72] and bone matrix synthesis and improve osseointegration [58]. It was shown that the height of nanoscale features could influence bone cell adhesion using nanoscale islands (heights of 18, 45, or 95 nm) created on surfaces by an approach called polymer de-mixing using polystyrene (PS) and polybromostyrene (PBrS) [73]. Higher islands (45 and 95 nm) initially caused an increase in the attachment of the cells compared to the shorter islands (18 nm); however, after 1 day the effect subsided and the number of adhered cells was the same on all surfaces [74].

On nanotextured silicon substrata with parallel ridges separated by grooves with equal width from 90 to 500 nm, it was shown that even though the nanosize is too small for osteoblasts, they stretched and aligned along the axis of nanogrooves of

silicon substrates with the actin cytoskeleton being parallel to nanogrooves [75]. They, however, could not spread laterally. As a biological activity change, it was reported that expression of focal contact point proteins osteopontin and osteocalcin was higher on titanium surfaces with nanopillars 15, 55, and 100 nm tall as compared to polished, smooth titanium control surfaces after 21 days in culture [76]. Tests with titanium dioxide nanotubes showed that differentiation of freshly isolated hematopoietic stem cells into osteoclasts, as shown by the formation of multinucleated cells, was promoted especially by 15–20 nm diameter nanotubes [77].

Other studies have also demonstrated that with osteoblasts on 85 nm high nanoislands, neither clear stress fibers nor focal adhesions could be observed [74], and the cell numbers were ca. 67% lower than cells on 13 nm high nanoislands.

The adhesion and spreading of osteoblasts on ZnO films and ZnO nanoflowers prepared by photolithography was studied and it was observed that osteoblast growth rate was higher on ZnO nanoflowers than ZnO films; osteoblasts covered the ZnO nanoflowers almost completely in 4 days [78].

Others [79] investigated the fabrication details to form large area, systematically changing multishape nanoscale structures including dots, ellipses, holes, and elliptical holes in both x and y directions on a chip by laser interference lithography (LIL). In the control experiments on smooth surfaces, adhesion sites, as indicated by vinculin, were randomly oriented, as was also rat calvarial osteoblast cell spreading, as shown by the F-actin of the cytoskeleton. On 300 nm high nanodots, adhesion sites were not random, they were on the peaks of the dots. The cell morphology was shown to not conform to the shape of the topography. On 1200 nm spaced lines, osteoblasts formed dense adhesions parallel to the long axis, and the overall morphology (f-actin) responded similarly. Thus, it was shown once again that nanopatterns could influence cell attachment and behavior significantly.

19.7.2 Fibroblasts

Fibroblasts have been widely investigated as model systems to explore the influence of surface topography on cellular response. It was shown that surfaces with nano-level roughnesses created with silica nanoparticles influenced fibroblast responses; cell morphology was changed, and cell adhesion, spreading, and proliferation were decreased during an in vitro test of 7 weeks [59] (Fig. 19.12).

Reduced fibroblast adhesion was also observed on polycarbonate substrates decorated with random and ordered nanopits 100 nm deep and 120 nm diameter produced by e-beam lithography [80]. This, and other studies, suggests that pitted nanotopographies interfere with the fibroblast-substrate interactions at the pit sites [81]. When nano-level hexagonal arrays were produced by e-beam lithography on PMMA, actin filaments were observed to develop less, and expression of tubulin and vinculin was lower compared to the smooth control surfaces [82]. In a study [69], human foreskin fibroblasts were studied on silicon surfaces with nanocolumns of 50–600 nm high, >10 nm in diameter, and a periodicity of 230 nm produced by interference lithography and deep reactive-ion etching. The fibroblasts had

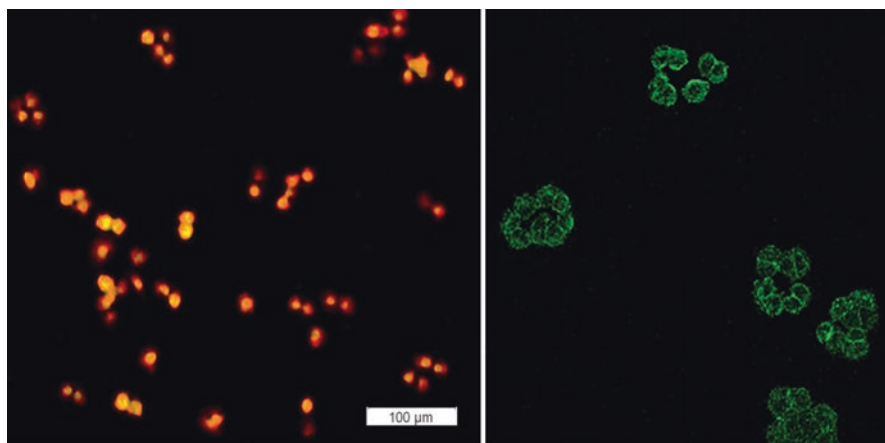


Fig. 19.12 Fibroblasts in growth medium (L929 cell line) (left, phalloidin and DAPI; right, phalloidin) [38]

significantly smaller cell size and lower proliferation on needle-like nanoposts. They were seen to extend their filopodia along the tips of the posts, and these nanoposts caused an approximately 30% reduction in the number of attached cells compared to those on smooth surfaces.

It was shown on surfaces decorated with nanoislands that their presence and size could reduce cell adhesion [74, 83]. These studies showed that the dimension of the nanoislands played an important role in modulating cell adhesion. For example, the cell area of the fibroblast (a measure of cell spreading) was decreased by nearly two-fold as the height and the diameter of the nanoislands were increased from 10 to 50 nm and 31 to 99 nm, respectively [84].

19.7.3 Endothelial Cells

Almost all tissues depend on endothelial cells for their blood supply because **endothelial cells** form the linings of all the blood vessels (Fig. 19.13). They have a remarkable capacity to adjust their number and arrangement to suit local requirements, and this is very important since the dimensions and conditions of the various vasculatures in the body are different [85]. Adhesion of endothelial cells onto surfaces with nano-level roughnesses created by colloidal silica nanoparticles was significantly reduced compared to the planar controls [86].

Similarly, nanotopographical features 27 nm high produced by de-mixing of poly(ϵ -caprolactone) (PCL) and poly(ethylene glycol) (PEG) led to reduced endothelial cell adhesion [87]. Both of these studies indicated that endothelial cells prefer to adhere onto smooth surfaces.

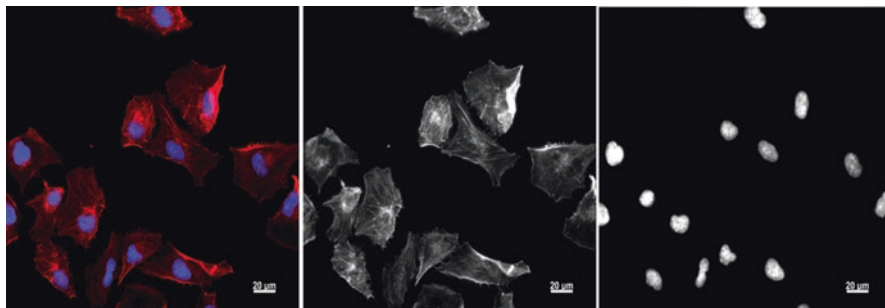


Fig. 19.13 Endothelial cells in spread morphology (HUVEC cell line, phalloidin, and DAPI) [38]

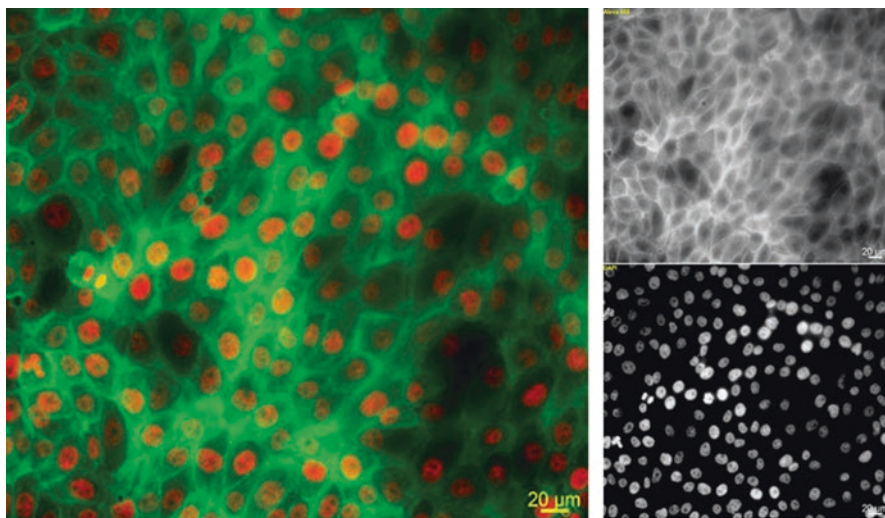


Fig. 19.14 Human mammary epithelial cells (MCF-10A cell line) (phalloidin and DAPI) [38]

19.7.4 Epithelial Cells

Epithelial cells cover the lining of the body cavities and glands like the colon, ovary, or mammary gland (Fig. 19.14). Epithelial cells require a substrate to adhere and for guidance. Cells must exert a tractive force on the substrate via focal adhesion complexes through the lamellipodia and filopodia in order to adhere and spread on a surface [88], and as a result of the presence of some topographic features, the cells could be expected to adhere better. However, this is not always the case. In one study, epithelial cells were seeded on channel-type nanogrooved silicon oxide substrates, produced by e-beam lithography with ridges 70 nm wide with 400 nm wide pitch, and were observed not to spread but stayed rather round, and only a few of

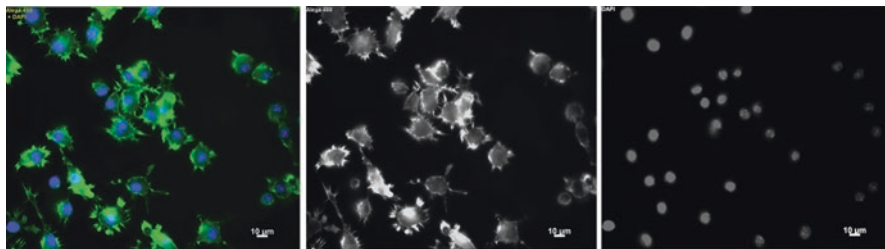


Fig. 19.15 Macrophages in cell culture [38]

them formed focal adhesions implying that there were no satisfactory interactions with the surface [89].

The focal adhesions formed were confined to the ridges of the grooves and significantly smaller than those observed on the planar control surfaces. Even though they did not spread over such surfaces, they were still shown to align with the channels on which they were seeded.

19.7.5 Macrophages

Macrophages are found in every tissue of the body under the names of microglia, Kupffer cells, or osteoclasts and engulf apoptotic cells, particulate matter, and pathogens (Fig. 19.15). They have a high capacity to change their functional phenotype depending on the environmental cues they encounter [90].

With these capabilities, macrophages constitute an important group of cells to study their behavior on nano- and micropatterns. The guidance and activation of macrophages from the P388D1 cell line and rat peritoneum were studied on topographic features (channels and steps) 30–282 nm deep and 2–20 μm on fused silica surfaces on a nanometric scale. The contact of cells with the nanopatterned surfaces activated cell spreading and adhesion and increased the number of protrusions of the cell membrane. Surface nanotopographies were also shown to direct macrophage adhesion and spreading. Accumulation of F-actin and vinculin at the edges of the topographic features was reported. Cells cultured on nanogrooves showed a higher phagocytotic activity than those cultured on smooth surfaces [91]. These show that macrophages respond to surface features as small as a collagen fiber.

19.7.6 Stem Cells (MSCs) and Other Cells

Stem cells are unspecialized cells that have the ability of self-renewal for much longer periods than mature cells, and unlike the mature cells, they have the capacity to differentiate into a variety of specialized cells with specific functions. Totipotent cells are stem cells that can give rise to any type of cell and have even the potential

to create a whole human being. Pluripotent cells have a narrower range of cells to turn into, and the other group, the multipotent stem cells, is restricted to produce only a specific type of cells. With this capacity to be able to differentiate to a variety of cells and also the many renewal cycles, they have been a favorite cell type for tissue engineers. Mesenchymal stem cells were originally identified in the bone marrow and since then they have been detected in other locations. These cells are multipotent and can be proliferated in vitro and can be guided to differentiate into certain cell types such as osteoblasts, chondrocytes, and cardiomyocytes. An area of importance for the stem cells in the biomaterials field is the study of surface-cell interactions which is very important for understanding cell behavior on substrates with different chemistries and topographies in 2D and 3D. In a study of production of biodegradable bone plates impregnated with nanocrystals of hydroxyapatite, mesenchymal stem cells of rat bone marrow origin were used to study the compatibility of the plate material. The cells showed indication of differentiating into osteoblasts (production of alkaline phosphatase, an enzyme specific for osteoblasts) and strongly adhered to the PLLA structures [92].

Studies of surface-cell interactions are carried out in micro- and nano-levels. The micro-level studies generally involve channels, pits, and protrusions starting at low micron values as low as few tens of nanometers, much lower than the cell dimensions. The following are some examples of these studies showing that surface designs much smaller than cell dimensions are able to influence stem cell behavior, or in other words, cells can sense nano-scale features.

In one such study, the behavior of mesenchymal stem cells along with other types on a variety of surfaces including wells, protrusions, and channels of various dimensions was reported [93]. Others [94] studied the influence of nanopores on polymeric films of PS, PLLA, PLGA, and PMMA on mesenchymal stem cell (MSC) growth. Their results indicate that the MSCs can sense the nanoscale topographic features and grow well on them.

In brief, cells of different origin behave differently on surfaces with the same chemistry and topography indicating the difficulty in generalizing the cell behavior on a given implant. It appears that every novel implant has to be tested thoroughly with cells before deciding on the most suitable surface features and chemistry.

19.8 Conclusion

In this chapter, the importance of nano- and micro-systems, as well as nano-modifications on biomaterials were discussed. Micro- and nanoparticles and fibers, physical, chemical, and biological modifications on implant surfaces, and their effects on cells were examined. Micro- and nano-modifications, such as patterning of the surfaces of implant materials, have a crucial role to play in medical applications. Cells, depending on their type and properties, exhibit different attachment and proliferation behaviors on these modified surfaces.

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