

Remineralization and antibacterial capabilities of resin-based dental nanocomposites **15**

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15.1 Introduction

15.1.1 Resin-based dental composites

More than half a century ago, resin-based composite materials were first introduced by Bowen in 1962. Resin composites came into the spotlight because of their desirable characteristics, such as being able to bonded to dental structures, minimal removal of tooth structure, and desirable aesthetics [1, 2]. These advancements have been largely focused on the mechanical and aesthetic properties of resin composites. Furthermore, more than 116 million restorations in 2005 and more than 200 millions in 1999 were placed using composites in the United States. This reinforces the fact that composites have become one of the most commonly used materials in dentistry [3].

In recent years, the structure of the polymer matrix, fillers, and even curing systems of resin composites has gone through vast changes in order to enhance the quality and longevity of composite restorations [4–6]. However, studies have reported that half of composite fillings failed in the first 10 years [7–11]. Numerous causes for failure of these restorations have been mentioned. The most common reasons include bulk and marginal failures due to low mechanical properties of the resin composite, and the prevalence of recurrent caries in the margins of the restoration. Caries in the margins of restorations are the main cause of replacing these restorations [7, 9, 12]. These failures comprise 50%–70% of restorations, and the replacement of composite restorations occupies a majority of dentists' time and incurs great costs. More than 5 billion dollars of damage is inflicted to the annual U.S. social health budget [13]. Therefore, research aimed at improving the mechanical properties and development of new methods for reducing recurrent caries' prevalence adjacent to restorations have a high priority in the field of resin composites. Dental caries are one of the most common infectious diseases in human beings [14, 15].

15.1.2 Dental secondary caries

The mechanism of caries is a dietary, carbohydrate-modified bacterial infectious disease. In 1999, Featherstone presented the concept of caries' balance, such that

the acute and arrested caries are based on the balance between the progression of pathologic factors (related to demineralization), and protective factors (related to remineralization) [16].

Based on Featherstone's theory, one of the pathologic factors is cariogenic bacteria, and the acids they produce [16].

The human oral cavity is a poly-microbial environment. Different individuals have more than 1000 different bacterial species, and numerous types of viruses, fungi, and yeast in their mouths. There are approximately 10^8 – 10^9 bacteria present in each milliliter of saliva, or each milligram of plaque [17–19]. The relationship between these bacteria and diseases of the oral cavity, such as gingivitis, periodontitis, candidiasis, endodontic infections, peri-implantitis, and dental caries has been proven [20]. These bacterial species can exist in planktonic form, or as a colony in bacterial plaque in the oral cavity. Research has shown that the kind that exist in bacterial plaque can lead to disease, and plaque-related-diseases are one of the most common types of illness in humans [21].

Dental pellicle is the initial protein-based precipitation that forms on oral tissues immediately after the oral tissue surface comes into contact with fluids of the oral cavity, and is derived from saliva and food debris. Due to the surface charges and properties of chemical groups of the pellicle, it changes the surface characteristics of the substrate on which it is located [17, 22]. After pellicle formation, the first colonizing bacteria accumulate on the acquired pellicle, and modify the conditions for acidogenic bacteria to colonize. The bacteria that initially accumulate on the pellicle determine the species of bacteria that will be dominantly colonized. The complex formed is called bacterial plaque. Bacterial plaque can form on all tooth surfaces, including dental structures, restorative materials, and even oral mucosa [17].

Dental plaque is the main cause of primary and secondary dental caries, and gingivitis adjacent to teeth. Cariogenic bacteria, which mainly include streptococci mutants and lactobacilli, metabolize carbohydrates by fermentation. This produces acid as a byproduct, which reduces the pH of the environment around dental structures. Demineralization of dental structures can lead to dental lesions, which, if they persist, can spread to the pulp chamber of the tooth [14, 15, 23–25].

Infections of the oral cavity, and the bacterial population related to them, not only compromise oral health, but also pose a threat to systemic health, and burden society with costs as a consequence [26–29].

The first ICNRA conference (International Conference on Novel Antiquaries and remineralizing agents) was held in 2008. In this conference, the aim of caries disease management was presented in two parts: (1) reducing bacterial challenges, and (2) increasing remineralization and enhancing natural repair of arrested lesions [30].

These approaches were presented for primary dental caries. However, considering the similar mechanism for prevalence of secondary caries, these approaches can be applied in prevention and control of secondary caries in areas adjacent to resin composite restorations.

One of the major challenges on resin composite restorations is the polymerization shrinkage that occurs in these materials. This shrinkage can lead to failure of the adhesive between the restoration and tooth structure if unsuitable materials and techniques

are used. This can create areas suitable for bacteria to accumulate and attach, and then caries to occur [31].

Considering this major drawback, resin composites not only fail to show antibacterial behavior, but also a higher prevalence of plaque formation in both in vitro and in vivo studies. This is related to the chemical, electrostatic, and physical properties of composites [32–35].

Therefore, producing the resin composites with antiplaque and antibacterial properties may contribute to reduction of secondary caries in areas adjacent to the composite restorations. The other side of the balance mentioned by Featherstone is the remineralization process. This preferred method for the demineralized dental tissue is to provide conditions in the oral cavity that promote regeneration of the dental structure as an alternative to the use of restorative materials, or removing the damaged tissue. This approach is part of the concept called “minimally invasive dentistry” [36].

Fluoride has been established as an antibacterial agent with the capability of promoting remineralization of dental mineral structures. It has been utilized in dental restorative materials such as glass ionomers, and some types of resin composites. Moreover, other substances with similar effects have been recently introduced, but their use in resin composites is still in the experimental phase.

15.1.3 Nanoparticles in resin-based dental composites

‘Nano’ is a Greek word meaning extremely small, but in scientific terms, “nano” refers to the substances that are smaller than 100 nm (10^{-7} m) in one dimension. Nanotechnology is an applied science that is related to the structure and properties of material on an atomic and molecular scale [37].

Nanomaterials possess different chemical reactivity, biological activity, and physical properties compared with their bulk form, and the reason behind this is their higher active surface area in nano form [31, 38].

The use of nanotechnology in dental materials, and specifically, in restorative materials, is not a new concept. Nanoparticles were added as fillers in resin-based composites 18 years ago for the first time. Colloidal silica particles were added as fillers to the microfilled composites with an average size of 40 nm, and marketed as nano-hybrid composites. Resin-based composites containing these particles have high aesthetic properties, good polishing characteristics, suitable wear resistance, and effective mechanical properties [31, 39].

Recently, in addition to the colloidal silica, ceramic nanoparticles such as ormocers have been used in the resin-based composites to reduce polymerization shrinkage and enhance aesthetic properties [40, 41].

Nanoparticles have also been used to improve properties of adhesives, and to reduce the cohesive fractures that occur in the adhesive layer [42–44].

However, utilizing nanomaterial as therapeutic agents in resin-based composites doesn’t go back a long way. Previously, nanotechnology had been used in preventive procedures, reversing the dental decay process, and repairing preliminary lesions via various methods such as mouthwashes, toothpastes, and other protective products [45, 46].

Using nanoparticles separately with desirable distribution in a single matrix phase (such as what is needed in resin-based composites) is a difficult task. Nanoparticles tend to become agglomerated, and when stable, are mostly available in the cluster form. On the other hand, it has been shown that wetting the nanoparticles using bifunctional primers is not very effective, and this poses a problem due to the fact that the bifunctional primers are a key part in creating a uniform resin and filler phases in resin-based composites. Scientists are progressing in overcoming these and other challenges that oppose the use of nanoparticles in resin [31].

Nanotechnology has been applied in two main approaches in dentistry; preventive and restorative, which are being developed further with the advances taking place regarding nanoparticles [47].

Nanoparticles have been added to adhesives and resin-based composites with two main goals:

1. Antibacterial and antiplaque accumulation effects
2. Remineralization of preliminary lesions [45]

15.1.4 Future of resin-based dental composites

In 2009, Klaus presented an overview of future advancements in dental materials with regard to the resin-based composite in five main topics:

- Nanotechnology
- Antimicrobial materials
- Stimuli responsive materials
- Self-repairing materials
- Materials for bone and dental hard tissue regeneration

Four of these topics are related to the subject in this chapter that emphasizes their importance [31].

In this chapter, the effect of integrating nanomaterials in resin-based composites has been discussed in two parts; antibacterial and remineralization agents.

15.2 Remineralization and antibacterial capability of resin-based dental nanocomposites

15.2.1 Nano-antibacterial agents in resin-based dental composites

15.2.1.1 Background

Antibacterial agents are a group of materials that fight against pathogenic bacteria. Thus, by killing or reducing the metabolic activity of bacteria, their pathogenic effect in the biological environments will be minimized [48].

Furthermore, these materials can prevent bacterial plaque accumulation in the oral environment and therefore, can reduce the prevalence of plaque-related diseases

such as caries. However, it should be noted that antibacterial agents do not necessarily possess antiplaque accumulation properties [31, 49].

Today, the manufacture and use of biomaterials with antibacterial effects in medical and dental treatment plans is rapidly progressing. Manufacturing products containing antibacterial agents, or coatings of the material with antibacterial properties, has become an interesting topic for research in the field of dentistry. Various types of implants, restorative materials, adhesives, denture-based materials, and tissue conditioners are being produced with antibacterial properties, and analyzed in laboratories; some have even been marketed.

The idea of adding antibacterial agents to the restorative materials such as resin-based composites or glass ionomers is not a new concept. However, introducing nanotechnology in the medical and dentistry fields has proven to be a promising concept. Previous efforts were made to produce resin-based composites with antibacterial properties by adding chlorhexidine; however, they had short-term effects [50–52].

Furthermore, the addition of chlorhexidine and antibiotics to the resin-based composites compromised their mechanical properties such that as these compounds came out of the composites and their mechanical properties deteriorated [52–55].

In addition, these organic antibacterial materials were highly susceptible to damage upon exposure to heat [50].

Numerous studies are being conducted to overcome these problems. Antibacterial agents can be added to either the fillers or resin matrix of composites. One thing that must be considered for all these innovative factors is that their integration into the composite material should not cause a decrease in the mechanical or esthetic properties [56–59].

In this section, we aimed to review the nanomaterials added to resin-based composites to obtain antibacterial properties.

15.2.1.2 *Antibacterial metallic agents*

Over the years, many antibacterial properties have been discovered in regard to pure metallic elements, or their compounds such as oxide or nitrides. Metallic elements such as silver, zinc, titanium, gold, copper, and magnesium have been found to show various antibacterial effects in different studies, and can be produced in nano form [60–63].

Some of these compounds have been added to resin-based composites.

Silver nanoparticles

Background Antibacterial properties of silver have been known for centuries. At 1000 BC, silver compounds were used as a balm for wounds and burns. Even today, silver nitrate and sulfadiazine are known as the gold standards for treating localized burns [63, 64].

Since 1800, silver has been used in medicine and dentistry due to its anticariogenic, antibacterial and antirheumatic properties. In 1840, silver compounds were used to reduce caries in deciduous teeth. Then, it was also used for permanent molars, and in disinfecting dental cavities before restoration, and as a desensitizing agent for dentine [65–68].

Various compounds of silver have been used for dental purposes such as silver nitrate, as a compound with fluoride, and also with tin [69].

Gradually, the use of silver in dentistry was set aside, as it caused discoloration of tissues, and resin- based composites containing silver [70]. However, silver has become popular again today and is being used in dental and medical products because of its broad spectrum, low toxicity, and lack of cross-spectrum bacterial resistance [69].

Antibacterial mechanisms of silver nanoparticles Silver has the highest antibacterial effect when it is in ionic form (Ag^+), and when it is metallic, silver is almost an inert element. When silver is used in its metallic form and placed in the mouth, it readily reacts with water available in the oral cavity and produces ionic silver, having antibiotic effects [71].

Basically, the ability to ionize silver is the factor that determines the antibacterial effect of a compound containing metallic silver [72]. Silver ions have three major effects: destruction of cell wall structure, denaturation of cytoplasmic enzymes, and inhibition of microbic DNA replication [72].

Ionic silver can bond to the disulfide compounds available in the cell membrane's proteins and allow easy penetration into the cell [73]. Moreover, ionic silver has a positive charge, which enables it to be bonded to the peptidoglycans available in the bacterial cell wall with a negative charge. Thus, the silver ion has disrupted the transfer process in the bacterial cell membrane and caused the bacterial cell to die [74]. Besides, these silver ions can be bonded to the sulfhydryl groups available in intracellular enzymes, and disrupted vital metabolic functions, which can also cause the cell to die [75, 76]. Silver ions can attack the base guanine that causes dysfunction in DNA replication [69]. In addition to the effect of silver on planktonic bacteria, it can also prevent the accumulation of biofilm. This characteristic of silver is put to use in catheters, cardiac devices, and orthopedic prosthesis to create a surface with minimal plaque formation [69].

Briefly, silver has antibacterial properties against a wide spectrum of bacteria, viruses, and other eukaryotic organisms [62].

The effectiveness of silver has been proven against *Streptococcus mutans*, one of the main bacteria involved in caries and periodontal diseases. Therefore, scientists have been trying to incorporate silver into dental cements, sealers, and restorative materials such as glass ionomers and resin-based composites in order to achieve antibacterial properties [57, 58, 77–79].

Some studies have reported an increase in hydrophobicity as a consequence of adding silver particles with sizes in the micro range to the resin-based composites [80].

Hydrophobic materials are renowned for being resistant to bacterial adhesion and colonization, and are called “self-cleaning material.” This can explain, to some extent, the antibiofilm characteristic of silver-containing materials [81, 82].

Other analyses have also verified the antiadherence property of resin-based composites that contain silver in regard to *S. mutans* [80].

There are two major drawbacks, even with all the benefits mentioned, for using silver in resin-based composite restorations adjacent to the gingiva: the silver ion released from these materials in demineralized areas of tooth structure is oxidized and

forms metallic silver. This causes discoloration of the tooth structure in close proximity with the restorative material containing silver [83, 84].

The other disadvantage is that the addition of silver to the resin-based composites can darken these materials in color. These problems were mostly solved after being able to produce composite on a nanosized area. Silver nanoparticles had higher antibiotic properties than that of other silver compounds used before [63]. The reason for this is the small size of the particles. As the size of the particles becomes smaller, the active surface increases, thus increasing their effectiveness. For example, a mass fraction as small as 0.08% of silver nanoparticles can cause a 40% reduction in the bacterial coverage of nanocomposites [85].

The mechanism of silver nanoparticles in regard to antibacterial activity is the same as larger silver particles. It has been shown that the smaller the particles are, the easier it is for them to adhere to the bacterial cell wall and membrane. It would also be easier to penetrate the bacterial membrane and other microorganisms [86–89]. Other studies have also demonstrated that particles smaller than 10nm have higher reactivity with the bacterial cell wall due to certain types of electric characteristics. Therefore, it is completely predictable that the bactericidal effects of silver are size-dependent [88, 90].

Research has been carried out on the relationship between the shape of nanoparticles and their bactericidal effects. For example, if the nanoparticles are truncated triangular, spherical, or rod shapes, in order to have bacterial inhibition effects, they have to be 1, 12.5, and 50–100 μg per thousand nanoparticles, respectively. This phenomenon is most likely because the silver nanoparticles have different efficiencies in different shapes on microorganisms' cell walls [88].

Silver nanoparticles in resin-based dental nanocomposites Silver has been used in various forms in the resin-based composites. For example, silica fillers with silver ions implanted onto them have been used in resin-based composites [57].

In other experiments, silver ions that were supported by other compounds such as silica, zirconium phosphate, glass alumina, and magnesia as carriers were used as fillers in resin-based composites. Analysis of these composites, besides verifying inhibitory effects of silver on *S. mutans*, showed that these composites had long-term (3 months) antibacterial effects [78, 91].

Attempts were made to even add YDA fillers to resin-based composites. YDA fillers are particles with an average size of 6 μm that contain 14.1% silver, 21.2 %aluminum, and 64.7% phosphorus [91].

The use of these compounds demonstrated two major problems:

1. These silver compounds could not be dispersed uniformly in the matrix.
2. They did not have a desirable color.

One suggestion to solve these problems was to use organic-functionalized silver nanoparticles in resin-based composites. Because these compounds have an organic part, they have better compatibility with the resin matrix. Therefore, they can achieve a more uniform dispersion of the nanoparticles containing silver, and the formation of clusters and agglomeration is prevented. The interaction of the organic part and resin matrix also leads to long term antibacterial effects.

Using particles on a nanoscale reduces the amount of silver compounds needed to achieve antibacterial properties, which eliminates the discoloration caused by large amounts of these compounds, and better aesthetics are acquired [92].

These silver-containing filler particles are in fact nanocrystals covered with oleic acid, which are analyzed along silica filler particles in resin-based composites.

The results obtained from analysis showed that by adding silver nanoparticles covered with an organic factor, the degree of polymerization is reduced. One reason for this may be the fact that the addition of silver will darken the material to some extent, preventing complete penetration of the blue light-curing device. However, even after reduction of the degree of polymerization in the experimental resin-based composites containing 50 ppm organic-functionalized silver nanoparticles, an improvement of mechanical properties was reported. This can be due to the crosslinking that occurs between the organic branch of the filler particles and resin matrix. Another reason for the improved mechanical properties can be because of the nanoparticles that fill up spaces that are usually left empty between fillers, which ultimately increases the filler packing density of the composite [93].

Another important characteristic of these compounds is the immobilization of silver in the resin-based composite. This means that silver ions are not released and the antibacterial effect of these compounds works mainly through the activation function of silver ions, which produces active oxygen species. In this process, silver plays the role of a catalyst [58].

This issue causes a weaker, but prolonged antibacterial effect in these resin-based composites.

Considering the growth inhibition zone around composite discs in the agar diffusion test, a small amount of silver release can be predicted [93].

As mentioned before, one of the major problems of adding silver nanoparticles to the resin-based composites is their weak inclination to disperse in organic material, and when added, they tend to agglomerate, rather than to be dispersed in the resin matrix [94].

Another method that has been used extensively in many studies to overcome this problem is to create the particles *in situ*. In this method, Ag 2-ethylhexanoate salt is solved in a monomer called TBAEMA, and then this complex is mixed with the other structural monomers in resin-based composites [85].

TBAEMA can form coordination bonds with silver particles, and increase the solubility of silver in the resin matrix. The nano-particles created in this method are 2.7 nm in size, and can be dispersed adequately in the resin matrix [95].

Studies have shown that the resin-based composites that contain these nanoparticles cause a reduction in the bacterial growth, biofilm colony forming units, viability, and metabolic activity of the biofilm. Another advantage of using this method is the covalent bonds that can be formed between the silver-containing monomers (TBAEMA) and other monomers present in the matrix, which can increase the crosslinking of the resin-based composites and improve the mechanical properties [85, 95]. These silver nanoparticles have been used alongside other promoting factors inside the resin-based composites. One example is the remineralization factors, such as amorphous calcium phosphate nanoparticles [96]. Another example is quaternary ammonium-containing nanoparticles, which have been proven to have antibacterial effects [97].

Considering that the primary objective of the resin-based composite restorations is providing function and aesthetics, the addition of silver nanoparticles should not undermine these objectives. Studies have confirmed that the addition of silver nanoparticles in quantities less than 0.02 mass fraction did not reduce the tensile strength of resin-based composites [85].

However, higher quantities, for example 0.1% (w/w) of silver benzoate, can reduce the surface hardness of resin-based composites significantly [98].

Regarding the esthetic properties, even though nanoparticles are extremely small, they can cause the resin-based composite to be darkened in color if a high amount of them is added due to their interaction with visible light rays [98].

Silver nanoparticles with a size of 2.7 nm can darken the resin-based composite if they exceed a 0.175 mass fraction [96]. Up to this point, all the suggested methods for improving the dispersion of silver nanoparticles have used organic vectors, which improved the solubility of silver nanoparticles in the matrix. Another method for better dispersion of silver nanoparticles is to use filler particles in a resin-based composite. In this method, silver and zinc cations are added to silica nanoparticles. It has been shown that the silica particles were not changed noticeably after the adsorption of these ions. In addition, the silver and zinc nanoparticles fell into the porosities on the surface of silica nanoparticles. Husheng's study showed that these silica fillers could have significant antibacterial effects [99].

Silver nanoparticles are also used vastly as adhesives and primers, as well as resin-based composites, due to their antibacterial effects [100–102].

ZnO nanoparticles

Background Previously, ZnO was used as a reinforcing and opaquer filler for the resin-based composites [103]. However, zinc oxide and many other metal oxides have antibacterial characteristics against both gram-positive and gram-negative bacteria [104–106]. Zinc oxide is used as an antibacterial agent in oral hygiene products such as toothpaste and other products related to gingival hygiene [107].

Antibacterial mechanisms of ZnO nanoparticles Multiple mechanisms have been suggested for the antibacterial effects of zinc oxide. ZnO is able to react with the moisture available in the oral cavity and creates active oxygen species such as H_2O_2 , inhibiting the planktonic bacteria. This mechanism is similar to some of the other metal oxides such as TiO_2 . Studies have shown that zinc oxide can have photocatalytic effects similar to TiO_2 , such that in the presence of ultraviolet light it can produce active oxygen species and promote their antibacterial properties [108, 109].

Another proposed mechanism that can justify the antibacterial properties of ZnO in conditions without any light present is the absorption of halogens by the surface of ZnO nanoparticles. These halogens function similar to the oxidizers and can disrupt bacterial functions. This mechanism is the principal approach in aerobic environments [110].

Another suggested mechanism is the release of zinc ions into the surrounding environment. Zinc ions have the ability to disrupt the active transportation of ions and elements in the bacterial cell membrane and thus, inhibit the metabolism of glucose [111].

Studies have shown that zinc replaces magnesium, which is a critical element in functions of bacterial enzymes and thus, disrupts the metabolic activities related to the formation of bacterial plaque [112].

Disrupting the metabolic functions of bacteria means inhibition of the function of the glucose transferase enzyme and reducing the acid produced from the metabolism of bacteria such as lactobacilli and *S. mutans*, which ultimately means less caries prevalence [113–115].

The antibacterial effects of zinc oxide are similar to silver in the sense that the smaller the particles are, the larger the surface to volume ratio becomes, and thus, the antibacterial effect becomes stronger [116–118].

ZnO nanoparticles in resin-based dental nanocomposites An important point about zinc oxide is its opacity. Therefore, in high quantities, it reduces the curing potential of light cured resin-based composites and deteriorates the mechanical properties. It is important to use this material in small and controlled amounts, and nanotechnology has managed this [116]. The addition of hexagonal zinc oxide nanoparticles with a size of 20 nm in quantities of 1, 2, 3, 4, and 5 wt.% does not affect the degree of polymerization of nanocomposites, but significantly reduces the depth of curing. The addition of 5 wt.% of zinc oxide nanoparticles reduces the depth of curing by half [116].

Studies on the resin-based composites containing ZnO have revealed that these composites do not have a growth inhibition zone around sample discs during agar diffusion tests. This shows that zinc ions are not released (not enough to be effective against bacteria) by these composites.

After submerging the composites in water and testing for leached antibiotic agents, none were detected, which was confirmed by the previous results. The fact that these antibacterial factors are not leached into the surrounding environment could mean a limited antibacterial effect when these factors come into contact with material [116, 119]. An advantage for using ZnO nanoparticles instead of silver nanoparticles in nanocomposites is the absence of discoloration [35]. Moreover, ZnO nanoparticles have a stronger inhibitory effect on streptococci mutans compared with that of silver nanoparticles [120]. Unlike silver, however, ZnO and other metallic oxides have been identified to be toxic for human cells in high concentrations [121, 122]. In terms of antibacterial effects, a resin-based composite containing 10% (w/w) ZnO nanoparticles has the same efficiency as the resin-based composites containing 9% (w/w) chlorhexidine [119]. However, in other studies, only 1 (wt.%) of ZnO nanoparticles has been reported to have sufficient antibacterial properties [120].

TiO₂ nanoparticles

TiO₂ were previously added to the resin-based composites and epoxy resins with the goal of improving mechanical properties [123, 124]. The advantage of using these nanoparticles is their chemical stability, biocompatibility, and lack of toxicity [125, 126].

Even though, their antibacterial properties have been confirmed especially in nano size, they have not been used in resin-based composites, and their mechanical and antibacterial properties have only been analyzed when they were added to the glass ionomers and resin-based composites used in orthodontics [127, 128].

It has been reported that adding TiO₂ nanoparticles in 3%, 5%, and 7% (w/w) to glass ionomers has increased the antibacterial effects in both short and long term. [127].

Even small amounts of titanium oxide (0.1–0.25 (wt.%)) can lower aesthetic properties such as translucency and discoloration of resin-based composites, which could be one of the reasons they are not added to these materials [129].

15.2.1.3 Antibacterial polymeric agents

Chitosan nanoparticles

Chitosan is a natural polymer obtained from chitin which makes up the bulk of the exoskeleton in crustaceans [130]. Chitosan has plenty of amine groups that are attached to a polysaccharide chain and have a high potential for reacting with the surrounding environment [131]. One of the areas that reacts readily with cation groups of chitosan is the bacterial cell membrane which provides antibacterial properties in this substance [132, 133].

Many studies have shown that the surface charge of chitosan can prevent bacterial plaque formation adjacent to it, an effect similar to polyethylenimine (PEI) which will be described later on. The nontoxic and antibacterial effects of chitosan have rendered this material to have multiple uses in dentistry [134, 135].

Some examples of products in which chitosan has been used are toothpastes and oral hygiene products, dental adhesives with antibacterial properties, metallic scaffolds, and so forth [136, 137]. Chitosan has been analyzed in resin-based composites and results showed that it promoted the antibacterial effects without deteriorating the mechanical properties such as surface hardness [138].

The antibacterial properties of chitosan have also been tested against *S. mutans* and the results showed that the biofilm formation was inhibited in the presence of this substance [139]. Several studies have showed that by decreasing the molecular weight of chitosan, its antibacterial properties were enhanced [140, 141].

One study carried out on the antibacterial properties of chitosan nanoparticles in the resin-based composites used in orthodontics that contained zinc oxide nanoparticles and chitosan nanoparticles. This resin-based composite was made by adding an equal amount of each of these nanoparticles. Based on the obtained results, the resin-based composites with 10% (w/w) of zinc oxide and chitosan nanoparticles (1:1 ratio) significantly inhibited bacterial growth. In this analysis, no change in the mechanical properties was observed with the addition of chitosan nanoparticles [142].

QPEI nanoparticles

Background Strategies that can add antibacterial effects to the resin-based composites are divided into two groups:

1. Antibacterial factors that are soluble in different medias in the mouth
2. Antibacterial factors that do not leach from the material and are immobilized

Leaching of these antibacterial agents has many limitations and disadvantages:

1. Causes defects in the bulk material, which reduces the bulk and surface characteristics of the resin-based composite.

2. The antibacterial effect of these resin-based composites is for a limited time and after the depletion of these factors, the resin-based factor would no longer show antibacterial effects.
3. Because the rate and amount of leaching for any of these compounds is difficult to control, an allergic reaction is possible [50, 56–58, 143, 144]. Considering these issues, the antibacterial agents that would remain in the structure of resin-based composite with long-term antibiotic effects are necessary.

Antibacterial mechanisms of QPEI (quaternary ammonium polyethylenimine) nanoparticles Previous studies have shown that polycations that contain quaternary ammonium groups have antibacterial characteristics. The antibacterial effects of these compounds can be related specifically to the inducing dysfunction in the bacterial cell membrane [145, 146].

Bacterial cells have a negative surface charge that attracts the N^+ (nitrogen) in quaternary ammonium compounds. Attachment to these compounds causes disruption of the membrane functions such as in-take and out-take of vital ions, which are the main function of the membrane. After disrupting the cytoplasmic diffusion, the bacterial cell bursts under osmotic pressure [147].

Prior to this, quaternary ammonium salts and their derivatives were used in water treatment in the food industry and manufacture of antibacterial covers. This vast range of use for these compounds is due to low toxicity and good antibacterial effects [148].

Based on this approach, one of the mechanisms to create antibacterial effects in the resin-based composites is the use of pyrimidium type polymers. These polymers are available in two forms, leachable and nonleachable (immobilized). The immobilized kind can be used to produce coatings and materials with bulk antibacterial properties that are stable [149–151].

QAS bromes and chlorides in different chain lengths have been used to create glass ionomers with antibacterial effects [152]. One of the most common monomers in this field is MDPB (methacryloxydodecyl pyridinium bromide), which is used as an antibacterial agent in products [33, 153].

Another compound used with this approach is QPEI (quaternary ammonium polyethylenimine). These particles are made in a nanoscale, and can be added to the resin-based composites to provide an antibacterial effect.

Synthesis and characterization of QPEI nanoparticles The method of producing these nanoparticles based on the suggested method by Beyth et al. [97] is shown as follows:

In brief, PEI (10 g, 0.23 mol monomer units) dissolved in 100 ml ethanol was reacted with dibromopentane at a 1:0.04 mol ratio (monomer units of PEI/dibromopentane) under reflux for 24 h. N-alkylation was conducted as follows: octyl halide was added at a 1:1 mol ratio (monomer units PEI/octyl halide). Alkylation was carried out under reflux for 24 h followed by neutralization with sodium hydroxide (1.25 equimolar, 0.065 mol) for an additional 24 h under the same conditions. N-methylation was conducted as follows: 43 ml of methyl iodide (0.68 mol) were added. Methylation was continued at 42 C for 48 h followed by neutralization with sodium bicarbonate (0.23 mol, 19 g) for an additional 24 h. The supernatant obtained was decanted and precipitated in 300 ml of double distilled water (DDW), washed with hexane and DDW

and then freeze-dried. The purification step was repeated using additional amounts of hexane and DDW. The average yield was 70% (mol/mol)

Beyth et al. [97].

In vitro analysis of QPEI nanoparticles in resin-based nanocomposites The antibacterial properties of these nanoparticles has been analyzed against a wide range of gram positive and negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* ATCC 8325-4, *S. mutans*. SEM analysis and direct contact tests showed that adding only 1% (w/w) of QPEI nanoparticles to the resin-based composites was enough to significantly reduce the amount of superficial bacteria. This effect lasted for the 4 weeks that the samples were submerged in water, which verifies the long-lasting effects of this material [97, 151]. However, the antibacterial effects of QPEI have been verified to last up to 6 months under in vitro conditions [154].

In the agar diffusion test which is based on solubility of compounds, no growth inhibition zone formed around samples containing QPEI, which showed the lack of leachability of this substance from the resin-based composite [155, 156].

This would count as an advantage for the resin-based composites which are used as restorative materials in a moist environment. Without leaching, the mechanical properties of the resin-based composite do not deteriorate [156]. According to studies, QPEI nanoparticles have bactericidal effects and not bacteriostatic. Whereas, the other monomer in this group, MDPB, has more bacteriostatic effects against *S. mutans* [157].

Other advantages of these polymeric antibacterial agents compared with the other agents are as follows: they are nonvolatile and chemically stable, can be chemically bound within the polymer carrier via active groups for improved integration in the composite, and cannot easily penetrate the skin and mucosa [97].

As mentioned before, an important point regarding the resin-based composite is maintaining their mechanical properties at an acceptable standard while having antibacterial effects. To analyze these properties after adding QPEI, Beyth et al. [97] added 1% (w/w) of these nanoparticles to the three resin-based products available on the market (one restorative resin-based composite, one flowable composite, and one dental adhesive) and tested their mechanical and antibacterial properties at 1 and 4 week intervals. The results showed that adding these agents to the resin compounds—regardless of their kind—created stable (4 weeks) antibacterial effects. Based on the results of the same study, adding these nanoparticles did not lead to deterioration in the mechanical properties and flexural modulus of the restorative resin-based composites.

As a new nanoparticle, the biocompatibility of this material has to be examined. The results of cell viability tests carried out on macrophages showed that the resin-based composites containing QPEI nanoparticles (1% and 2%, w/w) were not significantly different in terms of biocompatibility with that of composites without these nanoparticles. The amount of α TNF cytokine secretion also was not significantly different between the resin-based composites with and without QPEI nanoparticles [156]. The reason for choosing macrophages in this study to analyze the biocompatibility was

the imperative role of monocytes and macrophages in immunologic and biologic responses of the pulp to material [158].

The importance of this analysis is due to the direct contact of resin-based composites to open dentinal tubules, which leaves the possibility of leaching for the unreacted material such as monomers or soluble components from the material into the pulpal area [159, 160].

It has also been reported that the main cause of cytotoxicity in the resin-based composites containing QPEI are methacrylate monomers present in the composite.

In vivo analysis of QPEI nanoparticles in resin-based nanocomposites In the next stage, in situ tests were performed on the resin-based composites containing QPEI. The reason for these tests was the complexity of the three dimensional structures and composition of bacterial plaque. Even though in these compounds, the highest efficiency is the inhibiting bacterial plaque formation, bacterial plaque cannot be simulated in in vitro tests, therefore, it is obliged to perform in situ tests. It has been shown that the bacteria present in the biofilm on the surface of the resin-based composites containing QPEI were mostly dead [161]. A comparison between the number of dead bacteria in the biofilm on the resin-based composites with and without QPEI revealed a statistically significant difference. However, when the thickness of biofilm formed on the surface of samples was analyzed, it was determined that biofilm formation was not prevented by adding QPEI to the resin-based composites. Some samples of the resin-based composites containing QPEI even showed a thicker layer of biofilm compared with the samples or resin-based composites without QPEI. However, the structure and integrity of bacterial cell membranes available in the plaque was more damaged in the presence of QPEI [161].

It is generally presumed that immobilized antibacterial materials administer their inhibitory effects against bacteria through contact. However, analyses showed that 50% of bacterial species in the biofilm (far from the surface of the material) were harmed. This can be justified by the findings of some studies which have showed when the bacteria in contact with antibacterial agents die, they release metabolites and byproducts. These are interpreted as stressful conditions for other bacteria and trigger programmed cell death (PCD) in surrounding bacterial cells [162–165]. Traditionally, PCD is associated with eukaryotic multicellular organisms [166]. Research has proved that under stressful conditions, bacteria show similar behaviors [161]. Through understanding the antibacterial effects of QPEI nanoparticles, their effects are being analyzed in other dental materials. For example, the use of QPEI has been analyzed in endodontics sealers and also cements [167, 168].

15.2.2 Nano remineralizing agent in resin-based dental composites

15.2.2.1 Background

The major part of the tooth structure is made from hydroxyapatite crystals. This compound contains two main ions, calcium and phosphate, which bond together to form the bulk of enamel and dentine's mineral structure. Research has shown that calcium

and phosphate ions can have a protective effect against demineralization. For example, toothpaste containing calcium is more effective in preventing demineralization compared with those containing SiO_2 [169]. It has been proven that calcium in mouthwashes and chewing gums can cause remineralization [170]. The remineralization effects of calcium and phosphate compounds that are used in pulp capping materials such as MTA have been determined [171].

Hydroxyapatite is the stable and final product of calcium and phosphate in neutral and alkaline conditions (pH 7–9) [172].

15.2.2.2 *Calcium and phosphate releasing resin-based nanocomposites*

Amorphous calcium phosphate nanoparticles (NACP)

Background Based on this approach, composites with the ability to release calcium and phosphate were manufactured. Prior to using nanoparticles, these compounds were used on a microscaled scale. For example, ACP was used with an average size of $55\text{ }\mu\text{m}$ in some resin-based composites, and in others, with an average size of $7.4\text{ }\mu\text{m}$. These resin-based composites released a significant amount of calcium [173–175] and phosphate, and facilitated the remineralization in in vitro conditions [173, 174, 176].

Other calcium phosphate compounds that were added to resin-based composites in micro sizes are tetracalcium phosphate (TTCP: $\text{Ca}_2(\text{PO}_4)_2\text{O}$) and dicalcium phosphate anhydrous (DPA: CaHPO_4) [173, 176].

These compounds had one major problem and that was their poor mechanical properties. Due to their poor mechanical properties, the application of these resin-based functions was limited to lining or pit and fissure sealant materials and they could not be used as bulk restorative materials [173, 177]. After producing calcium phosphate compounds in nano scales (50–100 nm), most of these problems were solved [178].

According to analysis carried out on the amorphous calcium phosphate nanoparticles with a size of 116 nm, not only the release of ions is comparable to previous calcium phosphate compounds with micrometer sizes, but also they could maintain the mechanical properties of resin-based composites high enough to be used as restorative materials [178, 179].

Synthesis and characterization of amorphous calcium phosphate nanoparticles (NACP) Amorphous calcium phosphate nanoparticles, which are one of the most common calcium phosphate compounds used in resin-based composites, are made using the spray drying method.

This method is described as follows:

To make nanoparticles of ACP (referred to as NACP), calcium carbonate (CaCO_3 , Fisher, Fair Lawn, NJ), and dicalcium phosphate anhydrous (CaHPO_4 , Baker Chemical Co., Phillipsburg, NJ) were dissolved into an acetic acid solution to obtain final Ca and PO_4 ionic concentrations of 8 and 5.333 mmol/L , respectively. This resulted in a Ca/P molar ratio of 1.5, the same as that for ACP. This solution was sprayed into a heated chamber and an electrostatic precipitator (AirQuality, Minneapolis, MN) was used to collect the dried particles.

In vitro analysis of amorphous calcium phosphate nanoparticles (NACP) in resin-based nanocomposites Different amounts of glass particles made from barium-boroaluminosilicate sized in the micrometer range that were silane treated were used as cofillers alongside calcium phosphate nanoparticles in the resin-based composites with the goal of improving mechanical properties.

Application of this method has enabled the composites that contain NACP to have mechanical properties twice that of the resin-based composites with micrometer-sized ACP [178].

Another method for increasing mechanical properties in these nanocomposites is using whiskers as cofillers [176]. Even though, the weight content of calcium compounds was reduced in the previous and modern resin-based composites, a similar amount (over saturated) of ions was leached into a water environment from both of the resin-based composites [173, 175].

The high amount of Ca and P in the bacteria plaque adjacent to these nanocomposites in in situ studies verified this issue [180]. The amount of ion leaching from these nanocomposites depends on two parameters:

1. *Ion source*: the higher the amount of NACP in the nanocomposite is, the more ions are released. However, this relationship does not function in a linear fashion for all filler quantities. There are limitations to the method of increasing the ion source. For example, increasing the nanoparticles beyond 20% volume will reduce the amount of glass fillers, leading to a decrease in the bulk mechanical properties of nanocomposites.
2. *Size of particles*: the smaller the particles are, the bigger the surface area they have to interact with in the environment, and thus, more ions will be released. Another mechanism that leads to the increased ion release is through creating smaller particles, such that the larger volume of air enters and gets trapped inside the compound when adding nanoparticles to the resin-based composites. This reduces the degree of polymerization in the composite which can facilitate water penetration into the bulk of the composite that increases ion release rates [172, 179].

Studies have shown that the resin-based composites containing NACP had similar mechanical properties to that of conventional commercial resin-based composites based on the results from thermal cycling, three-body wear, and water aging experiments [181]. Therefore, calcium and phosphate ion release over a long-term period (180 days) did not cause a decrease in the mechanical properties, such as flexural strength [182].

It has been also proven that adding NACP did not affect the polymerization depth in the resin-based composites [183].

In terms of remineralization potential of these composites, microradiographic analysis of human enamel revealed that the demineralized enamel adjacent to these resin-based nanocomposites had high degrees of remineralization (approximately four times commercially available composites with fluoride releasing abilities) [183]. The depth of demineralized areas was considerably less in proximity to these composites, compared with that of other commercially available composites. The remineralizing effects of NACP were traceable in depths of 200 μm [180].

An important point in the analysis of remineralization during pH cycles is that in low pH (cariogenic conditions), these composites release high amounts of calcium and

phosphate ions. However, these ions are not used for remineralization; rather, they are used to neutralize and reduce acidity around the enamel [184]. The released ions can only lead to remineralization in neutral pH conditions [185].

Remineralization mechanism of resin-based dental nanocomposites containing amorphous calcium phosphate nanoparticles (NACP) The NACP-containing resin-based composites can suppress caries' prevalence in two ways: (1) reducing the rate of demineralization by neutralizing the acid produced from bacteria. (2) Increasing the rate of remineralization in neutral pH conditions [183].

NACP-containing nanocomposites are classified as smart materials. This is because the release of calcium and phosphate ions from these composites is 5–10 times higher in acidic conditions. Therefore, the rate of release also increases, meaning more ions are available in critical conditions [172].

Studies have found that NACP resin-based composites are able to increase the pH from 4 to 6 in a small period of time; whereas, the conventional resin-based composites lack this ability [185].

Resin-based dental nanocomposites containing amorphous calcium phosphate nanoparticles (NACP) and antibacterial agents In order to produce resin-based composites with optimum properties, a combination of antibacterial and remineralization factors are used. For example, Cheng et al. produced a resin-based composite containing NACP and quaternary ammonium dimethacrylate and silver nanoparticles, which had a combination of antibacterial and remineralization effects [185]. One of the appreciable results of their study was the minor inhibitory effect of NACP on biofilm formation and acid production. One reason proposed for this effect was that the alkaline environment surrounding these materials can reduce biofilm formation, even though the main purpose behind adding NACP was calcium and phosphate production [185]. However, in situ analysis did not show any antibacterial effects related to NACP [180].

NACP has also been used in combination with other antibacterial monomers such as dimethylaminohex-decyl methacrylate (DMAHDM) and dimethylamineododecyl methacrylate (DMADDM) [186, 187].

In vivo analysis of amorphous calcium phosphate nanoparticles (NACP) in resin-based nanocomposites Based on the only in vivo study carried out in rats' teeth, the adhesives and resin-based composites containing NACP that were placed adjacent to the pulp showed better biocompatibility. In proximity to these composites, there was less chronic and acute inflammation, and more dentin formation [187].

This can be due to the inherent bioactivity of calcium-phosphate based materials [173, 174, 188]. Prior to this, it was verified that calcium phosphate cements have osteogenic properties [189] and can promote odontoblastic proliferation and differentiation in human pulp [190]. Ion release around these compounds can make the pH of pulp more alkaline locally which enhances calcination conditions [191].

All of these items can justify the larger thickness of tertiary dentin formed near NACP-containing nanocomposites. Also, the healing effects of calcium phosphate-based material on the pulp has been shown [188]. The precipitation of

calcium phosphate compounds into dentinal tubules can prevent the penetration of resin monomers into the pulp and improve the biocompatibility of NACP-containing nanocomposites in proximity to dentinal tissues.

The effect of NACP has also been analyzed in self-healing resin-based composites. Although, in this study, more emphasis has been placed on the self-healing behavior of resin-based composites and the remineralization properties were not analyzed comprehensively [192].

Rechargeable resin-based dental nanocomposites containing amorphous calcium phosphate nanoparticles (NACP) One of the biggest challenges facing NACP-containing nanocomposites is their limited time of ion releasing. Ion release occurs from a few weeks to a few months and the amount of leaching decreases over time [173, 179, 183]. Considering that the restorative treatments stay in place for long periods of time, the rechargeability of these nanocomposites is very important.

To create these nanocomposites, two types of resin monomer are used to create the polymer matrix:

Pyromellitic glycerol dimethacrylate (PMGDM) and ethoxylated bisphenol A dimethacrylate (EBPADMA) at 1:1 ratio [193].

Both of these monomers are acidic monomers used in self-etching adhesives [194, 195]. The recharge mechanism of these resin-based composites is related to the carboxylate groups of these acidic monomers, which have a high tendency to chelate with calcium and phosphate ions present in the environment [193]. The mechanical properties of these nanocomposites was not significantly different from that of conventional commercially available composites. It has been shown that the decline in the rate of ion release over time was compensated by being in contact with solutions rich with calcium and phosphate ions. The amount of ions released did not change after six consecutive charge and discharge cycles [193]. It has been concluded that there was an equilibrium in the bond between PMGDM and Ca that was regulated by the pH of the environment. In acidic conditions, the bond is inclined to break and in alkaline conditions (such as using mouthwashes), the bond forms between Ca and PMGDM [193].

In other studies, an antibacterial monomer, dimethylaminohexadecyl methacrylate and a protein accumulation inhibitor monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) were added to these rechargeable nanocomposites [196]. These new nanocomposites had antibacterial properties, as well as remineralization properties; however, their flexural modulus was lower than that of conventional composites and similar to the flowable resin-based composites [196, 197]. This issue limited their use to class V cavities and restorations on the roots surfaces [198].

Dicalcium phosphate anhydrous nanoparticles (DCPA)

Background Another nanoparticle that can be used in resin-based composites with the ability to release calcium and phosphate ions is dicalcium phosphate anhydrous. These nanoparticles are added to resin-based composites with an average size of 112 nm [179]. Previously, DCPA had been used in a resin-based composite used in pulp capping with a size of 1.1 μm [173].

In these resin-based composites, silicon nitride whiskers are used to improve the mechanical properties by twice as much [199].

Synthesis, characterization, and properties of dicalcium phosphate anhydrous nanoparticles (DCPA) DCPA is also made by the spray drying technique:

A single solution was prepared by dissolving 1.088 g of a traditional DCPA powder (J.T. Baker, Phillipsburg, NJ) in 1 L of an acetic acid at 16 mmol/L concentration to obtain a Ca and PO_4 ionic concentration of 8 mmol/L. The solution was sprayed through the nozzle into the heated column and the dried nanoparticles of DCPA were collected by the electrostatic precipitator [176, 200, 201].

It has been shown that reducing the size of DCPA nanoparticles was related to increasing the rate and amount of Ca and P ion release; however, by reducing the size of the nanoparticles, the mechanical properties of nanocomposites was also reduced. The reason for this is that more air penetrates in the structure of composite when smaller particles are mixed into the compound. This reduces the degree of polymerization, which reduces the mechanical properties of the nanocomposite [179]. Introducing whiskers into the composite can compensate for this reduction in the mechanical properties.

Another important point is the increase in the mechanical properties that occurs by silanizing DCPA particles. Silanizing these particles makes a more uniform composite; however, it reduces the amount of ion leaching that occurs in these resin composites. Therefore, it was suggested to use nonsilanized calcium phosphate nanoparticles, and to maintain mechanical properties' cofillers, such as whiskers. Using material like this can improve the mechanical properties to standards required for the resin-based composites used in posterior teeth [179].

One suggested solution for using bioactive fillers without silane is to use a porous alumina as a filler. These alumina particles integrate closely with the resin matrix by micromechanical retention without the need for silane. A special application for these filler particles is the ability to make bioactive compounds, such as calcium phosphate nanoparticles inside these filler particles [202].

Studies have recommended using fluoride mouthwashes alongside these Ca- and P-releasing nanocomposites. This enhances the remineralization effect and creates fluorapatite in the tooth's structure. This compound is more resistant to acidic conditions [172].

15.2.2.3 Fluoride- and calcium-releasing resin-based nanocomposites

Calcium fluoride (CaF_2)

Background Fluoride has been identified and used as an antibacterial and remineralization agent for many years in dentistry. Initially, silicate cements, and then glass ionomers had fluoride-releasing abilities. Gradually, resin-modified glass ionomers, compomers, and resin-based composites with fluoride-releasing abilities were introduced [203–207].

Fluoride's releasability counts as an advantage for restorative material, as it can enrich the adjacent mineral structure to be more resistant to caries [208, 209]. Fluoride can precipitate bacterial plaque and can also increase Ca and P precipitation in the demineralized areas [209, 210].

Glass ionomers are the main restorative material that release fluoride; however, their mechanical properties are poor and adding resin to them did not completely solve this problem [178, 211].

Therefore, there is still a need for a restorative material with fluoride-releasing ability.

Synthesis and characterization of calcium fluoride (CaF₂) Calcium fluoride (CaF₂) nanoparticles with fluoride-releasing ability are made using the spray drying method [178, 201, 212]. This method is summarized as follows.

A two-liquid nozzle was employed. This allowed two solutions to be mixed during atomization: Ca(OH)₂ and NH₄F. The two solutions were atomized, leading to the formation of CaF₂ nanoparticles: $\text{Ca(OH)}_2 + \text{NH}_4\text{F} \rightarrow \text{CaF}_2 + \text{NH}_4\text{OH}$.

The NH₄OH was removed as NH₃ and H₂O vapors [178, 212]. The properties of these nanoparticles are:

The specific surface area of these nanoparticles was measured to be $A = 35.5 \text{ m}^2/\text{g}$. With the density of CaF₂, $\rho = 3.18 \text{ g/cm}^3$, the CaF₂ particle diameter was calculated to be $d = 6/(A\rho) = 53 \text{ nm}$.

In vitro analysis of calcium fluoride (CaF₂) in resin-based nanocomposites To improve the mechanical properties of these nanocomposites, whiskers are used as cofillers. Studies showed that the mechanical properties of fluoride-releasing nanocomposites are similar to the standard hybrid resin-based composites; whereas their fluoride releasing ability was higher than that of conventional resin-modified glass ionomers [201].

In some studies, CaF₂ nanoparticles were used as fluoride-releasing agents and DCPA was used as Ca- and P-releasing agents together. One of the major flaws of these nanocomposites was their opacity which prevented light curing. Using glass fillers can solve this problem to some extent [178, 201]. The theory behind these nanocomposites is providing all three ions involved in remineralization of the dental structure. Under these conditions, the formation of fluoroapatite is more likely than hydroxyapatite. Fluoroapatite is more resistant against acidic conditions and caries compared with hydroxyapatite [201]. No significant deterioration in the mechanical properties was observed in the nanocomposite after releasing of ions because of the whiskers used to reinforce the mechanical properties [201].

An interesting phenomenon in these nanocomposites is the lower calcium content compared with the media's phosphorus content. The reason for this is the precipitation of fluoroapatite. In this molecule, there are five calcium ions and only three phosphorus ions. Therefore, it is predictable that after formation of this molecule and its precipitation, the calcium content of the environment will be lower than the phosphorus content [201]. In order to achieve the antibacterial properties in Ca-, P-, and F-releasing nanocomposites, chlorhexidine was also added. The Ca- and P-releasing particle in this study was ACP, and F releasing particles were CaF₂ which were made by the spray drying method. These nanocomposites used glass fillers made from barium aluminosilicate glass to improve the mechanical properties. In this study, the CaF₂-containing nanocomposite could not be light-cured due to its high opacity and had to be cured chemically. However, the nanocomposite-containing ACP

could be light cured. Both of these composites had twice the mechanical properties of resin-modified glass ionomers, even after being submerged in water for 28 days [213, 214]. Analysis of the acid produced by bacteria around these nanocomposites showed that if the resin-based composite contains CaF_2 , it is more effective in preventing acid production than those composites containing ACP. This can be related to the antibacterial properties of fluoride. Studies have shown that fluoride can reduce lactic acid formation by inhibiting the metabolic path of acidogenic bacteria [214, 215].

15.2.3 Nanocomposites for class V restorations with inhibitory effects on pathogens responsible for periodontitis

The nanocomposites mentioned so far were all focused on the antibacterial effects against cariogenic bacteria. However, the plaque accumulated adjacent to class V restorations can cause gingivitis and initiate gingival recession, further accumulation of plaque, and ultimately, caries in the areas around the restoration, which intensifies periodontitis [216]. These nanocomposites have a composition similar to the NACP-containing nanocomposites with rechargeability [193]. In order to be effective against the six species of pathogens responsible for gingival disease, they had an antibacterial monomer called dimethylaminohexadecyl methacrylate (DMAHDM). This nanocomposite had the most antibacterial effect against *Porphyromonas gingivalis*, and the least effect against *E. fecalis*. This difference in efficiency can be related to the different structure of cell walls in different bacteria [217]. To sum up the mechanical and antibacterial properties of these nanocomposites, this has rendered them as a suitable replacement for glass ionomers in class V restorations. Moreover, the resin-modified glass ionomers unlike these nanocomposites do not show enough inhibitory properties to prevent the growth of bacteria and biofilm formation [218].

15.3 Conclusions

The use of dental resin-based composite as a restorative material has been increasing in recent years. Therefore, improvement the properties of these materials to address previous foibles is among the research priorities. One of the major weaknesses of these materials is the occurrence of decay in the vicinity of old restorations.

Nanotechnology has helped to overcome this problem by adding the antibacterial and/or remineralizing agents in nanometer sizes to the resin-based dental composites. It can be expected that the occurrence of secondary caries in the vicinity of the restorations will decrease, and thus, the durability of composite restorations will be improved.

Due to the integrity disruption of dental resin-based composites by addition of these compounds, one of the limitations is the reduction of mechanical properties in these restorative materials. Nowadays, this problem is being resolved by adding newer filler compounds in different forms and sizes such as whiskers and fibers.

Another limitation for the use of these bioactive compounds in the resin-based dental composites is the limited duration of their release. This limitation has been relatively resolved by the production of rechargeable resin-based dental composites.

Today, the use of remineralizing and antibacterial compounds together is another novel and interesting field in the dental research. Finally, it can be predicted that the resin-based dental composites with the ability to inhibit bacterial activity, as well as the possibility of remineralizing of primary dental lesions will be introduced to the market. Therefore, along with dentin bonding systems with similar properties, the durability of these novel resin composites will be promoted in the future.

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