9 Multifunctional Restorative Dental Materials: Remineralization and Antibacterial Effect

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Abstract

Remineralizing biomaterials have been a long-time pursuit in dentistry as a strategy to prevent or at least postpone the development of caries lesions around existing restorations, fissure sealants, and orthodontic brackets. Glass-ionomer cements, with a track record spanning four decades, have shown good results *in situ*. However, their low mechanical properties and bond strength to the tooth structure are limiting factors in several clinical situations. In the last decade, calcium orthophosphates (e.g., amorphous calcium phosphate/ACP), bioactive glasses (e.g., 45S5), and calcium silicates (e.g., mineral trioxide aggregate/MTA) have been tested as ion-releasing fillers in dentin bonding systems and resin composites. *In vitro* testing showed unequivocal evidences of hybrid layer remineralization, which reduces permeability and collagen degradation, therefore contributing to the longevity of bonded interfaces. On enamel, composites containing calcium orthophosphates were shown to promote mineral recovery *in vitro* and reduce mineral loss *in situ*. Besides fostering remineralization, some of these particles may also grant antimicrobial activity to resin-based materials, making them "multifunctional restorative materials." Studies show that bioactive glasses are effective against some bacterial species due to their alkalinity and effect on osmotic gradient. For calcium silicates, however, there seems to be no consensus among authors regarding antimicrobial effect, while calcium orthophosphates and glass-ionomers show no evidence of intrinsic antimicrobial activity.

9.1 Introduction

Restorative materials with remineralizing and antibacterial properties are not unknown to dentistry. For instance, calcium hydroxide and glass-ionomer cements (GIC) have a very long history of clinical use. The last few decades, however, have seen a surge in research of new multifunctional resin-based materials combining both effects in a vast range of applications including desensitization of exposed cervical dentin, biomimetic dentin remineralization, atraumatic restorative treatment (ART), orthodontic cements, pulp capping, and as direct restorative materials. These new materials contain bioactive glass, calcium silicate, or calcium orthophosphate particles dispersed in a dimethacrylate-based resin matrix. Because the antibacterial effect is not necessarily the primary feature of these ion-releasing fillers, other antibacterial agents (e.g., chlorhexidine or silver nanoparticles) can be associated.

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Multifunctional materials are intended to promote apatite deposition in hard dental tissues (e.g., ART and remineralization of resin-infiltrated dentin) or to prevent the development of caries lesions by increasing mineral uptake after a demineralization event (e.g., bracket bonding and direct restorative materials). In either case, antibacterial activity is important to facilitate the intended outcome. Depending on the clinical situation, these effects are necessary only for relatively short periods of time or may be needed for as long as possible.

In this chapter, the current and most relevant findings on the research leading to the development of remineralizing and antibacterial materials are presented.

9.2 Calcium Orthophosphates

The first attempts of using calcium orthophosphates (CaP) as fillers in restorative composites date back to the 1980s. Interestingly, these studies focused not on remineralization, but on the development of coupling agents to improve adhesion to the tooth structure [1]. Later on, hydroxyapatite (HAP) particles were tested as reinforcing fillers, as its relatively low hardness could reduce composite wear damage [2]. It was also in the mid-1990s that amorphous calcium phosphate (ACP) started to be tested as bioactive filler in resin-based materials [3]. ACP is an intermediate phase in HAP precipitation and that, along with its relative solubility, makes it suitable as ion-releasing filler. Other orthophosphate phases, such as dicalcium phosphate anhydrous (DCPA), dicalcium phosphate dihydrate (DCPD), and tetracalcium phosphate (TTCP) are also found in the literature.

The development of remineralizing composites containing CaP particles is not without its drawbacks. For instance, the incorporation of CaP particles in dimethacrylate matrices leads to significant reductions in fracture strength due to the lack of a strong particle-resin interaction [4]. Therefore, there is a trade-off between bioactivity and mechanical strength [5]. Other mechanical properties, such as elastic modulus and fracture toughness, are less sensitive to the presence of CaP particles [6]. In order to improve the interaction between CaP particles and resin matrix and minimize the loss in strength, it is possible to functionalize these particles with organic molecules, such as carboxylic acids, silanes, or dimethacrylates [7–9]. These molecules work as coupling agents, binding to calcium and copolymerizing with the monomers in the matrix, and also improving the wettability of the resin on the particles.

Another point of concern is the long-term degradation of these materials. Since calcium and phosphate release occurs at the expense of particle surface dissolution, it is licit to assume that over time oral fluids would find opened pathways at the filler-matrix interfaces to penetrate the material and increase matrix degradation. There are very few studies that investigated this topic, with contradictory findings. While a more severe degradation was verified in composites containing DCPD after 28 days in water in relation to the control material [6], no differences were observed due to the presence of ACP after 2 years in water [10].

9.2.1 Remineralization Studies

Resin-based materials foster remineralization by releasing calcium and phosphate ions in supersaturating levels. Also, the presence of calcium in the biofilm increases fluoride retention, which also helps to prevent demineralization [11]. Ion release is determined by a number of factors, such as solubility of the calcium orthophosphate phase [12], particle surface area [9], CaP volume fraction in the composite [13], hydrophilicity of the resin matrix [14], and pH of the immersion medium [15]. *In vitro* studies have demonstrated that ionic concentrations released by CaP-containing composites are capable of promoting apatite precipitation [16, 17]. However, ion release does not occur indefinitely. Experimental composites containing 20 wt% of ACP showed ion release up to 70 days under very acidic condi-

tions (pH 4), which increase particle erosion and, consequently, boost ion release [18].

Composites containing calcium orthophosphate particles were shown to promote mineral recovery in enamel artificial caries lesions *in vitro*. Small fractions of hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) and methacryloyloxyethyl phthalate (MEP) were added to the matrix to enhance fluid access to the particles. A dimethacrylate-based material containing 40 wt% of ACP (without reinforcing fillers) was able to recover 38% of the mineral content of the lesion (quantified by transverse microradiography, TMR) and reduce lesion depth by 23% after 2 weeks of pH cycling. In the same study, a composite containing HAP was as ineffective as the control composite with silica particles due to its reduced solubility [19, 20]. When compared to a commercial orthodontic cement containing fluoride, an ACP composite (40 wt%) promoted a mineral recovery of 14%, against 4% of the fluoride composite. Interestingly, in the top third of the lesion the mineral gain was higher for the fluoride material, while the ACP composite promoted higher mineral deposition at the deeper regions [19, 21]. Another study showed that after 30 days of pH cycling, an experimental composite containing 40 wt% of ACP and 20 wt% of reinforcing glass promoted 22% of mineral gain, in comparison to 6% of a commercial restorative composite containing ytterbium trifluoride [22].

The experimental model utilized in the studies mentioned in the previous paragraph does not truly represent the intended use of these materials, though it does serve as a "proof of concept" [21]. In a more clinically relevant *in situ* experiment, the protective effect of an ACP-containing composite on the surrounding enamel was verified as mineral loss and lesion depth was significantly lower than around a conventional composite after 14 days. Also, calcium and phosphate concentrations in the biofilm formed on the specimens were statistically higher [23].

In a series of studies, a two-paste resin cement containing approximately 40 wt% of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) intended for indirect pulp capping or atraumatic restorative treatment (ART) material was tested *in vitro* and *in vivo*. Under static conditions (immersion in saliva-like solution, SLS, for 5 weeks), the cement was shown to promote a 38% recovery in dentin mineral content [24]. The use of a bonding agent between the demineralized dentin and the cement reduced remineralization, possibly due to calcium binding by the acidic monomers in the adhesive [25]. *In vivo*, the cement was applied directly on caries-affected dentin and under a conventional resin composite. After 3 months, calcium and phosphorous content was significantly higher in the treated dentin in comparison to the untreated control and similar to sound dentin levels up to a 30 μm depth [26].

Finally, in another example of *in vitro* top-down dentin remineralization, experimental composites containing 40 wt% of ACP and 20 wt% of either silanated glass or TTCP promoted 43–48% mineral recovery after 8 weeks of pH cycling [27].

9.2.2 Antibacterial Activity

The addition of calcium orthophosphate particles in resin-based materials does not seem to provide any significant protection against biofilm formation. For example, the aforementioned *in situ* study found no reduction in the number of *Streptococci* and *Lactobacilli* colony-forming units (CFUs) grown on an ACP-containing composite in relation to the control [23]. Notwithstanding, the same material showed some acid-neutralizing activity, promoting a raise in pH from 4 to 7, which could reduce the growth of acidogenic bacteria [28]. The buffering capacity of CaP-containing resin materials, though insufficient to reduce biofilm growth, was confirmed in a subsequent study [29].

Multifunctional composites and bonding agents associating calcium orthophosphates with quaternary ammonium monomers [30–32], silver (Ag) nanoparticles [32, 33], or chlorhexidine [34] were tested with overall good results in

terms of antibacterial activity. Particularly in the case of adhesive systems, antibacterial agents can be added to both the primer and the bonding resin, while ACP was added to the latter. Bond strength tests showed that the addition of antibacterial agents and calcium phosphate particles did not reduce bond strength after 28-day storage [31]. Transmission electron microscopy revealed the presence of ACP agglomerates and Ag nanoparticles in the resin tags [35].

Recently, silver phosphate/calcium phosphate particles were synthesized. These particles are capable of producing metallic Ag nanoparticles *in situ* when exposed to UV-Vis radiation (<530 nm), therefore, in the range emitted by dental light-curing units. Calcium release from resin materials containing 20–30 wt% of these mixed phosphate particles was similar to that of calcium phosphate only, while *S. mutans* CFU count was reduced by three log units [36].

9.3 Bioactive Glasses

Silica-based (SiO_2) glasses have been widely studied in the past 50 years, after Hench and colleagues found out that certain compositions can chemically bond to bone [37]. The most studied glass composition (Bioglass™ 45S5, $45SiO_2$, 24.5CaO, $24.5Na_2O$, $6P_2O_5$, in wt%; "5" meaning a 5:1 calcium-to-phosphorus molar ratio) showed good results when used for pulp capping in animal models [38] and is found in products indicated for the repair of alveolar bone defects [39] and treatment of dentin hypersensitivity [40].

Similarly, 45S5 glass plates were shown to bond to etched dentin (35% phosphoric acid for 15 s) after 3 weeks in artificial saliva [41]. The basic bonding mechanism can be summarized as follows: at initial stages of glass dissolution, side groups on type I collagen fibers can bind to the negatively charged particle surface; at later stages, an interfacial layer of hydroxycarbonate apatite (HAC) nucleates on top of a silica gel layer containing silanol groups (Si–OH), with interpenetrating collagen fibers [42]. Interestingly, apatite-wollastonite (A/W) glass-ceramic (4.6MgO, 44.9CaO, $16.3P_2O_5$, $34.2SiO_2$, in wt%) did not bond to dentin. Other bioactive glass (BAG) compositions, such as S53P4 ($53SiO_2$, $23Na_2O$, 20CaO, and $4P_2O_5$, in wt%), were capable of promoting HAC deposition and obliterate the dentin tubules after 24-h immersion in a BAG suspension followed by 2 weeks of incubation [43].

9.3.1 Remineralization Studies

BAGs have been tested for dentin remineralization with promising results. Demineralized dentin bars were shown to recover the carbon-to-mineral ratio (determined by thermal analyses) of natural dentin after 30-day immersion in a suspension of nanometric 45S5-type particles (30–50 nm, surface area: 64 m^2/g). However, the flexural strength and elastic modulus remained similar to those of demineralized dentin [44]. In fact, more recent studies have demonstrated that acidic polymers are necessary as biomimetic precursors in order to guide dentin remineralization and recover its mechanical properties (i.e., bottom-up remineralization) [45]. Apatite deposition, with obliteration of dentinal tubules, was also observed on demineralized dentin samples treated with 45S5 (20 mg for 1 min) after 7-day storage in artificial saliva [46].

Adhesive systems containing BAGs have been tested as a way to reduce the long-term degradation of the bonded interface. The incorporation of 30 wt% of 45S5 particles (<10 μm) in a Bis-GMA/HEMA resin adhesive was able to maintain the microtensile bond strength to dentin after 6 months in phosphate-buffered solution (PBS), compared to a 37% reduction displayed by the control adhesive [47]. One of the mechanisms proposed to explain the lower degradation was that mineral deposition would replace water-rich domains within the hybrid layer, reducing hydrolysis. The precipitation of calcium phosphates also interferes with metalloproteinase (MMP) and cathepsin activity,

reducing enzymatic degradation of the collagen [48, 49].

Apatite deposition from composites containing both silanized reinforcing glass and BAG particles was also tested. When 15 wt% of BAG particles with Ca/P = 4 was added to a commercial flowable composite, apatite formation was verified after 20 days in simulated body fluid (SBF) [50]. Experimental resins containing 37.5–50.0 wt% of 45S5 were shown to prevent enamel demineralization after 45-day immersion in lactic acid. The proposed mechanism was acid neutralization by the ions released from the glass [51].

Similarly to what was described for CaP-containing composites, the effect of bioactive glass particles on the mechanical properties of experimental composites is a clinically relevant concern. The replacement of reinforcing fillers by 10 or 15 wt% of bioactive glass (65% SiO_2, 31% CaO, 4% P_2O_5 in mols, particle size: 0.04–3.0 μm) did not lead to significant reductions in flexural strength or fracture toughness in comparison to the control composite. Also, the presence of bioactive glass did not increase composite degradation after 2-month immersion in brain-heart infusion medium [52].

9.3.2 Antibacterial Effect

The antibacterial effect of bioactive glasses is a topic of great interest in orthopedics, as a way to prevent medical device-associated infections (MDAIs) in joint and bone implant surgeries [53], and a consensus seems to exist among authors about BAG efficacy against bacteria. Bioactive glasses are considered materials with intrinsic antimicrobial activity due to the release of ions such as Na^+ and Ca^{2+} that leads to a local increase in osmotic concentration and pH. As a result, there is an unbalance in bacterial intracellular Ca^{2+}, leading to membrane depolarization and bacterial death [54]. In dentistry, S53P4 (20 μm) was tested against cultures of Gram-positive (*A. naeslundii*, *S. mutans*, and *S. sanguis*) and Gram-negative pathogens (*A. actinomycetemcomitans* and *P. gingivalis*). Except for *S. sanguis*, the other species lost viability after 60 min of incubation in the presence of S53P4. Besides a pH raise to 10.8, the increase in osmotic pressure resulting from Na^+ release and bacterial agglutination (for *P. gingivalis*) in the presence of Ca^{2+} are also listed as antibacterial mechanisms [55]. A similar increase in pH was verified for 45S5 glass (90–710 μm), which led to a 93–99% reduction in supragingival bacterial cultures after 3 h of incubation (*A. viscous*, *S. mutans*, and *S. sanguis*). Subgingival bacterial species (*A. actinomycetemcomitans*, *F. nucleatum*, *Prev. intermedia*, and *P. gingivalis*) presented 91–100% of reduction in viability, while the control (non-bioactive) glass resulted in reductions of 8–62% [56].

An experimental composite containing 15 wt% of bioactive glass ($65SiO_2$, 31CaO, and $4P_2O_5$, in mols, particle size: 0.04–3 μm) was able to reduce bacterial penetration along the tooth/restoration interface. While for the control composite the entire axial wall was infiltrated by *S. mutans* after 2 weeks in a bioreactor under cyclic loading, for the composite with bioactive glass, only 61% of the gap depth showed bacterial penetration [57].

Bioactive glasses can have their antibacterial effect enhanced by the incorporation of metallic elements such as silver, copper, strontium, or zinc. Among them, Ag-doped glasses are the most studied [53]. Ag-doped bioactive glass particles ($58.6SiO_2$, $24.9CaO$, $7.2P_2O_5$, $4.2Al_2O_3$, $1.5Na_2O$, $1.5K_2O$, $2.1Ag_2O$, in wt%, particle size: 25 μm) were incorporated into a commercial flowable composite. After 8 days in PBS, the extract of the composite containing 15 wt% of Ag-doped glass was able to completely inhibit *S. mutans* growth. Interestingly, PBS pH (7.4) was not affected by the immersion of the composite specimens, indicating that antibacterial activity was due to Ag release from the material [50].

9.4 Calcium Silicates

Calcium silicates share some of the characteristics and mechanisms described for bioactive glasses, in terms of both remineralization and antimicrobial activity. The use of calcium sili-

cates in dentistry gained mommentum in the mid-1990s, with the development of MTA cements (mineral trioxide aggregate) for use in endodontics as root-end and furcal perforation filling material [58]. MTA is a mixture of dicalcium silicate (belite), tricalcium silicate (alite), tricalcium aluminate and tetracalcium aluminoferrite (Portland cement, amounting to 75% of the entire mass), bismuth oxide (20%), calcium sulfate (gypsum, 5%), and trace amounts of other metallic silicates and oxides. Hydration of MTA produces a calcium silicate hydrate gel and crystalline calcium hydroxide [59].

The good results shown by calcium silicates in several applications, including as pulp capping materials, may explain why much of the research on dentin remineralization produced in the last few years has focused on these particles as ion source. Unfortunately, no reports have been published on the mechanical properties of resin-based materials containing calcium silicates or reinforcing glass fillers associated with calcium silicates. Therefore, their reinforcing effect on the resin matrix is still to be verified. Experimental materials containing no reinforcing fillers and 56 wt% of calcium aluminosilicate particles in a light-curable hydrophilic resin matrix showed water sorption four times higher than a commercial flowable composite, and similar to a resin-modified glass-ionomer [60].

9.4.1 Remineralization Studies

The availability of calcium ions in a highly alkaline environment created by the hydroxyl ions, associated with the phosphate present in physiologic fluids, favors the precipitation of poorly crystalline calcium-deficient carbonated apatite [61]. This characteristic was first identified in relation to calcium silicates' sealing ability when used as root-end filling material [62]. When applied to mineral-depleted dentin, calcium silicates associated with analogues of acidic non-collagenous proteins (e.g., polyacrylic acid) allow for the production of metastable amorphous calcium phosphate nanoprecursors and, at a later stage, intrafibrillar and interfibrillar apatite deposition (biomimetic, "bottom-up" remineralization) [63]. Evidences of remineralization within the hybrid layer kept in contact with Portland cement discs were detected after 2–4 months of immersion in SBF containing polyacrylic acid and polyvinylphosphonic acid [64].

The incorporation of Portland cement-based particles in experimental adhesives was able to maintain the bond strength of dentin-composite interfaces after 6 months in SBS. According to the authors, besides mineral deposition, the increase in pH within the hybrid layer may interfere with MMP activity [65]. The increase in nanohardness and elastic modulus of the hybrid layer and the reduction in micropermeability as evidences of remineralization were also observed after 3 months in SBS [66]. It is important to remember that, since calcium silicates do not have phosphorus in their composition as they rely on external phosphate sources to promote apatite deposition [48].

The efficacy of calcium silicates in relation to bioactive glasses was evaluated in several studies. For example, bioactive glass 45S5 was found to inhibit MMP activity in dentin demineralized by both phosphoric acid and EDTA, while particles containing 90% of Portland cement and 10% of β-tricalcium phosphate were only efficient when applied to EDTA-treated dentin. Since EDTA is not capable of removing all the phosphoproteins from the collagen, the residual phosphate favored calcium phosphate precipitation in samples infiltrated with modified calcium silicate particles. Calcium phosphates, in turn, are capable of inhibiting MMP activity, as well as forming CaP:MMP complexes with restricted mobility, preventing collagen enzymatic degradation [67]. In another study, discs of resin containing 33 wt% of polycarboxylated bioactive glass or Portland cement were kept in contact with dentin samples, immersed in artificial saliva. After 14 days, no difference in remineralization was observed between both groups [68]. The use of experimental adhesives containing bioactive glass or MTA particles (40 wt%) resulted in similar bond strength values after 10 months of storage in phosphate-buffered saline, both statistically

higher than the control adhesive without ion-releasing particles, ascribed to the maintenance of the hybrid-layer integrity [69].

9.4.2 Antimicrobial Effect

Most of the available literature evaluating the antimicrobial effect of calcium silicates tested MTA against species usually identified in periapical and root infections. Similar to bioactive glasses, MTA also shows a rapid increase in pH after mixing due to the formation of calcium hydroxide as one of the hydration products. Its initial pH is 10.2, reaching a plateau at 12.5 after 3 h [58].

In spite of its alkalinity, MTA antimicrobial activity is controversial. For example, an agar diffusion test showed no inhibitory effect over seven anaerobic bacteria and limited effect on five out of nine facultative bacteria usually found in infected root canals, either immediately or 24 h after mixing [70]. In another study using the direct contact test, MTA showed growth inhibition of *E. faecalis* and *C. albicans* when placed in the culture media 20 min or 1 day after mixing [71]. The reason for the lack of consensus in the literature has been attributed to differences in methods and microbial strains as well as material-related variables, such as power-to-liquid ratio and source of the MTA [72, 73]. An interesting finding was reported where authors found that *E. faecalis* inhibition increased when specimens made of crushed set MTA were incubated with dentin powder [74]. This phenomenon had been reported in relation to S53P4 glass and seems to be related to a higher dissolution rate of the particles in the presence of dentin powder [75].

In order to improve their antimicrobial activity, calcium silicates have been associated with other compounds. Ag-doped and chlorhexidine-loaded calcium silicate nanoparticles showed good substantivity against *E. faecalis* due to its retention on the dentin surface by means of an apatite layer between the nanoparticles and the dentin surface [76, 77]. A quaternary ammonium monomer (QAM), 2-methacryloxylethyl dodecyl methyl ammonium bromide (MAE-DB), added to an experimental resin containing Portland cement particles showed significant antibacterial activity against *S. mutans* [78].

9.5 Glass-Ionomers

Glass-ionomer cements (GIC) are acid-base cements with widespread use in dentistry as restorative and luting material, orthodontic cement, and sealant. Their mechanical properties, however, are not high enough to allow their use in large cavities on stress-bearing areas. Their remineralizing and antibacterial effects are attributed to the presence of fluoride in the silicate-based glass particles, which is initially released upon their reaction with the polyalkenoic acids in the cement liquid and, in smaller concentrations, over time due to particle dissolution. From the cariology standpoint, it is important to point out that fluoride does not prevent caries lesion development, but it does slow down its progression. In fact, the incorporation of fluoride into enamel and dentin is a consequence of the caries process [79]. Still, the possibility of remineralizing caries-affected dentin, associated with ease of use and good marginal sealing, makes GIC the material of choice in atraumatic restorative treatment (ART) techniques.

There is a vast amount of literature on remineralization and antibacterial properties of GIC cements evaluated *in vitro* and *in situ* but, unfortunately, the clinical evidences are scant and most often point to the absence of significant effects of fluoride-releasing materials regarding the prevention of caries lesion development.

9.5.1 Remineralization Studies

GICs, both conventional and resin-modified versions, were shown to reduce enamel demineralization *in situ*. When pH drops below 5.5 due to acid production by bacteria in the biofilm, HAP dissolution takes place. In the presence of fluoride, however, this process is counteracted by the deposition of fluorapatite, which does not disso-

ciate at pH values above 4.5. Therefore, increases in fluoride content in the enamel adjacent to the restoration are actually the result of the de- and remineralization process, and should not be regarded as an indication of an enamel-"strengthening effect" granted by the fluoride-releasing material [79]. An important aspect that must be taken into consideration in *in situ* studies evaluating GIC is the association or not with other sources of fluoride. When GIC restorations were evaluated in patients making use of fluoridated dentifrices, no differences in plaque fluoride or mineral loss around the restoration were observed in comparison to a resin composite [80, 81].

Nevertheless, fluoride release from GIC seems to be an effective way to increase mineral content of caries-affected dentin in ART procedures. *In vivo* studies revealed that fluoride from GIC penetrates partially demineralized, caries-affected dentin through an ion-exchange process taking place to buffer the low pH of the fresh cement. This process seems to be driven by a concentration gradient between the GIC and the demineralized dentin, where fluoride and strontium (if present in the cement particles) would precipitate within the demineralized dentin [82, 83]. However, ultrastructural studies failed to encounter evidences of actual remineralization rather than simple mineral uptake [84].

9.5.2 Antibacterial Effect

Studies evaluating the effect of GIC on biofilm formation may show contradictory results due to differences in test methods and, most importantly, to aging conditions of the specimens. In general, freshly mixed cements show antibacterial effect due to its initial high fluoride release and low pH [85, 86]. However, this effect is lost with time. For instance, biofilm collected from the surface of aged (1 year) resin-modified glass-ionomer, compomer, resin composite, and intact enamel *in vivo* showed similar counts for streptococci and lactobacilli [87].

Fluoride 0.53 mmol/L (10 ppm) was shown to change biofilm composition and reduce *S. mutans* count in the presence of glucose due to a direct inhibition of its metabolism, which reduces acid production and favors the growth of less aciduric species [88]. However, it is unlikely that a material could provide such levels of fluoride to the biofilm in the long term [79], as fluoride concentration in the biofilm formed *in situ* on resin-modified GIC or on dentin after acidulated phosphate fluoride application showed values not higher than 0.01 mmol/L [81, 89].

The antibacterial effect of fluoride released from GIC is considered secondary to its effect on demineralization and the clinical effectiveness of high fluoride levels on the biofilm metabolism is unclear at best [90]. In order to increase the antibacterial effect of GIC, several approaches have been tested, including well-known antibacterial agents such as chlorhexidine [91], antibiotics [92], titanium oxide nanoparticles [93], chitosan [94], and silver nanoparticles [95].

9.6 Final Remarks

There are a multitude of compositional variables involved in the performance of remineralizing/antibacterial restorative materials. Among the ion-releasing particles being investigated, bioactive glasses (45S5 in particular) and calcium silicates show good results regarding dentin remineralization. On the other hand, most of the research on calcium orthophosphates focuses on their use in enamel remineralization. Overall, the bioactivity of these materials is expected to decrease over time, as observed with glass-ionomers, which may limit their clinical effectivity in some applications.

These ion-releasing fillers show different levels of antibacterial activity, granted by their effect on the osmotic gradient and alkalinity. Among them, bioactive glasses seem to be the most effective, while calcium orthophosphates seem to have a very limited antibacterial effect. Several antibacterial agents can be associated with these particles to enhance antibacterial activity.

The intense research on multifunctional restorative materials reveals their potential in several clinical applications. Notwithstanding, it

is of fundamental importance to explore the long-term performance of these materials. The experience obtained with *in situ* and *in vivo* research on glass-ionomers emphasizes the importance of increasing the level of evidence in the near future, as evaluations conducted *in vitro* usually do not reproduce the complexity of the oral environment.

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