



## Toward dental caries: Exploring nanoparticle-based platforms and calcium phosphate compounds for dental restorative materials

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### ABSTRACT

Millions of people worldwide suffer from a toothache due to tooth cavity, and often permanent tooth loss. Dental caries, also known as tooth decay, is a biofilm-dependent infectious disease that damages teeth by minerals loss and presents a high incidence of clinical restorative polymeric fillings (tooth colored fillings). Until now, restorative polymeric fillings present no bioactivity. The complexity of oral biofilms contributes to the difficulty in developing effective novel dental materials. Nanotechnology has been explored in the development of bioactive dental materials to reduce or modulate the activities of caries-related bacteria. Nano-structured platforms based on calcium phosphate and metallic particles have advanced to impart an anti-caries potential to restorative materials. The bioactivity of these platforms induces prevention of mineral loss of the hard tooth structure and antibacterial activities against caries-related pathogens. It has been suggested that this bioactivity could minimize the incidence of caries around restorations (CARS) and increase the longevity of such filling materials. The last few years witnessed growing numbers of studies on the preparation evaluations of these novel materials. Herein, the caries disease process and the role of pathogenic caries-related biofilm, the increasing incidence of CARS, and the recent efforts employed for incorporation of bioactive nanoparticles in restorative polymer materials as useful strategies for prevention and management of caries-related-bacteria are discussed. We highlight the status of the most advanced and widely explored interaction of nanoparticle-based platforms and calcium phosphate compounds with an eye toward translating the potential of these approaches to the dental clinical reality.

### 1. Introduction

Dental caries, or tooth decay, represents one of the most significant and prevalent problems in oral health [1]. According to the WHO, proximally 60–90% of children and nearly 100% of adults worldwide have had caries. This high rate for dental caries causes dental expenditures to be around \$124 billion in 2016 in the US [2,3]. An imbalance between minerals loss (demineralization) and minerals gain (remineralization) from saliva triggers this multifactorial disease [4]. Microorganisms, substrate, host/teeth and time are the main factors leading to the terminal stage of continuous minerals loss is marked by

the destruction of tooth structure, as known as “dental caries” [4].

The acidic attack from cariogenic bacteria found in dental plaque biofilm growth over the tooth is responsible for the consecutive net minerals loss [5]. This acid attack is initiated at low pH, which allows for fermentable carbohydrate to be consumed by the cariogenic bacteria, at the site of biofilm accumulation for a prolonged-time [5]. Oral microorganisms' function in dental biofilms has been defined as interactive complex microbial communities [6]. Cariogenic biofilm is characterized as dysbiosis where acidic microenvironment (low pH levels) favors bacteria species that can multiply and survive in acidic environments [7]. This process involves acidogenic plaque bacteria

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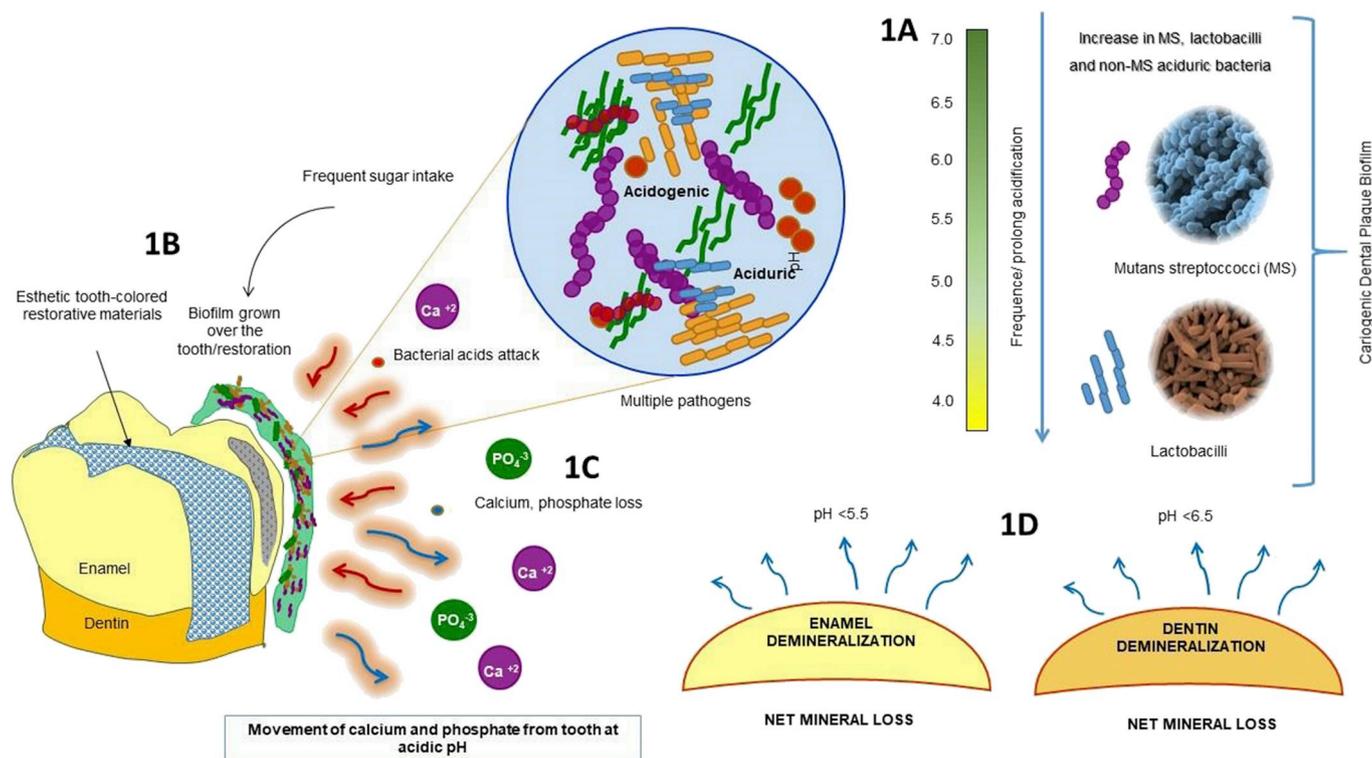
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**Fig. 1.** Schematic drawing illustrating the cariogenic biofilm formation. 1A) Cariogenic dental plaque biofilm, where mainly *Mutans streptococci* (MS), *lactobacilli* and non-MS acid-producer bacteria are responsible for the acidic attack; 1B) The cariogenic biofilm is found in dental plaque that grows over the tooth and esthetic tooth-colored restorative materials; 1C) The acidic attack is responsible for the continuous net mineral loss; and 1D) For enamel and dentin, the net mineral loss is present when the pH is lower than 5.5 and 6.5, respectively.

including *Streptococcus mutans*, the major caries-related species, that represents the dominant streptococci genus in the dental plaque biofilm (Fig. 1A–D).

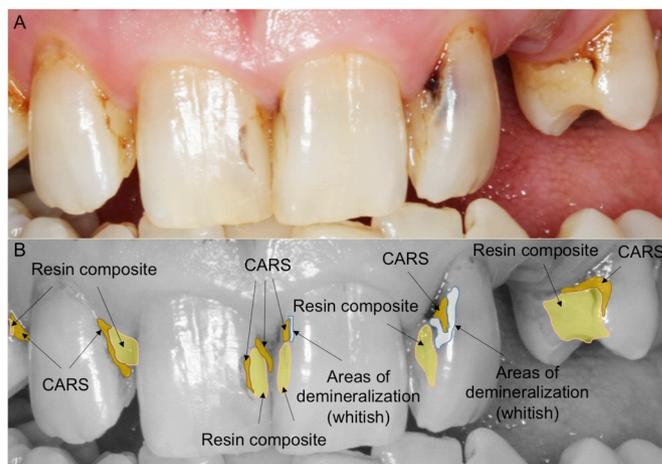
The primary mineral component of the hard dental tissues (enamel and dentin) is hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ), which is the primary target for the bacterial acids. Thus, pH is the driving force governing the loss or gain of Ca and  $\text{PO}_4$  from the mineral structure of the teeth [7]. At physiological conditions, the oral fluids (saliva, biofilm fluid) have calcium (Ca) and phosphate ( $\text{PO}_4$ ) in supersaturated concentrations concerning the mineral composition of enamel and, as a result, these ions are continually deposited on the enamel surface [8]. With the presence of bacterial acids, the pH decreases and the biofilm fluid becomes undersaturated concerning the enamel minerals [9]. The demineralization occurs by chemical dissolution of Ca and  $\text{PO}_4$  when pH remains below around 6.5 for dentin and 5.5 for enamel (Fig. 1D).

In the presence of advanced demineralization that is associated with cavities formation, esthetic tooth-colored restorative materials are the chosen materials due to improved esthetic properties and more conservative preparation techniques. Clinical restorative polymer materials are represented by a set of materials including direct resin composite restorative, enamel-dentin adhesives and dental primers (adhesion promoters) with similar primary chemical composition [10]. These materials have been used as direct restorative materials to replace missing biological tissue for more than 40 years and do not exert any detrimental effect on caries-related pathogens or positive impact in the mineral balance of hard tissues. Preceding reports have highlighted that resin-based restorative materials facilitate cariogenic biofilm growth [11–13]. The suggested rationale is based on degradation products from dental monomers such as bisphenol A glycidyl dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGMA), which may alter the metabolism and promote the proliferation of caries-related bacteria such as *Streptococcus mutans* [14].

The resulting cariogenic biofilm triggers the destruction of the

mineral structure of any dental surface – intact, sealed or restored – where biofilm remains accumulated and regularly exposed to sugar. Hence, it influences the initiation and progression of carious lesions, not just in its primary development but also its recurrence [14].

Recurrent caries or caries around restorations (CARS) develops at the interface between the restoration and the prepared cavity as a result of restoration failure (Fig. 2A). The rates of CARS for restorative polymer materials are very high at, approximately 60% and it has been identified as one of the major reasons for the failure of resin composite restorations [15,16]. Fig. 2A and B illustrate the clinical aspect of CARS and demineralized areas around multiple resin composite restorations



**Fig. 2.** A) Clinical aspect of secondary caries lesions (CARS) and demineralized areas around multiple resin composite restorations in a young adult; B) Black and white version of the same figure illustrating the location of esthetic tooth-colored restorative materials, CARS, and demineralized areas.

in a young adult. Replacement rates of failed restorations have been reported to be 37%–70% with consequences that can seriously compromise the oral health status [17,18]. CARS are frequently located at the gingival margins of the proximal restorations, which are common areas for biofilm accumulation (Fig. 2B). Given these challenges, the introduction of novel treatment approaches, supplementary to the conventional therapeutic strategies, is considered crucial for the efficient control of CARS.

## 2. Restorative polymer materials as carriers for nanoplatforms

In Restorative Dentistry, restorative polymer materials represent a set of materials including bulk resin composite and dental bonding agent with similar primary chemical composition [6]. The bulk resin composite is bonded to the tooth by an intermediate layer of bonding agent to connect the dental substrates to different resin-based materials. Typical restorative polymer materials are composed of a mixture of dental polymer and inorganic fillers. The polymeric phase of this mixture has emerged as a clinically viable means of incorporating nanosized bioactive compounds. The optimization of formulation designs of dental materials represents an opportunity for imparting bioactivity to restorative polymer materials.

Nanotechnology in the development of bioactive dental materials has been evolving for many decades. Several innovative approaches, such as metallic nanoparticles with antibacterial activity and calcium phosphate-based nanoparticles for the balance of the mineral loss, has revealed potential tangible benefits for Restorative Dentistry [19] (Fig. 3). The possibility of therapeutic achievement associated with nanoparticles depends mainly on the properties of these nanoparticles, e.g., their active surface area, chemical reactivity, and biological activity.

The review addresses the inherent feasibility of various types of calcium phosphate compounds and metal nanoparticles for application to restorative polymer materials treatment concerning the anti-carries performance based on bacterial reduction and Ca and PO<sub>4</sub> ion release. Also, the limitations and future applications of these materials are discussed.

## 3. Bioactivity for clinical restorative polymer materials

Medical and dental researchers have an ongoing interest in creating bioactivity for clinical restorative polymer materials. Currently available materials can return the form, function, and appearance of the natural tooth; however, they do not present any bioactivity. The capability of a dental material to positively affect its biological surroundings seems like an avenue for improving the longevity and clinical service inside the mouth.

Nanoscience and nanotechnology have the potential to provide new solutions in the development of dental materials by the inclusion of bioactive compounds without affecting the functional and aesthetic performance. Different types of nanostructures can be incorporated into clinical restorative polymer materials to introduce new functionalities (Fig. 3A and B). The bioactivity toward the dental caries prevention focus on two pathways: 1) Control of tooth mineral loss and 2) Reduction/modulation of biofilm formation. Nanoparticles of different sizes, shapes, and functionality can be synthesized to meet the two specific pathways. Dental researchers are potentially exploring these benefits to overcome both the physical and chemical limitations of incorporating bioactive compounds in dental polymeric formulations.

Approaches to impart bioactivity to clinical restorative polymer materials cannot be accomplished by various methods, such as coating or impregnation, due to the mechanical load that these materials are subjected inside the mouth. Incorporation into the polymeric matrix, a core constituent of the clinical restorative polymer materials, is the main venue, which is naturally accompanied by some advantages and disadvantages. From a technological perspective, rapid advances in nanoscale engineering provide opportunities to develop new nanomaterials with bioactivity. Nanotechnological interventions could be utilized for increasing the efficiency of preventing demineralization and biofilm formation as well as overcoming the challenges of incorporation. Therefore, novel nanosized formulations have a considerable edge over conventional formulations.

### 3.1. Control of tooth mineral loss: inorganic calcium phosphate (CaP) bioactive compounds

Ongoing interest has been directed toward calcium phosphates

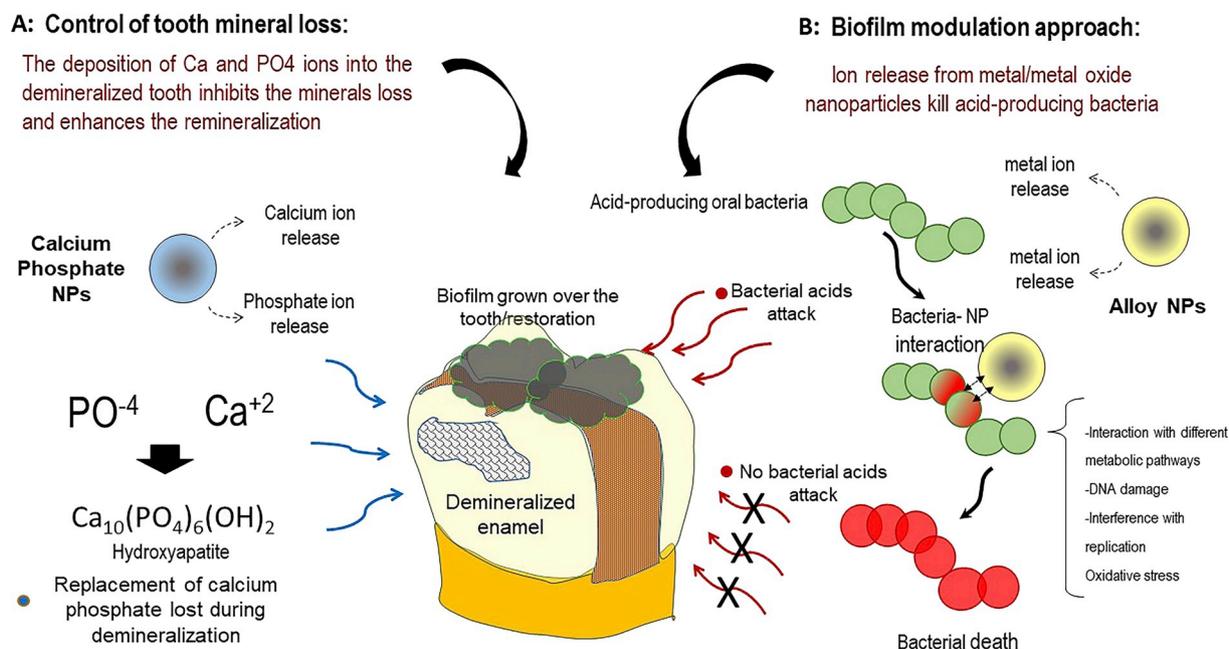


Fig. 3. Schematics is indicating the pathways of bioactivity toward dental caries prevention via dental restorative materials. A) Control of tooth mineral loss via nanosized particles of CaP, highlighting NACP and bioactive glass and B) Reduction and modulation of biofilm formation via antibacterial metallic nanoparticles.

(CaP) compounds as these materials demonstrate remineralization capabilities [20–34]. In dentistry, there are multiple applications of CaP compounds because this inorganic material is composed of human bone and teeth in the form of hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] [23]. CaP can be used as a bone graft to restore bone defects that are caused by periodontal disease. Also, CaP is used as scaffolds in tissue engineering for bone or dentin regeneration, used as orthodontic cement, used in dentifrices, bleaching agents and coating material for dental implant surface [35–37].

Currently, one of the most promising applications of CaP particles in restorative dentistry is to incorporate into direct restorative materials. CaP particles induce anti-carries activities by releasing sufficient levels of calcium and phosphate ions to form a stable apatitic tooth mineral [26,33,34,38]. With efforts toward incorporating and adjusting of CaP formulations must be conducted carefully to avoid detrimental effects on the mechanical and physical properties of the restorative materials. The resin composites are the main target restorative material. These filling materials present a wide surface area that favor interaction materials-microenvironment to further ion release. Dental adhesives can also be considered a source of bioactive compounds. Overall analysis showed bioactive bonding systems were statistically similar to the control at immediate evaluation (after 1 day). Very few studies show long-term evaluation by water aging or accelerated artificial aging processes. Several CaP phases have been tested as bioactive components in dental materials. With a variation on the Ca/P molar ratio and the stability of the salt phase, CaP compounds such as dicalcium phosphate dihydrate (DCPD), dicalcium phosphate anhydrous (DCPA) and tetracalcium phosphate (TTCP), tricalcium phosphate (TCP) [20–34] and amorphous calcium phosphate (ACP) were considered. A comprehensive structure-composition-property relationship considering a variety of interfering factors are needed to reach the desirable anti-demineralization/remineralization therapy effect. Ions release from CaP resin-based material depends on many factors. One of the crucial factors is the critical pH value. At that point, the solution is saturated with calcium phosphate, and below which it starts to dissolve [37]. Particle size is another relevant factor as the nano-scale CaP particles released ions more than the micron scale due to increased surface area and improved the mechanical properties [37]. Moreover, the higher volume fraction of CaP particles in the resin composite will increase the ions release and enhance water and ions diffusion by increasing the filler-matrix interfacial area [33]. Reinforced fillers also have a role in ion release as the ratio of these fillers increase, the mechanical properties increase and the ion release decrease [33]. Silanization of the CaP particles also hinders the ion release [33].

### 3.1.1. Dicalcium phosphate dihydrate, dicalcium phosphate anhydrous, and tetracalcium phosphate

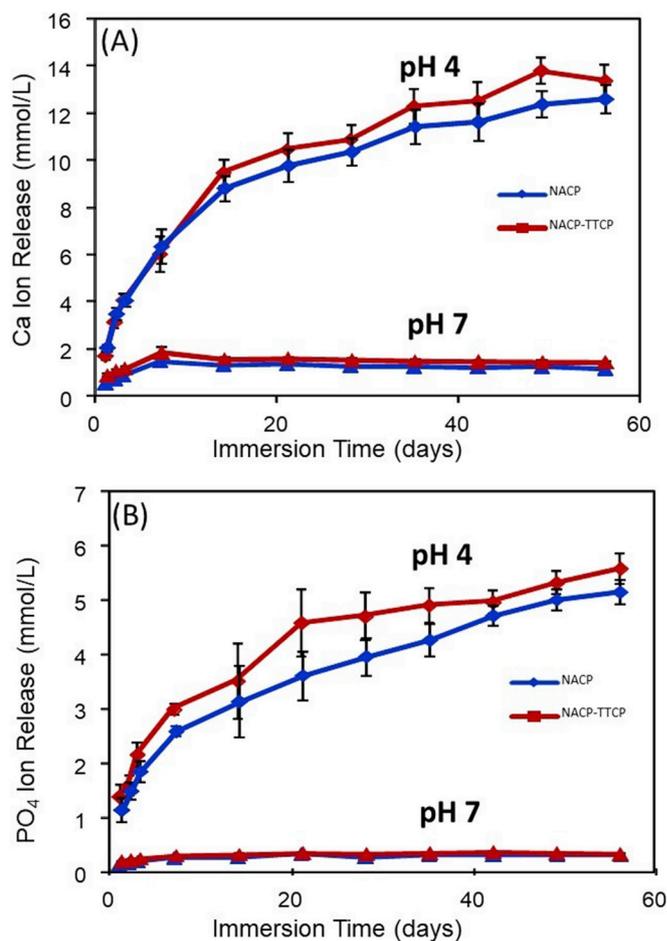
Dicalcium Phosphate Dihydrate (DCPD,  $\text{CaHPO}_4\cdot\text{H}_2\text{O}$ ) is one of calcium orthophosphate phases. It was initially investigated in the development of Ca and  $\text{PO}_4$ -releasing polymer materials. DCPD enters its stable end phase when the pH of the setting reaction is less than 4.2 [39]. Dicalcium Phosphate Anhydrous (DCPA,  $\text{CaHPO}_4$ ) has the same Ca/P ratio of DCPD, but it is less soluble. Table 1 illustrates calcium orthophosphate phases that have been used in dentistry and their

**Table 1**

Calcium orthophosphate phases that been used in dental restorative materials concerning Ca/P ratios.

Calcium Phosphate	Chemical formula	Ca/P Ratio	$\log(K_{sp})$ at 25°	$\log(K_{sp})$ at 37°
Dicalcium phosphate dihydrate (DCPD)	$\text{CaHPO}_4\cdot\text{H}_2\text{O}$	1.0	6.59	6.63
Dicalcium phosphate anhydrous (DCPA)	$\text{CaHPO}_4$	1.0	6.90	7.02
Amorphous calcium phosphate (ACP)	$\text{Ca}_x\text{H}_y(\text{PO}_4)_{2z}\cdot\text{H}_2\text{O}$	1.2–2.2	<sup>a</sup>	<sup>a</sup>
Tetracalcium phosphate (TTCP)	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	2	38–44	42.4
Hydroxyapatite (HA)	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	1.67	58.4	58.6
$\beta$ -Tricalcium phosphate	$\beta\text{-Ca}_3(\text{PO}_4)_2$	1.5	28.9	29.5

<sup>a</sup> ACP solubility cannot be measured accurately.



**Fig. 4.** Calcium (Ca) and phosphate (P) ion release from dental resin composite containing 40% NACP, and the resin composite containing 40% NACP and 20% TTCP and the timeline of their Calcium (Ca) and phosphate (P) ion release. (A) Ca and (B) P ion releases (mean  $\pm$  SD; n = 3). Low pH is associated with the higher amount of ion release. The greater release in lower pH is promising to respond to the acid attack and low pH environment, which then might neutralize the pH and prevent demineralization around tooth-colored restorative materials. Adapted from Ref. [42], with permission from © 2017 Elsevier.

different ratios [40,41]. Both salts are considered acidic salts. When they are combined with a basic salt (e.g., TTCP), an acid-base reaction in the process of appetite is formed [39]. Tetracalcium phosphate [TTCP,  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ] is the most soluble at pH < 8.5 and the highest Ca/P ratio (2.0) among different CaP phases [40]. In contrast to DCPD and DCPA, TTCP is the most alkaline among all Ca- $\text{PO}_4$  phases. It elucidates the potential of using it in resin composite to buffer the cariogenic acids and prevent dental caries [32,39,40].

Efforts have been made to incorporate DCPA with TTCP in micron size into resin composite to be used as a base, liner material or bonding agents [26]. However, to reduce the amount of incorporation using

DCPA particles into resin composite restorative material and reach the same rates of Ca and PO<sub>4</sub> release, nano-particles of DCPA were synthesized via a spray-drying technique and reinforced with whisker fillers [36,42]. The result of *in-vitro* studies on this material was promising. Fig. 4 shows the release of Calcium (Ca) and phosphate (P) ions from a dental composite comprising 40% nanosized ACP (NACP), and the resin composite containing 40% NACP and 20% TTCP. Significant ions release was associated with low pH compared to neutral pH. The ion release increased with time before reaching a plateau due to the increased surface area of the particle [36,42]. Fig. 4 shows the performance for ion release from resin composites with incorporated NACP and TTCP. These materials were able to release Ca and PO<sub>4</sub> in response to the acid attack and low pH environment, which then might neutralize the pH and prevent demineralization.

DCPA reinforced whisker-resin composites generally had comparable mechanical properties with those of currently available hybrid resin composites [34]. However, whisker-reinforced nanocomposite proposes an esthetic problem as this material is opaque due to refractive index mismatch between the filler and the resin [36,42]. TTCP particles synthesized using ball-milled fine were also combined with reinforced nano-whisker [32]. The ions release of this resin composite increase by about 6-fold when the pH was reduced from neutral to 4 in addition to preserving the mechanical properties compared to commercial hybrid resin composite, which is why they call it ‘smart material’ [32]. Figs. 5 and 6 illustrate the resulting performance of these materials in the remineralization of the caries-like lesion in dentin. Remineralization with NACP and NACP-TTCP nanocomposites demonstrated the highest remineralization values compared to the commercial control (TPH) (Figs. 5 and 6). TTCP mixture with reinforced glass particles was investigated to develop a mechanically durable and remineralizing composite resin [23]. The final composite containing-TTCP had flexural strength similar to commercially available composite [23].

### 3.1.2. Amorphous calcium phosphate and nano-amorphous calcium phosphate

Amorphous Calcium Phosphate (ACP, Ca<sub>x</sub>H<sub>y</sub>(PO<sub>4</sub>)<sub>z</sub>nH<sub>2</sub>O) is the most efficient and commonly CaP phase studied with resin composite and adhesive system in the dental literature [37]. ACP resin composites in various biostable matrices release calcium and phosphate ions in a manner that effectively buffers free calcium and phosphate ions activities and, in turn, maintains the desired state of supersaturation concerning tooth mineral [37,38]. ACP is the first phase to form before the final thermodynamically stable product, which is hydroxyapatite (HAP) when CaP precipitation occurs in high-saturated solution [38]. ACP was discovered accidentally in the 1960s by Aaron Posner [43]. ACP has a unique structure among CaPs phases, which is the lack of crystalline order [43].

Furthermore, ACP is an unstable phase that makes it highly soluble [40,43]. It is easily transformed into crystalline phases such as octacalcium phosphate and apatite due to microcrystalline growth [44]. Therefore, ACP is suitable as a potential remineralizing agent in dental applications as it might raise the pH when it is placed into an acidic solution that could demineralize the tooth structure [40,43].

Restorative polymer materials containing ACP as part of the inorganic filler content have been synthesized and evaluated for restorative applications [29,30]. Although this material released a significant amount of calcium and phosphate ions, it showed weak mechanical properties [29,30]. As dental material subjected to masticatory loads when patients bite over the fillings, this is a significant drawback for clinical application.

The nanotechnology has provided the pathway to address this challenge. NACP has the advantage of better ion-release profiles due to the small size and increased surface area for chemical interactions. NACP can release Ca and P ions in higher concentration than micro-sized particles [51]. The enrichment of polymer materials with reinforcement fillers and NACP can promote the remineralization without

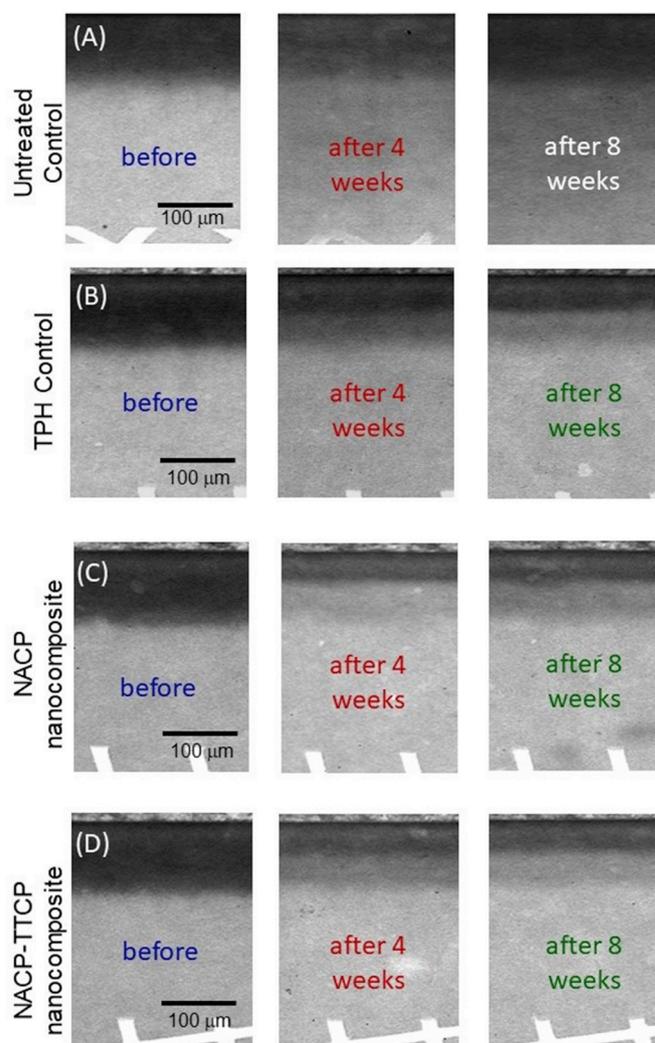


Fig. 5. Microradiographs of dentin lesions before and after the cyclic demineralization/remineralization regimen. The left column: the initial dentin demineralization created in the acidic solution. The middle column: four weeks of the cyclic demineralization/remineralization regimen. The right column: after eight weeks of the cyclic demineralization/remineralization regimen. Adapted from Ref. [42], with permission from © 2017 Elsevier.

loss of the mechanical characteristics of flexural strength, presenting similar values to microfill composite resins [20,21,27,29,30]. As a result, NACP has become the focus of attention for the recent and ongoing studies due to its improved Ca and PO<sub>4</sub> release. NACP is used as co-filler in the inorganic content of a variety of restorative polymer materials such as dental bonding, sealants, resin composites, and cement [20,21,27,29,30]. NACP has been synthesized in particles sizes of about 100 nm via a spray-drying technique and loaded into dental resin composites [31]. NACP resin composite raises the pH and neutralizes the environment, which directly affects the growth of caries-related bacteria [28,31]. In term of mechanical properties, 10–30% NACP is equivalent to the strength of the commercial hybrid resin composite [28]. Also, the nanocomposite at 40% NACP was found comparable to the strength of a microfill composite [28]. Another study measured the durability of NACP glass reinforced resin composite. It was found that the long-term mechanical performance of NACP nanocomposites was relatively higher than the control resin composite, and the wear behavior was within the range of commercial controls [45]. Table 2 outlines previous studies on applications of calcium phosphate compounds into dental resin composites. The specific biological response of these materials relies on the deposition of Ca and PO<sub>4</sub> ions into the

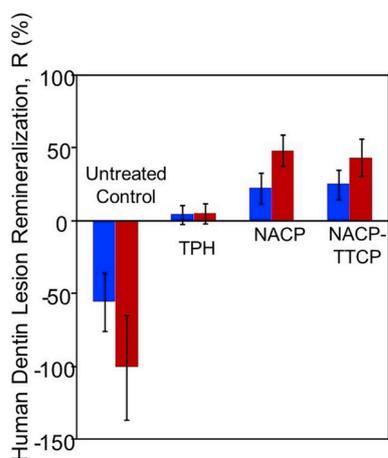


Fig. 6. Remineralization (mean  $\pm$  SD; n = 15) of human dentin lesions in the cyclic demineralization/remineralization regimen *in vitro*. Remineralization with NACP and NACP-TTCP resin composites had the highest values compared to lesions with no resin composite or lesions restored with commercial resin composites (TPH, Caulk/Dentsply), values indicated by different letters are significantly different from each other ( $p < 0.05$ ). Adapted from Ref. [42], with permission from © 2017 Elsevier.

demineralized tooth to inhibit the minerals loss and enhance the remineralization.

For Ca and PO<sub>4</sub> release performance, an *in-situ* study showed that there is a significant increase in ions release from NACP resin composite compared to control at low pH (Fig. 7). Although there is no significant antibacterial effect expressed by biofilm colony-forming unit (CFU) between the two resin composites, microradiographs showed lower demineralized area at enamel around NACP compared to the control [42,46] (Fig. 7). Recently, NACP has been developed to be rechargeable via solutions, such as mouthwashes, to have long-term remineralization effects [47]. New rechargeable NACP has been synthesized using acidic monomers that can chelate Ca and P ions from a recharging solution with comparable mechanical properties to the control resin composite [47]. Nano-scale CaP particles integrated into inorganic fillers seem to be promising to use as an adhesive or as a direct resin composite material based on *In-vitro* studies. However, there are no clinical studies to evaluate how significant is the effect of the ions release in CARS prevention, how long this effect will be maintained and what the impact of the rechargeability of these new materials is on ion release.

### 3.2. Modulation of biofilm formation via metallic nanoparticles

Metallic nanoparticles, as nanostructured agents, have a high surface-to-volume ratio which demonstrates higher and closer interaction with the bacterial membrane to induce the killing in addition to the release of metal ions [48]. Therefore, incorporating metallic nanoparticles has become a field of interest in dentistry. Several studies have demonstrated the ability of metallic nanoparticles to enhance the mechanical and physical properties, and also improve the antimicrobial properties to compact certain oral species [48,49]. Table 3 summarizes previous studies using metal/metal oxide nanoparticles to stimulate a detrimental biological response to bacterial growth over dental materials. All the cited studies present are *in vitro* studies highlighting the ongoing stage on the development of these dental materials.

#### 3.2.1. Silver and gold nanoparticles

Silver (Ag) has been used in the medical field for centuries because of its antimicrobial activities [50]. Ag ions interact with the cell membrane of microorganisms, inhibit the enzymatic system of the respiratory chain, and alter the DNA conformation causing DNA damage of such bacterium [51–53]. The antimicrobial effect of silver and silver

compounds is proportional to the quantity of released silver ions and their availability to interact with the bacterial cell membrane. Each silver source, macro or nano-sized, should release silver ions and therefore might be useful in fighting pathogenic microorganisms. Moreover, Ag particles have low toxicity and low risk of causing any complications by inhalation, ingestion or allergy [44].

Ag nanoparticles decrease the amount of bacterial colonization, *C. albicans* growth [54], and *S. mutans* [55]. While gold (Au) nanoparticles demonstrated inferior antibacterial properties [56], incorporation of Ag and Au nanoparticles increases the surface hardness of resin composite with a comparable tensile strength to control [57]. Two types of the silver mixture were used 1) silver 2-ethyl hexanoate salt dissolved in 2-(tert-butylamino) ethyl methacrylate (Ag-TBAEMA), and 2) mixed with photo-activated BisGMA–TEGDMA resin [58]. The incorporation of 0.028% Ag nanoparticles reduces the amount of colony-forming unit of total streptococci and *S. mutans* by 75% without compromising the mechanical properties compared to resin composites with no silver nanoparticles [58]. Fig. 8 illustrates the antibacterial effect of nano-silver assessed via live/dead staining assay. *S. mutans* and *Lactobacillus acidophilus* biofilms were significantly inhibited with 0.35% of Ag nanoparticles incorporated into resin composites with similar mechanical properties compared to control [59]. Ag nanoparticles with and without the incorporation of NACP demonstrate a significant biofilm inhibition of caries-related microorganisms [60] (Fig. 9).

The designing of Ag bromide-cationic polymer nano-scale resin composite resulted in a resin composite with a sustained release of silver ions, higher surface hardness, and higher antibacterial activity against *S. mutans* [61]. 1% of Ag nanoparticles significantly reduce the amount of *S. mutans* and *Lactobacillus* colonies growth [62]. Another study investigated the conjunction of bioactive glass ceramic with Ag nanoparticles, which significantly decreased the growth of caries-related microorganisms [63]. These results indicate the possibility of using a minimal amount of Ag nanoparticles to achieve desired clinical outcomes that minimize the risk of CARS.

Some disadvantages of using Ag nanoparticles were reported in the literature such as the presence of brown discoloration correlated with high Ag nanoparticles load [62], and a reduction in the wettability of the incorporated resin composite [64]. A decrease in light transmission was observed when silver incorporated resin composites were cured, which might affect the polymerization process [65] and increase the roughness of the material [66]. A higher amount of valuable monomers was observed as the concentration of Ag nanoparticles increased [65].

**3.2.1.1. Zinc oxide nanoparticles.** Zinc oxide particles (ZnO) has been found to inhibit multiple oral species growth [67], and the use of nano-scale ZnO particles was found effective against gram negative and gram positive bacteria [68,69]. In dentistry, ZnO was firstly used as opaque reinforcing fillers in resin composite [70]. ZnO demonstrated some antibacterial activity against *S. mutans* when it was included in the dental adhesive [71]. ZnO was firstly incorporated in resin composite in 2010 [72]. Tetrapod-like ZnO whisker reveals antibacterial activities without affecting the mechanical properties of resin composites. 5% of incorporated Tetrapod-like ZnO whisker has demonstrated the optimum antibacterial activity and mechanical properties compared to adding 3% and 10% of ZnO whisker. However, the antibacterial properties are diminished with the aging of the composite resin [72].

Resin composites with 1–10% incorporated uncoated ZnO nanoparticles significantly reduce the amount of mature biofilm formation compared to resin composites with no ZnO nanoparticles [73]. 80% biofilm reduction was reported with 10% of ZnO nanoparticles compared to control [73]. It was also found that 1% nonpolar ZnO nanoparticles reduce the amount of biofilm significantly compared to polar coating [73]. The incorporation of ZnO nanoparticles into flowable resin composite was achieved with an average particle size of 20 nm [74]. 1–5 wt% of incorporated ZnO resin composites reveal higher compressive strength and flexural modulus compared to unmodified

**Table 2**  
Summary of preceding studies on applications of calcium phosphate compounds into resin composites. The specific biological response of these materials relays on deposition of Ca and PO<sub>4</sub> ions into the demineralized tooth to inhibit the minerals loss and enhances the remineralization.

Agent	Study (authors)	Concentration	Mechanical properties	Remarks
ACP ZrOCl <sub>2</sub> -ACP TEOS-ACP	Skrtic et al., 1996 [29]	40%	Biaxial flexure strength values were significantly lower compared to control samples	Sustained release of Ca and PO <sub>4</sub> ions that is able to induce remineralization
ACP reinforced with silica or zirconia	Skrtic et al., 2000 [30]	40%		
Nano DCPA	Xu et al., 2006 [36]	60%	Mixed with nano silica fused whisker, flexural strength values were comparable to control samples and higher than previous CaP compounds	Comparable or higher amount of Ca and PO <sub>4</sub> ion release compared to previous CaP compounds.
Nano DCPA	Xu et al., 2007 [34]	Varied from 0 to 75%	Compared to control, nano DCPA demonstrated higher elastic modulus and hardness, but comparable flexural strength values	
TTCP	Xu et al., 2009 [32]	Varied from 0 to 75%	TTCP with whisker reinforcement demonstrated flexural strength values that were not significantly different compared to control hybrid resin composites. TTCP with whisker reinforcement demonstrated significantly high flexural strength compared to TTCP alone.	Ca and PO <sub>4</sub> ion release increased by about 6-fold when the pH changed from 6 to 4. TTCP resin composites demonstrated higher ion release compared to TTCP with whisker reinforcement.
NACP	Xu et al., 2011 [31]	10, 15 and 20%	No significant differences were found in flexural strength and elastic modulus between all NACP samples and control	Increasing NACP amount was associated with higher ion release.
NACP	Moreau et al., 2011 [28]	10–40%	10–30% NACP resin composite demonstrated comparable flexural strength and elastic modulus to hybrid resin composite control. 40% NACP resin composite demonstrated significantly low flexural strength and elastic modulus compared to control but was similar to microfill resin composite control.	NACP resin composites raised the pH and neutralized the acid, higher capability to raise the pH and neutralize the acid was observed and the NACP concentration increased. NACP resin composite demonstrated a significant ability to resist the adherence of <i>S. mutans</i> compared to control samples.
NACP	Moreau et al., 2012 [45]	10, 15 and 20%	Flexural strength and elastic modulus were higher or matching that of control samples before and after thermal cycling. With water aging, the flexural strength of NACP samples decreased significantly, but they were higher than their control counterparts. Increasing the NACP mass fraction significantly increased the amount of wear compared to control, but the values were lower than that of resin-modified glass ionomer	–
NACP	Melo et al., 2013 [46]	40%	–	This <i>in situ</i> study demonstrated that biofilms collected from NACP restored samples had a higher amount of Ca and PO <sub>4</sub> ions compared to control. NACP samples demonstrated fewer subsurface enamel lesions compared to control. 10% mass fraction of DCPD demonstrated a constant ion release for 28 days.
DCPD	Chiari et al., 2015 [25]	Varied from 0 to 20%	Adding DCPD filler did not affect the degree of conversion of resin composites. Increasing the mass fraction of filler negatively compromised the material strength. However, the optimum mass fraction DCPD that demonstrated proper mechanical properties after water aging was 10% modulus between PE-NACP and control samples.	
NACP	Zhang et al., 2016 [47]	20%	No significant differences were found in flexural strength and elastic modulus between PE-NACP and control samples.	Resin composite containing NACP demonstrates the ability to be recharged with Ca and PO <sub>4</sub> ions
NACP + TTCP	Weir et al., 2017 [42]	40% NACP 20%TTCP	–	NACP-TTCP resin composite was able to remineralize dentin and neutralizes pH. However, no significant differences were found in ion release and remineralization capability between NACP and NACP-TTCP.
NACP	Al-Dulajian et al., 2018 [20,21]	20%	Flexural strength and elastic modulus were similar to control	Recharging capability and ion release after was demonstrated after 3 recharging cycles.

ZrOCl<sub>2</sub>-ACP: zirconyl chloride-modified ACP.  
TEOS-ACP: tetraethoxysilane-modified ACP.  
DCPA: Dicalcium phosphate anhydrate.  
DCPD: Dicalcium phosphate dehydrate.  
NACP: Nano Amorphous Calcium Phosphate.  
TTCP: Tetracalcium phosphate.

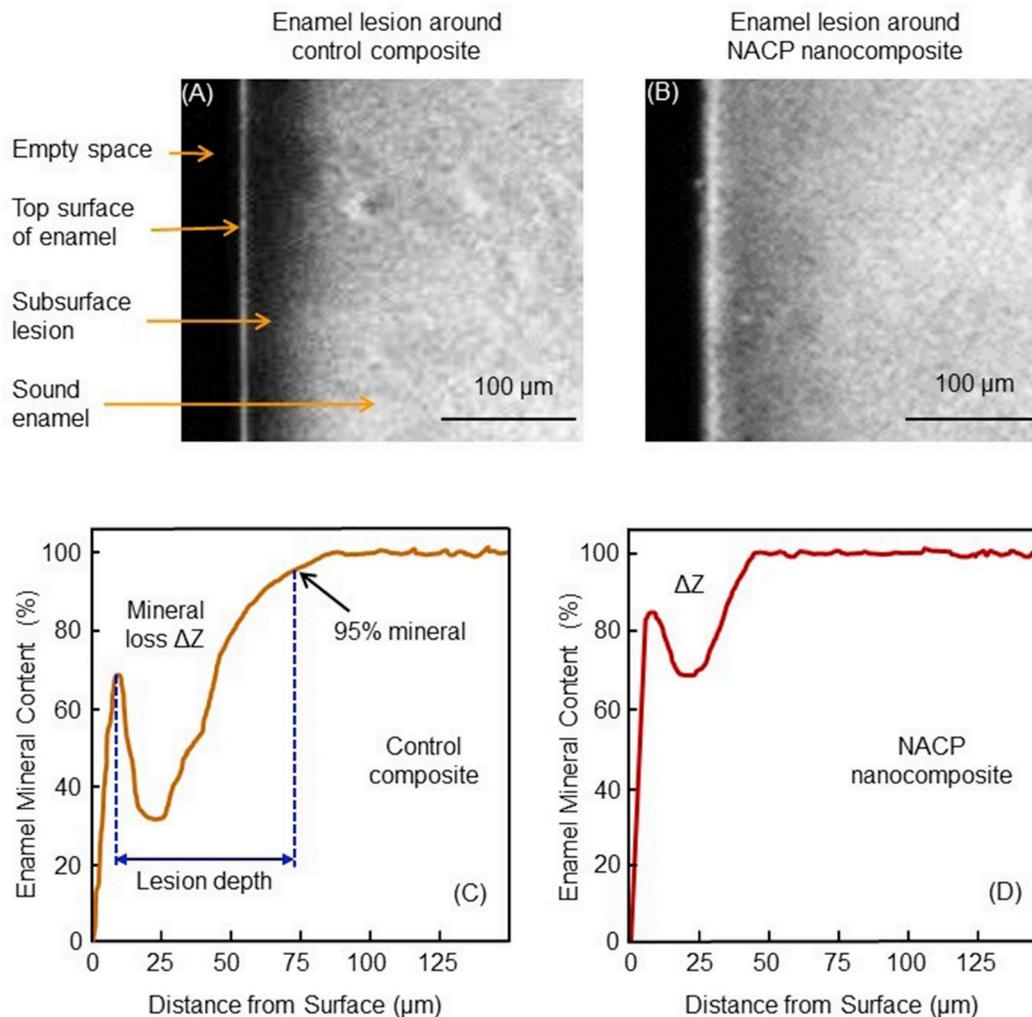


Fig. 7. Transverse microradiography analysis for subsurface enamel lesions around (A) controls resin composite, and (B) NACP resin composite. (D) Exposed enamel (no varnish cover) under biofilms *in situ* had much less lesion around NACP resin composite compared to control in (C). Adapted from Ref. [46], with permission from © 2013 Elsevier.

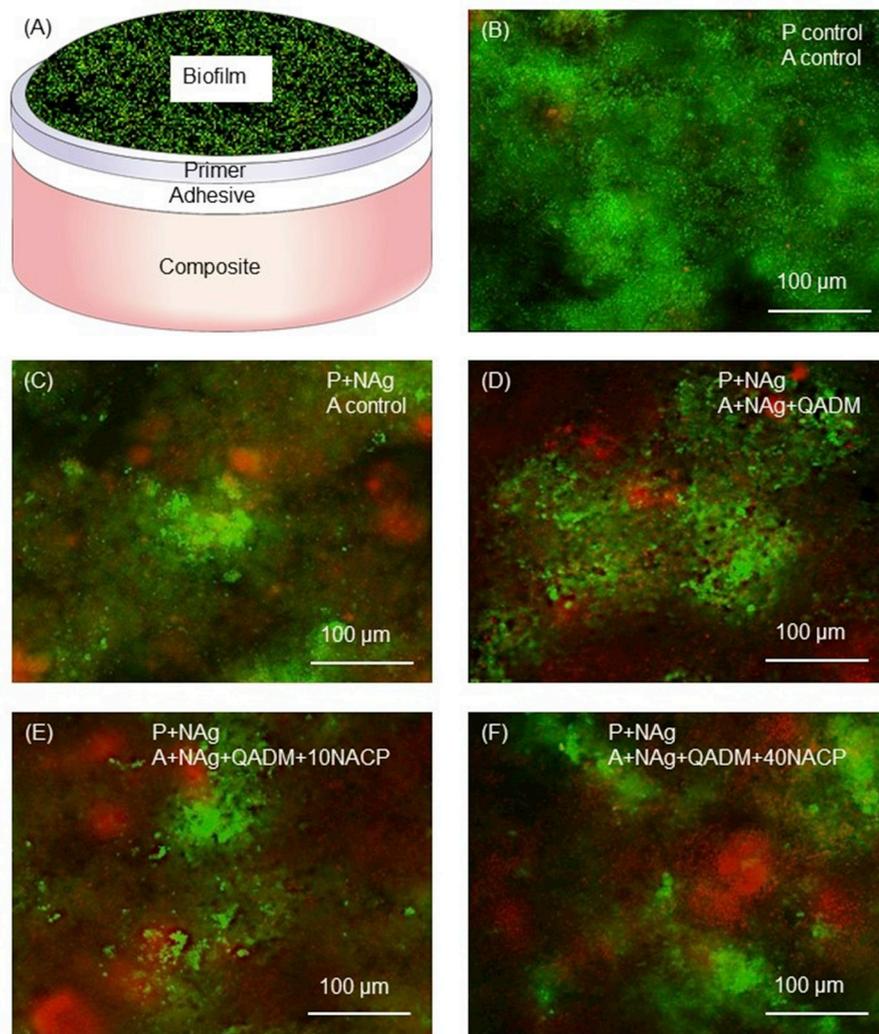
counterparts [74]. ZnO nanoparticles demonstrate better but not significant antibacterial activities against *S. mutans* and *Lactobacillus* compared to Ag nanoparticles [62]. Some limitations were reported in regards the use of ZnO nanoparticles. The ZnO incorporated resin composite did not demonstrate significant biofilm inhibition when a three-species model was used [73] or when the materials were suspected to age [74]. Also, lower depth of cure was observed compared to control [74].

**3.2.1.2. Copper oxide (CuO).** Copper Oxide (CuO) has a different antibacterial effect against multiple gram-negative and gram-positive species such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Escherichia coli* [75]. The use of copper oxide nanoparticles was limited to orthodontic brackets and dental adhesives [76,77]. CuO incorporated orthodontic brackets significantly reduce the amount of *S. mutans* biofilm [76]. In the adhesive, higher bond strength was observed without compromising the other mechanical and physical properties such as ultimate tensile strength, the degree of conversion, water sorption and solubility [77]. The use of copper in resin composite was attempted to reduce the shrinkage stress of the resin matrix. Photo-initiated copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) polymerization demonstrates three times lower shrinkage stress and more antibacterial activities against *S. mutans* compared to control restorative polymers [78,79].

**3.2.1.3. Bioactive glass (BG).** Bioactive glass (BG) is composed of a mixture of reactive glasses with different compositions that could manipulate its properties [80]. In the orthopedic field, it has become evident that BG can participate hydroxyapatite, which then leads to minerals gain and reduces bacterial colonization [81]. The remineralization activity of BG occurs due to the deposition of calcium and phosphate ions over the demineralized tooth structure [82], and fluoride conjugated with BG could enhance the remineralization of dentin and decrease the risk of dentin-matrix degradation [83]. Also, BG demonstrated antibacterial activities against several oral microorganisms by the release of calcium and phosphate ions and increasing the pH of the oral cavity [84,85]. The first study that incorporated BG in resin composite was conducted by designing resin composite samples with 5, 10, or 15-wt% non-silanated BG. The total fillers content of all samples was 72%. After two months of exposure to aqueous media and a bacterial challenge, BG resin composites displayed higher flexural strength and fracture toughness compared to control [86]. The cytotoxicity of BG was investigated as BG did not demonstrate any toxicity except for the release of unreacted monomers which was comparable to that found in commercial resin composites [87]. Incorporating BG in flowable resin composite demonstrated significant inhibition of *E. coli* and *S. mutans* without compromising the bonding strength [88]. BG resin composites had less bacterial penetration compared to free-BG resin composite. This outcome was observed in other laboratory study using cyclic

**Table 3**  
 Outline of previous studies using metal/metal oxide nanoparticles for stimulate a detrimental biological response to bacterial growth over dental materials.

Agent	Bioactive function	Study (authors)	Concentration	Mechanical properties	Remarks
Silver nanoparticles	Silver ion release with bacterial damage and cell death	Cheng et al., 2012 [58]	0.028%	Flexural strength and elastic modulus were comparable to commercial control	Significant reduction of <i>S. mutans</i> metabolic activity, lactic acid, and colony-forming units by around 50%, 60%, and 90% respectively
Silver nanoparticles		das Neves et al., 2014 [59]	0.35%	Roughness and the compressive strength were comparable to the control samples	Inhibition of <i>S. mutans</i> and <i>Lactobacillus acidophilus</i> by around 90%.
Silver nanoparticles		Kasraei et al., 2014 [62]	1%	–	Proximally 95% and 80% significant colonies reduction of <i>S. mutans</i> and <i>Lactobacillus</i> , respectively
Ag bromide-cationic polymer (AgBr/BHPVP)	Silver ion release with bacterial damage and cell death	Cao et al., 2017 [61]	< 0.1%	Flexural strength and elastic modulus were not affected with higher Vicker's hardness compared to control	Greater antibacterial activities against <i>S. mutans</i> compared to control
Tetrapod-like ZnO whisker	A specific reaction that releases H <sub>2</sub> O <sub>2</sub> and reactive oxygen species forming hydroxyl radicals that limit the bacterial growth	Niu et al., 2010 [72]	5%	Higher flexural, compressive and diametral tensile strength compared to control	Enhanced antibacterial activity against <i>S. mutans</i>
ZnO		Aydin Sevinc et al., 2010 [73]	1–10%	–	80% bacterial count reduction against <i>S. mutans</i> , but low antibacterial activities against multispecies biofilm
ZnO nanoparticles		Kasraei et al., 2014 [62]	1%	–	Approximately > 99% and 70% significant colonies reduction of <i>S. mutans</i> and <i>Lactobacillus</i> , respectively
CuO	Generation of reactive hydroxyl radicals which are toxic to the bacterial cells	Zajdowicz et al., 2018 [79]	0.5–4%	–	Around 90–95% reduction of <i>S. mutans</i>
BG	Ca and PO <sub>4</sub> ion release followed by increasing in the local pH.	Chatzistavrou et al., 2015 [88]	5 wt% and 15 wt%	Bonding strength was comparable to the control samples	> 99% reduction against <i>S. mutans</i> was associated with 15 wt% BG
BG	BG also induces remineralization with and without fluoride	Khvostenko et al., 2016 [81]	15 wt%	–	Around 40% Less bacterial penetration compared to free-BG resin composite
Fluoride-containing phosphate-rich BG		Tezvergil-Mutluay et al., 2017 [89]	50 wt%	–	Significantly higher capability to remineralization of dentin and higher protection of dentin-matrix interface from degradation compared to control samples
Nanodiamonds	Negatively or positively charged particles change the membrane permeability which might cause bacterial death	Cao et al., 2018 [93]	0.1%–1.5% wt.%	Higher Vicker's hardness, flexural strength, modulus of elasticity. Higher toxicity was reported as the concentration of nanodiamonds increased	The number of viable <i>S. mutans</i> decreased by about 80–90% compared to unmodified samples



**Fig. 8.** Live/dead staining assay for different composition resin composite disks. (A) Schematic of biofilm on cured disk with three layers: Primer, adhesive, and composite. (B–F) Illustrate live/dead images as live bacteria were stained green, and dead bacteria were stained red. Orange/yellow colors are an illustration of live and dead bacteria when they come close to each other. Control disks were associated with alive biofilms, while disks incorporated with nanoparticles of silver (Nag) and nanoparticles of silver-quaternary ammonium dimethacrylate (Nag-QADM) had large amounts of dead bacteria. Adapted from Ref. [60], with permission from © 2012 WILEY PERIODICALS, INC.

mechanical loading, where the *S. mutans* diffusion into the interface tooth-materials was observed over the whole depth of restoration (100%) in the free-BG resin composites, the *S. mutans* penetrated (61%) of the marginal gap in the BG resin composites [81]. Incorporation of BG and fluoride with resin composites was found to enhance the remineralization of dentin, and also prevent the solubilization of C-terminal cross-linked telopeptide (ICTP) and C-terminal telopeptide (CTX), which eliminate the degradation of the dentin-matrix interface [89].

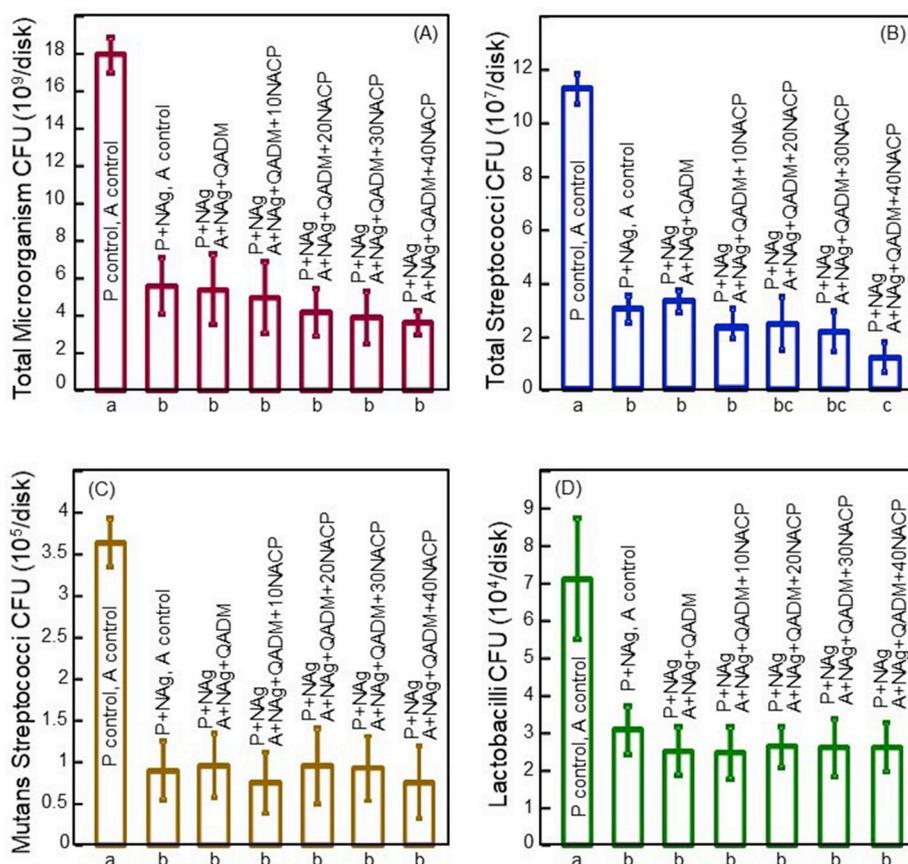
**3.2.1.4. Diamond nanoparticles.** Nanodiamond is a member of the carbon-based nanomaterials, and it has been used in many industries and technologies to enhance the mechanical and antibacterial properties of several materials, especially in polymer engineering [90]. The main advantage of nanodiamonds is related to their biocompatibility and stability [91]. Also, nanodiamond has demonstrated diverse antibacterial activities against several microorganisms, which expands its use in medicine [92].

The incorporation of nanodiamonds into resin composite was attempted [93]. A 0.1%, 0.3%, 0.6%, 1.0% and 1.5% w/w of silver-loaded polycation functionalized nanodiamonds (Ag/QNDs) were added into a mixture of Bis-GMA and TEGDMA monomers. A homogeneous dispersion of Ag/QND's was observed using transmission electron microscopy. Ag/QNDs resin composites demonstrated an improvement in the mechanical properties compared to unmodified resin composites. The addition of 0.3–1.5% of Ag/QNDs significantly

increased in the Vicker's hardness, flexural strength, and modulus of elasticity. While the 0.1% of Ag/QNDs added to resin composite improve the mechanical properties, that was significant only with the Vicker's hardness. Also, a synergetic effect of Ag/QNDs particles was observed decreasing the viability of *S. mutans* [93]. The cytotoxicity test of Ag/QNDs resin composites demonstrated significant higher cytotoxicity except for the 0.1% Ag/QNDs resin composite, which was comparable to the control group [93].

#### 4. Biocompatibility of bioactive resin composite materials

The cross-linking process of resin composites never achieves a complete polymerization reaction, which then may lead to the release of unreacted monomers [94,95]. Also, incomplete polymerized resin composites are more susceptible to degradation [96,97]. Therefore, unreacted monomers may leach from polymeric materials and induce some amount of cytotoxicity that affects the surrounding cells [98,99]. Also, resin composite degradation could be accelerated by bacterial biofilms [100]. Bioactive resin composites with antibacterial activities demonstrated promising *in-vitro* results in reducing biofilm formation, and subsequently, resin composite degradation. Also, some studies investigated the cytotoxicity behavior of bioactive resin composites and revealed that these materials did not induce any significant toxicity or degradation more than what is found in unmodified resin composites [44,83,87,89,93]. However, some other bioactive materials were not assessed in regard to their toxicity and degradation behavior. Also, even



**Fig. 9.** Colony-forming unit (CFU) counts for biofilms on resin composite disks (mean six sd; n 1/4 6). (A) Total microorganisms, (B) total streptococci, (C) mutans streptococci, and (D) lactobacilli. The CFU counts for biofilms on the experimental bonding agents were reduced to about 20%–30% compared to control, values indicated by different letters are statistically different from each other ( $p < 0.05$ ). Adapted from Ref. [60], with permission from © 2012 WILEY PERIODICALS, INC.

though ion release from bioactive resin composites could diminish the amount of biofilm formation, it also may compromise the whole polymeric structure making it more susceptible to degradation, monomer release, and future acid attack. There is an almost total lack of literature concerning the long-term performance of these bioactive resin composites assessing mechanical/physical properties, adhesion, biocompatibility, and antibacterial activities. Future studies may focus on conducting comprehensive short and long-term evaluations related to the biocompatibility of invented bioactive resin composite materials. Also, the relation between ions release and long-term clinical performance of such material should be investigated. Recharging bioactive materials to compensate the volume and function of the released ions could be a method to maintain the clinical performance over time. However, rechargeability and its capability to preserve the materials' performance could be one of the future directions in the dental biomaterial field.

## 5. Summary

Available evidence has indicated the importance of considering bioactive restorative polymer materials in clinical practice. These materials have demonstrated a strong potential to reduce and modulate the metabolic activities of caries-related bacteria without compromising the mechanical and physical properties. However, the clinical applications of bioactive restorative polymer materials might be more complicated than expected. The optimum restorative polymer material should have the capabilities to survive inside the mouth, where the material is susceptible to more challenges compared to long-term *in vitro* studies. Many limitations of the current evidence base are evident by the lack of clinical data. Therefore, randomized control clinical trials should be conducted to investigate the performance of these materials and validate the extent of their bioactivity and interaction with the microflora in the oral cavity. It is expected in the few coming years that many clinical studies will be performed to investigate the ability of

bioactive restorative polymer materials to eliminate the risk of CARS and reduce the activities of caries-related microorganisms.

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