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Calcium phosphates as ion-releasing fillers in restorative resin-based materials

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ABSTRACT

Calcium phosphates (CaP) are the main constituents of the mineral phase in bones and teeth and, along with calcium silicates and bioactive glasses, have been extensively investigated in remineralization of enamel and dentin. When used as ion-releasing fillers in resin-based materials, they could contribute to extend the service life of adhesive restorations, remineralize caries-affected dentin or prevent caries lesions under sealants and orthodontic brackets. However, the development of resin-based bioactive materials is not straightforward because of the several compositional variables involved in ion release. Also, CaP particles do not reinforce the material; therefore, if high mechanical properties are required, the ratio between CaP particles and reinforcing fillers must be observed. Several research groups have investigated how CaP phase, particle size and content, as well as resin matrix formulation affect remineralization, ion release kinetics and mechanical properties of these materials. This review presents an overview of the main findings reported in the literature.

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1. Introduction

Most restorative materials currently used in clinical practice are considered “inert” in the sense that they do not elicit a physiological response from the host tissue. Calcium hydroxide and hydraulic calcium silicate cements (*e.g.*, mineral trioxide aggregate, MTA) are exceptions, as these pulp capping materials promote the release of signaling molecules from the dentin extracellular matrix, responsible for tissue repair [1]. A broader concept of “bioactive materials” includes those with the ability of promoting apatite precipitation when immersed in phosphate-rich physiological medium [2,3]. Bioactive glasses and calcium phosphates, extensively used as bone graft substitutes due to their biocompatibility, osteoconductivity and/or osteoinductivity, are included in this definition [4]. When placed in bone defects, calcium silicates have also demonstrated osteoinductive properties, which involves ion release and apatite deposition on the surface of the host tissue [5].

In Dentistry, there has been an emerging trend towards the development of bioactive restorative materials, and remineralizing resin-based materials are often mentioned as a possible way to increase the durability of bonded restorations [6–10]. Examples of possible clinical applications of resin-based materials containing ion-releasing fillers include: (1) restorative composites, sealants and orthodontic cements, to reduce or postpone the development of carious lesions at the tooth-material interface [11], (2) in conservative approaches for management of caries lesions (*e.g.*, atraumatic restorative treatment, ART), to increase the mineral content of caries-affected dentin [12] and (3) adhesive systems, as ion release was shown to reduce hydrolytic degradation of the hybrid layer and inhibit enzymatic activity [13,14].

Though calcium silicates and bioactive glasses have been extensively investigated in enamel and dentin remineralization [13,15], calcium orthophosphates (CaP) are arguably the most studied group of compounds used as ion-releasing fillers in resin-based materials. The first studies on enamel remineralization using dimethacrylate-based resins containing amorphous calcium phosphate (ACP) particles were published in the 1990s [16–18], but it was in the last decade that a substantial amount of information was produced, including the use of different CaP phases as co-fillers in experimental composites [19,20].

This text presents relevant topics related to the development and use of resin-based materials containing calcium orthophosphates, providing an overview of the current knowledge on this subject. The available literature is discussed, with focus on studies dealing with enamel and dentin remineralization, variables affecting ion release, mechanical properties of CaP-containing materials, and particle functionalization as a strategy to improve their performance.

2. Calcium orthophosphates (CaP): definition and uses in restorative dentistry

Calcium phosphates can be differentiated according to the type of phosphate anion as ortho- (PO_4^{3-}), meta- (PO_3^{-}), pyro- ($P_2O_7^{4-}$) and poly- ($(PO_3^{-})_n^{n-}$) [21]. Orthophosphates are derived from phosphoric acid (H_3PO_4, sometimes referred as orthophosphoric acid to differentiate from polyphosphoric acids), which is triprotic, *i.e.*, presents three dissociation constants (pK_a). The first hydrogen atom dissociates at pK_{a1} 2.12, resulting in the dihydrogen phosphate anion ($H_2PO_4^{-}$). The other dissociation constants (pK_{a2} and pK_{a3}) are 7.21 (HPO_4^{2-}, hydrogen phosphate) and 12.67 (PO_4^{3-}, phosphate; all values at 25 °C). Hence, calcium orthophosphates can be defined, in broad terms, as salts of calcium derived from orthophosphoric acid [22].

Calcium orthophosphates have crystalline structures stabilized by a PO_4 tetrahedral network, making them sparingly soluble in water. The different anions ($H_2PO_4^{-}$, HPO_4^{2-} or PO_4^{3-}) and the presence of hydroxyl groups in some compounds allow for different calcium-to-phosphorous (Ca/P) molar ratios. In general, lower Ca/P ratios correspond to more acidic and relatively water-soluble CaP phases [21]. Apart from crystalline forms, amorphous calcium phosphate (ACP) is a metastable phase lacking the long-range atomic organization that, in supersaturated conditions, precedes the precipitation of crystalline apatite [23]. Table 1 shows the Ca/P ratio and solubility of the known non-ion-substituted calcium orthophosphate phases. Notice that hydroxyapatite (HA) has the highest solubility product constant (pK_s) meaning it is the least soluble of calcium orthophosphates, which explains why this is the major CaP compound encountered in normal and pathological calcifications.

The first reports on the use of CaP particles in Dentistry date back to the 1980s. Interestingly, the initial studies described the addition of HA particles to resin-based materials with the purpose of testing coupling agents capable of bonding to the tooth structure. For instance, surface-active amine accelerators were indirectly evaluated as adhesion promoters by testing the mechanical strength of dimethacrylate resins containing different accelerators (both surface-active or not) and HA particles [24]. Around the same time, coating agents for HA particles were being investigated aiming solely to use them as reinforcing phase in composites [25]. In 1986, a hydraulic calcium phosphate bone cement consisting of 73% tetracalcium phosphate (TTCP) and 27% dicalcium phosphate anhydrous (DCPA) was developed [26]. In solution, under slightly alkaline conditions, both compounds dissolve and reprecipitation occurs in the form of HA [27]. A similar formulation is currently used as dentin desensitizer [28]. It was not until the mid-1990s that the remineralizing potential of CaP-containing composites started to be assessed [18].

ACP is the most commonly found CaP phase in studies with experimental composites and adhesive systems [17,29–32]. Its high solubility in water (in relation to other CaP phases) make ACP a suitable option as ion-releasing particle [18]. ACP particles can be found in commercially available dentifrices, bleaching agents, as well as resin-based materials such as sealants, orthodontic cements and restorative composites. ACP is metastable and in order to prevent spontaneous conversion to HA and extend ion release for longer periods, particles must be stabilized, for instance, by adding pyrophosphate ($P_2O_7^{4-}$) in the synthesis [17,18]. Casein phosphopeptide (CPP) can also be used to stabilize ACP. CPP–ACP is arguably the most

Table 1 – Calcium orthophosphates phases, with the respective Ca/P molar ratios and solubility (at 25 °C, $pK_s = -\log K_s$) [21,22,83,84]. "Category" refers to the temperature necessary to synthesize the CaP phase. Those in bold letters indicate phases tested as ion-releasing fillers in experimental resin-based materials.

Category	Compound	Formula	Ca/P molar ratio	Solubility (pK_s)
Low-temperature CaPs	Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2\,H_2O$	0.5	1.14
	Dicalcium phosphate dihydrate (DCPD)	$\mathbf{CaHPO_4H_2O}$	**1.0**	**6.59**
	Dicalcium phosphate anhydrous (DCPA)	$\mathbf{CaHPO_4}$	**1.0**	**6.90**
	Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4\,5H_2O$	1.33	96.6
	Precipitated, calcium-deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}(0 < x < 1)$	1.5-1.67	~85
	Amorphous calcium phosphate (ACP)	$\mathbf{Ca_xH_y(PO_4)_{z}nH_2O}$	**1.2-2.2**	**
High-temperature CaPs	Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	0.5	1.14
	α-tricalcium phosphate (α-TCP)	$\alpha\text{-}Ca_3(PO_4)_2$	1.5	25.5
	β-tricalcium phosphate (β-TCP)	$\boldsymbol{\beta}\text{-}\mathbf{Ca_3(PO_4)_2}$	**1.5**	**28.9**
	Sintered hydroxyapatite (HA)	$\mathbf{Ca_{10}(PO_4)_6(OH)_2}$	**1.67**	**116.8**
	Tetracalcium phosphate (TTCP)	$\mathbf{Ca_4(PO_4)_2O}$	**2.0**	**38-44**

** According to Dorozhkin [21], ACP solubility cannot be measured accurately, but is assumed to be close to that of α-TCP.

Table 2 – Refractive indices of crystalline calcium orthophosphates [85].

CaP phase	Mineralogical name	Refractive index
DCPD	Brushite	1.54–1.55
DCPA	Monetite	1.59–1.65
β-TCP	Whitlockite	1.63
HA	Hydroxyapatite	1.64–1.66
TTCP	Hilgenstockite	1.64–1.65

studied CaP-based material in preventive and restorative dentistry of the last two decades. Its effect on remineralization of enamel lesions is credited to a strong ionic gradient towards the enamel granted by CPP–ACP complexes [33]. CPP–ACP can be found in several commercial products, but it is not suitable as bioactive filler in restorative materials. Data from clinical studies testing CPP–ACP-based products are inconclusive and there has been some debate regarding the quality of the available scientific evidence supporting its clinical use [34,35].

Other CaP phases have been tested as ion releasing fillers in resin-based materials, such as DCPA [36,37], TTCP [19] and dicalcium phosphate dihydrate (DCPD) [20,38]. An important aspect on the development of light-cured materials is the refractive index (RI) mismatch between the matrix and the filler particles, which may negatively affect the material's degree of conversion. Table 2 shows the RI values of crystalline CaP phases. Considering that strontium and barium glass fillers have RI of 1.51 and 1.53, respectively and Bis-GMA/TEGDMA mixtures show RI between 1.50 and 1.56 [39], DCPD can be considered a suitable option in terms of optical properties of the resulting material. In fact, problems related to light transmission in HA-filled experimental composites have been mentioned in previous studies [40,41]. The RI of amorphous calcium phosphates is directly related to their CaO content, with values ranging between 1.53 and 1.58 [42].

3. Remineralization studies

Enamel remineralization is accomplished when, in supersaturated conditions, calcium and phosphate ions penetrate through the pores of the lesion surface and precipitate into the HA crystal voids in body of the lesion [43]. The result of this top-down remineralization mechanism is often referred to as "rehardening", denoting that enamel microhardness is recovered (at least partially) as a result of mineral precipitation. Dentin remineralization, on the other hand, is a much more complex matter and requires mineral precipitation to occur in the collagen intrafibrillar and interfibrillar spaces in order to recover its mechanical properties (bottom-up remineralization) [15].

The literature on enamel and dentin remineralization using dimethacrylate-based materials containing CaP particles is not very extensive, and the findings of available *in vitro* and *in situ*/*in vivo* studies are discussed below. In spite of the differences in methods and tested formulations, all studies seem to agree that is possible to increase the mineral content of demineralized enamel and dentin with the use of resin-based materials containing CaP particles. At the time of this writing, no clinical studies involving CaP-containing composites were found in the literature.

3.1. *In vitro studies*

Remineralization of enamel and dentin has been quantified using transverse microradiography (TMR) after various types of calcium phosphate containing composites were applied directly on top of previously demineralized surfaces and the specimen was subjected to pH cycling, simulating a cariogenic challenge (Table 3). The validity of this method for testing enamel remineralization can be criticized, as the placement of the material on top of a white spot lesion does not reproduce its intended use as restorative material. Furthermore, in most studies the composite was tested without an interposed adhesive layer. In fact, when an adhesive system was applied on the demineralized dentin prior to the composite, remineralization

Table 3 – *In vitro* remineralization studies with experimental CaP-containing resin-based materials (human teeth were used except when noted otherwise).

Substrate	Reference	Composite formulation		pH cycling or static immersion conditions[a]	Outcome[b]
		Resin matrix	Fillers (wt%)		
Enamel	Skrtic et al. [16]	BisGMA, TEGDMA, HEMA	40% $P_2O_7^{4-}$-stabilized ACP	0.5 h DE, 12.5 h RE, 14 days	+38 % Control composite: −9% (Obs: bovine enamel)
	Langhorst et al. [45]	EBPADMA, TEGDMA, HEMA, MEP	40% Zirconia-hybridized ACP (55 μm)	1 h DE, 23 h RE, 30 days	+(14.4 ± 16.7) % Control composite: + (4.4 ± 14.2)%
	Weir et al. [86]	EBPADMA, TEGDMA, HEMA, MEP	40% ACP (116 nm); 20% barium glass (1.4 μm)		+(21.8 ± 3.7)% Control composite: + (5.7 ± 6.9)%
Dentin	Dickens et al. [36]	EBPADMA, PMGDM	A: 17.9% DCPA (1.1 μm), 55.1% TTCP (16 μm); B: 19.3% DCPA, 59.5% TTCP	Saliva-like solution 5 weeks (no pH cycling)	A: +(38 ± 10)% B: +(47 ± 16)%
	Dickens and Flaim [44]	EBPADMA, PMGDM	A: 19.6% DCPA (1.1 μm), 60.4% TTCP (16 μm); B: 19.3% DCPA, 59.5% TTCP	Saliva-like solution 6 weeks (no pH cycling)	A: +(38 ± 10)% (without adhesive) +(23 ± 11)% (with adhesive) B: +(35 ± 19)% (without adhesive) +(13 ± 7)% (with adhesive)
	Weir et al. [87]	EBPADMA, PMGDM	A: 40% ACP (116 nm); 20% barium glass (1.4 μm); B: 40% ACP; 20% TTCP (0.8 μm)	1 h DE, 23 h RE, 8 weeks	A: +(48.2 ± 11.0)% B: +(43.2 ± 12.6)% Control composite: +(5.0 ± 7.2)%

BisGMA: bisphenol A glycidyl dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; EBPADMA: ethoxylated bisphenol A dimethacrylate; MEP: methacryloyloxyethyl phthalate; PMGDM: pyromellitic glycerol dimethacrylate.

[a] DE: demineralizing solution (pH 4.0), RE: remineralizing solution (pH 7.0).

[b] Positive values: mineral recovery, negative values: mineral loss.

was significantly reduced [44]. These aspects, associated with other limitations inherent of *in vitro* studies, make their clinical significance questionable though they are still valid as proof of concept [45]. Also noteworthy is the fact that all experimental formulations listed in Table 3 included hydrophilic monomers to increase the transit of fluids through the composite and facilitate ion release. Overall, mineral recovery was higher in dentin (35–48%) than in enamel (14–38%). Also, it is interesting to notice that the control groups (*i.e.*, composites without CaP) also showed some mineral gain (around 5%) due to precipitation of calcium and phosphate ions from the remineralizing solution.

Calcium and phosphate precipitation in enamel seems to present a different pattern in relation to fluoride. While a resin cement containing fluoride promoted higher mineral gain in the first 25 μm of the lesion, the ACP composite was more efficient at greater depths [45]. A similar observation was made in an *in situ* study comparing the remineralization of lesions with a glass ionomer cement and a CPP–ACP gel [46]. However, when enamel demineralization around brackets was assessed by laser fluorescence after 21 days of pH cycling, no difference was found between a commercial ACP-containing composite and a RMGI, while both reduced demineralization in comparison to a conventional resin cement [47].

Resin composites containing ACP nanoparticle agglomerates were also tested in association with organic molecules as templates for mineral nucleation. Demineralized dentin treated with a poly(amino amide) (PAMAM) solution was placed in contact with an experimental composite containing 40 wt% of ACP and 20 wt% of barium glass particles (1.4 μm) dispersed in a EBPADMA/PMGDM matrix, and kept immersed in saline solution acidified with lactic acid (pH 4) for 21 days. Mineral deposition in the dentinal tubules was observed up to 30 μm beneath the dentin surface and the microhardness of sound dentin was recovered. Different from studies using pH cycling solutions or artificial saliva as immersion media, the only ion source for remineralization was the ACP in the composite, emphasizing its effectiveness in promoting remineralization [48].

3.2. *In situ and in vivo studies*

A resin cement formulation containing DCPA (1.1 μm, 29.1 wt%) and TTCP (16 μm, 36.8 wt%) was tested as a base material on caries-affected dentin *in vivo*. After three-months, teeth were extracted and calcium and phosphorous contents in dentin were quantified by electron probe micro-analysis. Dentin that received the CaP-containing cement showed similar mineral contents from non-carious teeth (positive control), and both groups had higher mineral content than the negative control (*i.e.*, teeth restored without the base material) [12]. However, dentin microhardness was not totally recovered, suggesting that the collagen interfibrillar and intrafibrillar areas were not remineralized [49].

Two *in situ* studies evaluated commercially available materials with ACP. The use of an ACP-containing orthodontic resin to bond brackets on premolars scheduled for extraction resulted in higher enamel crossectional microhardness up to a 20 μm depth in comparison to a conventional resin cement after 30 days [50]. In another study, sealants containing either ACP or fluoride applied on artificial enamel caries lesions showed similar increases in surface microhardness (32–34%) after five days *in situ*, against 18–19% in exposed enamel samples [51].

The protective effect on the surrounding enamel of an experimental composite containing 40 wt% of ACP agglomerates and 20 wt% of silanized glass particles (1.4 μm) dispersed in a BisGMA/TEGDMA matrix was verified *in situ*. After 14 days in the presence of cariogenic biofilm, mineral loss around the ACP composite was reduced to one third and lesion depth was reduced by 45% in comparison to the control composite without ACP. Biofilm analysis revealed higher calcium and phosphate concentrations in the ACP composite group [11].

4. Ion release from resin-based materials containing CaP particles

The determination of calcium and phosphate concentrations released from resin-based materials in different immersion media (*e.g.*, saline solution or artificial saliva) is often reported in the literature. Though it constitutes an important parameter to assess the influence of variables involved in this process and the release kinetics, the relevance of this information is somewhat limited because no dose-response relationship has been established for these ions, as it has been with fluoride [52]. Also, the lack of a standardized testing procedure impedes the comparison among studies, as they differ in several aspects such as the volume ratio between specimen and immersion medium, data collection (cumulative *versus* non-cumulative) and analytical methods.

Several factors interact to determine ion release from resin-based materials and, therefore, it is difficult to make general statements. Notwithstanding, the available information seem to agree that ion release increases exponentially with the material's CaP content, regardless of particle type and size, or formulation of the resin matrix [37]. This non-linear relationship was first established in a study showing that resin-based materials containing ACP particles presented a marked increase in ion release around 0.37–0.40 mass fractions [17]. Also, experimental composites containing DCPD particles (17–20 μm) showed a significant increase in ion release between volume fractions of 10% and 20% [53].

Ion release increases with CaP particle surface area, as the interface with water becomes larger [54]. Concentration and rate of release are also significantly increased under acidic pH conditions [19,55], similar to what was observed with fluoride release from glass ionomers [56]. This is an interesting feature as, theoretically, more ions would be available for remineralization after a pH challenge event. This higher ion release, though resulting from more severe particle erosion, does not seem to affect the composite's flexural strength or modulus in comparison to specimens stored under neutral pH conditions [19,55]. Finally, the presence of hydrophilic monomers (such as HEMA, 2-hydroxyethyl methacrylate) and less densely cross-linked networks, as found in EBPADMA-based composites compared to BisGMA- and UDMA-based composites also increased ion release [57].

Calcium and phosphate ions released from resin-based materials containing CaP particles may reach supersaturated concentrations leading to HA precipitation on the specimen surface *in vitro* [58]. The tendency of a CaP-containing material to induce HA precipitation is expressed by Gibbs free energy change (ΔG, in KJ mol^{-1}), which can be calculated as follows [59]:

$$\Delta G = -\frac{RT}{n}\ln\left(\frac{IAP}{K_{sp}}\right)$$

where R is the gas constant ($8.314\,\text{J K}^{-1}\text{mol}^{-1}$), T is the temperature (in Kelvin), IAP is the ionic activity product for HA (*i.e.*, the ionic product considering the actual ionic concentrations available to precipitate from the solution), *n* is the number of ions in HA ($Ca_{10}(OH)_2(PO_4)_6$, $n = 18$) and K_{sp} is the solubility product constant (*i.e.*, the maximum possible amount of a solute that can be dissolved in a given solvent). HA spontaneous precipitation is thermodynamically possible if the ionic concentrations found in the solution result in $\Delta G < 0$. Indeed, negative ΔG values were reported in several studies, with different formulations [36,60–62]. Though the ionic concentrations found in closed systems most likely do not correspond to those found in a clinical scenario, negative ΔG values provide theoretical basis for remineralization observed *in vitro* and *in situ*.

Table 4 shows ion release data from studies that also evaluated enamel or dentin remineralization. Without taking into account the differences in specimen dimensions and volume of immersion solutions, typical *cumulative* values for calcium release range from 0.5–3.0 mmol L^{-1} (pH 7) to 12 mmol L^{-1} (pH 4). Phosphate values are usually lower, ranging from 0.1–0.5 mmol L^{-1} (pH 7) to 6 mmol L^{-1} (pH 4). The lower phosphate release is explained by the structural role of the phosphate tetrahedra and also by the establishment of hydrogen bonds between the oxygen atoms in the phosphate groups and structural or adsorbed water molecules [63].

In order to estimate the actual remineralizing potential of resin-based materials containing CaP particles and identify possible clinical uses, it is important to comprehend the kinetics of ion release. Unfortunately, the available information was obtained under static conditions and there are no studies evaluating ion release in a continuous flow system. The existing literature suggests a diffusion controlled mechanism similar to what has been described for glass ionomer cements [56], with initially a high release rate that continuously decreases over a few weeks [55]. Under acidic conditions (pH 4), composites containing 20 wt% of ACP nanoparticle agglomerates and 50 wt% of glass particles (1.4 μm) were shown to release calcium and phosphate for five to six weeks before a plateau was reached, with maximum cumulative values of approximately 2–4 mmol L^{-1} for calcium and 1.25–1.75 mmol.L^{-1} for phosphate [64]. A composite containing 15 vol% of DCPD (25 μm) and 45 vol% of barium glass (2 μm) in a BisGMA and TEGDMA matrix showed *non-cumulative* calcium/phosphate concentrations varying from 0.10/0.05 mmol.L^{-1} at 15 days of immersion to 0.04/0.01 mmol L^{-1} after 60 days under neutral pH [65].

One final aspect related to ion release is the ability of phosphate ions to neutralize acids produced by bacteria, as an additional mechanism to protect teeth against mineral loss.

Table 4 – Ion release concentrations from studies on experimental CaP-containing resin-based materials that also evaluated remineralization (all *in vitro* studies except ref. [11], which was conducted *in situ*). Please, refer to Table 3 for details on composite formulations of *in vitro* studies.

Reference	Duration (days)	Immersion conditions	Quantification method	Cumulative concentrations released (mmol/L)
Langhorst et al. [45]	28	Saline, pH 7	Atomic emission spectroscopy	Ca: 0.744 ± 0.069 P: 0.543 ± 0.057
Melo et al. [11]*	28	Saline, pH 4.0, 5.5 and 7.0	Spectrophotometry	Ca: 12 (pH 4.0), 4 (pH 5.5) and 3 (pH 7.0) P: 6 (pH 4.0), <1 (pH 5.5 and 7.0)
Dickens et al. [36]	90	Saline, pH 7.4	Spectrophotometry	Ca: 0.3 (cement A), 0.5 (cement B) P: 0.05 (cement A), 0.1 (cement B)
Dickens and Flaim [44]	45	Artificial saliva, pH 7.0	Spectrophotometry	Ca: 1.5, P: 0.5 (Obs: total Ca and P concentrations, i.e., including the concentration from artificial saliva)
Weir et al. [87]	56	Saline, pH 4.0 and 7.0	Spectrophotometry	Ca: 12 (pH 4.0), 1 (pH 7.0) P: 5 (pH 4.0), <1 (pH 7.0)

* Composite formulation: BisGMA/TEGDMA matrix, 40 wt% of ACP nanoparticle agglomerates and 20 wt% of silanized glass particles (1.4 μm).

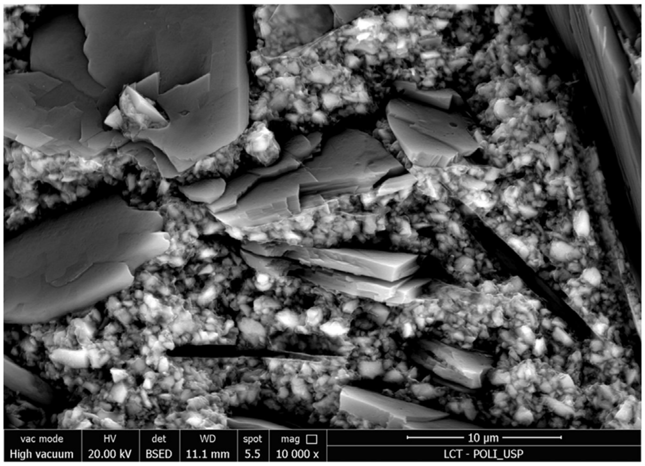

Fig. 1 – Scanning electron microscopy image of the fractured surface of a composite containing 40 vol% of barium glass and 20 vol% of DCPD particles (large plates). It is possible to identify empty slots previously occupied by DCPD particles, that detacched during crack propagation, as well as fractured particles.

Under static conditions, a composite containing 40 wt% of ACP was shown to increase the pH of the immersion medium from 4.0 to 5.7 after 10 min to a maximum of 6.8 after 90 min and cause a moderate reduction in *Streptococcus mutans* growth on the composite surface [66].

Orthophosphate anions ($H_2PO_4^{-}$, HPO_4^{2-} and PO_4^{3-}) can reversibly bind H^+ ions in solution and, therefore, form good chemical buffers (*i.e.*, solutions resistant to pH variations). Therefore, in the range of pH values associated with acid production by *S. mutans* (between 4 and 5) [67] the presence of orthophosphate anions (particularly $H_2PO_4^{-}$, with $pK_2 = 7.21$) will help bring the pH back to neutral. However, this is not enough to provide an antibacterial effect. Calcium silicates and bioactive glasses, on the other hand, form calcium hydroxide in water. This strong base is predominantly dissociated in solution [$Ca^{2+} + 2(OH–)$], raising the pH to values above 10, which is largely responsible for their antimicrobial activity [68,69]. Though there is some evidence that calcium release from bioactive glasses may also contribute for their antibacterial effect [70], the previously mentioned *in situ* study did not find differences in the number of *Streptococci* and *Lactobacilli* colony forming units (CFUs) on a composite containing 40 wt% ACP and 20 wt% reinforcing glass in relation to the control [11]. Another study did not see differences in *S. mutans* biofilm formation on composite specimens containing 20 vol% of DCPD (17–20 μm) in comparison to the control composite under a continuous flow system [71].

Experimental resin-based materials with both remineralizing and antibacterial properties have been developed combining CaP particles with silver nanoparticles [72], quaternary ammonium compounds [31] or protein repellents (for example, dimethylaminohexadecyl methacrylate, DMAHDM) to reduce bacterial attachment on the material surface [73].

5. Mechanical properties

Calcium orthophosphate particles do not reinforce the resin matrix. In fact, the fracture strength of unfilled resins is significantly reduced by the incorporation of CaP particles [17,74]. The lack of a strong chemical and micromechanical interactions between the resin matrix and the particles does not allow for an effective stress distribution between both phases. In fact, CaP particles behave as defects (inclusions), creating several potential crack initiation sites within the material and increasing the risk of crack propagation under relatively low stress levels (Fig. 1). Elastic modulus, on the other hand, increases with the incorporation of CaP particles to the unfilled resin, as this property is determined mostly by direct (physical) contact between particles; in fact, particle fractions as low as 20 vol% were shown to result in materials with significantly higher modulus in relation to that of the unfilled resin [19,75].

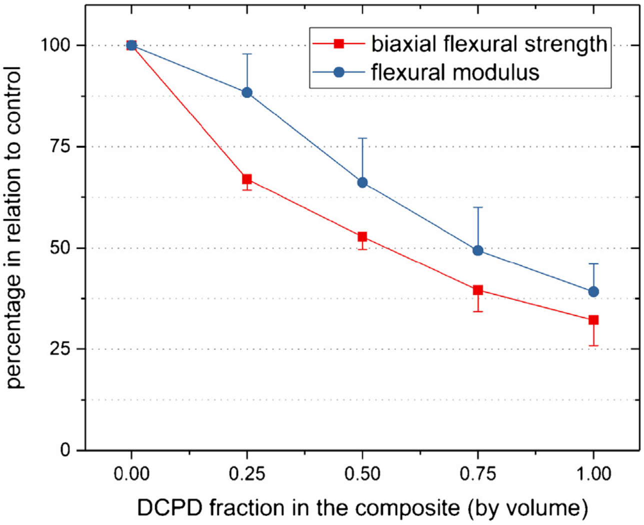

Fig. 2 – Effect of “barium glass: DCPD ratio” on the biaxial flexural strength and flexural modulus of experimental composites with a total filler content of 50 vol%. Values represent percentages in relation to the composite containing only glass particles. Error bars represent +1 standard deviation (modulus) and −1 standard deviation (strength).

According to Griffith’s law, strength is inversely proportional to flaw size. Therefore, larger CaP particles should be more detrimental to the material’s fracture strength. This was confirmed by a study comparing resin materials containing 75 wt% of TTCP particles, where particles with 0.97 μm resulted in 30% higher strength than the material with particles with 16.2 μm. The material with small particles showed strength similar to that of a resin-modified glass ionomer [55]. However, the opposite was observed when materials containing 65 wt% of DCPA particles with 112 nm, 0.88 μm or 12 μm were compared. According to the authors, the smallest particles led to an increase air entrapment in the material, reducing its degree of conversion and, consequently, its strength [54].

Mechanical requirements are dictated by the material’s intended use. If a material is expected to withstand severe loading, such as a direct restorative material, CaP particles must be used in association with reinforcing fillers [19]. The effect of “DCPD: glass ratio” on the flexural strength and modulus of composites with a total filler content of 50 vol% is shown in Fig. 2. Notice that the complete replacement of the reinforcing glass by DCPD particles (8 μm) reduced the strength to 32% and the modulus to 40% of the value displayed by the control composite. The reduction in modulus, more pronounced above 0.25 DCPD, is likely due to an increase in interparticle spacing, associated with the lack of chemical bond between the DCPD particles and the resin phase. DCPD particles are relatively large plates (15–20 μm in the long axis). Therefore, increasing DCPD volume fraction will result in areas sparsely filled with glass particles. As expected, there is a trade-off between fracture strength and ion release [53] and in order to maximize ion release, more of the inorganic fraction will have to be occupied by CaP particles, resulting in a material with relatively lower strength.

Very few studies investigated the fracture toughness (K_{1c}) of CaP-containing composites. An interesting finding was that replacing 15–20 vol% of the conventional glass fillers by DCPD particles in experimental composites with a total inorganic content of 60 vol% increased K_{1c} measured after 24 h storage in water by 25% [20,65]. This effect is likely the result of a reduction in stress intensity factor (K) at the crack tip when the crack front propagates around or through the relatively large plate-like DCPD particles, postponing its unstable growth.

Studies comparing experimental composites containing both CaP and glass fillers with commercial composites show that materials containing up to 40 wt% of ACP nanoparticle agglomerates and 20 wt% of reinforcing glass have fracture strength similar to that of microfilled composites [29,48], while a material with 40 wt% of ACP (6 μm) and 10 wt% of barium glass particles (0.77 μm) showed strength similar to a resin-modified glass ionomer [61]. Conversely, when DCPD (15 μm) was limited to 10 vol%, an experimental composite with another 50 vol% of barium glass particles (2 μm) reached strength similar to that of a microhybrid composite [38]. CaP-containing composites show elastic modulus similar to those displayed by heavily filled microhybrid composites, granted that at least part of the total filler fraction is occupied by glass particles in order to reduce interparticle spacing [38,66].

Considering that ion release is the result of CaP particle dissolution, it is intuitive to assume that CaP-containing composites undergo more severe degradation over time than conventional composites. Moreover, the lack of a strong coupling between particles and the resin matrix would facilitate the transit of fluids within the material. Indeed, the presence of ACP particles was shown to increase water sorption of unfilled BisGMA/TEGDMA resins after 30 days, which was not observed with the addition of silanized silica particles [74]. Composites containing 10 vol% or 20 vol% of DCPD particles in total filler contents between 40 vol% and 60 vol% presented higher percent reductions in strength after 28 days in water than the control [20,38]. Also, flexural modulus and K_{1c} values were reduced after 28-day storage only for the composites with DCPD, but not for the control [20]. However, when stored for longer periods (60 days to 2 years) no evidence of more severe degradation due to the presence of CaP particles was observed [65,76]. Therefore, though CaP-containing composites seem to undergo a more severe drop in mechanical properties in the short term, the available information suggest that this difference seems to fade over longer storage periods.

Finally, even considering the higher ion release observed under acidic conditions (suggestive of more severe particle erosion), flexural strength and modulus of composites containing 10–20 wt% of ACP agglomerates (70–75 wt% total filler content) or 40 wt% TTCP (0.8 μm, 70 wt% total filler) were not different after 28-day storage under pH 4 and pH 7 [19,29].

6. Functionalization of CaP particles

Ideally, CaP particles intended for use as ion-releasing fillers in resin-based materials should fulfill the following requirements: (1) size similar to that of the reinforcing fillers (*i.e.*, a few micrometers or less), (2) high surface area, (3) dispersible in the resin phase, and (4) capable of chemo–mechanical interac-

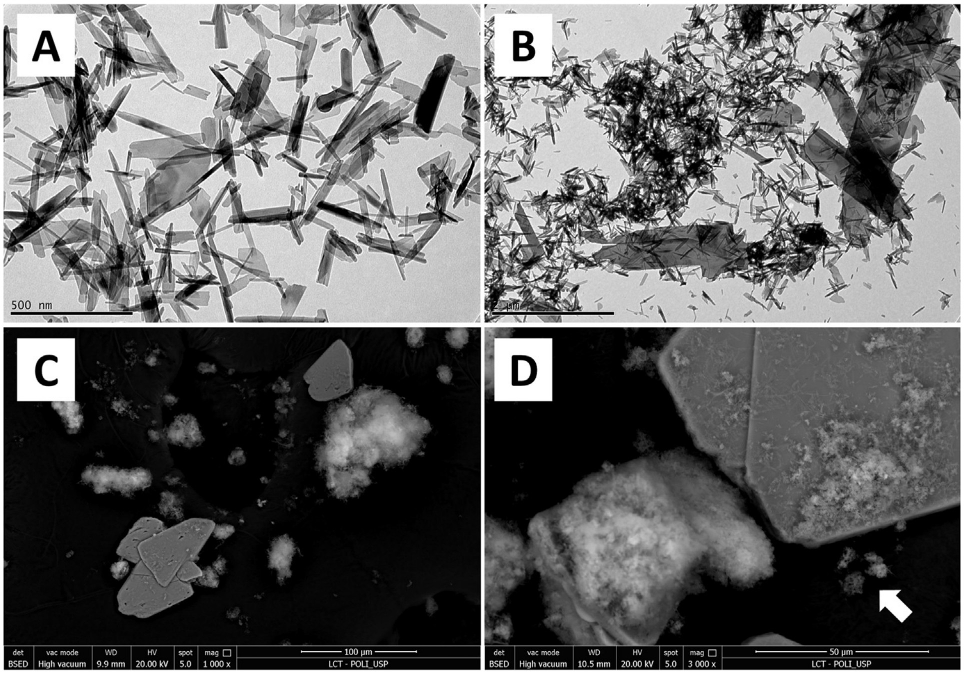

Fig. 3 – A: DCPD nanoparticles observed under transmission electron microscopy (TEM); B: TEM image showing nanoparticles and much larger particles obtained in the same synthesis; C: scanning electron microscopy images showing large particles next to nanoparticle agglomerates with different dimensions. D: nanoparticles deposited on the surface of a large particle. Arrow: small nanoparticle agglomerates.

tion with the resin phase. In spite of the recent advancements in nanoparticles synthesis, it is notorious that they have the tendency to agglomerate due to interactions involving surface Ca–OH and P–OH groups that create Ca–O–Ca and P–O–P bonds between particles. Practically speaking, nanoparticle agglomerates behave as micron-sized particles (nonetheless, usually retaining a high surface area). Also, it must be mentioned that depending on the synthesis method, the product is a mixture of nanoparticles with particles several micrometers large (Fig. 3).

Particle functionalization with organic molecules has been investigated as a way to develop CaP particles with the above-mentioned characteristics. Functionalizing agents contain atomic groups capable of interacting with the CaP surface through primary (*i.e.*, ionic) or secondary forces (*i.e.*, ion-dipole, van der Walls) and, due to its organic nature, they improve the contact between the particle and the resin phase, enhancing micromechanical interlocking [77]. Furthermore, these molecules may present polymerizable carbon double bonds, which can co-polymerize with monomers in the matrix. In some cases, they may present an excess of ionizable groups (such as hydroxyl, carboxyl or hydrogen phosphate groups) so the particle becomes electrically charged. Repulsive forces among similarly charged particles are responsible for reducing agglomeration and particles are considered "stabilized" (not to be confounded with stabilization as in preventing phase modification). Citric acid, for example, with three ionizable carboxyl groups –COOH, is an effective particle stabilizer [78]. CPP–ACP nanoclusters are an example of stabilized CaP particles. In fact, they are "stabilized" in both senses of the word, *i.e.*, ACP conversion to apatite is hindered and electrically charged amino acids in the peptides also prevent nanocluster agglomeration [35].

Particle functionalization can be accomplished by stirring the particles in a solution containing the surface-active agent, similar to the procedure usually adopted for silanization of glass particles. Alternatively, the organic compound can be added to one of the ion-precursor solutions, *i.e.*, previous to particle nucleation. In this case, functionalization occurs concomitantly to particle growth (*ab initio* functionalization) and characteristics such as morphology, size and surface area may be substantially modified depending on chemical structure and concentration of the functionalizing agent (Fig. 4).

The first reports on CaP particle functionalization associated with resin-based materials used 3-methacryloxypropyltrimethoxysilane (MPTMS). Silanization of HA particles (60 wt% or 67 wt%, 7 µm) significantly increased the flexural and diametral tensile strength of experimental self-cured composites [40]. However, silane hydrophobic character may hinder the access of water to the particle and reduce ion release. Still, a better particle dispersion in the matrix as a result of silanization was used to justify the higher ion release presented by a material with silanized ACP (40 wt%, particle size not specified) [18]. Conversely, calcium release by an experimental resin material containing 65 wt% of silanized DCPA nanoparticles (agglomerate size not specified) was half of that released by the material with non-silanized DCPA, while strength doubled [54]. The use of a more hydrophilic and less reticulated resin network seems to compensate for the silane hydrophobicity [77].

Experimental resin materials containing HA particles functionalized with carboxylic acids were tested in a series

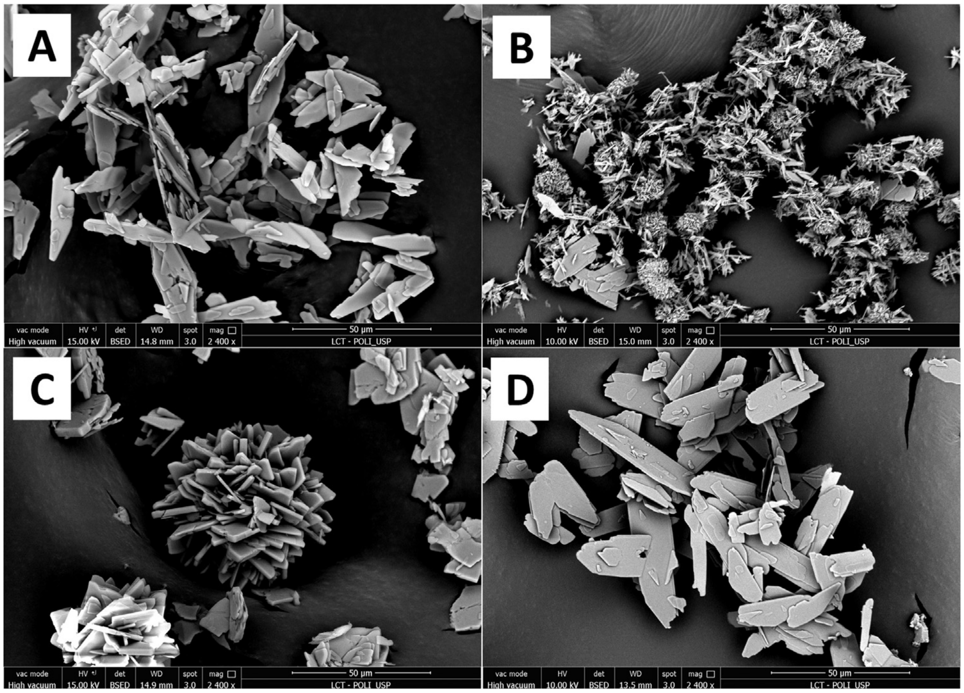

Fig. 4 – Scanning electron microscopy images of DCPD particles functionalized *ab initio* with different organic compounds. A: acrylic acid, B: citric acid, C: methacryloyloxyethyl phosphate (MOEP), D: non-functionalized. All images are in the same magnification.

of studies [41,79–81]. When added to BisGMA/TEGDMA or BisGMA/HEMA resins, HA particles (1–5 µm, 60 wt%) functionalized with acrylic, methacrylic or citric acids resulted in materials with higher flexural strength and modulus in comparison to those with non-functionalized HA [80]. As mentioned above, besides binding to the HA surface *via* carboxylic groups, particles functionalized with acrylic and methacrylic acid also can co-polymerize with monomers from the matrix. Though not discussed by the authors, the positive result achieved by the material with particles treated with citric acid may be explained by their reduced agglomeration. In subsequent studies, it was shown that the material containing HA particles functionalized with acrylic acid reached similar flexural strength to the BisGMA/TEGDMA unfilled resin [41] and similar flexural properties to a material with silanized HA particles [81]. Also, water sorption and solubility were similar to that of a commercial microhybrid composite [81].

DCPD particles functionalized *ab initio* with TEGDMA were developed as ion-releasing fillers for resin-based materials [82]. Besides being relatively soluble, DCPD particles do not interfere with the material's optical properties, due to the similar refractive index to barium and strontium glass (1.51–1.53) and resin matrices (1.50–1.56) [75]. TEGDMA can bind to calcium ions via ion-dipole interactions with oxygen atoms from ether groups and also co-polymerizes with dimethacrylates in the resin phase. Since TEGDMA is non-polar, functionalization does not reduce nanoparticle agglomeration and particle medians were 17–20 µm. TEGDMA content on the particle can be modulated by varying the amount used in the synthesis, ranging from 4% to 30% (by weight). Usually, particles with higher TEGDMA contents result in composites with higher mechanical strength. Also, because TEGDMA is relatively hydrophilic and functionalization is not uniform across the entire particle surface, ion release is not hindered [38]. Recently, other dimethacrylates have also been tested in *ab initio* DCPD functionalization. Diethylene glycol dimethacrylate (DEGDMA) has shown higher retention to DCPD than TEGDMA, lower particle size and higher surface area in comparison to both non-functionalized and TEGDMA-functionalized DCPD particles. These group of characteristics are likely to explain the higher flexural strength displayed by composites containing 15 vol% of DEGDMA-functionalized DCPD (12 µm) and 45 vol% of barium glass (2 µm) in comparison to those containing TEGDMA-functionalized (25 µm) and non-functionalized particles (19 µm) [65].

7. Future perspectives and final remarks

Calcium orthophosphates represent an interesting alternative for the development of remineralizing resin-based materials. Because ion release decreases over time and seems to cease after 2–3 months, these materials are particularly suitable for applications where a short-term action is needed, for example, in the remineralization of caries affected dentin and dentin-resin interdifusion zones. Notwithstanding, it would be interesting to investigate the possibility of extending their bioactivity by combining CaP phases with different solubilities and/or developing controlled release approaches to expand their use in caries prevention. Functionalization with organic molecules is an important strategy to improve particle compatibility with the resin phase and, consequently, enhance the mechanical properties of the final material. Though there is still a lot of work to be done in the basic aspects of particle synthesis and materials development, more information must be gathered from *in situ* and clinical studies. In doing so, it will be possible to identify aspects that need to be addressed to improve upon current experimental and commercially available remineralizing resin-based materials.

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