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Title: Zn-doping of silicate and hydroxyapatite-based cements: dentin mechanobiology and bioactivity.

Authors: Manuel Toledano[1*], Raquel Osorio[1], Marta Vallecillo-Rivas[1], Estrella Osorio[1], Christopher D. Lynch[2], Fátima S. Aguilera[1], Raquel Toledano[1] and Salvatore Sauro[3].

Institution: [1] University of Granada, Faculty of Dentistry, Dental Materials Section. Colegio Máximo de Cartuja s/n, Granada 18071, Spain.

[2] University Dental School & Hospital/University College Cork, Wilton, Cork, Ireland.

[3] University of CEU-Cardenal Herrera, Dental Biomaterials and Minimally Invasive Dentistry, Department of Dentistry, Faculty of Sciences and Health, Valencia, Spain.

*Corresponding author: Manuel Toledano
University of Granada, Faculty of Dentistry
Dental Materials Section
Colegio Máximo de Cartuja s/n
18071 – Granada - Spain.
Tel.: +34-958243789
Fax: +34-958240809
Email: toledano@ugr.es

Abbreviations

AFM	Atomic force microscopy
ALP	Alkaline phosphatase
BAG	Bioactive glass
BF	Bioactive microfiller
BG	Bioglass
CS	Calcium silicate
DMA	Dynamic mechanical analysis
DT	Dentinal tubules
FESEM	Field emission scanning electron microscopy
FS	Flexural strength
FT	Fracture toughness
FWHM	Full width at half maximum
GIC	Glass ionomer cement
HAp	Hydroxyapatite
HCA	Hierarchical cluster analysis
HCPMM	Aluminum-magnesium-carbonate hydroxide hydrates Portland cement
Hi	Nano-hardness
HOPC	Sodium-calcium-aluminum-magnesium silicate hydroxide Portland cement
HPCTO	Titanium oxide Portland cement
ID	Intertubular dentin
MMP	Matrix metalloproteinases
MSC	Mesenchymal stem cells
MTA	Mineral trioxide aggregate
OPC	Ordinary Portland cement
PAA	Polyacrylic acid
PD	Peritubular dentin
Res	Resin
SAED	Selected area electron diffractions
SBF	Simulated body fluid
SRa	Nano-roughness
TCP	Tricalcium phosphate
TCS	Tricalcium silicate
TD	Intratubular dentin
ZCs	Zinc polycarboxylate cements
ZPCs	Zinc phosphate cements

ABSTRACT

The objective was to state zinc contribution in the effectiveness of novel zinc-doped dentin cements to achieve dentin remineralization, throughout a literature or narrative exploratory review. Literature search was conducted using electronic databases, such as PubMed, MEDLINE, DIMDI, Embase, Scopus and Web of Science. Both zinc-doping silicate and hydroxyapatite-based cements provoked an increase of both bioactivity and intrafibrillar mineralization of dentin. Zinc-doped hydroxyapatite-based cements (oxipatite) also induced an increase in values of dentin nano-hardness, Young's modulus and dentin resistance to deformation. From Raman analyses, it was stated higher intensity of phosphate peaks and crystallinity as markers of dentin calcification, in the presence of zinc. Zinc-based salt formations produced low microleakage and permeability values with hermetically sealed tubules at radicular dentin. Dentin treated with oxipatite attained preferred crystal grain orientation with polycrystalline lattices. Thereby, oxipatite mechanically reinforced dentin structure, by remineralization. Dentin treated with oxipatite produced immature crystallites formations, accounting for high hydroxyapatite solubility, instability and enhanced remineralizing activity.

Key words: Zinc, cement, dentin, remineralization, bioactivity.

1. Introduction. Concept of cement.

Cement is a material that origins an adhesive or mechanical interlocking effect between surfaces upon hardening (Zwemer, 1993). Cements are employed primarily for cavity lining, luting applications or are specifically formulated as filling or restorative materials (O'Brien, 1997). Other, more specialist products, are used for sealing root canals as part of a course of endodontic treatment (McCabe and Walls, 2006). One of the principal goals in radicular canal therapy is to reinforce the treated dentin, to strengthen the dentin substrate, and to restore both chemical and mechanical characteristics (Toledano et al., 2017b, 2018) with bioactive materials (Watson et al., 2014).

2. Structure of the dentin and its peculiarities

Dentin is the main constituent of the tooth. It is formed by type I collagen (18%), an inorganic reinforcing phase of nanocrystalline carbonated apatite (70%), and water (12%). Tubules, radiating from the canal to the cementum, support the hierarchical microstructure of dentin (Kinney et al., 2005). This structure of dentin is understood as a cylindrical fiber reinforced compound, with the matrix as intertubular dentin plus the supports, as the tubule lumens and the associated peritubular dentin cuff (Nalla et al., 2003) (Figure S1). The composition of dentin is not the same across its anatomy, but it changes from the cervical down to the apical zones in radicular dentin; it also changes from the outer to the inner dentin (Verdelis et al., 1999). Therefore, root dentin can performs distinctly depending on the kind of the sealing cement and the particular study region. So, differences among dentin stages should be evaluated (Toledano-Osorio et al., 2020a) (Figure S2).

Hence, the mechanical characteristics of radicular dentin were supposed to change with the site of analysis (Xu et al., 2014), and this assertion has been confirmed later (Toledano et al., 2019, 2020a).

3. Concept of bioactivity throughout the potential for dentin remineralization

The terms "biomineralizing" or "bioactive" have been overemployed in marketable publicizing purposes (Vallittu et al., 2018). Calcium silicates (so-called mineral trioxide aggregate, MTA) and glass polyalkenoate cements as those employed in endodontics (Chen et al., 2013; Vallittu et al., 2018) represent restorative dental materials that can release ions into the surrounding environment with a possible function in biomineralization.

In order to biomimetically reinforce intact dentin, researchers have been trying to biomineralize dentin beneath restorations (Sinclair-Hall, 1964; Vallittu et al., 2018). Material's bioactivity is defined as *"the ability of a material to induce apatite formation on its surface after immersion in a simulated body fluid (SBF) solution"* (Kokubo and Takadama, 2006). But the term bioactivity has several meanings depending on context. In the widest meaning, *"materials that are able to have a biological effect or be biologically active, and form a bond between the tissues and the material, are called bioactive materials"* (Vallittu et al., 2018). In biomaterial science, with bioceramics and bioactive glasses, *"bioactivity of a material usually denotes that the material is capable of forming hydroxyl apatite mineral on its surface: in vitro and in vivo"* (Hench, 1998; Vallittu et al., 2018). Two classes of different cement materials are going to be analyzed in this manuscript. Their interactions with the peripulpal dentin, in particular assessing their potential for biomineralization will be examined.

The use of bioactive materials with the capability to leach specific ions at the bonding interface determines the concept of therapeutic restoration.

4. Silicate and hydroxyapatite-based cements for dentin remineralization.

Dentin protection and remineralization of endodontically treated teeth with a considerable degree of coronal destruction and radicular affectation should be attained by the new biomaterials (Gandolfi et al., 2010; Osorio et al., 2016). Glass-ionomer cements, that are vulnerable to water sorption and leaching, are controversial to seal canal root (Monticelli et al., 2007). The use of AH-Plus, acknowledged as "gold standard" epoxy resins among endodontic materials, aimed to provide adhesion to the radicular dentin surface is questioned because some plausible toxicity (Hakki et al., 2013).

Silicate cement was originally used as restorative material in anterior teeth. Due to their bioactivity and their relatively long working and setting times, calcium silicate cements have been essentially applied for endodontic uses such as apexification, pulp capping or perforated roots (Torabinejad and Chivian, 1999; Dammaschke et al., 2005; Camilleri and Pitt Ford, 2006). This was supported on the creation of "the mineral infiltration zone", (Watson et al., 2014).

Hydroxyapatite, the most reported calcium phosphate cement available nowadays, may be employed, in bulk modality, as a cement and/or as coating (Moursi et al., 2002). It shows remarkable osteoconductive and bioactive properties. Therefore, it has been extensively proposed as the biomaterial of election in some dentistry fields (Sakkers et al., 1997; Mendelson et al., 2010). They include some endodontic treatments, such as repair of mechanical bifurcation perforation, pulp capping, restoration of periapical defects and apical barrier formation (Liu et al., 1997).

The calcium silicate cement (MTA) (Torabinejad et al., 1993) has been employed in endodontics. However, such Portland-based materials have shown high solubility (Fridland and Rosado, 2005), long setting time (Gandolfi et al., 2007), potential staining of teeth (Parirokh and Torabinejad, 2010), reduced handling and a high cost (Islam et al., 2006). Moreover, microleakage over time has been recently reported (De Bruyne et al., 2005). On the other hand, calcium hydroxide [$Ca\ (OH)_2$] used as intracanal medicament in regenerative endodontics, provokes root fracture (Kitikuson and Srisuwan, 2016). To repair and promote regeneration of hard tissues, calcium hydroxide powder in combination with HAp has been postulated (Al-Sanabani et al., 2013). Nonetheless, HAp has scarce mechanical strength and fracture toughness. This poses a shortcoming for its use in high-load areas (Al-Sanabani et al., 2013).

5. Advantages of Zn-doping the traditional canal sealers based on silicate and hydroxyapatite cements.

Zinc is an essential trace element, ubiquitous in the body. It is present in saliva and the teeth. Relatively large amounts of zinc are incorporated into teeth prior to eruption, and zinc concentration increases after eruption (Lynch, 2011). Zinc has been widely used in Dentistry for more than two hundred years with the only evidence of high clinical success of zinc containing/releasing materials *i.e*, silver amalgam, zinc oxide and zinc phosphate cements or setting calcium hydroxide. Currently, research about zinc in Dentistry has been widely developed, from 2010 to 2020; about 200 manuscripts per year have been published and all of them demonstrated zinc benefits. Between others, zinc facilitates: 1) antibacterial and antibiofilm formation properties against cariogenic and periodontal pathogens (Gu et al., 2012; Sánchez et al., 2019; Toledano-Osorio et al., 2020b), 2) dentin demineralized collagen protection from MMPs through binding at the collagen sensitive cleavage sites of MMPs (Osorio et al., 2011; Toledano et al., 2012), 3) demineralized collagen stabilization through crosslinking formation (Osorio et al., 2014), 4) dentin protein phosphorylation and calcium deposition (Osorio et al., 2014), 5) dentin and enamel remineralization (Toledano et al., 2012, 2013; Mohammed et al., 2015), 6) zinc-substituted apatite (scholzite) formation on dentin (Osorio et al., 2014), 7) acid resistant dentinal tubule occlusion by minerals precipitation (Gu et al., 2012; Osorio et al., 2018), 8) osteoinductive and osteoconductive properties (Toledano et al., 2020b). Therefore, zinc addition to dental cements is encouraged (Li et al., 2009; O'Connor et al., 2020; Toledano et al., 2020a).

Zinc-doping of endodontic sealers may assure for the formation of new crystalline HAp and non-crystalline amorphous-like apatite species (Toledano-Osorio et al., 2020a). Zinc performs as a therapeutic agent for hard tissue regeneration. Zn-doped silicate and Zn-substituted HAp cements have demonstrated enhanced bioactivity (Sauro et al., 2013; Toledano-Osorio et al., 2020a).

MMPs are a family of zinc-dependent endopeptidases (Tjäderhane et al., 1998) that fulfill a pivotal role in dentin remineralization (Osorio et al., 2011). Both organization and the ensuing mineralization, *i.e*, modulation of the dentin matrix are determined by MMPs 2, 8, 9 and 20. MMPs also play a role in collagen degradation at the dentin interfaces (Tjäderhane et al., 1998; Boukpessi et al., 2008), comprising the lifespan of dentin bonding (Carrilho et al., 2009; Breschi et al., 2010). MMPs are displayed as inactive pro-enzymes. The catalytic domain is protected by a pro-domain that reacts with the zinc ion in the catalytic pole *via* a cysteine residue. A wide variety of functional groups (*i.e.* hydroxamates or sulphonamides) can interact straightly with the zinc ion, providing appropriate targets for anti-MMP drugs (McCall et al., 2000; Osorio et al., 2011).

5.1 Zn-doped calcium silicate cements for therapeutic restorations.

The improvement of novel bioactive/biomimetic ion-leaching restorative materials poses one of the main objectives in research to increase the quality and the longevity of the restorations (Tay and Pashley, 2008; Ryou et al., 2012; Profeta et al., 2012). Two experimental calcium silicate-based micro-fillers (TCS) modified with β-tricalcium phosphate (TCP) or β-TCP ZnO/polyacrylic acid (PAA) were proposed to promote therapeutic remineralizing effect (Sauro et al., 2013). The remineralization and the improvement of the mechanical performance of mineral-depleted collagen fibers (Marshall et al., 1997; Bertassoni et al., 2009; Sauro et al., 2012), may have been induced by the continuous delivery of phosphates ($PO_4{}^{3}{}_{-}$) and calcium (Ca^{2+}) ions. This was particularly patent at the interface promoted by using the βZn-TCS filler. This compound may have released polycarboxylated complexes ($R–COO^{-}$ species) by the βZn-TCS filler. In presence of remnant calcium alongside the demineralized dentin, remineralization may have been enhanced (Tay and Pashley, 2008). The βZn-TCS filler also has a high ZnO-content (20 wt%); therefore, the contribution to collagen protection and remineralization is evident (Sauro et al., 2013) (Figure 1).

FIGURE 1

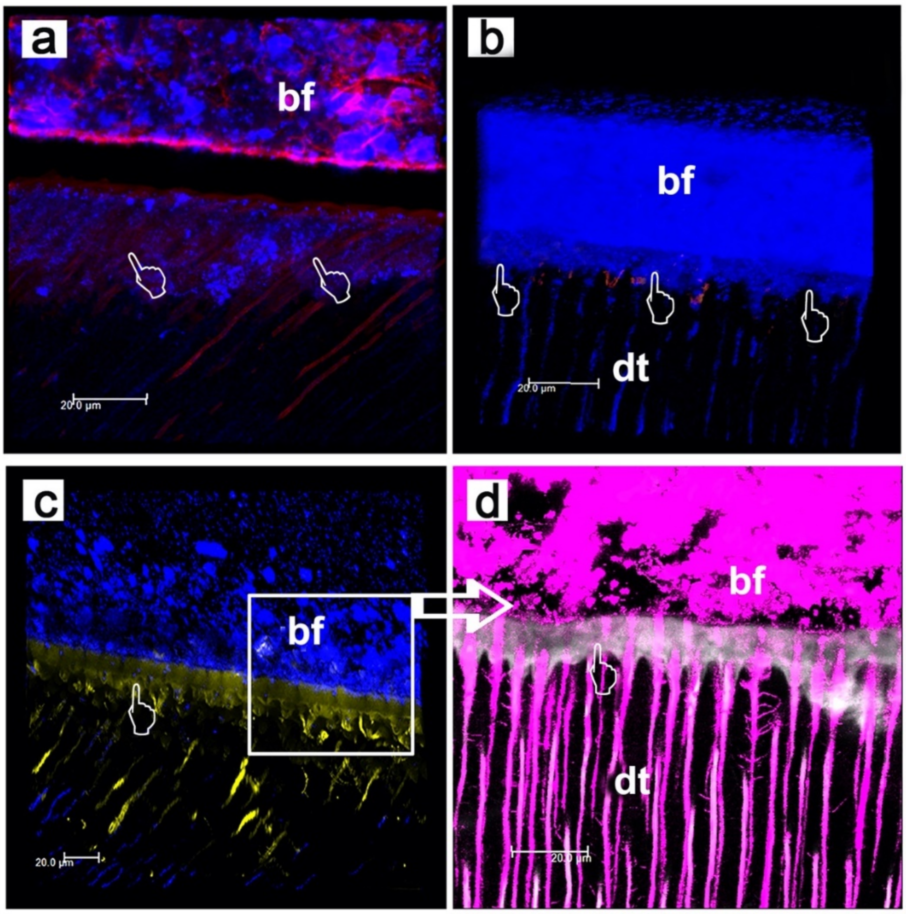

Figure 1. Confocal images of a resin-dentin interface created with an experimental resin adhesive doped with bioactive zinc-doped calcium microfiller (bf). Single projection that permits to see how the polycaboxylate zinc-doped calcium silicate microfiller, after 3 months of storage in saliva, was able to induce a clear deposition of minerals along the hybrid layer (pointers) devoid of any micropermeability (**a**). 3D confocal projection that clearly shows the presence of minerals deposited within the hybrid layer (pointers) and dentinal tubules (dt) by the zinc doped bioglass microfillers (**b**). Confocal images (**c**, **d**) of a resin-dentin interface created with an experimental resin adhesive doped with polycaboxylate zinc-doped calcium silicate microfiller (bf). It is possible to see a hybrid layer and dentinal tubules (dt) still porous and micropermeable to fluorescein (pointer), at 24h of storage in saliva (**c**). At higher magnification, a single projection shows even clearly the presence of dentinal tubules and a hybrid layer (dt)

still porous and micropermeable to fluorescein (pointer), underneath a resin adhesive doped with polycaboxylate zinc-doped calcium silicate microfiller (bf) (**d**).

The bioactivity and remineralization effects of the experimental resins may have been enhanced by the combination of Zn^{2+} ions with the PO_4^{3-} and Ca^{2+} ions released by the βTCP and calcium silicate-based micro-fillers. These materials also exert an antibacterial action within the bonded-dentin interface, concerning an hydroxyl (OH^-) and Zn^{2+} ions release, developing a further protective effect (Sipert et al., 2005; Swetha et al., 2012). The MMP activity (pH dependent) related to the breakdown of dentin collagen may have been interfered through the alkaline conditions caused by the OH^- release (Pashley et al., 2004).

As stated above, an ideal bioactive cement should be not only highly biocompatible, but it needs to be bio-stimulating in order to induce a response for tissue regeneration (Abdullah et al., 2002; Sauro et al., 2015; Innes et al., 2016). Indeed, clinicians nowadays are struggling to get bioactive materials for direct and indirect pulp protection, as well as for specific endodontic treatments (*e.g.* apicectomy/retrograde obturations). These biomaterials could maintain the vitality of pulp tissue, facilitating healing/repair of hard tissues (*e.g.* bone and dentin) (Kokubo and Takadama, 2006; Kim et al., 2010). The most common cements used in clinic for such purposes are calcium hydroxide and calcium silicate cements, as they can promote gain of minerals and dentin bridge formation without pulp inflammation (Camilleri et al., 2005; Camilleri, 2013). However, these latter materials present several shortcomings concerning mechanical performance, setting time and dissolution rate (Bogen and Kuttler, 2009; Porter et al., 2010). For those reasons, several researchers have hypothesized that a generation of innovative resin-modified bioactive cements should be implemented, to represent an optimal strategy for getting the best of mechanical properties, stability and bioactivity. However, conventional resin-modified calcium silicate cements (*e.g.* Theracal, Bisco, USA) are characterised by a reduction in protein expression and cellular metabolism. Moreover, a greater cytotoxicity has been observed in comparison to conventional calcium silicate cements (Hebling et al., 2009).

Some experimental light-curable resin-based cements formulated with conventional (calcium silicate or Bioglass 45S5) and zinc-doped bioactive micro-fillers have also been assessed. Mechanical properties such as fracture toughness (FT) and flexural strength (FS), cell differentiation, biocompatibility and bioactivity were evaluated. In addition, cytotoxicity of the tested materials by using specific assay, cell differentiation and mineralization through gene expression assays (alkaline phosphatase (ALP) and Runx-2) by human mesenchymal stem cells (MSCs) was besides analyzed (Sauro et al., 2018). Four ion-releasing micro-fillers were organized and added into a resin-based light-curing cement: 1) Bioglass 45S5 (BG); 2) zinc-doped bioglass (BG-Zn); 3) β-TCP-modified calcium silicate (βCS); 4) zinc-doped βCS (βCS-Zn). At baseline, the lowest FS and FT were obtained with βCS, while the other cements showed a decrease in FS. Stable FT was obtained by the βCS-Zn cement, over time. The incorporation of the tested bioactive micro-fillers did not influence biocompatibility of the experimental combinations. The expression of the osteogenic genes Runx2 and ALP and the cellular remineralization potential increased when zinc-doped fillers were included (Kim et al., 2010).

The benefit of having zinc-doped micro-fillers in resin-based cements is that zinc ions may trigger a sustained cationic polymerization in the polymer matrix (O’Donnell et al., 2009). Moreover, calcium and zinc ions from the experimental fillers might have reacted with the carboxylate groups of some specific acid such as polyacrylic acid; this would have caused an increase of the mechanical properties of zinc-doped resin-based cements due to a two-step mechanism: *i*) primary hardening *via* zinc-polyacrylate reaction; and *ii*) water sorption, inducing maturation of calcium-polyacrylate as a second step after hardening (Kamitakahara et al., 2001).

5.2 Zinc-modified hydroxyapatite-based cements for endodontic purposes

Probably, the apical third of dentin is one of the regions more widely analyzed of the canal root (Toledano-Osorio et al., 2020a). The strains at the apical third of the root is proximately 3- and 6-fold greater than the strains at middle-root and cervical thirds, respectively (Brosh et al., 2018). In our previous studies, it has been demonstrated that the inner apical dentin attains the highest Young's modulus that is associated to dentin remineralization (Balooch et al., 2008; Bertassoni et al., 2012) (Figure 2) when oxipatite (Zn-doped HAp) is used over time (Toledano et al., 2019), at intratubular, peritubular and intertubular locations (Figure 3). This remineralizing effect (Toledano et al., 2017a) has also been corroborated at the cervical third of the dentin, that showed a general nanohardness increase (Toledano et al., 2020a). It has been, beforehand, assumed that nanohardness improvement at dentin will only be obtained after functional or intrafibrillar remineralization (Balooch et al., 2008; Bertassoni et al., 2012; Toledano et al., 2018) (Figure S3).

FIGURE 2

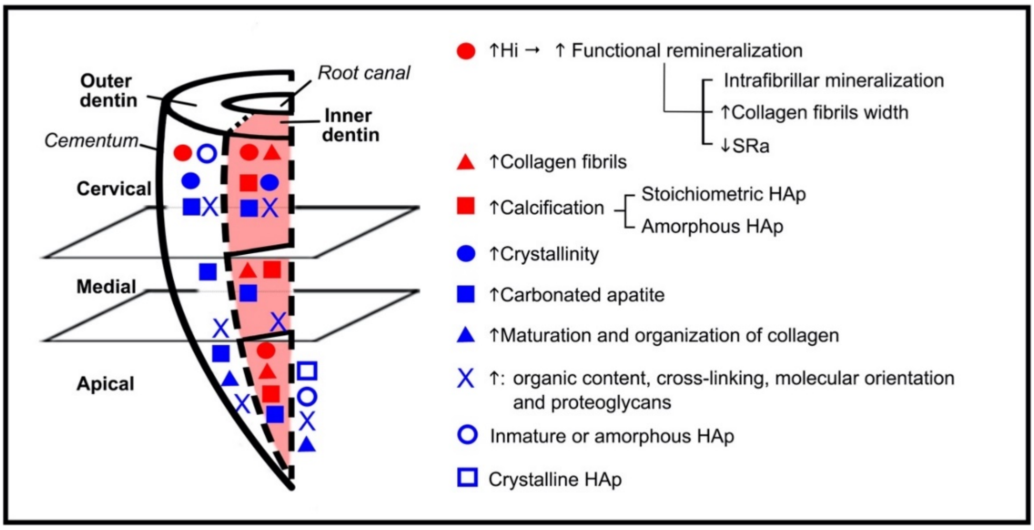

Figure 2. Schematic representation of a vertical section at radicular dentin showing the inner and outer areas of dentin, both limited by the root canal and the cementum. Arrows (↑/↓), Hi, SRa, and HAp, are indicative of increase/decrease, nano-hardness, nano-roughness and hydroxyapatite, respectively. The mild red color at the inner dentin represents a reinforced zone at the expenses of an increase of the Young's modulus, complex modulus and sealing ability, and a decrease of the tan delta (δ), as a result of a raise in functional remineralization. The generalized presence of immature or amorphous HAp at both cervical and apical dentin is related with crystal imperfections, small crystallite size and lattice distortion, low chemical stability, high micro-strain and broadening. The higher presence of crystalline HAp at apical dentin reflects the finding of greater crystalline size. These encodes outcomes proceed from Toledano et al. (Toledano et al., 2019, 2020a) and Toledano-Osorio et al 2020 (Toledano-Osorio et al., 2020a).

FIGURE 3

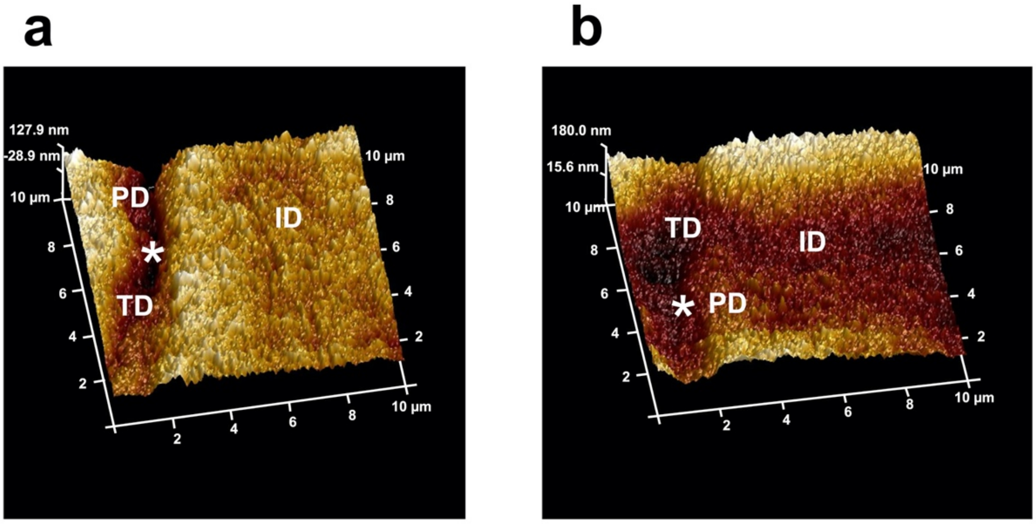

Figure 3. 10 × 10 μm top-view and surface plot images of apical root dentin, inner zones, with partially (**a**) or totally (**b**) remineralized dentinal tubules (asterisks) obtained by atomic force microscopy (AFM) after applying oxipatite, at 12 m of storage time. Peritubular (PD) and intertubular (ID) dentin mineralization is evidenced. Intratubular dentin (TD) appears occluding the dentinal tubules.

At the inner region of root dentin, the highest collagen fibril width coincided with a mineralization increase (Toledano et al., 2020a) (Figure 4). The lowest nanoroughness and the highest intensity of the phosphate peaks (960 cm^{-1}) at Raman analyses, were also determined at the same location, thus confirming significative presence of this mineral (Figures 2, 5). A decrease in roughness is commonly associated with a maturation of mineral, complying with intrafibrillar remineralization (Zurick et al., 2013; Toledano et al., 2017b) (Figure S3). These values have become related to strong diffraction rings after diffraction analysis (Figure 6) and to the highest calcification of the dentin matrix (peak at 954 cm^{-1}) (Kunstar et al., 2012) due to both amorphous-like apatite classes (peak at 956 cm^{-1}) and stoichiometric hydroxyapatite (HAp) (963 cm^{-1})

at Raman analyses (Figure 2). Amorphous phase formation is continuously followed by a phase transition and stabilization of calcium phosphate within the remineralization event. Amorphous Ca/P affords a local ion-rich environment which is advantageous for *in situ* formation of pre-nucleation clusters (Liu et al., 2011b; Toledano et al., 2020a). Equally, oxipatite helped for the highest maturity and organization of collagen, mainly supported on collagen, pyridinium, proteoglycans, amide III and ά-helices, at the apical section of dentin (Toledano et al., 2020a) (Figure 5). The preferred grain orientation from polycrystalline lattices combined with amorphous structures is produced after treating apical dentin with oxipatite (Moshaverinia et al., 2008; Toledano-Osorio et al., 2020a). Apical dentin treated with oxipatite also attains favorable texture. The distribution of crystallographic orientation of a polycrystalline sample (texture) determines changes in microstructure. Texture may, besides, influence crack resistance (Liss et al., 2003), capital condition in endodontic treatment (Toledano et al., 2018).

FIGURE 4

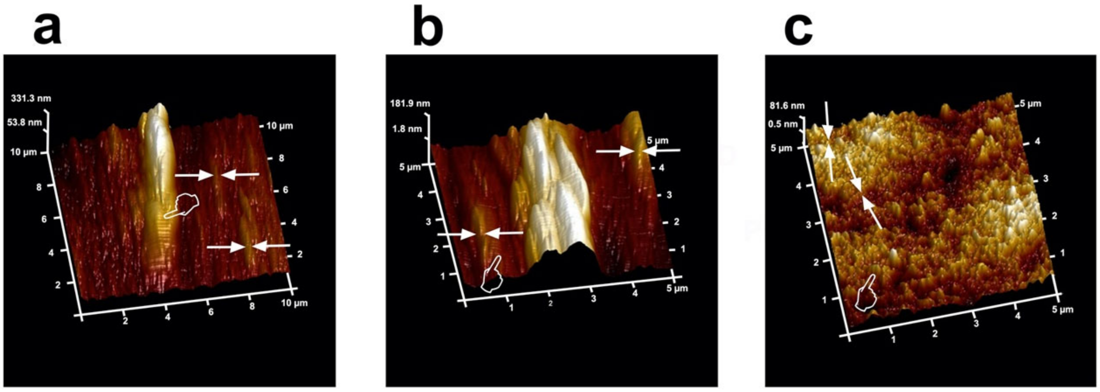

Figure 4. 10 × 10 µm top-view and surface plot images of root dentin, inner zones. Collagen fibrils, the bandwidth of the collagen fibrils, and the wider bandwidth (faced arrows) with the staggered pattern of collagen fibrils are shown (pointers) at the cervical (**a**), medial (**b**) and apical (**c**) thirds of the root treated with oxipatite.

FIGURE 5

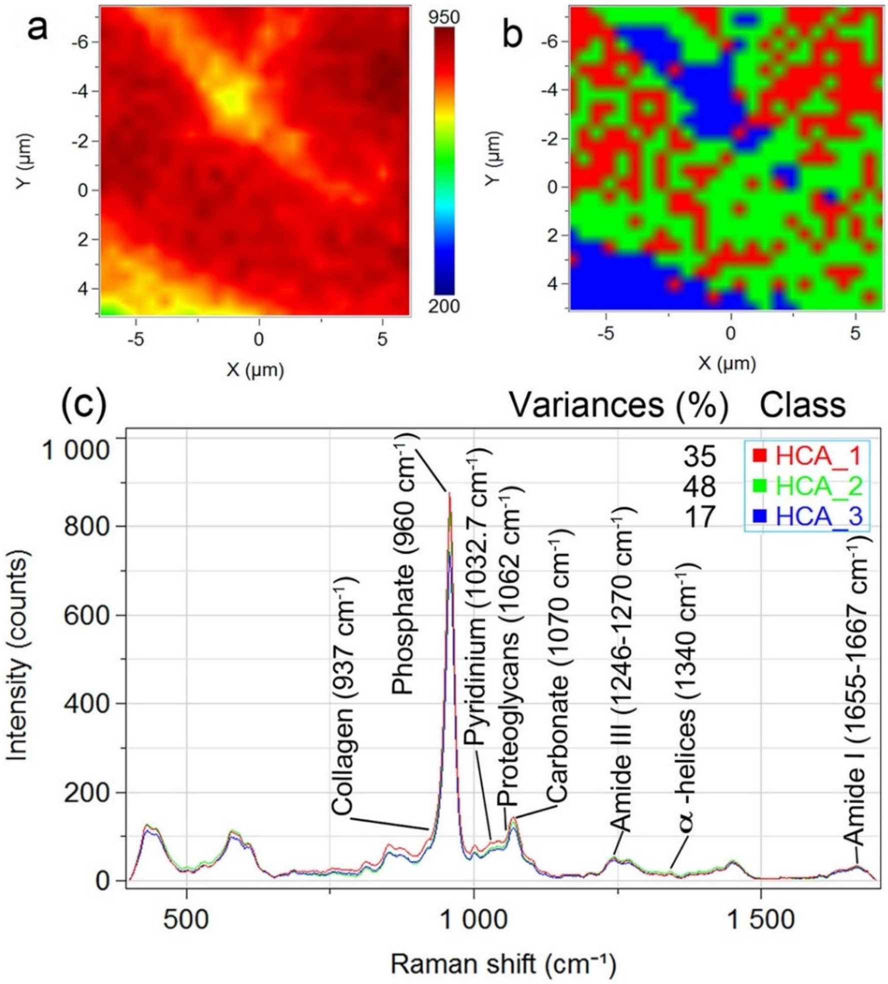

Figure 5. 2D micro-Raman map of the phosphate peak (961 cm^{-1}) intensities (red color) at the group of samples treated with oxipatite (**a**) of the inner zone at the apical third of radicular dentin, after 12 m of storage. Color mapping from hierarchical cluster analysis **(**HCA) images corresponding to dentin surfaces treated with oxipatite (**b**) in conditions similar to those reflected in (a). Three levels of HCA clustering are shown. Areas of distinct colors have differences in Raman spectral distribution and chemical composition. Each cluster, corresponding to a different dentin remineralization stage, is assigned to a different color (red, green, and blue), thus obtaining a false color-image of the substrate on the basis of similar spectral features. Spectra from hierarchical cluster analysis (HCA) results of dentin surfaces treated with oxipatite (**c**) in conditions similar to those reflected in (a).

FIGURE 6

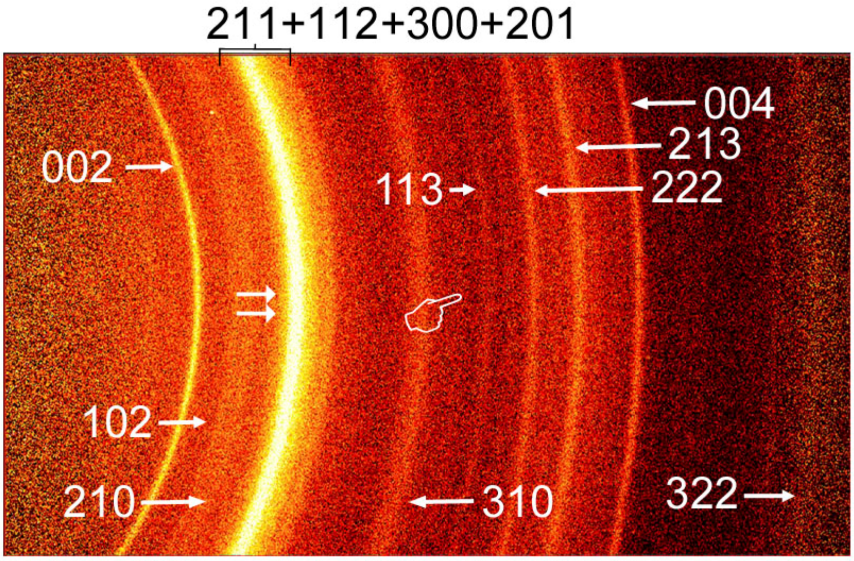

Figure 6. Debye-Scherrer rings of the inner zone at the apical third of radicular dentin treated with oxipatite, after 12 m of simulated body fluid solution storage. Pointer and double arrows mean weak and strong diffraction rings, respectively. The reflections of different peaks are expressed as numbers.

After Raman analysis, the most noticeable carbonate band at 1070 cm^{-1} and carbonate substitution for phosphate was obtained by zinc oxide-based HAp cements (Toledano et al., 2020a) (Figures 5, 2). When carbonated apatite, as precursor of HAp, precipitates in the presence of zinc an interchange between Zn^{2+} and Ca^{2+} happens, by isomorphous substitution, creating a substituted apatite complex (Mayer et al., 1994). Even at a very low concentration, Zn can compete with Ca for binding, as binding constant of Zn is 8.7 and it is 6.8 for Ca. Based on the crystal structure theory, Son et al., 2011 (Son et al., 2011) reported that if the radii of doped ions (Zn: 0.074 nm) are lower than Ca (0.099 nm), in HAp, it is likely to fill them in the free interstitial places of the crystal apatite. The amorphous HAp is linked to crystal imperfections in nano crystalline materials, small round crystallite size and lattice distortion with augmented microstrain. It is believed that zinc may have facilitated amorphous calcium phosphate stabilization, by performing as crystal growth inhibitor (Timlin et al., 2000) and providing both bioactivity and intrafibrillar mineralization (Toledano et al., 2015, 2020a) (Figure S3) at the expenses of polyhedral apatite crystals (Toledano-Osorio et al., 2020a) (Figure 7). Amorphous HAp has the greatest bioactivity, biodegradability and biocompatibility, in comparison with the stoichiometric HAp. At the same time, a reduction of the full width at half maximum (FWHM) of the phosphate peak at 960 cm^{-1}, *i.e.*, higher crystallinity, at cervical dentin was determined and associated to an enhancement of mechanical performance. Crystallinity, wider and shorter crystallites, and maturity of the formed HAp are associated (Toledano-Osorio et al., 2020a).

FIGURE 7

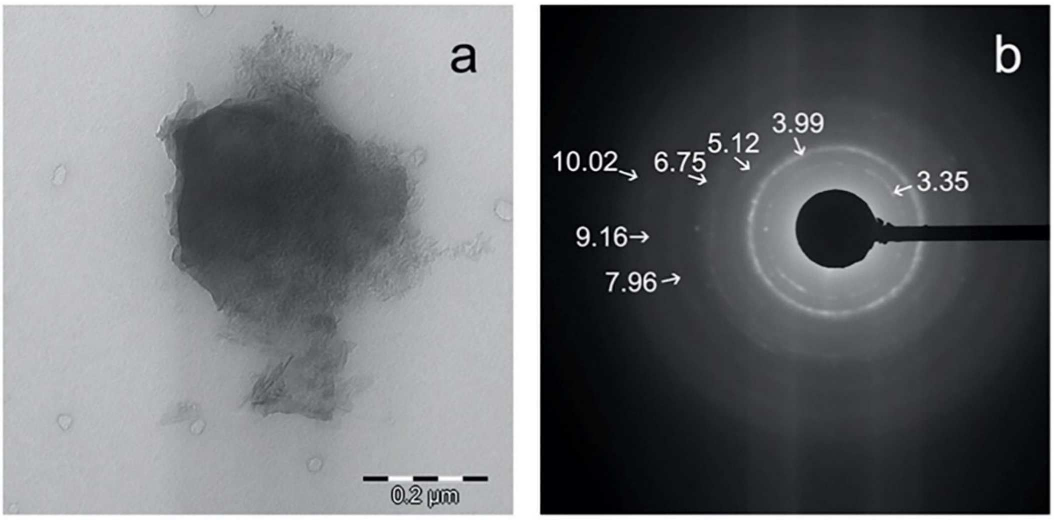

Figure 7. Bright-field of an assembly of polyhedral apatite crystals of apical root dentin, at the inner zone treated with oxipatite, after 12 m storage (scale bar: 0.2 µm) (**a**). The agglomerated crystals of plate-like polygon crystallites characterized these basic phases of mineral formation. Dark-field (**b**) of selected area electron diffractions (SAED) of the crystals shown at microscale. They exhibit clear halo rings and diffraction *d* spacing values of submicron size crystals (nm^{-1}), indicating the presence of crystalline matter, whose pattern show relatively uniform reflections

for all variants. They also confirm the presence of hexagonal apatite and a highly polycrystalline structure.

In order to avoid crack generation and transmission through the dentin substrate, an effective remineralizing approach would also involve the ability to absorb mechanical shock waves. Cracks influence microleakage facilitating the penetration of acids and fluids. Then, HAp becomes demineralized, generating fracture and recurrent caries (Leal et al., 2017; Toledano et al., 2019). The lowest microleakage among groups has been obtained by radicular dentin treated with oxipatite. This better sealing ability is linked to new deposits of HAp that would obliterate pores, voids, and capillary channels (Gandolfi et al., 2007; Toledano et al., 2019) (Figure S2). Minerals precipitation inside tubules acts as a barrier that hinders bacterial penetration preventing pulp inflammation. Dentin remineralization, in these circumstances, is complex because dentin possesses a heterogenous structure (Mocquot et al., 2020a). Both the complex modulus and tan δ have been assessed in radicular dentin treated with oxipatite (Figures 2, 8). The resistance of a material to dynamic deformation is measured by the complex modulus (Ryou et al., 2013). Tan δ reveals how well a material can get rid of the energy. The lower tan δ, the greater the quantity of energy presented in the structure for failure and/or recoil (Gopalakrishnan and Zukoski, 2007). Oxipatite produced homogeneous viscoelastic performance that became linked to any sign of energy concentration (Agrawal et al., 2013). Both peritubular and intertubular dentin showed practically similar tan δ values, without signs and zones of energy concentration revealing tightly bonded dentin interfaces and structures. It is speculated that a rise in mineralization may have increased dentin cohesion, decreasing interfacial sliding and crack growing (Bajaj et al., 2006) (Figure 3). Clinically, this mineralization was *in vitro* expressed through low microleakage and permeability with high sealing ability after treating dentin with oxipatite (Toledano et al., 2019) (Figure S2). A representative diagram as a summary can reflect the benefits and clinical advantages of both zinc-doped silicate and HAp-based cements (Figure 9).

FIGURE 8

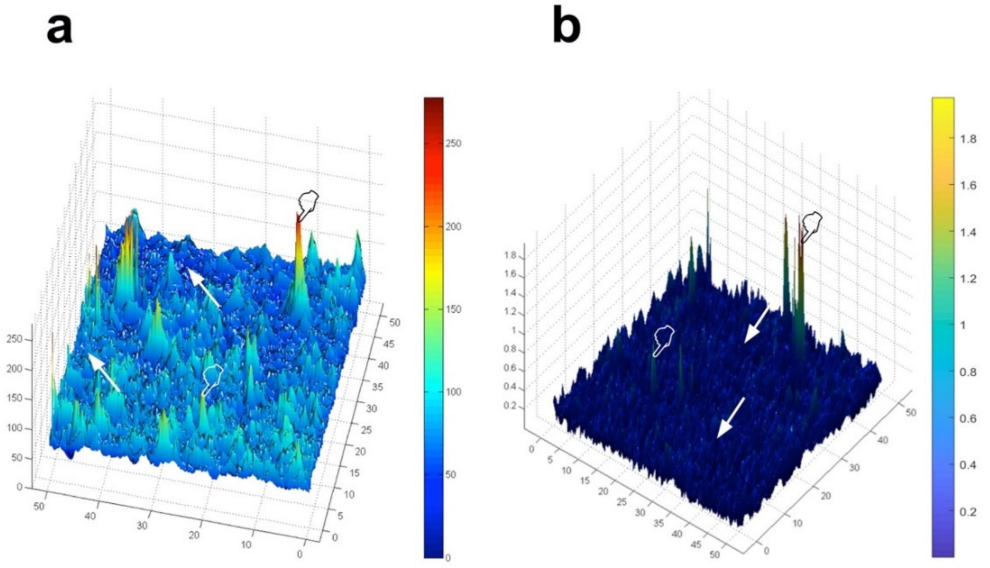

Figure 8. 3-D contour map mode nano-DMA analysis of the complex modulus (**a**) and tan *δ* (**b**) at the inner zone of the apical dentin treated with phosphoric acid plus oxipatite, obtained at 6 months storing in simulated body fluid solution. In the color scheme shown, the redder color corresponds to higher values of the locally complex modulus and tan *δ* value moduli, potentially associated to the presence of mineral precipitates. In a, the intertubular dentin was represented by the blue color plateaus (arrows). Peritubular dentin was associated with the blue-green to yellow color elevations (pointers). In b, the highest tan *δ* is potentially associated *to* intratubular mineral precipitation (pointers). The capacity for getting rid of the energy at peritubular dentin is represented by the clear blue ring marks (arrows) at the mapping.

FIGURE 9

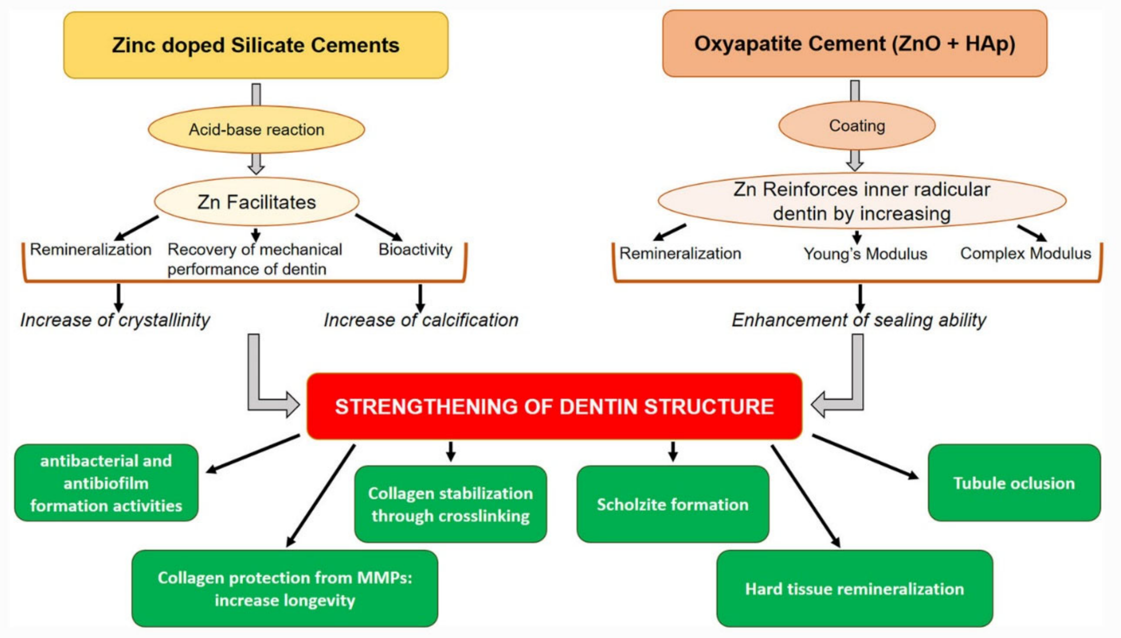

Figure 9. Infographic scheme representing both setting and advantages of using the proposed Zn-doped cements.

6. Conclusions.

The continuous delivery of phosphates (PO_4^{3-}) and calcium (Ca^{2+}) ions facilitates the remineralization and the recovery of the mechanical performance of mineral-depleted collagen fibers when using zinc-doped silicate cements. The polycarboxylated complexes (R–COO– species) possibly released by the β-Zn-microfillers interact with the residual calcium existent along the demineralized dentin. This potentiates the bioactivity, enhancing the remineralization process perhaps due to the high ZnO-content.

The inner root zone of radicular dentin became reinforced after sealing radicular canals with oxipatite, a mixture of zinc oxide and HAp particles. This is attained by increasing remineralization, Young's modulus and resistance to dynamic deformation. As a result, sealing ability of these zinc-doped cements is enhanced. Such a specific concert is associated to precipitation of Ca, P and Zn (both amorphous lattices and polycrystalline minerals) within the demineralized organic matrix.

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