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Title: Polymeric zinc-doped nanoparticles for high performance in restorative dentistry.

Short title: Zn-doped NPs in dentistry

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Key words: zinc, polymeric nanoparticles, remineralization, bioactivity, adhesion.

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ABSTRACT

Objectives: The aim was to state the different applications and the effectiveness of polymeric zinc-doped nanoparticles to achieve dentin remineralization.

Data, Sources and Study selection: Literature search was conducted using electronic databases, such as PubMed, MEDLINE, DIMDI, Embase, Scopus and Web of Science. A narrative exploratory review was undertaken.

Conclusions: Polymeric nanospheres (NPs) were efficiently loaded with zinc. NPs sequestered calcium and phosphate in the presence of silicon, and remained effectively embedded at the hybrid layer. NPs incorporation did not alter bond strength and inhibited MMP-mediated dentin collagen degradation. Zn-loaded NPs remineralized the hybrid layer inducing a generalized low-carbonate substitute apatite precipitation, chemically crystalline with some amorphous components, and an increase in mechanical properties was also promoted. Viscoelastic analysis determined that dentin infiltrated with Zn-NPs released the stress by breaking the resin-dentin interface and creating specific mineral formations in response to the energy dissipation. Bacteria were scarcely encountered at the resin-dentin interface. The combined antibacterial and remineralizing effects, when Zn-NPs were applied, reduced biofilm formation. Zn-NPs application at both cervical and radicular dentin attained the lowest microleakage and also promoted durable sealing ability. The new zinc-based salt minerals generated covered the dentin surface totally occluding cracks, porosities and dentinal tubules.

Clinical significance: Zinc-doped NPs are proposed for effective dentin remineralization and tubular occlusion. This offers new strategies for regeneration of eroded cervical dentin, effective treatment of dentin hypersensitivity and in endodontically treated teeth previous to the canal filling. Zn-NPs also do reduce biofilm formation due to antibacterial properties.

Key words: zinc, polymeric nanoparticles, remineralization, bioactivity, adhesion.

1. Introduction

Resin-based composite restorations represent the cutting edge in current dentistry due to their ability to bond to the structure of enamel and dentin structure, mechanical properties and aesthetic appearance [1]. The most significant factor that defines the longstanding success of these restoratives is their adhesive bond layer integrity [2–5]. Resin–dentin bonding, clinically, is promoted after dentin demineralization from acid etching and additional infiltration of resin. The collagen matrix is exposed after mineral ions removal during dentin demineralization. When the resin adhesive is infiltrated and polymerized into the demineralized collagen, the hybrid layer (HL) is created. At the bottom of the hybrid layer (BHL), a volume of demineralized and exposed collagen prone to degradation endorsed to the action of host-derived matrix metalloproteinases (MMPs) [6–8], remains. This conducts to a compromise of the bonding efficacy of restorations over time [9,10]. Therefore, the BHL represents a microgap and the weakest bond at the composite restorations [3]. The BHL, thereby, is also prone to degradation by matrix or bacterial collagenases [7].

Durable adhesion to dentin and protection of the collagen fibers, at the resin-dentin interface, from degradation through remineralization are perused when designing dental adhesives [11]. Bioactive materials, such as carboxylic acid-containing polyelectrolytes, phosphoproteins, bioactive glass particles, casein phosphopeptide-amorphous calcium phosphate, colloidal nanobeta-tricalcium phosphate, have been used for functionalization of adhesives, and to provide for mineral precipitation within the HL and subsequent remineralization of the underlying dentin. All of them have participated in the regrowth of minerals at the demineralized dentin. Several fillers, as hydroxyapatite (HAp) and some nanoparticles (NPs), performing as ceramic bioactive nanospheres [12], have also been proposed, but they do not have a well-regulated release nor ideal degradation kinetics [13]. Incorporation of zinc into polymeric NPs represents a breakthrough and a boost to innovation in dental regenerative biomaterials. For planning the research, a historical timescale, at international range, has been followed. The aim of the present study was to state the effectiveness of polymeric zinc-doped nanoparticles to achieve dentin remineralization and to propose the different applications in restorative dentistry.

2. Methods

2.1 Question addressed by this review

What different applications and further benefits can be attributed to Zn-doped NPs in the restorative dentistry field?

2.2 Literature search

A narrative exploratory review was undertaken. Literature search was conducted using electronic databases, such as PubMed, MEDLINE, DIMDI, Embase, Scopus and Web of Science. Hand-searching of the literature was also conducted, including the references lists of related and similar studies. The main search terms were “zinc”, “dentin”, “NPs”, “remineralization” and “adhesion”. Only English-written articles were selected and no time limit was established. Articles selected and included in the present narrative review gathered those in which Zn-doped NPs were studied in order to establish a possible application in the restorative dentistry field.

Correct generation of the randomization sequence and allocation concealment of samples was evaluated in all the selected articles in order to eliminate those with a high risk of bias. After deep reading of the selected manuscripts, performance of Zn-doped

NPs at the resin-dentin interface was evaluated by means of bioactivity and remineralization, ICTP determination, hybrid layer morphology, permeability, antibacterial properties and bonding efficacy. Zn-doped NPs used as antibacterial agent on dentin surface was also encountered as well as remineralization at cervical and radicular dentin.

3. Results

3.1 Novel non-resorbable polymeric nanoparticles for the dentin interface

Innovative polymeric NPs (100-nm in diameter) with anionic carboxylate (*i.e.*, COO^-) groups located along the polymer have been synthesized to enable dentin remineralization. The proper definition is supported on the fact that these NPs have polymeric nature and are surrounded by carboxylate radicals (Figure S1). The proposed NPs have been synthesized by precipitation with polymerization in a non-solvent medium, and further characterized [14]. The zeta potential and the hydrodynamic radius of NPs were -41 ± 5 mV, and 120 ± 15 nm, respectively. The polydispersity index (PDI) was of 0.02 ± 0.0015 assessed in water at pH = 7 by dynamic light scattering [15]. For the synthesis of NPs, methacrylic acid as a functional monomer, 2-hydroxyethyl methacrylate as a backbone monomer, and ethylene glycol dimethacrylate as a cross-linker were used [16].

3.2. Relationship between collagen fibers and polymeric NPs

substrate was obtained [16,17]. Normally, different kind of NPs may be seen without agglomerate (because of their negative zeta potential) on the hybrid layer and inside the dentinal tubules [11,16].

3.3. Zinc-modified nanopolymers improve the integrity of resin-dentin bonded interfaces by preserving collagen and inhibiting cariogenic biofilm formation

Polymeric NPs infiltrated in demineralized dentin drastically reduced MMPs activity on dentin collagen. MMPs activity was even lower if demineralized dentin was infiltrated with Zn-doped polymeric NPs. [16]. The characteristic 67-nm periodicity banding and fibers, on the other hand, are not denatured or fractured when NPs are applied.

In planktonic cultures treated with Zn-doped NPs, Zn release from NPs clearly performs as an antimicrobial agent over several cariogenic bacteria [18,19]. In case of Zn-doped NPs, the release of zinc is 0.02 $\mu g\ ml^{-1}$ (per mg of NPs), and the cumulative liberation for zinc is 0.3% at 48 h; increasing release of 0.8% for zinc after 28 d has been stated [18]. Zn-NPs relate easily with the cell membranes of bacteria developing anionic charges (at pH = 7, zeta potential roughly −20 mV) [20,21]. Both antibacterial properties and remineralization diminish viability and biofilm formation at the interface after applying Zn-NPs on acid conditioned dentin. Bacteria have been difficultly shown at any of the bonded interfaces when Zn-NPs were applied [22].

3.4. Therapeutic restorations throughout Zn-doped nanoparticles application

NPs application in dentin prior to resin infiltration increased the bonding efficacy over time if compared to conventional resin-dentin interfaces [23]. The surface analyses have shown an extended bundle of mineral precipitates at the entrances of some tubules and throughout the thick network of plate-like crystals, in multilayers, on intertubular dentin. An extensive labyrinth of anastomoses, hollows, and cavities observed at nano-metric scale could also be shown (Figure 1). On the other hand, when NPs were not included into the formulation of the adhesive resin, bond strength was

reduced over time and dentin collagen fibrils appeared broken and morphologically affected at the debonded interface (Figure 2). The failure that corresponds with the debonding was reproduced below the hybrid layer, where resin uncovered collagen was present. Some dentin collagen fibrils, at this location, appeared partially demineralized [24], exhibiting the characteristic 67 nm periodicity banding, and showing diameters below 100 nm.

3.5. Sealing ability and mechanical performance of both cervical and radicular dentin treated with NPs

The reduction of dentinal fluid flow has been ~90% at 7 d of storage, after treating cervical dentin with Zn-NPs [74]. This finding concurred with a rise in the percentage of total occluded tubules (100%). Dentinal tubules resulted occluded by mineral tags with, principally, Ca and P [25]. The lowest nanoroughness was achieved by cervical dentin surfaces treated with Zn-NPs [26]. Zn-NPs application inducted an improvement of the sealing ability [27]. Greater fibrils thickness after NP binding to the partially demineralized dentin have been found. Specimens treated with Zn-NPs showed a big area of mineral precipitates through the dense network of crystals, located at all tubules' lumen and at intertubular dentin. This new surface is far from that attained in the absence of NPs (Figures 3a, 3b) [95]. Viscoelastic analysis have shown zones of stress concentration that appeared as minerals attached to the peritubular dentin, when Zn-NPs treated apical root dentin (Figure 4). Stick-slip images at root dentin treated with Zn-NPs were also revealed after topography mapping achieved with AFM. These mineral deposits were displayed as numerous rod-like structures connecting the intratubular minerals to peritubular dentin (Figure 3c).

3.6. Chemical and physical performance of NPs-treated cervical and radicular dentin

A general growth of the phosphate peak (Figure 5) and crosslinking of collagen have been produced in samples treated with Zn-NPs [28–30]. A sharp carbonate band around 1070 cm^{-1} has been unveiled. This matched with the narrowing of the full width half maximum (FWHM) of the phosphate *v1* peak (961 cm^{-1}), when dentin was treated with Zn-NPs. Zn-NPs improved collagen quality, structural differences [31] and better organization [32]. Cervical dentin at 7 d storage has less amorphous HAp [33] than that measured at 24 h of storage. This HAp has also great lattice distortion and crystallite size.

3.7. Dentin hypersensitivity treatment through Zn-NPs application.

Tubules were 100% occluded in cervical dentin treated with zinc-loaded NPs, analyzed at 7 d. Dentin treated with zinc-NPs attained the highest reduction of dentinal fluid flow. When treating dentin with zinc-NPs, complex modulus values attained at intertubular and peritubular dentin were higher than other ion-doped NPs. Dentin treated with Zn-NPs attained the highest nanomechanical properties, mineralization and crystallinity among groups. Nanoroughness was lower in Zn-treated surfaces in comparison to dentin treated with un-doped gels. Crystals at the dentin surface were identified as hydroxyapatite with the highest crystallographic maturity and crystallite size in dentin treated with Zn-NPs based-gel. Polyhedral, plate-like and drop-like shaped apatite crystals constituted the bulk of minerals in dentin treated with Zn-NPs gel, after 7 d [25,28,34].

Zinc-NPs are then supposed to fasten active dentin remodeling, with increased maturity and high mechanical properties.

3.8. Zinc-loaded bioactive and cytocompatible NPs for the treatment of periodontal disease

Zn-loaded NPs have been non-apoptotic and non-cytotoxic in fibroblasts of humans alongside any test, except in the Live/Dead assay, where 100% of Zn-NPs (30 mg/ml) concentrations exerted a faintly greater (10% increase) cytotoxicity in comparison with other groups. But, the viability of cells was permanently beyond 80% [11]. Zn-doped NPs produce a negligible degree of cytotoxicity [11]. Zn-NPs have also generated low reactive oxygen species (ROS) activity and thereby minor stress reaction and oxidative damage [35].

4. Discussion

4.1 Novel non-resorbable polymeric nanoparticles for the dentin interface

The incorporation of nanotechnology into operative dentistry was primarily focused on the reinforcement of the mechanical properties the resin-based composites [36]. The mechanical properties of resin-based composites are related to their clinical application and the longevity of the restoration [37]. Modern research is therefore constantly looking for solutions to improve these properties, with a prospect on the latest developments from various research areas. In line with recent studies, nanotechnology could provide new strategies in operative dentistry [38]. At the resin-dentin interface, nanotechnologies not only have favored our understanding of dental tissues at the nanoscale and enabled the design of materials with ultrafine architecture, but have persued regeneration and remineralization of dental hard tissues. Even more, due to the similarity in composition between dentin and bone, any novel insights in the mineral/protein interactions that govern the structure and functionality of dentin may be applicable in reparation of hard tissues in general. With this target, nanoparticles are playing a pivotal role [39,25]. Engineered NPs have become the key-point of much research in remineralization [40]. The employ of polymeric particles as phosphate and calcium attracting biomaterials (*i.e.*, carboxylate-functionalized polymer particles that fix calcium) were primarily offered to shelter the former crystallite-sparse collagen fibers of the scaffold from degradation. It was achieved by re-taking of mineral into the demineralized collagen matrix, offering durable adhesion to dentin. Restoring dentin with bioactive materials should be facilitated through both intrafibrillar and interfibrillar remineralization [11]. Thereby, the knowledge gap covered by the present review is to state the exact contribution of novel polymeric zinc-doped nanoparticles to dentin remineralization.

Precipitation polymerization is a polymerization process in which a monomer and a initiator are dissolved in a given solvent (in the absence of any stabilizer or additive), and this continuous phase is a non-solvent for the formed polymer chains beyond a critical molecular weight [41,42]. The process of precipitation is controlled by the Hansen's solubility parameters supported on the flurry Hugings model which was inspired in a thermodynamic approach. The basis of this model are the interactions of solvent molecules with growing polymeric chains [14]. The most remarkable properties are the characteristic monodisperse copolymer microspheres that are obtained.

Other capital property and advantage concerns the fact that these particles show a strong chelating effect due to their carboxyl-terminated polymers [16,43]. Thereby, a

mineral precipitation and an ion exchange within the hybrid layer is wanted but only possible at regions in which a partial resin infiltration happened [44]. Hence, the deposited mineral may perform as a place for future mineral precipitation, and the remineralized substrate can be less affected by degradation [45]. A functional remineralization procedure of the conditioned resin-bonded dentin may be only taken into account if a rise of the mechanical properties is obtained at the bonded interfaces [46,47].

The presence of NPs at the dentin bonded hybrid layer warrants the recovery of mechanical properties and functional remineralization at the Achilles heel of the resin dentin interface, *i.e*, the BHL (Figure 6). In order to exert the remineralization effect, NPs collagen binding is necessary (Figure 7). A limited remineralization potential will be obtained because of the incapability of the NPs to adhere to the fibers [12,43]. Binding of the present polymeric NPs to collagen may be explained by (1) the linking of COO^- groups from NPs to NH^+ locus at dentin collagen by a peptide covalent binding or due to (2) the result of the high affinity between negatively charged polymeric NPs (−43.3 mV) and the positively charged demineralized dentin collagen [17] (Figure S2). Collagen fibers are hierarchically structured biological entities that perform as the primary tensile elements in tissues and perform as relevant factor for the preservation of standard tissue strength. These NPs, at the bonded interface, are able to link to the collagen and to favor the deposition of amorphous calcium-phosphate precursors [11,25,16,23]. The polymeric NPs may permit a controlled ion release rate, or even NPs may perform as carriers of other biological factors, helping to promote tissue mineralization [13,32,33].

Zinc has been demonstrated to act not only as inhibitor of matrix metalloproteinase (MMP) reducing MMPs-mediated collagen degradation [48] but also as a stimulator of hard-tissue mineral precipitation [49,50] and dentin demineralization inhibitor [51]. Zinc favors a metabolic effect in hard tissue mineralization, influencing signaling pathways [50,52]. Zinc promotes the setting of a bond between material and tissue, eliciting a specific biological reaction at the interface [53]. Including zinc into the chemical composition of the adhesives, intrafibrillar remineralization at the dentin matrix will be augmented [54]. Employing Zn-doped polymeric NPs instead of Zn-NPs may not only facilitate a controlled release at the collagen site but also reduce zinc toxic effects [18], decrease bacterial biofilm formation and growth [21], and have exerted an antimicrobial role in former *in vitro* analyses [21,19].

Even when zinc is essential for life, it may also cause cell toxicity, trough cell membrane disruption, enzyme inhibition, or by competing mechanisms with Fe, Ca, or Cu [55,56]. Zinc improves height and weight development in humans [57]. Zn-NPs also stimulate both different metabolic effects in innate immune system [58] and diverse organisms [49]. Zn-doped NPs perform as a real drug nanodelivery system. Nanocarriers place the drug at the disease location in a sustained mode and for an extended time (Figure S3). Hence, systemic toxic effects and so rapid clearance can be avoided [59,60]. Achieving reduced toxicity by sustained effect of the drugs is one of the pivotal objectives of drug delivery systems [61]. Toxicity of particles is rightly associated to their sizes. Usually, greater toxicity is developed by smaller size particles [62,63].

Zn-NPs have also been proposed as therapeutic agents in periodontal disease [21,19], as they are antibacterial [21,19] and may avoid collagen degradation by MMPs at the periodontal complex (periodontal ligament, dental cement and bone) [18,21]. Besides, Zn-NPs increase experimental animal growth (worms), thanks to the MMPs

inhibitor characteristic [18], and make decrease the intracellular hydrogen peroxide concentration. Zinc has also been shown to attenuate the oxidative stress [64–67].

4.2. Relationship between collagen fibers and polymeric NPs

Due to the functional diameter of the tested NPs (approximately 350 nm), higher than interfibrillar space breadths, they cannot pass into the resin-dentin interface [17]. Agglomeration of particles and/or particle linking to demineralized collagen is produced when particles lesser than 20 nm are used. Some NPs appear covered by Ca, P and/or silicon. Silicon enables CaP precipitation on polymeric particles [68], playing a pivotal role in the linking of CaP to the collagen web [12]. Silica NPs mediate the formation of precursors of CaP, that act as a nucleating mineral [69], necessary for further mineralization. Demineralized dentin has been, previously, treated with silica and HAp nanoparticles [12] and it has been hypothesized that phosphorus reacts with silica NPs, thus favoring remineralization after mixing with ionic calcium.

One more advantage of these polymeric NPs is that they do not alter hybrid layer formation or adhesive penetration [16]. In addition, NPs do not reabsorb or dissolve, but are capable to form amorphous calcium phosphate agglomerates onto their surface [16], facilitating mineral deposits formation, as stated above. They also will stay adhered to collagen fibers, being assimilated within the remineralized substrate. Even more, the growth of calcium phosphates may be template by carboxylate groups ($COOH^-$). This interface preservation has also been demonstrated after the total absence of any filtration when performing sealing ability tests [11]. Furthermore, Zn-NPs produce progressive scaffolding of collagen and superior potential for further remineralization [11,31].

4.3. Zinc-modified nanopolymers improve the integrity of resin-dentin bonded interfaces by preserving collagen and inhibiting cariogenic biofilm formation

The carboxy terminal telopeptide of type I collagen (ICTP) measurement has been defined as the most reliable procedure for the quantification of the enzymatic activity of MMPs on type I collagen [70–74]. MMPs are inhibited by NPs, because of possible interactions between the acidic functional groups (COO^-) at NPs surfaces and the active-site zinc of MMPs [16]. Zinc may, additionally, exert a MMPs a competitive inhibition [75]. During dentin acid conditioning it is produced a losing of extra and intrafibrillar mineral, which exposures some gap zones in the collagen fibril enabling local telopeptidase action to split C-terminal telopeptide. This leads to a partial loss of collagen periodicity and intermolecular cross-linking. Furthermore, fibrils have shown (100 to 200 nm) if infiltrated by polymeric NPs, which commonly happen when cross-linking is maintained or intrafibrillar mineralization does exist [76].

Bacteria, in the oral environs, organize a micro-ecosystem recognized as a biofilm [3]. More specifically, at the BHL, the bacteria colonize the demineralized dentin collagen [77]. As a consequence, bacteria could infiltrate through the demineralized micropores, cracks, voids, and open dentinal tubules [25] making present the mature biofilm within the interface, and promoting secondary tooth caries formation. The resin-dentin interface is then degraded and the underlying dental tissues get infected [78]. At the border of the composite resin the pathogenic power of the biofilm restoration can be diminished by introducing anti-cariogenic materials. The proposal of non-reabsorbable and biocompatible NPs, exhibiting a gently release of antibacterial agents and high local bioactivity must be pursued in order to control infection [79,80].

Zn-complexed on different biomaterials has also shown anti-biofilm formation and antibacterial effects in multispecies biofilm stocks [21,81–84,22]. On the

consortium, zinc ions markedly enhance the adhesion and growth of serum and salivary proteins on bacteria membranes, inhibiting co-aggregation within the biofilms [19]. On bacteria, Zn-NPs produce rougher cell membranes with distinct morphologies. Zinc also inhibits some proteinase activity and glycolysis in numerous oral bacteria [85]. Even more, zinc can condition acid generation by distinct microbes and, besides, has performed as a plaque-inhibiting complex [21,86]; oxidative damage may also happen [22]. Other metal ions can be displaced by cationic elements (Zn) from the outer membrane of bacteria, conducting to cell death (Figure S4).

4.4. Therapeutic restorations throughout Zn-doped nanoparticles application

Main objectives of the current therapeutic restorations involve the strengthening of the treated dentin, the reestablishment of the dentin chemical and mechanical properties and the recovery of the dentin micro-structure [87,88], as intertubular and peritubular dentin (Figure 3a). This finding, *per se*, is a milestone. This was attained due to an increase of functional dentin remineralization, as the modulus of Young achieved higher values than when NPs were not present [23]. The increase of the mechanical properties of the partially demineralized collagen fibers is linked to mineralization at the resin-dentin interface [89], and more explicitly to mineralization at intrafibrillar level [90,91]. Furthermore, dentin surfaces treated with Zn-NPs have achieved a 96% of mixed failures after microtensile bonding tests, indicating the strong attachment at the resin-dentin interface. When NPs were not present, it did appear collagen degradation by MMPs, which fractures under tension [92]. This fact explains the presence of unattached collagen fibrils found within the broken hybrid layers in the absence of NPs at the interface. These interfaces also have shown permeability, as one more sign of hybrid layer degradation (Figure 8). The altered organic components, and a decrease of crystallinity, the relative presence of minerals, and carbonate substitution are also participating in this progressive state of interface degradation [93].

4.5. Sealing ability and mechanical performance of both cervical and radicular dentin treated with NPs

Both permeability testing and scanning electron microscopy analysis of dentin surfaces pose the two main reported methods in the literature for the *in vitro* assessment of possible dentin desensitizing agents [94]. Zn-doped NPs have been demonstrated to be effective in dentin sealing ability. Calcium-phosphate salts deposits provoked this significant effect on the declining in the flow rate. This greater sealing ability, that is associated to mineral deposition (Ca, P, and Zn) in the demineralized dentin [95] is accompanied by not a single remineralization effect, but with a general improvement of both nano-hardness and nano-modulus of Young [23,28]. It has been previously assumed that only if interfibrillar remineralization is followed, nanomechanical properties' recovery at the dentin substrate may be effective [90,91]. The lowest nanoroughness became associated to mineral maturation and intrafibrillar remineralization,. Thereby, the clinical erosive challenge is hindered by this new mineral coat, which acts as a mechanical barrier [96].

All in all, Zn-NPs based-gels must be understood as a feasible therapy for DH, as after inducing calcium-phosphate precipitation they occluded dentinal tubules. These are their main clinical applications in this field. Aimed to prevent crack propagation across the surface, this remineralized dentin necessitates the capacity to process mechanical shock waves. Microfractures in the cervical area of dentin are a controversial topic. Clinical or experimental evidences of these fractures are weak or even absent, but description of fractures based on finite elements analysis are abundant

[97]. Cracks and fractures would facilitate the penetration of acids, demineralizing the surrounding hydroxyapatite, even producing caries disease [97]. Cervical dentin specimens infiltrated with Zn-NPs have shown, besides, mineral deposits at the limit between peritubular-intertubular dentin. The topography mapping analyzed restates the finding of a neat mineralized stick-slip appearance at this location. This mineral precipitation might be an outcome from the discrepant nanomechanical behavior of close structures [98]. Discrepant values of the tan δ distribution between intertubular and peritubular dentin may justify, at the 3-D contour map, this performance [25].

The enhancement in the sealing ability that has been achieved by NPs in general and by Zn-NPs in particular after treating root dentin is related to hydroxyapatite formation that may close pores, voids and capillary channels [27] by deposits of calcium-phosphate salts (Figure 9). This burst of mineralization also occurred at the intrafibrillar compartment of the collagen fibers, as the Young's modulus significantly improved [23,99,29]. If the energy stored at dentin is great enough the energy excess dissipate through tissue cracking in the area of stress concentration [23]. These little rod-like minerals, the stick-slip images, appeared as bridge-like structures on the dentin surface [98]. This stick-slips formation on dentin depends on the Zn^{++} existence in NP formula, which generates new precipitation of crystals [25]. The amorphization of the mineral component may be another source of the sticks-slips formation as it provides ions for remineralization and reparation of dentin [100].

4.6. Chemical and physical performance of NPs-treated cervical and radicular dentin

The interest for strengthening the radicular dentin, after endodontic treatment, has increased in the last ten years [101]. Indicated materials have ranged from the employ of silicate aluminum based cements [102], the glass-ionomer [103], to epoxy resins for radicular canal sealers such as AH-Plus (Dentsply Maillefer, Ballaigues, Switzerland) [104], but most of them have presented different clinical limitations [105]. Controlled delivery of drugs is determinant for the radicular dentin treatment trying to reinforce the tissue [101]. Currently, this can be attained as a result of the increased use of NPs and the rapid advances of nanotechnology [106,107]. Specifically, it is recommended the application of Zn-NPs in the endodontic treatment before the sealing step, due to the gain in minerals and the attained lowest microleakage values. This intertubular, intratubular and peritubular dentin remineralization, that produced reinforcement of mechanical properties of radicular dentin, totally covered the dentin surface occluding dentinal tubules and cracks [29]. These are their main clinical applications in this field.

The carbonate band increase was shown and complies with the augmented carbonate replacement in the lattice of apatite [31]. FWHM is indicative of crystallinity and crystallographic maturity at the dentin substrate [28,108]. When FWHM increases, HAp crystals exhibit lower degree of substitutions and imperfections [109,110]. Treatment with Zn-NPs have also influenced the collagen components. After treating with Zn-NPs based-gel, diffractography patterns gradually change from wide diffuse to sharper peaks, after 7 d. This confirms that the amorphous phase is dynamic. Nevertheless, the diffraction analysis permitted to show brighter rings, meaning low line broadening of peaks, after 7 d [34]. Normally, findings of selected area electron diffraction (SAED) permit to observe that the mineral phase, at cervical dentin is crystalline HAp [111] and amorphous, because of the presence of both neat and diffuse rings, respectively [34,112,113].

The complementary physical evaluation also confirmed an augment of the mean crystallite size with large and straight crystalline peaks (Figure 10), tissue maturation

and low nanoroughness, commonly associated [28,26,29,30,114,115]. In endodontics, the use of Zn-NPs should be indicated, as radicular dentin is a potential failure site due to deformation, stress concentration and strain [116]. All in all, Zn-NPs should be considered as a feasible alternative for the advancement of root dentin sealing. Nevertheless, it is worth emphasizing that any disadvantages have been found when NPs were applied in dentin, but outcomes in clinical studies and in carious lesions are absent.

4.7. Non-carious cervical lesions and dentin hypersensitivity treatment based on NPs application

Loosing dental hard tissues close the cement-enamel junction, without caries, characterizes the non-carious cervical lesions processes. They usually predispose to dentin hypersensitivity (DH) (Figure S5) [117], which is produced by an extracellular matrix demineralization generating dentinal tubules exposure. DH is an oral health problem on a global scale in adults. Under normal conditions, dentinal tubules are surrounded by intertubular dentin. It consists of apatite crystals, similar to those of peritubular dentin [118], reinforcing a collagen matrix. DH is clinically defined as a localized, non-spontaneous, intense and brief pain that finishes when stimuli are eliminated [119]. Erosion is capable of enlarging and opening the dentin tubules; thereby, it has been recognized as the main etiological factor for DH [120]. In these conditions, the nerve termination in the dental pulp is excited by pressure changes coming from the fluid flow into the open dentinal tubules [121]. One of the main purposes of DH treatment [122,123] is the occlusion of tubules. Erosion exposes the organic matrix after a former dissolution of the mineral [124]. Then, degradation of the dentin matrix occurs later [96]. New materials should enable dentin remineralization [18,125]. They should focus on facilitating regenerative procedures of the extracellular matrix to release dentin bioactive compounds [126].

Different materials have been destined to treat these lesions. Resins, varnishes and remineralizing agents were proposed as desensitizing products for tubule occlusion, but with undefined results [127,128]. Others, have shown high rates of solubility, as calcium sodium phosphosilicates which form hydroxycarbonate apatite compounds [122]. Ferric oxalate, calcium and potassium have also been employed to treat DH, but oxalate precipitates were dissolved by saliva [129]. Nano-hydroxyapatite forms soluble complexes that weaken its long term effectiveness [130]. There are evidences of the bioactivity of NPs applied into demineralized dentin, in apatite formation, which may be determinant for treating DH through remineralization effects [25,131]. During caries and erosion processes the pH is lowered [124] and MMPs are activated. Host enzyme MMPs degrade the exposed organic matrix [48], as explained before. In erosion, MMPs reduce dentin loss [132]. In partially demineralized dentin, Zn intensely reduces MMP-mediated collagen degradation [48]. Additionally, MMPs normally act in the functional remineralization process [48].

4.8. Zinc-loaded bioactive and cytocompatible NPs for the treatment of periodontal disease

NPs may also be applied in the dental environment to treat periodontitis. This process has a multifactorial character with an important infectious component. A bacterial biofilm initiates periodontitis, provoking an inflammatory and immune response in the contiguous tissues [133]. The stability of periodontal repaired tissue represents a controversial matter at the moment [134]. The current resorbable materials used in periodontal treatments (*e.g.* nano/micro-sized calciumphosphate, HAp or beta-

tricalciumphosphate) may represent unfavorable options, as dissolution performance is not all the times as long-lasting as necessary [135]. Even more, degradation materials may not be totally cytocompatible [136,135]. Thereby, bioactive materials should then determine the success in periodontal therapy. If a material is capable to make bone-bonding with host bone material, then it is considered bioactive.

Bone graft biomaterials attain a moderately fast degree of biodegradation. The proposal of a biocompatible and efficient, nanoscaled, non-reabsorbable material capable to undertake regeneration of the periodontium is required. Nanosized biomaterials show irreplaceable features in contrast with its bulk-phase equivalent. As stated before, series of anionic carboxylate (*i.e*. COO-) are located alongside the body of the proposed NPs. The contained functional groups favor the likelihood of zinc quelation; this fact becomes more important in some processes such as periodontitis. Chronic infections may be overcome through the change of antibiotics by other antibacterial mediators, as metal oxides [11]. Furthermore, biomimetic remineralization of the experienced NPs has been pivotal for future regeneration of the periodontum.

Polymethylmethacrylate NPs are noncytotoxic [137]. Considering these features, assuming that unpolymerized monomers are lacking and dissolution of these particles is improbable, all hypothetical toxicity detected will not be associated with the NPs as such, being due to zinc ions over NPs. In a whole, Zn-loaded NPs have been non-apoptotic and non-cytotoxic in fibroblasts of humans alongside any test, except in the Live/Dead assay, where 100% of Zn-NPs (30 mg/ml) concentrations exerted a faintly greater (10% increase) cytotoxicity in comparison with other groups. But, the viability of cells was permanently beyond 80% [11]. In spite of this, the cytotoxicity of ZnO-loaded NPs assessed on fibroblast of human periodontal ligament resulted strongly depend on concentration, and it was harmful at concentrations between 50-100 μg/ml [138]. Ionic zinc loaded in the NP structure, therefore, does show lower cytotoxicity than when ZnO NPs were found [11,83,84]. As a consequence, the proposed NPs employed in this research pose a challenging tool for therapy in regeneration of human periodontum. These are their main clinical applications in this field.

Future perspectives include the extension of the clinical applications of NPs as drug-carriers to help with other different oral diseases. Currently, it has been proposed the design of new biocompatible and non-resorbable nano and micro hydrogels containing similar particles but with high drug-charge capability and response to environmental stimuli (smart hydrogels). Previously, the surface modification of the chemical properties of our former designed spheres was used to improve the tissue-biomaterial integration, achieving bioactivity and remineralization [101]. The new objectives will consist on the designing and fabrication of rechargable hydrogels doped with bioactive drugs.

The present manuscript is a narrative review. It is a comprehensive summary that synthesizes previously published scientific evidence. However, it does not follow any rules about the search for evidence, and decisions about relevance of included studies were not systematically performed. Therefore, conclusions should be taken with caution. There is no doubt about the strengths of the systematic review: the search for evidence, the criterion-based selection of literature, the rigorous appraisal of validity, and the evidence-based inferences. However, for some review topics, the narrow focus of the question of systematic reviews is a crucial limitation, as it does not allow for comprehensive literature coverage.

5. Conclusions

Polymeric nanoparticles do not affect immediate bond strength but facilitate bond strength maintenance, at the coronal resin-dentin interface, in the long term studies. Zinc-loaded nanoparticles induced collagen preservation, facilitated remineralization and increased mechanical properties within the resin-dentin interface. Coronal, cervical and radicular dentin infiltrated with Zn-NPs released the stress by breaking the resin-dentin interface creating specific mineral formations, that were mostly crystalline, in response to the energy dissipation. Thereby, zinc-doped dentin adhesives may have therapeutic/protective effects for stabilizing dentin hybrid layers. Besides, application of Zn-NPs, at both cervical and root dentin, is encouraged due to its mechanical reinforcing capability and to its enhanced durable sealing ability. In general Zn-doped NPs have contributed to collagen preservation and inhibition of cariogenic biofilm formation, following *in vitro* studies.

Additionally to the stated aims of this narrative review, it has also been observed that the Zn-doped NPs could have a potential applicability in other dental fields apart from operative dentistry. They might be considered as a viable treatment for dentin hypersensitivity and root canal treatments. Further studies should also encourage the application of Zn-NPs to treat periodontitis.

Declaration of Competing Interest

The authors report no declarations of interest.

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Figure 1. Debonded dentin surface where Zn-NP infiltration was performed before bonding. Intertubular dentin is observed, with a dense collagen network in which fibrils exhibit a growing width (100 to 200 nm), and the typical 67-nm periodicity (arrows). NPs may be seen attached to collagen fibers (open arrows). Some tubules are evident (asterisks). Mineral deposits are patent.

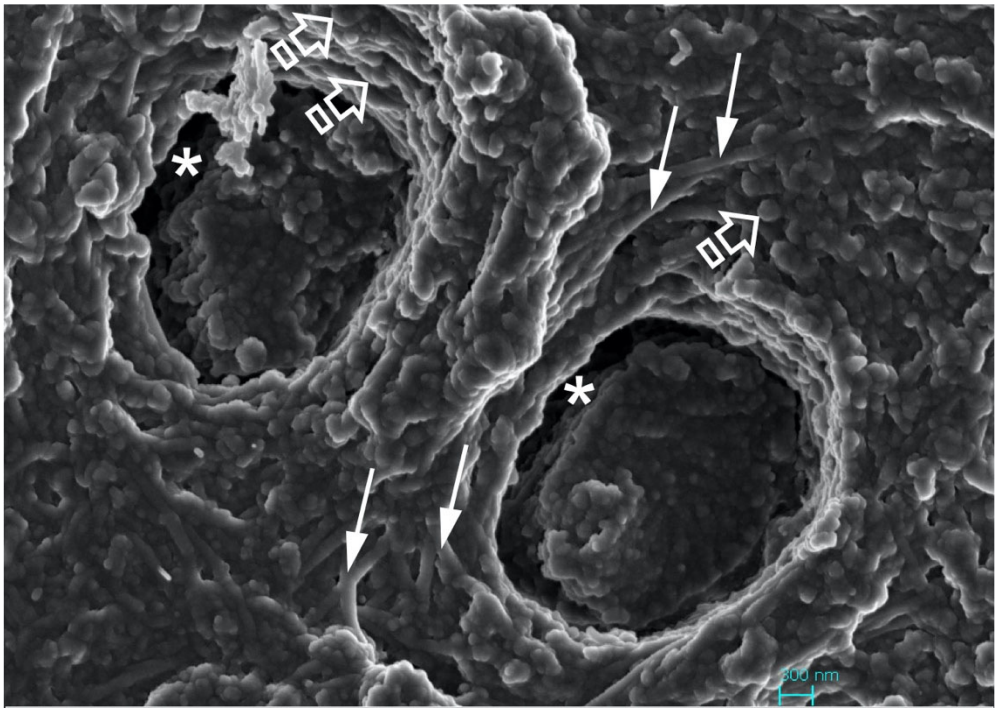


Figure 2. Field emission scanning electron microscopy (FESEM) images of a debonded resin-dentin specimen tested after 6 months of artificial saliva storage. The debonded dentin surface was created after bonding with Single Bond and water/ethanol as primer. Dentinal tubule with fractured resin tag (arrow) and demineralized collagen fibers (pointers) is visible. Fractured collagen fibers (arrow heads) that are not mineralized or resin protected are apparent. Collagen fibers show loose ends, a reduced diameter and seem to be disrupted.

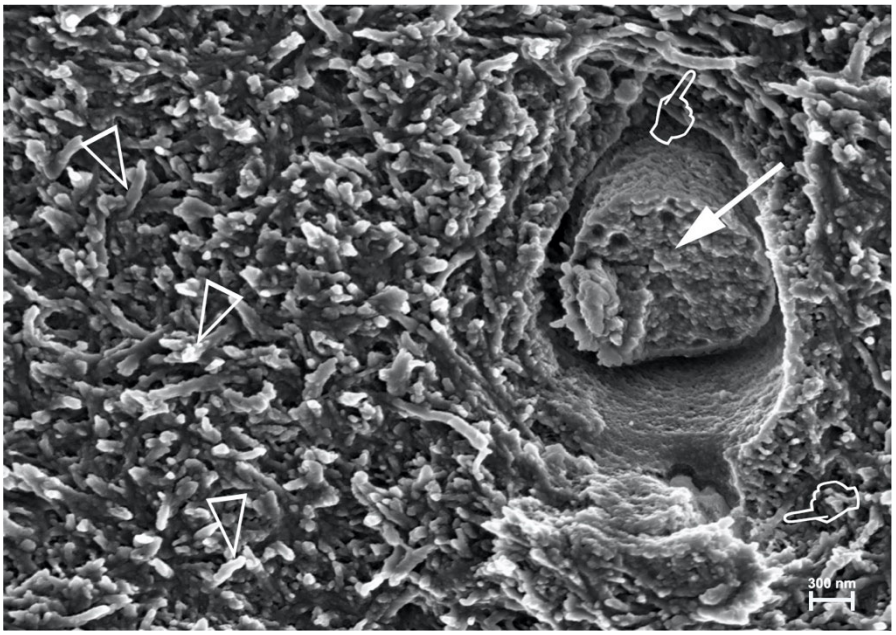


Figure 3. (**a**) 2 x 2 µm topographic mapping obtained by atomic force microscopy (AFM) at the inner zone of the apical dentin in a sample of the control group, after 6 m of storage. Peritubular (PD) and intertubular (ID) dentin are evidenced. (**b**) 2 x 2 µm topographic mapping obtained by AFM at the inner zone of the apical dentin treated with Zn-NPs, after 6 m of storage. Tubules were completely occluded (asterisks). Wider bandwidth of the collagen fibrils (faced arrows) and the staggered pattern of collagen fibrils (pointers) are shown. (**c**) 15 x 15 µm topographic mapping obtained by AFM at the inner zone of the apical dentin treated with Zn-NPs, after 6 m of storage. Peritubular dentin appeared strongly remineralized (double arrows). Stick-slip images in radial direction of nucleated minerals resulted observable at the intertubular dentin, as sight of energy dissipation (pointers). The crack deflection and branching around the peritubular cuff, may be observed at the dentin wall of an unfilled tubule (single arrows). Strong processes of intertubular and intratubular mineralization, with protruding rounded forms were also observed (arrowhead). Some bridge and rod-like new mineral formations were observed surrounding the intratubular crystals (asterisks). These crystals precipitated beams held the intratubular deposits of mineral to the peritubular dentin.

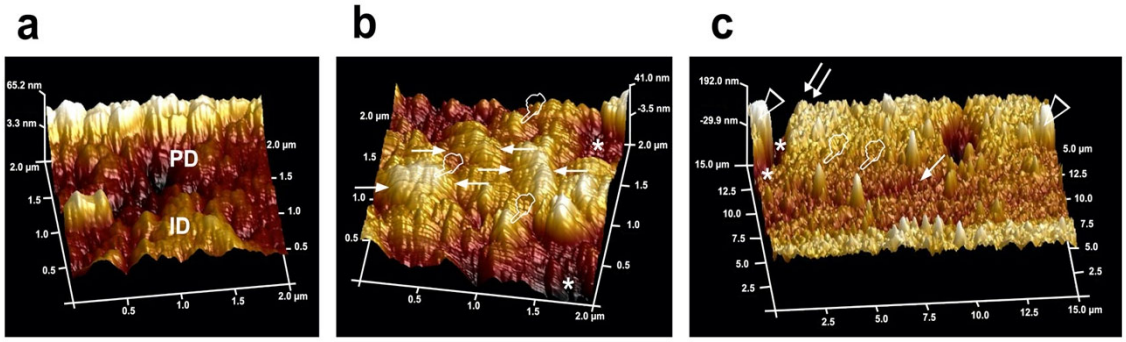


Figure 4. 3-D contour map of the complex modulus (E^*) at the cervical dentin surface treated without NPs (control group) (**a**) or with Zn-NPs (**b**) Zn-NPs, 6 m. In the color scheme shown, the red color corresponds to the highest value of the locally measured moduli. The mapping reflects regular and continuous rings of higher complex modulus (faced arrows), which correspond with the resistance to deformation of peritubular dentin. Extended areas of low values (pointers), but higher at "b" consistent with advanced mineralization may also be adverted, corresponding to the complex modulus of the intertubular dentin. In the color scheme shown, the red color corresponds to the highest value of the locally measured moduli, potentially associated to E^* of peritubular and intratubular mineral precipitation, especially shown in "b" (asterisks).

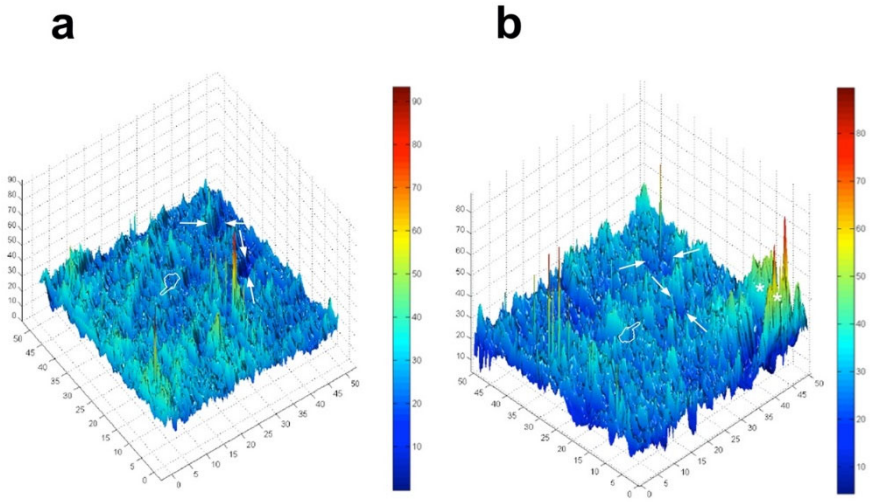


Figure 5. 2D micro-Raman map of the phosphate peak (961 cm^{-1}) intensities at the dentin bonded interface infiltrated with Single Bond adhesive (**a**) and Zn-NPs (**b**), and stored in SBFS for 21 days. Truncated Raman spectra from the phosphate PO_4^{-3}, at 961 cm^{-1} of principal components (HCAs) at the interface of the Single Bond adhesive (**c**) and Zn-NPs (**d**) groups. Abbreviations: HCA, Hierarchical Cluster Analysis; ADH, adhesive; HL, hybrid layer; BHL, bottom of hybrid layer; DEN, dentin.

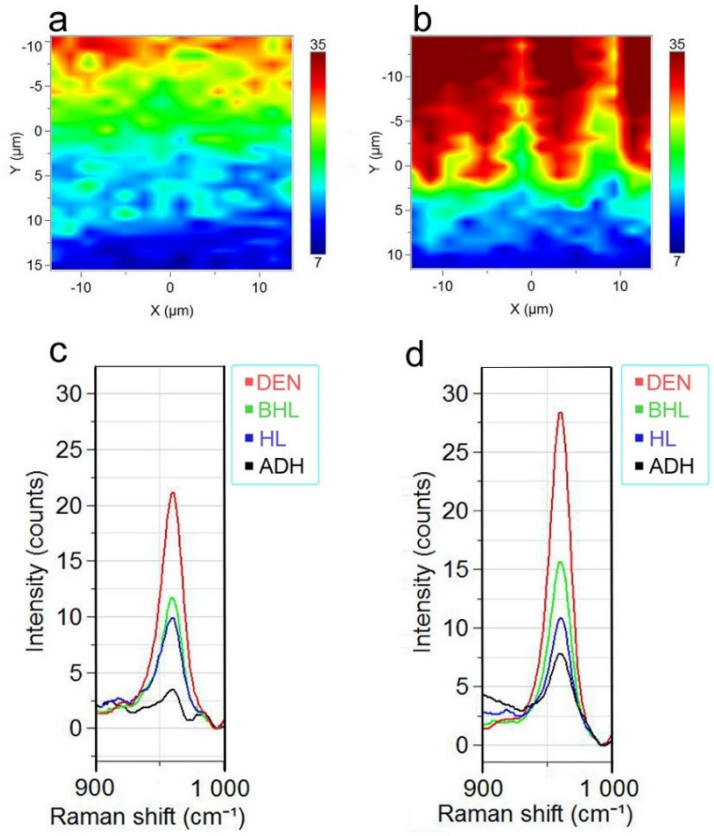


Figure 6. Mean and standard deviation (SD) of Young's modulus of elasticity (*Ei*) in GPa, at the experimental dentin bonded interface treated with zinc-loaded nanoparticles (Zn-NPs) after 24 h and 3 months storage periods. Same numbers indicate no significant difference between 24 h/3m (Adapted from Osorio et al, 2016).

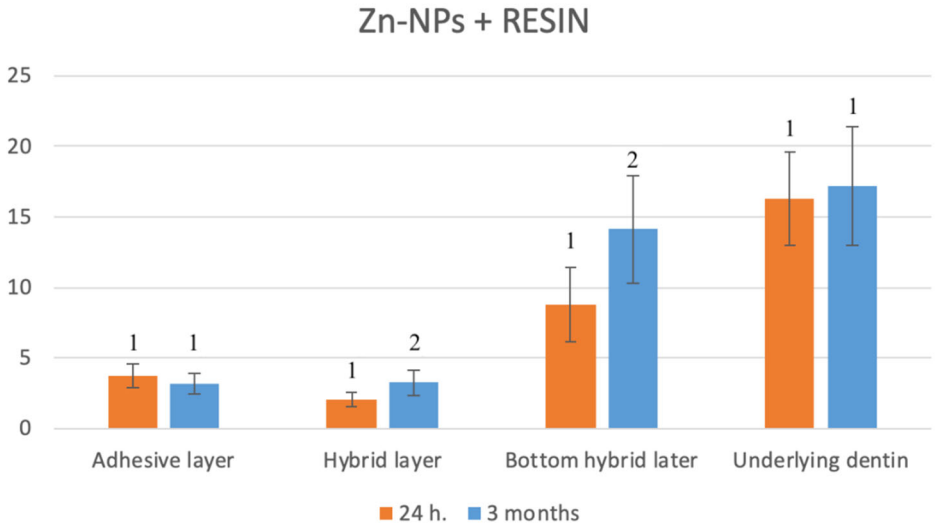


Figure 7. Scanning electron microscopy (SEM) micrographs of debonded dentin surfaces infiltrated with nanoparticles (NPs) and Single Bond. NPs remained embedded in the hybrid layer and were homogeneously distributed on the dentin surface (arrows). NPs did not agglomerate but penetrate dentin tubules (pointers). Some NPs were increased in size (arrow heads) and measured up to 1 micron, apparently covered by calcium and phosphorus.

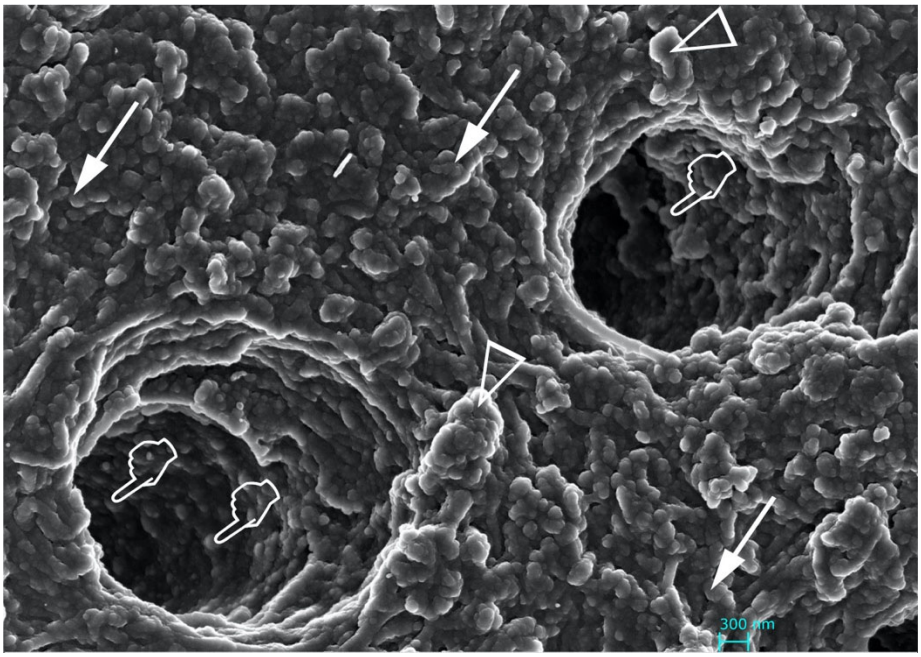


Figure 8. Confocal laser microscopy analysis single projection, after 3 months, of the resin–dentin interface created without NPs application. Proper adhesive penetration and resin tags formation are observed. Fluoresceine was able to pass through the hybrid layer creating a continuous line of emission (arrows) at the adhesive layer (al). It indicates the existence of a degraded hybrid layer. Remnant resin tags are present (rt) but seems to be degraded at the top of the hybrid layer.

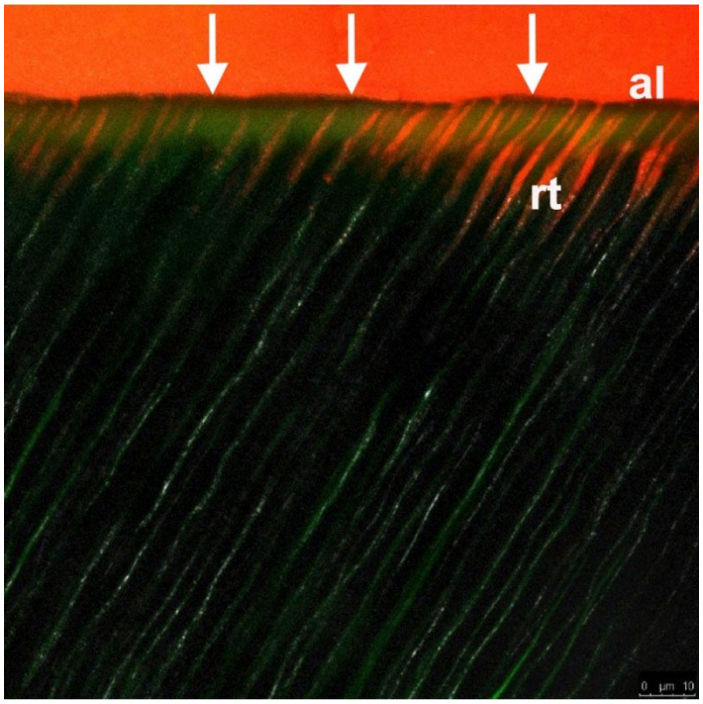


Figure 9. (**a**) FESEM image of apical root dentin treated with Zn-NPs at 6 m of storage. The dentin surface exhibited multiple amorphous clumps of buttons-like materials, rounding or amorphous (asterisks). Dentin tubules appeared completely filled (pointers), and intertubular (ID) and peritubular (PD) dentin were entirely remineralized. (**b**) Spectra from energy dispersive analysis, attained at presented zone, (corresponding with the sign "+" at the image). Elemental composition of phosphorous (P), calcium (Ca), and zinc (Zn), as main components, were encountered.

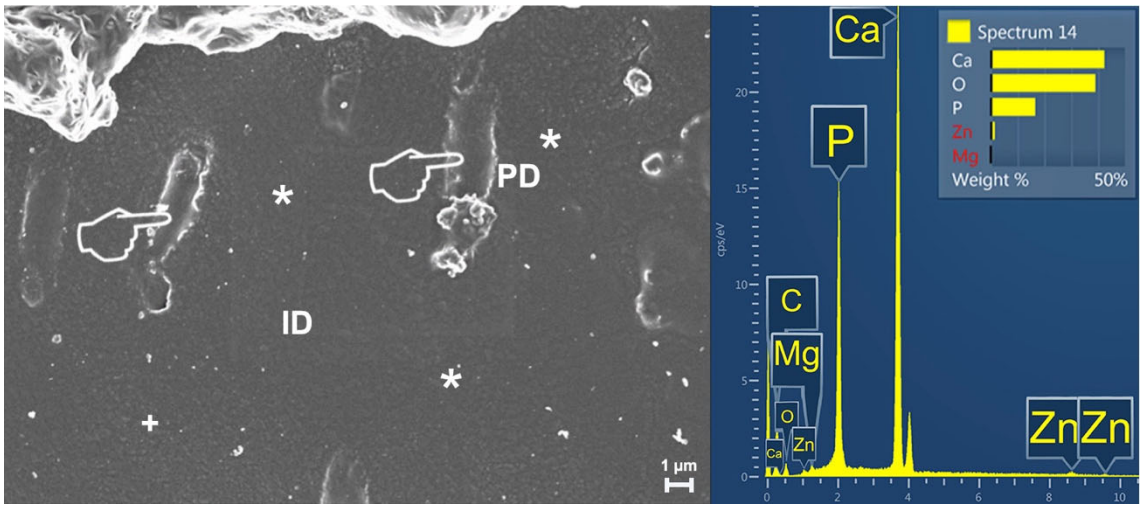
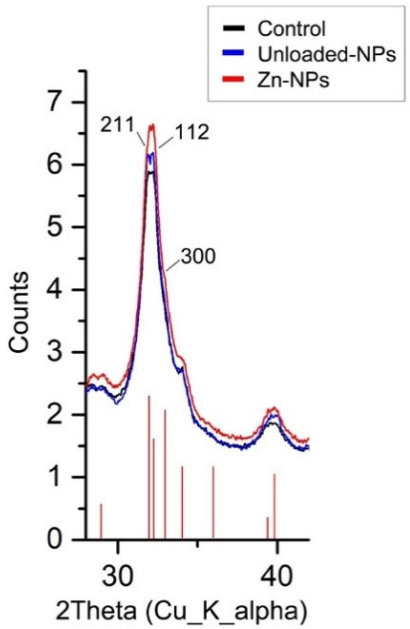


Figure 10. Refined and truncated µXRD^2 profiles of apical root dentin after 6 m of storage. The figure is centered at 211, 112 and 300 reflections. Vertical bars represent HAp peaks.



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