The use of bioactive particles and biomimetic analogues for increasing the longevity of resin-dentin interfaces: A literature review

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Protecting resin-dentin interfaces from hydrolytic and enzymatic degradation is critical for the longevity of adhesive restorations. In recent years, several strategies have been tested in vitro to induce apatite precipitation within interfibrillar and intrafibrillar collagen spaces, as well as in resin-sparse regions where the adhesive infiltration was incomplete. Also, the presence of calcium ions and other metallic ions has shown an inhibitory effect on enzymatic activity. Ion-releasing particles and biomimetic analogs have been studied for hybrid layer remineralization. Overall, remineralization strategy is dependent on the remaining mineral content. In partially demineralized dentin, residual apatite crystallites serve as nucleation sites for calcium and phosphate ions precipitation and crystal growth (“top-down” remineralization). In completely demineralized dentin where crystallites are absent (e.g., acid etched dentin) the use of mineral nano-precursors assisted by non-collagenous proteins analogs are necessary (“bottom-up” remineralization). This article reviews the approaches for hybrid layer remineralization and resin-dentin interface preservation.

Keywords: Remineralization, Dentin, Collagen, Degradation, Adhesion

INTRODUCTION

Contemporary adhesive systems are incapable of completely infiltrating the collagen network with resin monomers\(^1\)\(^-\)\(^3\), resulting in water-rich spaces along the unprotected collagen fibrils that are responsible for nanoleakage and micropermeability within hybrid layers\(^4\)\(^-\)\(^6\). The partially exposed collagen fibrils network is subjected to enzymatic degradation from endogenous MMPs and cysteine cathepsins, activated by the acidic components of adhesive systems\(^9\). The combination of hydrolysis of the resin component, collagenolytic activity, temperature, bacterial acids, and mechanical stresses compromise the long-term interfacial integrity of bonded restorations\(^6\)\(^-\)\(^7\).

Remineralization of resin-dentin interfaces has been studied aiming to replace water from intrafibrillar gaps, as well as from water-rich, resin-sparse regions of the hybrid layer, with apatite crystallites. By doing so, it would be possible to increase the mechanical properties of the dentin-resin interphase and protect the exposed collagen from external challenges\(^8\)\(^-\)\(^12\). Dentin remineralization strategy is dependent on the remaining mineral content of the tissue\(^13\). In partially demineralized carious dentin, apatite crystallites left in the intrafibrillar regions serve as nucleation sites for calcium and phosphate ions precipitation, followed by epitaxial crystal growth. This is the classical ion-based crystallization concept, also known as “top-down” remineralization\(^14\)\(^,\)\(^15\).

Yet, this approach may not be applicable for remineralization of completely demineralized dentin where seed crystallites are absent, e.g., in hybrid layers created by phosphoric acid etching or aggressive self-etch adhesives. In order to induce apatite precipitation in the intrafibrillar collagen spaces of completely demineralized dentin, a non-classical remineralization approach has been suggested, based on the use of metastable mineral nano-precursors assisted by biomimetic analogs of non-collagenous proteins. These molecules serve as stabilizers for the mineral precursors (avoiding premature extra-fibrillar crystallization) and also as templates to guide apatite growth in an oriented and hierarchically organized manner within collagen matrix. This is referred to as the “bottom-up” remineralization approach\(^16\)\(^,\)\(^17\).

This article reviews the recent literature on both epitaxial (top-down) and biomimetic (bottom-up) remineralization strategies are for extending the longevity of dentin-resin interfaces.

STRATEGIES INVOLVING BIOACTIVE PARTICLES

Ion-releasing (“bioactive”) particles have been tested as additives in experimental\(^18\)\(^-\)\(^23\) and commercial adhesives\(^24\). Also, they have been applied to acid-etched dentin prior to adhesive application\(^19\)\(^,\)\(^25\)\(^,\)\(^26\) or used in air-abrasion systems for selective caries removal\(^27\). The purpose of adding these particles in adhesive procedures is two-fold: besides replacing the lost mineral within the collagen network, metallic ions protect the collagen fibrils from degradation by reducing collagenase activity\(^28\). Overall, the ion-releasing particles tested in resin-based restorative materials can be divided in three groups: bioactive glasses (BAG), calcium silicates (CaSi) and calcium orthophosphates (CaP). Zinc oxide (ZnO) particles and zinc-loaded polymeric nanoparticles have also been tested as additive in adhesive system due to the protective role of zinc ions against collagen degradation; additionally, it has been shown to induce...
precipitation of poorly crystalized apatite, in a similar fashion to other metallic oxides. Among the silica-based BAG, the original 45S5 Bioglass, developed by Hench in the late 1960’s, is the composition most commonly tested, either unmodified or doped with zinc, copper, or treated with polyacrylic acid (PAA). Other compositions have been tested, including Niobium-based glass and a silica-based glass with higher Ca:P molar ratio than 45S5.

CaSi-based particles are the hydration products of tri-calcium and di-calcium silicates (i.e., mineral trioxide aggregate or MTA). Their bioactivity is related to the alkalization promoted by hydroxyl ions released from the crystalline calcium hydroxide phase. The increase in alkalinization promoted by hydroxyl ions released from aggregate or MTA). Their bioactivity is related to the enzymatic activity between MMP-2 or MMP-9 and CaP precipitates form MMP-1 and MMP-3 in bone.

Several mechanisms have been proposed to explain the reduced collagen degradation observed in demineralized dentin samples infiltrated or in contact with adhesives containing ion-releasing particles, which can be briefly described as follows: 1) ions released from the glass particles can bind to specific sites on the exposed collagen fibrils and modify its spacial configuration, protecting sensitive cleavage sites; 2) Electrostatic interactions between MMP-2 or MMP-9 and CaP precipitates form high molecular weight, low-mobility aggregates, reducing enzymatic activity; 3) HA was shown to inactivate MMP-1 and MMP-3 in bone; 4) local alkalization promoted by ion release (Ca²⁺, Na⁺) is likely to down-regulate MMP activity, normally triggered in acidic conditions.

The damage to the adhesive interface caused by MMPs and cathepsin K can be estimated by quantifying the concentrations of C-terminal cross-linked telopeptide (ICTP) and C-terminal telopeptide (CTX), respectively, released from the demineralized dentin sample. Totally demineralized dentin beams with either phosphoric acid or EDTA and infiltrated with a dimethacrylate-based adhesive containing 40 wt% of 45S5 particles released significantly lower amounts of ICTP after four weeks in AS than the control. In the same study, an adhesive containing CaSi/β-TCP particles was able to reduce collagen degradation only in EDTA-demineralized samples, probably because mineral remnants on the collagen were able to supplement the particles’ lower P content and the absence of Si and increase mineral nucleation. In a follow-up study, it was verified that the incorporation of ZnO to the CaSi/β-TCP particles reduced collagen degradation in samples demineralized with phosphoric acid due to the direct effect of zinc on MMP activity and also due to changes in pH that would favor apatite precipitation. Lower ICTP and CTX concentrations were released by dentin beams totally demineralized with PA that were kept in contact with polymerized resins containing 50 wt% of 45S5 or a fluoride-containing BAG for 30 days in AS compared to the unfilled control, with the adhesive containing the fluoride glass showing higher MMP inhibition than 45S5.

Fluoride, zinc and copper ions have been identified as MMP-2 and MMP-9 inhibitors, as they can bind to specific sites and induce conformational changes that impede their action (i.e., non-competitive inhibition). Also, zinc was shown to compete with MMPs for the collagen cleavage site in demineralized dentin beams. In fact, the addition of 10 wt% ZnO particles to a commercial two-step, total etch adhesive system significantly reduced ICTP concentrations released from infiltrated dentin beams after four weeks in AS, in comparison to the unmodified adhesive. Copper-doped glass nanoparticles have been tested as a strategy to increase the inhibitory effect achieved with calcium ions. An experimental adhesive containing 2 wt% of copper-doped nano-scale mesoporous bioactive particles tested in demineralized dentin beams (10% phosphoric acid for 12 h) showed higher MMP inhibition in relation to the adhesive containing non-doped BAG. Differently from the previously described studies, the dentin beams were immersed in a solution containing the non-polymerized adhesive and acetone for 5 min. Also, MMP inhibition was determined after a short incubation period (1 h). In comparison to micro-sized particles, nanoparticles have the advantages of a high surface area, allowing for significant ion release in relatively lower concentrations. Also, they have a lower thickening effect on the adhesive, which would improve infiltration in the demineralized collagen network and dentin tubules.
Interfacial micro-permeability and gap formation
Long-term integrity of dentin-resin interfaces has been evaluated in terms of their micro-permeability, using confocal laser scanning microscopy (CLSM). The experimental method consists in adding rhodamine (a fluorescent dye) to the resin prior to the bonding procedure. After storage, the samples are immersed in fluorescein for 24 h. A microscope equipped with laser sources of different wavelengths is used to excite the fluorescent molecules, allowing the analysis of rhodamine-doped adhesive penetration into the demineralized dentin and the dentin tubules (resin tags) and also the presence of gaps and voids at the interface (disclosed by fluorescein uptake). Overall, interfaces using adhesives with 30 to 40% of bioactive glass or CaSi-based fillers showed reduced micro-permeability after three to six months in phosphate-buffered saline (PBS) or simulated body fluid (SBF) than those using unfilled resins, likely due to mineral precipitation within the hybrid layer. Using a calcium-chelator fluorescent dye (xylene orange), intense mineral deposition was also observed within the demineralized dentin samples (37% orthophosphoric acid for 15 s) kept in contact with pre-polymerized resin discs containing 40 wt% bioactive fillers (BAG, Zinc-polycarboxylated BAG, CaSi/β-TCP or Zinc-polyacrylated CaSi/β-TCP) after one-month immersion in SBF (confirmed by X-ray diffraction), with no evident differences among fillers. The use of a slurry containing particles of set MTA cement on acid-etched dentin, prior to adhesive application, was shown to increase the percentage of continuous margin at the gingival wall of class II restorations after six months in PBS under simulated pulpal pressure, in relation to the control (respectively, 69% and 47%). Samples observed under the scanning electron microscope showed gaps predominantly between the hybrid layer and dentin and particles were identified inside the dentin tubules in groups treated with the MTA particle suspension.

Mechanical properties of demineralized dentin and adhesive interfaces
Nanindentation techniques have been used to assess the elastic modulus and hardness of the adhesive interface. This method employs loads in the micronewton (µN) to milinewton (mN) range, allowing for multiple indentations a few micrometers apart across the entire interface. Since it is a non-destructive test, the same specimen can be evaluated after different periods of immersion. Experimental adhesives containing either 40 wt% BAG (45S5 or Zinc-polyacrylated BAG) or 33 wt% CaSi/β-TCP (unmodified or Zinc-polyacrylated CaSi/β-TCP) were used to increase the elastic modulus of the hybrid layer after three months in SBF, suggestive of remineralization, while the control adhesive (without particles) showed the opposite behavior. However, the mechanical properties at the adhesive (i.e., bonding resin) layer decreased in most of the groups, possibly due to hydrolytic degradation of the polymer. Increases in elastic modulus of the hybrid layer after three months in PBS were also observed with suspensions containing Niobium-based glass or 45S5 BAG particles or zinc-loaded polymeric nanoparticles applied as primers on demineralized dentin, followed by the application of a commercial adhesive.

Studies verifying the effect of pre-polymerized resin-based materials containing ion-releasing fillers placed on demineralized dentin samples provide further evidence of mineral precipitation within the collagen network. A composite containing 15 wt% of BAG and 57 wt% of reinforcing fillers were able to increase by 25% the surface microhardness of demineralized bovine dentin (37% phosphoric acid gel for 60 s) after two week of immersion in SBF or PBS, although not to the same level displayed by non-demineralized dentin samples. Fully demineralized human dentin beams (10% phosphoric acid gel for 12 h) kept in contact with composite bars containing 50 wt% of fluoride-containing BAG for 30 days in AS showed a 13% increase in elastic modulus, while those in contact with the control resin showed a reduction of 32%. Though not related to adhesive interfaces, two complementary in vivo studies dealing with remineralization of caries-affected dentin need to be mentioned. Following selective caries removal, a resin cement containing TTCP and DCPA (approximately 65% by mass) was applied directly to the demineralized dentin as a base material prior to restoration with a commercial composite. After three months, teeth were extracted and dentin remineralization was assessed using electron probe microanalysis and transversal Knoop microhardness. In teeth treated with the experimental cement, calcium and phosphate contents were recovered to levels similar to the non-carious dentin. Dentin microhardness was higher than in the negative control samples, but did not reach values similar to those of sound dentin.

Effect on long-term bond strength
The incorporation of ion-releasing particles in experimental adhesives shows inconsistent results on the long-term bond strength to dentin. While studies evaluating adhesives containing BAG, CaSi-based or ZnO particles, as well as primers consisting of glass suspensions reported no loss in microtensile bond strength after three or six months of storage (as opposed to the control groups without particles), others did not find differences between adhesives containing particles and the unfilled controls. A study comparing the application of BAG particles to the demineralized dentin surface before the adhesive application or added to the adhesive (30 wt%) led to different outcomes after six months in PBS. BAG particles helped to preserve bond strength only when mixed to the resin. Besides the quality of the hybrid layer, long-term bond strength results are influenced by adhesive hydrophilicity and its susceptibility to hydrolysis. The hydrophilicity of the particles also seems to play a role in the long-term bond strength. Some studies explain the drop in bond strength in the presence of mineral deposition as the
result of a change in interfacial characteristics, as the remineralized hybrid layer would create an interface resembling that established between demineralized dentin and glass-ionomer cements. Finally, since resin formulations differ among studies, it becomes very difficult to draw conclusions regarding the role of bioactive particles on bond strength.

STRATEGIES INVOLVING BIOMIMETIC ANALOGS

Biomimetic remineralization relies on backfilling the demineralized dentin collagen with liquid-like ACP nanoparticles (referred to as mineral precursors) stabilized by biomimetic analogs of non-collagenous proteins. These analogues are characterized by the presence of aspartic acid and glutamic acid-rich domains, as well as phosphoproteins that may act as nucleators or inhibitors, growth modifiers, anchoring molecules, or as scaffolds for mineral deposition. More than ten different analogs have been tested for dentin remineralization. Among them, those tested for resin-dentin interfaces remineralization include PAA, polynvinylphosphonic acid (PVA), sodium trimetaphosphate (STMP), polyaspartic acid (PAS), and other engineered peptides. The majority of the studies added these analogs to the storage medium (usually SBF or AS), while few studies employed them as water-based primers, and one study tested an experimental adhesive containing ACP nanoparticles stabilized by PAA.

PAA acts as a calcium-binding molecule. It is employed as analog of acidic non-collagenous proteins such as dentin matrix protein 1 (DMP 1) for stabilizing and controlling the dimensions of amorphous calcium carbonate and calcium phosphate phases. PVA is a polyelectrolyte that has also been used as analog for collagen-binding matrix proteins, such as phosphoproteins DMP 1 and dentin phosphoproteins. STMP is a phosphophoryn analogue that binds to collagen fibrils producing phosphorylated and negatively charged sites that attract nanoprecursors, promoting the homogeneous nucleation of apatite crystallites. Other peptides with affinity to minerals and specifically to HA also have been demonstrated to mimic the role of the natural proteins, specially shorter peptides sequences, binding to collagen via electrostatic interactions and sequestering calcium and phosphate from the media to initiate mineral deposition.

Remineralization of resin-infiltrated dentin interfaces

Interfibrillar and intrafibrillar remineralization of phosphoric acid-etched dentin using biomimetic analogs was first demonstrated by Tay and Pashley. They were able to generate metastable ACP nanoprecursors when PAA was included in a phosphate-containing fluid. When associated to PVA, these nanoprecursors were attracted to the acid-demineralized collagen matrix and transformed into polyelectrolyte-stabilized apatite nanocrystals. The transition from nanocrystals to larger apatite platelets was observed in transmission electron microscopy images. In a follow-up study, interfibrillar and intrafibrillar apatite crystals became readily discernible within the hybrid layers of incompletely infiltrated resin-dentin interfaces created by etch-and-rinse adhesive systems after 2 to 4 months of immersion in SBF containing PAA and PVA. In addition, apatite clusters were observed within the porosities of the adhesive resin. Other studies showed evidences of mineral precipitation within the adhesive-infiltrated dentin when PAA, PVA or STMP analogs were added to SBF storage medium. Interestingly, in control specimens (i.e., absence of analogues in remineralization medium) some remineralization was observed in partially demineralized collagen matrices, probably due to epitaxial growth.

It is noteworthy that in these studies the main ion source for resin-dentin interface remineralization was a set Portland cement block placed in contact with the sample within the remineralization solution. However, this design is not clinically relevant, and delivery systems that incorporate the ion source either in the adhesive or the restorative material have to be employed, in order for remineralization to proceed via diffusion of the ACP nanoprecursors.

Nanoleakage and micro-permeability of hybrid layer

Voids within the hybrid layer can be assessed by nanoleakage using ammoniacal silver nitrate solutions, later identified by microscopy techniques. Interfaces remineralized in SBF containing PAA and PVA analogs showed mineral precipitation in the water-rich channels within the hybrid layer. Also, apatite deposits were unexpectedly created within the pores of the adhesive resin. Air-dried collapsed collagen fibrils cannot be remineralized to the same hierarchical order and extent seen in structurally intact dentin collagen; therefore, the re-expansion of the demineralized collagen matrix is not only important to avoid nanoleakage of poor resin infiltrated areas but also to allow for mineral precipitation within inter- and intra-fibrils spaces.

CLSM was also used to demonstrate long-term integrity of dentin-resin interfaces regarding micro-permeability. Smaller fluorescent areas within the hybrid layers (i.e., lower micro-permeability) were observed for samples immersed in PAA- and PVA-containing solutions in contact with Portland-cement block after 4 months. The importance of calcium and phosphate ions in remineralization approaches using biomimetic analogues was demonstrated when a reduction in micropermeability was observed with primers containing PAS and STMP associated to an adhesive containing CaSi particles. The same effect was not observed for the unfilled adhesive, which actually presented visible signs of degradation at the bottom of the hybrid layer.

Mechanical properties of remineralized interfaces

Though microscopy images provide valuable information regarding the ultrastructure of the analogs-assisted remineralized interface, it is fundamental to access
its mechanical properties to verify if the biomimetic remineralization strategy was able to promote intrafibrillar mineral precipitation. Increases in mechanical properties of the collagen fibrils occur not only by intrafibrillar remineralization, but due to the hierarchically organized interfibrillar crystallites\(^5\). Because hybrid layers created by contemporary adhesives are usually less than 10 µm thick, nanoindentation is the most suitable method to evaluate hardness and elastic modulus of this structure. Primers containing PAS and/or STMP applied previously to bonding with an adhesive containing CaSi particles were shown to contribute to some extent to hybrid layer elastic modulus recovery within three months, especially when both analogs were associated\(^5\). Increases in nano-dynamic mechanical properties of hybrid layers created with an etch-and-rinse adhesive were observed after six months in SBF medium containing PAA and STMP. Conversely, in the absence of biomimetic analogs the specimens showed resin-sparse, water-rich regions and significant decline of mechanical properties\(^6\).

Changes in flexural modulus of resin-infiltrated dentin beams can also be used to access remineralization. Dentin beams with 300-µm thick resin-infiltrated layer were stored in PAA- and PVA-containing SBF\(^7\). The flexural modulus of the control specimens remained low throughout 4-month evaluation period, while the specimens immersed in remineralization medium containing biomimetic analogs increased nearly two-fold during the first six weeks. This gain is still assumed as modest when compared with fully mineralized dentin properties, and these results were attributed to the diffusion-controlled effect of ACP through the polymerized adhesive resin matrix forming a gradient in mineralization, beyond the presence of water-plasticized adhesive resins that have very low stiffness within the collagen matrix of the experimental specimens\(^8\).

Long-term microtensile bond strength

There are only few studies assessing microtensile bond strength of resin-dentin interfaces using biomimetic remineralization. The use of a water-based primer containing PAS and STMP analogs associated with an adhesive containing ion-releasing particles exhibit no decrease in bond strength after three months. On the other hand, when the bioactive adhesive was used without the primer or in combination with PAS only, bond strength decreased after storage\(^9\). The same analogs were used associated with a zinc-doped Portland-based resin cement, and in this case PAS associated or not with STMP was able to maintain bond strength after 6 months\(^10\). Particularly in this study, higher bond strength values were attained when PAS was applied without STMP. In fact, findings were attributed to an effect of STMP in retarding the polymerization reaction of the resinous cement\(^11\). Also, Zinc may form polyphosphate complexes with STMP, which may reduce the analogue efficiency\(^12\).

**FINAL REMARKS**

There is an abundance of *in vitro* evidence showing that there is possible to promote mineral precipitation within the hybrid layer and reduce collagen degradation with the use experimental primers containing biomimetic analogues and adhesives containing ion-releasing fillers, including BAG, CaSi, ZnO, and CaP. However, studies are necessary in order to define clinically useful protocols and verify its effectiveness in the long-term.

**REFERENCES**

1) Breschi L, Mazzoni A, Ruggeri A, Cademoro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008; 24: 90-101.
2) Sauro S, Watson TF, Manocci F, Miyake K, Huffman BP, Tay FR, et al. Two-photon laser confocal microscopy of micropermeability of resin-dentin bonds made with water or ethanol wet bonding. J Biomed Mater Res B Appl Biomater 2009; 90: 327-337.
3) Carvalho RM, Manso AP, Geraldes S, Tay FR, Pasley DH. Durability of bonds and clinical success of adhesive restorations. Dent Mater 2012; 28: 72-86.
4) Suppa P, Breschi L, Ruggeri A, Mazzotti G, Prati C, Chersoni S, et al. Nano-leakage within the hybrid layer: a correlative FEISEM/TEM investigation. J Biomed Mater Res B Appl Biomater 2005; 73: 7-14.
5) Mazzoni A, Saffa P, Carrilho M, Tjaderhane L, Di Lenarda R, Polimeni A, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res 2013; 92: 82-86.
6) Breschi L, Maravic T, Cunha SR, Comba A, Cademoro M, Tjaderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018; 34: 78-96.
7) Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability: A literature review. Dent Mater 2016; 32: e41-53.
8) Akimoto N, Yokoyama G, Ohmori K, Suzuki S, Kohno A, Cox CF. Remineralization across the resin-dentin interface: in vivo evaluation with nanoindentation measurements, EDS, and TEM. Quintessence Int 2001; 32: 561-570.
9) Gu LS, Huffman BP, Arola DD, Kim YK, Mai SI, Elsalanty ME, et al. Changes in stiffness of resin-infiltrated demineralized dentin after remineralization by a bottom-up biomimetic approach. Acta Biomater 2010; 6: 1453-1461.
10) Wu Z, Wang X, Wang Z, Shao C, Jin X, Zhang L, et al. Self-Etch Adhesive as a carrier for ACP nanoprecursors to deliver biomimetic remineralization. ACS Appl Mater Interfaces 2017; 9: 17710-17717.
11) Garcia IM, Leitune VCB, Samuel SMW, Collares FM. Influence of different calcium phosphates on an experimental adhesive resin. J Adhes Dent 2017; 19: 379-384.
12) He L, Hao Y, Zhen L, Liu H, Shao M, Xu X, et al. Biomimetic remineralization of dentin. J Struct Biol 2019; 207: 115-122.
13) Niu LN, Zhang W, Pasley DH, Breschi L, Mao J, Chen JH, et al. Biomimetic remineralization of dentin. Dent Mater 2014; 30: 77-96.
14) Bertassoni LE, Habelitz S, Pugach M, Soares PC, Marshall SJ, Marshall GW Jr. Evaluation of surface structural and mechanical changes following remineralization of dentin. Scanning 2010; 32: 312-319.
15) Veis A, Dorvee JR. Biomimetic remineralization mechanisms: a new
paradigm for crystal nucleation in organic matrices. Calcif Tissue Int 2013; 93: 307-315.

16) Tay FR, Pashley DH. Guided tissue remineralisation of partially demineralised human dentine. Biomaterials 2008; 29: 1127-1137.

17) Liu Y, Mai S, Li N, Yiu CK, Mao J, Pashley DH, et al. Differences between top-down and bottom-up approaches in mineralizing thick, partially demineralized collagen scaffolds. Acta Biomater 2011; 7: 1742-1751.

18) Osorio R, Yamauti M, Sauro S, Watson TF, Toledano M. Experimental resin cements containing bioactive fillers reduce matrix metalloproteinase-mediated dentin collagen degradation. J Endod 2012; 38: 1227-1232.

19) Profeta AC, Mannocci F, Foxton RM, Thompson I, Watson TF, Sauro S. Bioactive effects of a calcium/sodium phosphosilicate on the resin-dentine interface: a microtensile bond strength, scanning electron microscopy, and confocal microscopy study. Eur J Oral Sci 2012; 120: 353-362.

20) Sauro S, Osorio R, Watson TF, Toledano M. Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface. J Mater Sci Mater Med 2012; 23: 1521-1532.

21) Sauro S, Osorio R, Fulg’encio R, Watson T, Cama G, Thompson I, et al. Remineralisation properties of innovative light-curable resin-based dental materials containing bioactive micro-filler. J Mater Chem B 2013; 1: 2624.

22) Tezvergil-Muthayu A, Sesoogullari-Dirihan R, Feitosa VP, Cama G, Brauer DS, Sauro S. Effects of composites containing bioactive glasses on demineralized dentin. J Dent Res 2017; 96: 999-1005.

23) Jang JH, Lee MG, Ferracane JL, Davis H, Bae HE, Choi D, et al. Effect of bioactive glass-containing resin composite on dentin remineralization. J Dent 2018; 75: 58-64.

24) Carneiro KK, Araujo TP, Carvalho EM, Meier MM, Tanaka A, Carvalho CN, et al. Bioactivity and properties of an adhesive system functionalized with an experimental niobium-based glass. J Mech Behav Biomed Mater 2018; 78: 188-195.

25) Bauer J, Silva ESA, Carvalho EM, Ferreira PVC, Carvalho CN, Manso AP, et al. Dentin pretreatment with 4iSSS and niobophosphate bioactive glass. Effects on pH, antibacterial, mechanical properties of the interface and microtensile bond strength. J Mech Behav Biomed Mater 2019; 90: 374-380.

26) Aggarwal V, Bhasin SS. Application of calcium silicate materials after acid etching may preserve resin-dentin bonds. Oper Dent 2018; 43: E243-E52.

27) Sauro S, Watson TF, Thompson I, Banerjee A. One-bottle self-etching adhesives applied to dentine air-abraded using bioactive glasses containing polyacrylic acid: an in vitro microtensile bond strength and confocal microscopy study. J Dent 2012; 40: 896-905.

28) Profeta AC, Mannocci F, Foxton R, Watson TF, Feitosa VP, De Carlo B, et al. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. Dent Mater 2013; 29: 729-741.

29) Toledano M, Yamauti M, Ruiz-Requena ME, Osorio R, A ZrO-doped adhesive reduced collagen degradation favouring dentine remineralisation. J Dent 2012; 40: 756-765.

30) Osorio R, Cabello I, Toledano M. Bioactivity of zinc-doped dental adhesives. J Dent 2014; 42: 403-412.

31) Osorio R, Cabello I, Medina-Castillo AL, Osorio E, Toledano M. Zinc-modified nanopowders improve the quality of resin-dentin bonded interfaces. Clin Oral Investig 2016; 20: 2411-2420.

32) Toledano M, Aguileras FS, Osorio E, Cabello I, Toledano-Osorio M, Osorio R. Self-etching zinc-doped adhesives improve the potential of caries-affected dentin to be functionally remineralized. Biointerphases 2015; 10: 031002.

33) Hench LL, Wilson J. Surface-active biomaterials. Science 1984; 226: 630-636.

34) Jun SK, Yang SA, Kim YJ, El-Fiqi A, Mandakhabyar N, Kim DS, et al. Multi-functional nano-adhesive releasing therapeutic ions for MMP-deactivation and remineralization. Sci Rep 2018; 8: 5663.

35) Wang Z, Shen Y, Haapasalo M, Wang J, Jiang T, Wang Y, et al. Polycarboxylated microfillers incorporated into light-curable resin-based dental adhesives evoke remineralization at the mineral-depleted dentin. J Biomater Sci Polym Ed 2014; 25: 679-697.

36) Sauro S, Osorio R, Watson TF, Toledano M. Novel light-curable materials containing experimental bioactive micro-fillers remineralise mineral-depleted bonded-dentine interfaces. J Biomater Sci Polym Ed 2013; 24: 940-956.

37) Braga RR. Calcium phosphates as ion-releasing fillers in restorative resin-based materials. Dent Mater 2019; 35: 3-14.

38) Osorio R, Yamauti M, Sauro S, Watson TF, Toledano M. Zinc incorporation improves biological activity of beta-tricalcium phosphate resin-based cement. J Endod 2014; 40: 1840-1845.

39) Osorio R, Yamauti M, Osorio E, Ruiz-Requena ME, Pashley DH, Tay FR, et al. Zinc reduces collagen degradation in demineralized human dentin explants. J Dent 2011; 39: 148-153.

40) Makowski GS, Ramsby ML. Differential effect of calcium phosphate and calcium pyrophosphate on binding of matrix metalloproteinases to fibrin: a comparison to a fibrin-binding protease from inflammatory joint fluids. Clin Exp Immunol 2004; 136: 176-187.

41) Kremer EA, Chen Y, Suzuki K, Nagase H, Gorski JP. Hydroxyapatite induces autolytic degradation and inactivation of matrix metalloproteinase-1 and -3. J Bone Miner Res 1998; 13: 1890-1902.

42) Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998; 77: 1622-1629.

43) de Souza AP, Gerlach RF, Line SR. Inhibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts. Dent Mater 2000; 16: 103-108.

44) Kato MT, Bolohan A, Zarella BL, Salo T, Tjaderhane L, Buzalaf MAR. Sodium fluoride inhibits MMP-2 and MMP-9. J Dent Res 2014; 93: 74-77.

45) Peters MC, Bresciani E, Barata TJ, Fagundes TC, Navarro RL, Navarro MF, et al. In vivo dentin remineralization by calcium-phosphate cement. J Dent Res 2010; 89: 286-291.

46) Bresciani E, Wagner WC, Navarro MF, Dickens SH, Peters MC. In vivo dentin microhardness beneath a calcium-phosphate cement. J Dent Res 2010; 89: 836-841.

47) Cao CY, Mei ML, Li QL, Lo EC, Chu CH. Methods for biomimetic remineralization of human dentine: a systematic review. Int J Mol Sci 2015; 16: 4615-4627.

48) Gu LS, Kim J, Kim YK, Liu Y, Dickens SH, Pashley DH, et al. A chemical phosphorylation-inspired design for Type I collagen biomimetic remineralization. Dent Mater 2010; 26: 1077-1089.

49) Liu Y, Li N, Qiu Y, Niu LN, Elshafiy S, Mao J, et al. The use of sodium trimetaphosphate as a biomimetic analog of matrix phosphoproteins for remineralization of artificial caries-like dentin. Dent Mater 2011; 27: 465-477.

50) Ryoo H, Niu LN, Dai L, Pucci CR, Arola DD, Pashley DH, et al. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. J Dent Res 2011; 90: 1122-1128.

51) Gu L, Kim YK, Liu Y, Ryoo H, Wimmer CE, Dai L, et al. Biomimetic analogs for collagen biomimeralization. J Dent Res 2011; 90: 82-87.

52) Wang Z, Ouyang Y, Wu Z, Zhang L, Shao C, Fan J, et al. A novel fluorescent adhesive-assisted biomimetic mineralization.
53) Lin HP, Lin J, Li J, Xu JH, Mehl C. In vitro remineralization of hybrid layers using biomimetic analogs. J Zhejiang Univ Sci B 2016; 17: 864-873.

54) Mai S, Kim YK, Kim J, Yiu CK, Ling J, Pashley DH, et al. In vitro remineralization of severely compromised bonded dentin. J Dent Res 2010; 89: 405-410.

55) Kim J, Arola DD, Gu L, Kim YK, Mai S, Liu Y, et al. Functional biomimetic analogs help remineralize apatite-depleted demineralized resin-infiltrated dentin via a bottom-up approach. Acta Biomater 2010; 6: 2740-2750.

56) Tay FR, Pashley DH. Biomimetic remineralization of resin-bonded acid-etched dentin. J Dent Res 2009; 88: 719-724.

57) Osorio R, Sauro S, Watson TF, Toledano M. Polyaspartic acid enhances dentine remineralization bonded with a zinc-doped Portland-based resin cement. Int Endod J 2016; 49: 874-883.

58) Sauro S, Osorio R, Watson TF, Toledano M. Influence of phosphoproteins’ biomimetic analogs on remineralization of mineral-depleted resin-dentin interfaces created with ion-releasing resin-based systems. Dent Mater 2015; 31: 759-777.

59) Bahari M, Savadi Oskoei S, Kimyai S, Pouralibaba F, Farhadi F, Norouzi M. Effect of casein phosphopeptide-amorphous calcium phosphate treatment on microtensile bond strength to carious affected dentin using two adhesive strategies. J Dent Res Dent Clin Dent Prospects 2014; 8: 141-147.

60) Ye Q, Spencer P, Yuca E, Tamerler C. Engineered peptide repairs defective adhesive-dentin interface. Macromol Mater Eng 2017; 302: 1600487.

61) de Sousa JP, Carvalho RG, Barbosa-Martins LF, Torquato RJS, Mugnol KCU, Nascimento FD, et al. The self-assembling peptide P11-4 prevents collagen proteolysis in dentin. J Dent Res 2019; 98: 347-354.

62) Olszta MJ, Odom DJ, Douglas EP, Gower LB. A new paradigm for biomimical formation: mineralization via an amorphous liquid-phase precursor. Connect Tissue Res 2003; 44 Suppl 1: 326-334.

63) Li X, Chang J. Preparation of bone-like apatite-collagen nanocomposites by a biomimetic process with phosphorylated collagen. J Biomed Mater Res A 2008; 85: 293-300.

64) Gungormus M, Fong H, Kim IW, Evans JS, Tamerler C, Sarikaya M. Regulation of in vitro calcium phosphate mineralization by combinatorially selected hydroxyapatite-binding peptides. Biomacromolecules 2008; 9: 966-973.

65) Nikolov S, Raabe D. Hierarchical modeling of the elastic properties of bone at submicron scales: the role of extrafibrillar mineralization. Biophys J 2008; 94: 4220-4232.

66) Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Corrilo M, et al. From dry bonding to water-wet bonding: A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007; 20: 7-20.

67) Zhang Y, Wang Y. Effect of application mode on interfacial morphology and chemistry between dentine and self-etch adhesives. J Dent 2013; 41: 231-240.