©Operative Dentistry, 2022, **47-3,** E152-E161

Development and Assessment of Bioactive Coatings for the Prevention of Recurrent Caries Around Resin Composite Restorations

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Clinical Relevance

The application of bioactive surface coatings potentially contributes to the *in vitro* prevention of recurrent caries in enamel and dentin—a major cause of failure of resin composite restorations.

SUMMARY

Objective: To develop hydrophilic resin-based surface coatings containing bioactive agents (proanthocyanidins from *Vitis vinifera* and calcium silicate) and assess their protective role at the dentin and enamel margins of cervical restorations against demineralization under simulated conditions of high and low caries activity. Methods: Suboptimal resin composite restorations were placed on cervical cavity preparations on buccal and lingual surfaces of thirty-two molars after a contamination protocol. Groups were divided according to the resin-based coatings (n=8): resin without bioactive (C), resin containing 2% enriched *Vitis Vinifera* (VVE), and resin coat containing 10% calcium silicate (CaSi). The control group did not receive a resin (NC). To simulate a hydrolytic-enzymatic degradation, specimens were subjected to 2-month storage followed by incubation in esterase at 37°C for 8 days. Afterwards, recurrent caries was induced using a pH-proteolytic model on half of the specimens to simulate high caries activity, and the other half remained in simulated body fluid (SBF). Measurements of cross-section

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http://doi.org/10.2341/20-299-L

microhardness (KHN) and infiltration with rhodamine-B assessed the micropermeability (MP), the extent of demineralization (ED), and the demineralization area (DA). Data were analyzed using analysis of variance (ANOVA) and post-hoc tests (α=0.05). Results: VVE and CaSi presented higher cross-sectional KHN values for enamel and dentin (p<0.001). The bioactive coatings resulted in lower MP, ED, and DA compared to NC (p<0.005) in enamel and dentin. CaSi coating preserved the enamel from demineralization (p=0.160). Conclusion: The application of bioactive coatings represents a potential strategy to protect the enamel-dentin margins of resin restorations.

INTRODUCTION

Recurrent caries is the main reason for the replacement of adhesive restorations (up to 59%), despite the advancements in restorative techniques and materials.[1,2] Randomized clinical trials have shown that cervical resin composite restorations represent a clinical challenge due to deterioration of marginal integrity, regardless of the adhesion strategy.[3] As a result, these restorations fail due to the development of recurrent caries and bulk or marginal fracture.[3,4]

Resin-based restorations are technique sensitive and do not tolerate contamination and mishandling. Suboptimal adhesive sealing associated with known biologically driven degradative factors may further compromise the integrity of the restoration interface.[5,6] The biologically driven degradation of the adhesive interface involves two important mechanisms—the degradation of collagen fibrils by host matrix metalloproteinases (MMPs)[7] and the hydrolysis of dental resins by bacterial and salivary esterases.[8,9] Hence, the by-products of biodegradation at the adhesive interface promote bacterial growth and proteins associated with biofilm formation and acid production, contributing to caries formation and development.[10,11]

The use of surface coatings has been identified as a potential strategy to seal microcracks and microgaps at the adhesive interface.[12] The resin coating forms a barrier-like film layer that protects the restoration from physical, chemical, and biological aggressions[13] and might reduce dentin demineralization.[14] The addition of bioactive agents to these coatings may further protect and assist in the repair tooth-restoration interface, and provide additional protection to the surrounding enamel and dentin. Thus, the objective of this *in vitro* study was to develop hydrophilic resin-based surface coatings containing bioactives (proanthocyanidins from *Vitis vinifera* and calcium silicate) and assess their protective role at the dentin and enamel margins of cervical restorations under simulated conditions of high and low caries activity. The hypotheses were that under conditions of high and low caries activity the release of bioactive agents contributes to the prevention of recurrent caries development by maintaining mineral homeostasis around margins of cervical restorations. Also, the bioactive agents can decrease the micropermeability and demineralization of the enamel and dentin adjacent to the restorations.

METHODS AND MATERIALS

Synthesis of Bioactive Resin-based Coatings

A hydrophilic resin-based formulation was developed to release bioactive materials due to high degradability in the oral environment. The formulation of the experimental hydrophilic coating was obtained from pilot tests and previous formulations of an experimental neat resin.[15] Concentrations of 34.55 wt% 2.2-*bis* [4-(2-hydroxy-3-methacryloylpropoxy)]-phenylpropane (*Bis*-GMA), 15.08 wt% triethyleneglycol dimethacrylate (TEGDMA), 0.075 wt% camphorquinone (CQ), 0.3 wt% 3 ethyl dimethyl-4-aminobenzoate (EDMAB), 40 wt% 2-hydroxyethyl methacrylate (HEMA), and 10 wt% ethanol were used for the formulation of the experimental coating. The concentration of hydrophilic monomer (HEMA) was established to increase the hydrolytic degradation of the coating. Also, the effect of the addition of solvent (10% ethanol) and air evaporation (2 minutes before light curing) was assessed. This formulation was determined to allow the gradual release of bioactive agents to the tissues adjacent to the restoration.

The investigated bioactive compounds were: oligomeric proanthocyanidin-enriched (OPACs) extract from grape *Vitis Vinifera* seeds (VV_E)[16] and calcium silicate (CaSi).[17] Thus, hydrophilic resin coatings were formulated as follows: Coating-resin without bioactive; VV_E-coating containing 2% (w/w) VV_E; CaSi-coating containing 10% (w/w) calcium silicate.

Preparation of Resin Composite Restorations and Coating Treatment

Thirty-two sound human third molars were selected from a pool of extracted teeth stored at −20°C for no more than 6 months. Sections 4.0 mm above and below the cementoenamel junction (CEJ) were made using a diamond blade (Series 15LC Diamond Saw, Buehler, Lake Bluff, IL, USA). Cervical cavity preparations of 4.0 mm width × 3.0 mm length × 1.5 mm depth were prepared at the CEJ using a flat-end cylinder diamond bur (#557D, 1-mm diameter, medium grit, ISO 15223,

Brasseler USA Dental, Savannah, GA, USA) attached to a high-speed handpiece with water irrigation. Enamel and root dentin margins were prepared at a 90° angle to the tooth surface, and burs were replaced by new ones every five preparations. Cavity preparations were assigned to experimental groups (n=16) and further divided into subgroups (n=8) of either high- or low-activity condition.

A contamination protocol[18,19] was employed to create suboptimal adhesive interfaces, allowing better visualization of the effect of bioactive coatings in critical areas. Fresh saliva was collected from one of the investigators at the same time of day, 2 hours after food intake.[19] The restorative protocol consisted of etching with 32% phosphoric acid (Scotchbond Universal etchant, 3M Oral Care, St Paul, MN, USA) the enamel and dentin for 30 and 15 seconds, respectively, followed by water rinsing for 30 seconds and drying with an absorbent tissue. The collected saliva (0.1 mL) was applied to the dentin surface[18] using a microbrush for 20 seconds, followed by air drying for 5 seconds.[19] A layer of adhesive (Adper Single Bond Plus, 3M Oral Care) was actively applied on the contaminated surface, air dried to remove the excess of solvent, and light cured for 20 seconds (1200 mW/cm^2, Elipar, Deep Cure-S, 3M Oral Care). Resin composite (Filtek Supreme Ultra - shade A2B, 3M Oral Care) was inserted in three increments, and each layer was light cured for 20 seconds. All restorations were polished using a slow-speed handpiece with aluminum oxide disks (Soft-Lex Pop-On, 3M Oral Care) and then stored in distilled water at 37°C for 24 hours. Teeth were sectioned axially in the mesial-distal direction to separate the buccal and lingual restorations (Figure 1).

The specimens were coated with nail polish, except for the restorations and 2 mm around the restorations. Experimental resin coatings were applied up to 1 mm beyond the restoration margins and light cured for 20 seconds. An additional group had no coating application.

Aging Protocol

All specimens were subjected to 2-month storage in SBF (5 mM HEPES, 2.5 mM $CaCl_2$, 0.05 mM $ZnCl_2$, and 0.3 mM NaN_3) at 37°C[20]; replaced every 2 weeks. After SBF storage, an enzymatic-driven resin degradation protocol was carried out using 30 U/mL of porcine liver esterase (PLE, Sigma-Aldrich, St. Louis, MO, USA) in 0.2 M phosphate buffer (pH 7.2) at 37°C in a shaker for 8 days.[21] The esterase solution was renewed every 3 days.

High and Low Caries Activity Simulation

After the simulated aging protocol was completed, the specimens were divided into two subgroups: high and low activity. The restorations on the buccal surface were allocated to the high-activity group and those on the lingual surface to the low-activity condition. The high-activity group was exposed to periods of imbalance of de-remineralization associated with dentin-targeted proteolytic processes.

In demineralization processes, the progression of dentin loss increases, with the presence of collagen degrading proteases present in the oral cavity. Therefore, the protocol included a 16-day pH/proteolytic cycle of 7-hour immersion in demineralization buffer (50 mM acetate, 2.25 mM $CaCl_2 \cdot 2H_2O$, 1.35 mM KH_2PO_4, 130 mM KCl, pH 5.0), followed by 17-hour immersion in neutral buffer (20 mM HEPES, 2.25 mM $CaCl_2 \cdot 2H_2O$,

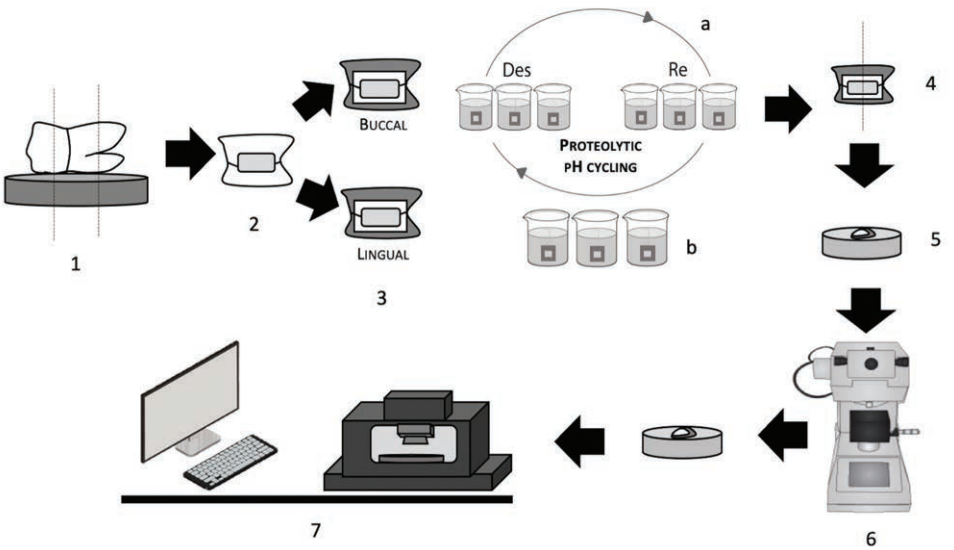

Figure 1. *Schematic of the experimental design. (1) Sections of the cervical portion of the tooth. (2) Cavity preparation with margins in enamel and dentin. (3) Surface blockage of areas away from the margin of restorations with nail polish. (a) Simulated high caries activity: buccal restorations follow pH-enzymatic/proteolytic cycles; (b) simulated low caries activity: lingual restorations were kept in simulated body fluid. (4) Restorations were sectioned in halves; (5) Specimens embedded in epoxy resin; (6) cross-sectional evaluation of microhardness (KHN), and (7) permeability at and around the margins of the restorations.*

1.35 mM KH_2PO_4, 130 mM KCl, pH 7.0) at 37°C.[22,23] Periods in demineralization buffer for 96 hours at 37°C,[22,23] were employed in order to guarantee lesion formation. For the biodegradation of the dentin matrix by exogenous protease, bacterial collagenase (*Clostridium histolyticum* 100 μg/mL; Sigma-Aldrich) was added to the neutral buffer.[22,23] All solutions were prepared daily. The specimens assigned to the low-activity group (lingual restorations, n=8) remained stored in SBF during the same period (remaining as control), without de-remineralization and dentin-targeted proteolytic challenges.

Assessment of Caries—Cross-section Microhardness (KHN)

The development of artificial recurrent caries was determined by the assessment of cross-section microhardness (KHN) measurements of enamel and dentin[22,24] (n=5). The buccal and lingual restorations were sectioned in half (IsoMet 1000, Buehler). The sections were embedded in epoxy resin and polished (EcoMet 3000, Buehler) sequentially using 600-, 800-, and 1200-grit silicon carbide paper. The KHN of enamel and dentin adjacent to the cervical restoration was measured using Knoop indentation (LM700AT hardness tester, Leco Corporation, Michigan, USA) at a 25 g load force for 15 seconds.[22] Indentations were performed at four depths from the outer surface of the tooth (enamel/dentin) (20, 40, 60, and 80 μm) at three distances from the restoration boundaries (20, 60, and 120 μm) (Figure 2A). Three measurements were performed at each depth, and the average was obtained for each distance.

Assessment of Interfacial Micro-permeability and Demineralization Around Restoration—Fluorescence Microscopy

A qualitative and quantitative evaluation of the enamel-dentin/resin margins was assessed by a permeability method using overnight incubation in rhodamine-B fluorescent dye solution (100 ml of 0.1-mM rhodamine B in phosphate buffer, pH 7.2).[25] After elapse time, specimens were rinsed in running water for 1 minute and dried with absorbent paper. Specimens were examined immediately under a fluorescence microscope (DMI 6000 B. Leica, Buffalo Grove, IL,

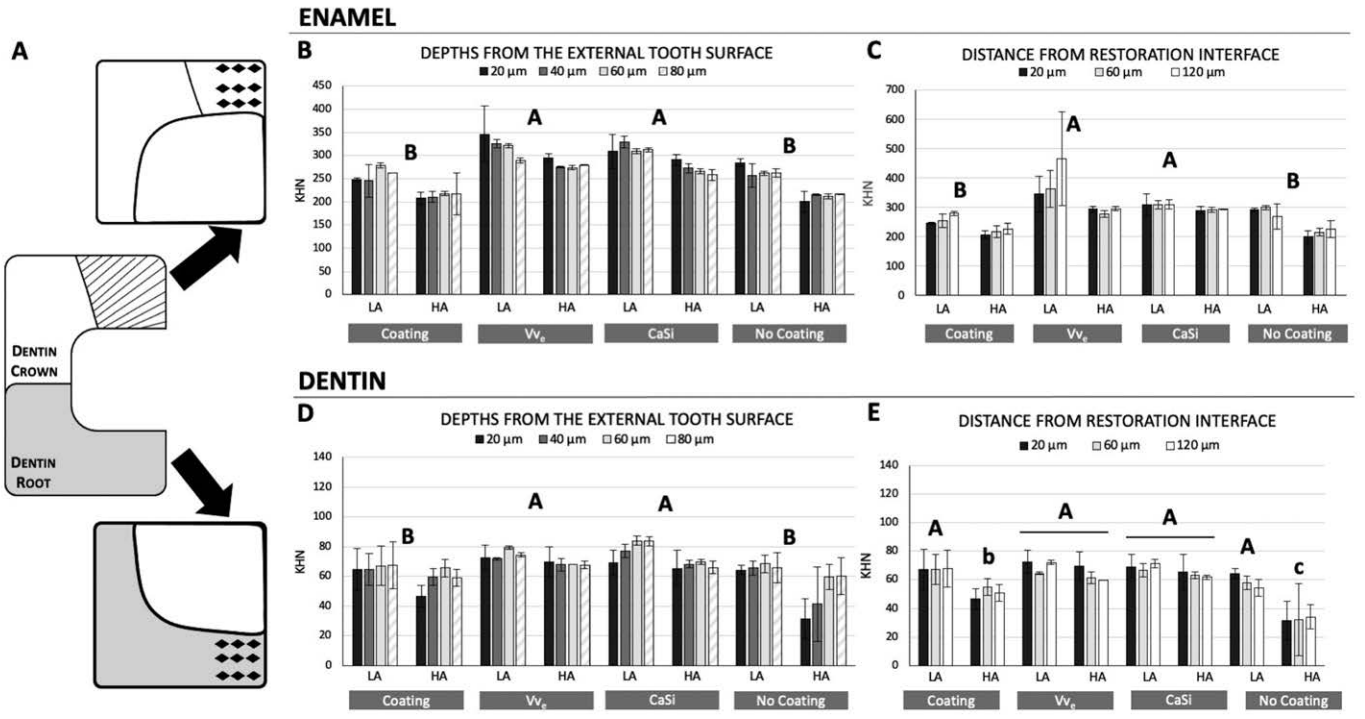

Figure 2. *Enamel and dentin microhardness (KHN). (A) Schematics of cross-sectional Knoop microhardness (KHN) measurements on enamel and dentin surrounding the restoration. KHN values of enamel (B) and dentin (D) treated with resin coatings as a function of depths (20, 40, 60, and 80 μm) from the outer surface to the enamel-dentin junction and inner dentin root. KHN values of enamel (C) and dentin (E) treated with resin coatings as a function of distance from the restoration interface (20, 60, and 120 μm). Different uppercase letters indicate differences between resin coatings (*p<0.05*). Different lowercase letters represent differences between low- (LA) and high-caries activity (HA) groups when the interaction effect between caries activity and resin coatings is significant (p<0.05). Bars represent lack of statistical difference between the low- and high-caries activity conditions (*p>0.05*).*

USA) using a connected digital camera (Hamamatsu, Skokie. IL, USA) and LAS AF software (Leica). The exposure time was 9.10 ms, gain: 30, FIM: 30%, IL-FID: 6 for red channel, and exposure of 25.28 ms, gain: 0, IL-FID: 9 using red emission and differential interference contrast (DIC) channels.

The margins of the restorations were identified in DIC images, and the measurements were done in fluorescence images.[25,26] After image acquisition of enamel and dentin, rhodamine infiltration was analyzed using the ImageJ software 1.48p (National Institutes of Health, Bethesda, MD, USA). Fluorescence emission intensity (FEI) was converted into numerical values, and values were calculated. Data analysis included quantification of interfacial micropermeability, the extent of demineralization depth from the restoration margin, and the demineralization area.

The interfacial micropermeability was measured using a parallel-line profile traced along with the adhesive interface, using ImageJ tools (Figure 3A).[26] The extent of demineralization from the restoration margin was measured by drawing a line parallel to the extent of rhodamine infiltration in the long axis of the tooth, within 300 μm from the margins of the restoration (Figure 3B). This evaluation verified if the coating protected the adhesive interface and the tissue adjacent to the restoration. Additionally, the area of infiltrated rhodamine-B was quantified to evaluate the influence of experimental coatings in the dentin and enamel adjacent to the restoration (Figure 3C).

Statistical Analysis

The assumption of homogenous data distribution was confirmed with Levene test and found not to be met for all the obtained results ($p<0.001$). The data were analyzed using three-way analysis of variance (ANOVA) for KHN and two-way ANOVA for FEI data, followed by Games-Howell post-hoc tests ($\alpha=0.05$) using SPSS software (SPSS V25, IBM Corp, Armonk, NY, USA).

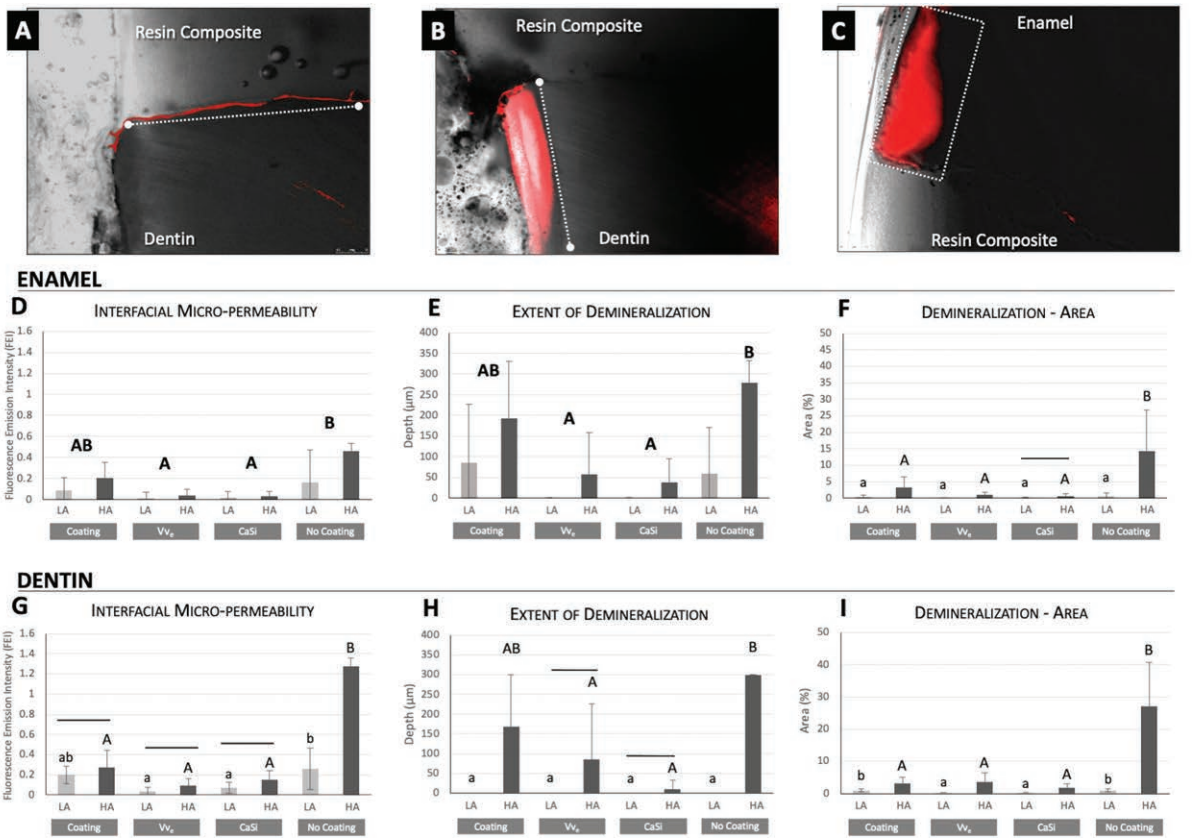

Figure 3. *Fluorescence microscopy representative images and results. Images depict measurements made on enamel and dentin margins to determine the (A) interfacial micropermeability, (B) extent of demineralization from the restoration margin, and (C) demineralization area. Results of the experimental groups after 2-month storage and pH-proteolytic cycling, under high- (HA) and low-caries activity (LA) conditions: (D,G) micropermeability at the tooth-restoration interface (%FEI), (E,H) the extent of demineralization from the restoration (mm), and (F,I) demineralization area (%). Different uppercase letters indicate differences between resin coatings* ($p<0.05$). *Different lowercase letters represent differences between low-caries activity treatment groups when the interaction effect between caries activity and resin coatings are present* ($p<0.05$). *Bars represent a lack of statistical difference between low- and high-caries activity* ($p>0.05$).

RESULTS

Degradation of Experimental Hydrophilic Resin-based Coatings

The experimental coating formulation containing 40% HEMA resulted in a significantly higher degradation as compared to the 6% HEMA concentration (p<0.001, Figure 4A). It was also observed that the mass loss (%) increased over 2 months (p=0.001). Knowing that the technique of applying ethanol-based resin can influence the degradation of the material, it was observed that the application of air evaporation of the solvent did not change the mass loss among groups; H40, with no ethanol; H40_10E (0), with 10% ethanol and immediate light-curing polymerization (no air evaporation); and H40_10E (2), 2-minutes air drying (p=0.070), even after 2 months (p=0.605, Figure 4B). Therefore, the selected base formulation was H40_10E (2): 2-minutes air drying to perform the hydrophilic resin coating.

Assessment of Enamel and Dentin Microhardness (KHN)

The cross-sectional KHN values of enamel and dentin are presented in Figure 2. Considering the KHN values of the depth from the external surface of the tooth (20, 40, 60, 80 mm), there was no significant interaction between all the variables tested (caries activity, depth, and experimental coatings) in enamel (p=0.561) and dentin (p=0.317). Overall, high activity resulted in lower values of cross-sectional KHN when compared to low activity, in both enamel and dentin (p<0.001). The groups with bioactive coatings (VVE and CaSi) resulted in significantly higher KHN for enamel and dentin (p<0.001) than coating and no-coating groups (Figure 2B,D). These KHN values refer to the depth of caries from the external surface of the tooth and thus can better explain the effects of the coatings that were applied on the restoration and the surrounding enamel/dentin.

The cross-sectional KHN values from restoration interface (20, 60, 120 mm) revealed the effect of coatings along with the internal wall of the restorations. No significant interaction was found between the caries activity groups, distance from restoration and experimental coatings for enamel (p=0.566), and dentin (p=0.910). In the enamel, VV_E and CaSi coatings presented significantly higher KHN values (p<0.001). Interestingly, there was a significant interaction between caries activity and experimental coating in the dentin (p=0.016). VV_E and CaSi coatings preserved the initial dentin KHN values even after high caries activity conditions, while coating and no-coating groups showed a reduction in KHN values when exposed to a high caries activity (Figure 2C,E).

Assessment of Interfacial Micropermeability and Demineralization Underlying Restoration

The results obtained from enamel margins are presented in Figure 3D-F. Results from the demineralization area in enamel showed significant interaction between the variables: experimental coating and caries activity (p<0.001). Although no significant differences were found among groups under a low-activity protocol (p=0.505), in high activity, the presence of coating showed less demineralization area when compared to the no-coating group (p<0.001). Only the CaSi bioactive coating preserved the enamel area in both the caries activity conditions (p=0.160) (Figure 3F). In enamel, no significant interaction between studied factors was

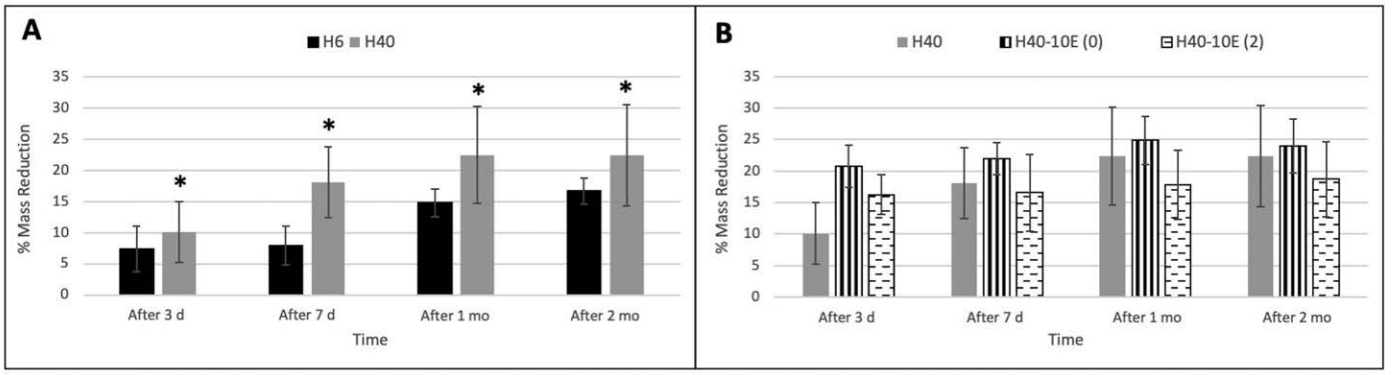

Figure 4. *Mass reduction of formulated resin coatings. (A) Initial results showed a higher mass reduction (%) for the most hydrophilic coating, containing 40% HEMA (H40) when compared to 6% HEMA (H6) (*p*<0.001), with a gradual increase over the 2 months (*p*=0.001). Asterisks (*) depict a statistical difference between the groups. (B) After the incorporation of ethanol (ETOH) and the use of solvent evaporation techniques, no difference was observed in the mass reduction (%) of developed coatings (*p*=0.070); H40-10E (0): coating with 40% HEMA + 10% ETOH (without solvent evaporation), H40-10E (2): 40% HEMA + 10% ETOH (with 2-minutes long solvent evaporation) or H40: adhesive with only 40% HEMA, even after 2 months (*p*=0.605).*

found for interfacial micropermeability (p=0.124) and the extent of demineralization (p=0.078). Bioactive coatings (VV_E and CaSi) reduced micropermeability (p<0.001) and extent of demineralization (p=0.001), when compared to the no-coating group (Figure 3,D-E).

In dentin, there was a significant interaction between the resin coatings and caries activity for interfacial micropermeability (p=0.003), the extent of demineralization (p<0.001), and area of demineralization (p<0.001). Different from the enamel, the application of bioactive coatings (VV_E and CaSi) had an effect under the low-activity condition, significantly reducing the demineralization area (p<0.001) and micropermeability (p=0.006), when compared to the no-coating group. In the high-activity condition, all coatings (Coating, VV_E, and CaSi) significantly decreased interfacial micropermeability (p=0.001) and dentin demineralization area (p<0.001) (Figure 3G,I). All the coatings prevented an increase of interfacial micropermeability when exposed to high-activity conditions, different from the no-coating group (p=0.02). Under high-activity conditions, less extension of demineralization was found in groups containing bioactive coatings (VV_E and CaSi) than in the no-coating group (p<0.001) (Figure 3H). However, none of the resin coatings was able to fully prevent the formation of recurrent caries.

DISCUSSION

Bioactive coatings with the potential to modify and protect the dental substrates may represent an effective strategy to prevent interfacial micropermeability and recurrent caries development around restorations. In this study, bioactive coatings assisted with preserving the KHN of the dentin and enamel surrounding the restoration. Hence, bioactive resin coatings contributed to the stability of the dentin and enamel without significant demineralization and an increase of interfacial micropermeability of suboptimal resin-based restorations. Therefore, the study hypotheses were confirmed.

The use of surface coatings has been shown to prevent tooth demineralization[14] and provide a seal of microcracks and microgaps in the resin.[12] Hence, the action of resin-based physical barrier[27] against root caries development is conditional to the wear resistance of the coating agent.[14] Also, greater exposure of the teeth to demineralization and by-products accelerates the coating's organic and inorganic degradation. Herein, an *in vitro* pH cycling model was designed to simulate high-activity conditions and reproduce the dynamic variations in mineral saturation and pH associated with the natural caries process.[28] Enzymatic degradation that occurs due to the presence of saliva esterases, microorganisms,[21] and proteolytic degradation[29] were also simulated under de-remineralization cycles.[22,23] As observed in this study, the enzymatic and proteolytic challenge induced a more significant dentin demineralization on uncoated or nonbioactive coating groups (Figure 3).

The effectiveness of the bioactive coating sustaining mineral homeostasis was assessed by detection of micropermeability. The penetration of a fluorescent dye into porosities of demineralized enamel/dentin enables quantitative analysis under fluorescence microscopy.[30] Higher micropermeability and demineralization values were observed under high-activity conditions compared to low activity (Figure 3). Also, the KHN results confirmed changes in the mineral gain or loss among the experimental conditions. A suboptimal adhesive interface[18,19] was utilized to simulate critical clinical challenges. Given the conditions evaluated in this study, the use of bioactive coatings (VV_E and CaSi) enhanced the protection of surface coatings on enamel and dentin surrounding restorations. Higher KHN values and lower micropermeability at the enamel adjacent to the restoration were observed with bioactive coatings, regardless of the bioactive agent (Figures 2 and 3).

Proanthocyanidins (PACs) are plant-derived dentin biomodification agents able to stabilize the organic matrix, improve mechanical properties,[31,32] and promote dentin remineralization.[22] Specifically, oligomeric PACs elicit greater bioactivity.[25] PACs' role on mineral homeostasis was observed earlier under a root dentin remineralization *in vitro* model.[33] Also, PACs from an enriched fraction of grape seed extract, when incorporated into adhesive systems, inhibited recurrent caries and contributed to the protection of the dentin-resin interface under a microbial model.[25] VV_E coating resulted in high KHN when compared to uncoated or nonbioactive surfaces (Figure 2). Also, reduced micropermeability and demineralization were observed for VV_E group (Figure 3).

Selective PAC-rich extracts preserve the integrity of the dentin and induce collagen crosslinks in the dentin extracellular matrix.[16] Furthermore, PACs decrease collagen digestibility by inhibiting proteases such as endogenous metalloproteinases.[34] These factors may have contributed to the slow progression of demineralization in the root dentin/enamel (Figure 3). The VV_E increases crosslinking of the collagen network, which represents a contributing factor preventing acid diffusion and mineral loss. Additionally, the calcium-binding ability of certain PAC compounds may contribute to mineral deposition during the remineralization process.[33] Previous studies have shown

that this process is associated with mineral deposition on the most superficial portion of the lesion, forming insoluble complexes in the presence of a remineralizing solution (pH 7.4).[22] These insoluble complexes have been shown to remain visually stable at pH in the range of 2.0-7.0.[22, 25]

Calcium silicate (CaSi) based materials assist in the formation of apatite,[35,36] increase the resistance to demineralization of dentin[36,37]; and for these reasons, have been used in dentistry as an endodontic sealer and for vital pulp therapy.[38] The tricalcium silicate was incorporated in the hydrophilic coating, because it releases calcium and forms hydroxyapatite crystals adjacent to the material on hydration, dissolving slowly in SBF solution[39,40] or oral fluids.[37] Ionic exchange of Na^+ or K^+ with H^+ or H_3O^+ occurs when tricalcium silicate materials are in contact with the SBF solution. A silica hydrogel [$Si(OH)_4$] layer is formed from this ionic exchange, increasing the solution's pH value. As the pH increases (alkalinizing activity), the silica hydrogel portion from the bioactive silica-based materials dissolves, leading to the breakdown of Si-O-Si bonds and silanol group formation (Si-OH). Thus, these silanol groups condense to create a SiO_2-rich layer on the surface. Ca^{2+} and PO_4^{2-} ions that cross a SiO_2-rich layer form heterogeneous nucleation of the initial calcium phosphate.[41] Over time, this process continues and is believed to help close gaps within the material-tooth interface.[42]

Caustic erosion of the dentin is caused due to the high alkaline nature of the calcium silicate-based materials, which helps penetrate the dentinal tubules and aggregate into the dentin. During this process, hydration promotes the expansion of tricalcium silicate, resulting in a good sealing ability.[42] These factors may explain the high KHN values in the enamel and dentin around the restoration protected with CaSi resin coatings (Figure 2). Additionally, the CaSi coating preserved the enamel from demineralization and did not allow the increase of micropermeability and extent of demineralization in the dentin (Figure 3). Energy-dispersive X-ray spectroscopy (EDX) composition of depth profile and IR analysis confirm that reactive calcium silicate placed in close contact with demineralized tissue helps in the formation of apatite by remineralizing without phosphorus,[37] reaching several depths of the dentin.[36] The presence of long parallel tags with minimal signs of degradation, and additional deposition of calcium and silicate particles into the dentinal tubules was shown to preserve the hybrid layer's integrity (resin-dentin interface).[17] However, our study results did not show significant differences in micropermeability after using CaSi coating compared to other coatings (Figure 3).

Based on the findings of this *in vitro* study, the mechanical barrier of a resin-based coating is not enough to prevent demineralization around restorations. The low caries activity condition does not promote major changes in cross-sectional KHN and tooth demineralization (micropermeability, demineralization from the surface, and demineralization area). However, the simulation of high caries activity can better demonstrate the influence of bioactive coatings (VV_E and CaSi) in the enamel and dentin. The release of bioactive materials, such as CaSi and VV_E, from the resin coating is a strategy that may enhance the prevention of recurrent caries in dental tissues. These study findings spotlighted the importance of caries activity assessment to provide high-risk patients with additional preventive measures against recurrent caries development.

CONCLUSION

Hydrophilic resin-based coatings containing calcium silicate or proanthocyanidins are potential strategies to prevent the development of recurrent caries. Bioactive resin coatings increased KHN, decreased micropermeability, and demineralization in enamel and dentin adjacent to resin composite restorations.

Acknowledgments

This work was supported in part by research grants from the National Institutes of Health [DE021040] and FAPEMA (Maranhão Research Grant Foundation) [BPD - 08173/17].

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies. The use of extracted human teeth was approved by Institutional Review Board of the University of Illinois at Chicago (protocol # 2018-0226).

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article. The authors alone are responsible for the content and writing of this paper.

(Accepted 25 May 2021)

REFERENCES

1. Nedeljkovic I, Teughels W, De Munck J, Van Meerbeek B, &

Van Landuyt KL (2015) Is secondary caries with composites a material-based problem? *Dental Materials* **31(11)** e247-e277.

2. Eltahlah D, Lynch CD, Chadwick BL, Blum IR, & Wilson NHF (2018) An update on the reasons for placement and replacement of direct restorations *Journal of Dentistry* **72** 1-7.
3. Kubo S, Yokota H, Yokota H, & Hayashi Y (2013) Challenges to the clinical placement and evaluation of adhesively-bonded, cervical composite restorations *Dental Materials* **29(1)** 10-27.
4. Beck F, Lettner S, Graf A, Bitriol B, Dumitrescu N, Bauer P, Moritz A, & Schedle A (2015) Survival of direct resin restorations in posterior teeth within a 19-year period (1996-2015): A meta-analysis of prospective studies *Dental Materials* **31(8)** 958-985.
5. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, & Katz JL (2010) Adhesive/dentin interface: The weak link in the composite restoration *Annals of Biomedical Engineering* **38(6)** 1989-2003.
6. Kermanshahi S, Santerre JP, Cvitkovitch DG, & Finer Y (2010) Biodegradation of resin-dentin interfaces increases bacterial microleakage *Journal of Dental Research* **89(9)** 996-1001.
7. Pashley D, Tay F, Yiu C, Hashimoto M, Breschi L, Carvalho RM, & Ito S (2004) Collagen degradation by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.
8. Delaviz Y, Finer Y, & Santerre JP (2014) Biodegradation of resin composites and adhesives by oral bacteria and saliva: A rationale for new material designs that consider the clinical environment and treatment challenges *Dental Materials* **30(1)** 16-32.
9. Huang B, Siqueira WL, Cvitkovitch DG, & Finer Y (2018) Esterase from a cariogenic bacterium hydrolyzes dental resins *Acta Biomaterialia* **71** 330-338.
10. Khalichi P, Cvitkovitch DG, & Santerre JP (2004) Effect of composite resin biodegradation products on oral streptococcal growth *Biomaterials* **25(4)** 5467-5472.
11. Sadeghinejad L, Cvitkovitch DG, Siqueira WL, Merritt J, Santerre JP, & Finer Y (2017) Mechanistic, genomic and proteomic study on the effects of BisGMA-derived biodegradation product on cariogenic bacteria *Dental Materials* **33(2)** 175-190.
12. Rizzante FAP, Bombonatti JSF, Vasconcelos L, Porto TS, Teich S, & Mondelli RFL (2019) Influence of resin-coating agents on the roughness and color of composite resins *Journal of Prosthetic Dentistry* **122(3)** 332.e1-332.e5.
13. Zhao X, Pan J, Malmstrom HS, & Ren YF (2016) Protective effects of resin sealant and flowable composite coatings against erosive and abrasive wear of dental hard tissues *Journal of Dentistry* **49** 68-74.
14. Tajima K, Nikaido T, Inoue G, Ikeda M, & Tagami J (2009) Effects of coating root dentin surfaces with adhesive materials *Dental Materials Journal* **28(5)** 578-586.
15. Farina AP, Cecchin D, Vidal CMP, Leme-Kraus AA, & Bedran-Russo AK (2020) Removal of water binding proteins from dentin increases the adhesion strength of low-hydrophilicity dental resins *Dental Materials* **36(10)** e302-e308.
16. Leme-Kraus AA, Phansalkar RS, Dos Reis MC, Aydin B, Sousa ABS, Alania Y, McAlpine J, Chen SN, Pauli GF, & Bedran-Russo AK (2020) Dimeric proanthocyanidins on the stability of dentin and adhesive biointerfaces *Journal of Dental Research* **99(2)** 175-181.
17. Aggarwal V & Bhasin SS (2018) Application of calcium silicate materials after acid etching may preserve resin-dentin bonds *Operative Dentistry* **43(5)** E243-E252.
18. Duarte SJ, Lolato AL, de Freitas CR, & Dinelli W (2005) SEM analysis of internal adaptation of adhesive restorations after contamination with saliva *Journal of Adhesive Dentistry* **7(1)** 51-56.
19. Cobanoglu N, Unlu N, Ozer FF, & Blatz MB (2013) Bond strength of self-etch adhesives after saliva contamination at different application steps *Operative Dentistry* **38(5)** 505-511.
20. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjaderhane L, Nishitani Y, Carvalho RM, Looney S, Tay FR, & Pashley DH (2010) The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices *Dental Materials* **26(11)** 1059-1067.
21. Park JG, Ye Q, Topp EM, Kostoryz EL, Wang Y, Kieweg SL, & Spencer P (2008) Preparation and properties of novel dentin adhesives with esterase resistance *Journal of Applied Polymer Science Symposium* **107(6)** 3588-3597.
22. Xie Q, Bedran-Russo AK, & Wu CD (2008) *In vitro* remineralization effects of grape seed extract on artificial root caries *Journal of Dentistry* **36(11)** 900-906.
23. Zamperini CA & Bedran-Russo AK (2018) Remineralization potential of mints containing bioactive agents in artificially induced root caries *Caries Research* **52(4)** 331-338.
24. Pedrosa VO, Flório FM, Turssi CP, Amaral FL, Basting RT, & França FM (2012) Influence of pH cycling on the microtensile bond strength of self-etching adhesives containing MDPB and fluoride to dentin and microhardness of enamel and dentin adjacent to restorations *Journal of Adhesive Dentistry* **14(6)** 525-534.
25. Kim GE, Leme-Kraus AA, Phansalkar R, Viana G, Wu C, Chen SN, Pauli GF, & Bedran-Russo A (2017) Effect of bioactive primers on bacterial-induced secondary caries at the tooth-resin interface *Operative Dentistry* **42(2)** 196-202.
26. Trevelin LT, Villanueva J, Zamperini CA, Mathew MT, Matos AB, & Bedran-Russo AK (2019) Investigation of five α-hydroxy acids for enamel and dentin etching: Demineralization depth, resin adhesion and dentin enzymatic activity *Dental Materials* **35(6)** 900-908.
27. Tsuchiya S, Nikaido T, Sonoda H, Foxton RM, & Tagami J (2004) Ultrastructure of the dentin-adhesive interface after acid-base challenge *Journal of Adhesive Dentistry* **6** 183-190.
28. White DJ (1995) The application of *in vitro* models to research on demineralization and remineralization of the teeth *Advances in Dental Research* **9(3)** 175-193.
29. Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhaes AC, Pereira JC, & Buzalaf MA (2012) Impact of protease inhibitors on dentin matrix degradation by collagenase *Journal of Dental Research* **91(12)** 1119-1123.
30. Gonzalez-Cabezas C, Fontana M, Dunipace AJ, Li Y, Fischer GM, Proskin HM, & Stookey GK (1998) Measurement of enamel remineralization using microradiography and confocal microscopy. A correlational study *Caries Research* **32(5)** 385-392.

31. Leme AA, Vidal CM, Hassan LS, & Bedran-Russo AK (2015) Potential role of surface wettability on the long-term stability of dentin bonds after surface biomodification *Journal of Biomechanics* **48(10)** 2067-2071.

32. Matuda LS, Marchi GM, Aguiar TR, Leme AA, Ambrosano GM, & Bedran-Russo AK (2016) Dental adhesives and strategies for displacement of water/solvents from collagen fibrils *Dental Materials* **32(6)** 723-731.

33. Pavan S, Xie Q, Hara AT, & Bedran-Russo AK (2011) Biomimetic approach for root caries prevention using a proanthocyanidin-rich agent *Caries Research* **45(5)** 443-447.

34. Koide T & Daito M (1997) Effects of various collagen crosslinking techniques on mechanical properties of collagen film *Dental Materials Journal* **16(1)** 1-9.

35. Profeta AC (2014) Preparation and properties of calcium-silicate filled resins for dental restoration. Part I: Chemical-physical characterization and apatite-forming ability *Acta Odontologica Scandinavica* **72(8)** 597-606.

36. Watson TF, Atmeh AR, Sajini S, Cook RJ, & Festy F (2014) Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: Biophotonics-based interfacial analyses in health and disease *Dental Materials* **30(1)** 50-61.

37. Gandolfi MG, Taddei P, Siboni F, Modena E, De Stefano ED, & Prati C (2011) Biomimetic remineralization of human dentin using promising innovative calcium-silicate hybrid "smart" materials *Dental Materials* **27(11)** 1055-1069.

38. Parirokh M & Torabinejad M (2010) Mineral trioxide aggregate: A comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action *Journal of Endodontics* **36(3)** 400-413.

39. Camilleri J, Sorrentino F, & Damidot D (2013) Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus *Dental Materials* **29(5)** 580-593.

40. Peng W, Liu W, Zhai W, Jiang L, Li L, Chang J, & Zhu Yaqin (2011) Effect of tricalcium silicate on the proliferation and odontogenic differentiation of human dental pulp cells *Journal of Endodontics* **37(9)** 1240-1246.

41. Dong Z, Chang J, Deng Y, & Joiner A (2011) Tricalcium silicate induced mineralization for occlusion of dentinal tubules *Australian Dental Journal* **56(2)** 175-180.

42. Solomon RV, Karunakar P, Grandhala DS, & Biragoni C (2014) Sealing ability of a new calcium silicate-based material as a dentin substitute in class II sandwich restorations: An *in vitro* study *Journal of Oral Research and Reviews* **6(1)** 1-8.