Effects of a coating resin containing S-PRG filler to prevent demineralization of root surfaces

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The purpose of this study was to evaluate the ability of a coating material containing the surface pre-reacted glass-ionomer (S-PRG) filler to protect the root from demineralization *in vitro*. The proprietary coating resin containing the S-PRG filler (PRG Barrier Coat) was applied to human root dentin and immersed in acid buffer at pH 4.5 for 3 d. Demineralization was evaluated by micro-CT scanning and the dentin-material interface observed by scanning electron microscopy. The ability of the coating resin to modify acid production by *Streptococcus mutans* was investigated by monitoring pH using an ion-sensitive field-effect transistor pH electrode. Application of PRG Barrier Coat produced a coating layer with the thickness of approximately 200 μm and completely inhibited demineralization. The bacteria-induced pH fall at the material surface was significantly inhibited. We conclude that S-PRG filler-containing coating resin may be an effective material for protecting exposed root from both chemical and biological challenges.

Keywords: Resin coating, Root caries, Demineralization, S-PRG

INTRODUCTION

Because of the increased awareness of oral hygiene and availability of dental care, as well as the improved preventive strategies, the elderly can now retain their teeth until much later in life[1]. However, gingival recession caused by normal aging, periodontal disease or traumatic toothbrushing habits renders the retained dentition susceptible to root surface caries[2-4]. Additionally, the risk for root surface caries is further increased with reduced salivary flow or xerostomia due to aging or medication[5]. According to the literature, the prevalence of root surface caries amongst non-institutionalized people over the age of 60 years in Western countries and Japan is higher than 50%[4].

Restoration of root surface caries can be problematic in many cases because of its proximity to the gingiva, which complicates the isolation and access of the site for placement of restorative materials[6]. Therefore, preventive measures are highly emphasized, and it is recommended that these measures should be taken immediately after exposure of root surfaces considering that lesion progression in roots is faster than that in enamel because of their relatively low mineral content[7,8]. Fluoride, which can interfere the physicochemical[9] and microbiological[10] processes of root caries initiation/development, is widely accepted as an effective preventative agent[11], and fluoride-containing mouthrinses, toothpastes and topical varnishes are recommended by clinicians as a prophylactic program to high-risk populations[12-15]. However, the effects of these preventative strategies are only temporary and successful prevention of root surface caries is usually hampered by the low compliance of the patients. Simple single-visit methods that can stop the development of root surface caries in the long-term are advantageous. Coating the root surface with a resin-based material that can release and recharge fluoride may be a simple and effective measure to protect it from physical, chemical and biological insults.

Surface pre-reacted glass ionomer (S-PRG) fillers are a novel class of particles that can be incorporated into resinous materials. They are prepared by an acid-base reaction between fluoroboroaluminosilicate glass and a polyacrylic acid aqueous solution[16]. A ligand exchange mechanism within the pre-reacted hydrogel endows S-PRG fillers with the ability to release and recharge fluoride[17-19]. In addition to fluoride, S-PRG fillers release multiple ions such as Al^{3+}, B^{-}, and Sr^{2+}, and may have efficacy in mineral induction[16,20]. Mukai *et al.* demonstrated that experimental denture base-resins containing S-PRG fillers have the capacity to substantially inhibit demineralization[21]. Recently, a resinous coating material containing S-PRG fillers became available for use in the prevention of caries, where it is hoped that it will enhance mineralization and reduce acidic attack by oral cariogenic bacteria. The purpose of this study was to evaluate the ability of this S-PRG filler-containing coating material to protect the root surface from demineralization *in vitro*.

The second and third authors contributed equally to this work.

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MATERIALS AND METHODS

Specimen preparation for micro-CT scanning

Six human extracted premolars or molars with intact roots were selected. The cementum was removed using a periodontal scaler to expose root dentin. The root was subsequently separated from the crown 1–2 mm below the cemento-enamel junction with a low-speed diamond saw (Isomet 2000, Buehler Ltd., Lake Bluff, IL, USA) under water coolant. Cubic root specimens (approximately 5 mm×5 mm×5 mm) were prepared and coated with four layers of an acid-resistant nail varnish, leaving a window of 3 mm×3 mm on the exposed dentin. The specimens were divided into two groups. Those in the experimental group were coated with the S-PRG filler-containing resinous coating material, PRG Barrier Coat (Shofu, Kyoto, Japan), according to the instructions of the manufacturer. Briefly, the powder and liquid were thoroughly mixed using a micro-brush, applied to the dentin surface, and cured by irradiation with a light activation unit (Optilux 501, Kerr Corporation, Orange, CA, USA) at the intensity of 500 mW/cm^2 for 10 s. The specimens in the control group were left uncovered. All specimens were immersed individually in 10 mL acidic demineralizing buffer solution (2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4, and 50 mmol/L acetic acid adjusted to pH 4.5 with NaOH)[22-24] for 3 d at 37ºC. The demineralization buffer was changed every 24 h. Three specimens were tested for each group.

Micro-CT scanning

For each specimen of the experimental group, micro-CT scanning was performed before and after application of coating material and after demineralization using a micro-focused X-ray CT (SMX-100CT-SV3, Shimadzu Ltd., Kyoto, Japan). The micro-CT system can generate polychromatic X-rays with a cone-beam geometry. A 0.2-mm-thick brass (Cu-Zn) filter was installed in the beam path to reduce beam-hardening effect[24,25]. The specimen was mounted on a computer-controlled turntable with the treated dentin surface parallel to the X-ray beam, and was covered with a wet cotton roll to prevent desiccation of the surface. The scanning was performed with the specimen being rotated 360° at rotation steps of 0.6°. The tube voltage was 90 kV at a current of 29 mA. The distance from X-ray source to the sample was 78.7 mm, and that from X-ray source to the detector was 694.3 mm. The three-dimensional image of each scanned specimen was reconstructed using commercial volume graphics software (VGStudio, Volume Graphics GmbH, Heidelberg, Germany) and cross-sectional images were obtained of the two perpendicular planes within the area where the material had been applied.

Scanning electron microscope (SEM) observation

The coating material was applied to the exposed dentin surface of the root as described above, and light-cured. The specimens were then embedded in epoxy resin and sectioned longitudinally through the center of the 'window'. The cross-sectional surfaces were polished using silicon carbide papers with increasing fineness (#120, 320, 600 and 1500 grit) under running water. After the specimens were fixed in half-strength Karnovsky's solution (2% paraformaldehyde and 2.5% glutaraldehyde, pH 7.4) and dehydrated in ascending grades of ethanol (50, 70, 80, 90, 95 and 100%), they were freeze-dried and coated with platinum. The interface of the coating material and root dentin was observed using scanning electron microscopy (JSM-5310 LV, JEOL, Tokyo, Japan; SEM). The thickness of the coating material covering the surface was measured on the SEM images (×100).

pH measurement at the interface between the bacteria and the coating material

The pH was measured by the method described by Mayanagi *et al.*[26]. *Streptococcus mutans* NCTC10449 was cultured at pH 7.0 and 37°C under anaerobic conditions (80% N_2, 10% H_2 and 10% CO_2). After centrifugation for 7 min at 16,000×*g* in a 1.5 mL tube, the bacterial pellets were collected and stored at 4°C before use. A polymethyl methacrylate (PMMA) plate (No.99997, Sanplatec, Osaka, Japan) was coated with PRG Barrier Coat according to the manufacturer's instructions and light-cured. A non-coated PMMA plate was used as control. For each experiment, a coated or non-coated plate was placed at the bottom of the well (4.0 mm in diameter and 2.0 mm in depth) in the experimental apparatus. An ion-sensitive field-effect transistor (ISFET) pH electrode (H^+ ion sensitive area, 2.0 mm in length, 1.0 mm in width and 0.2 mm in thickness; model PH-60T1; Nihon Koden, Tokyo, Japan) was placed on the plate. *S. mutans* cells were packed onto the plate and pH electrode using a spatula and syringe. The whole assembly was maintained at 37°C for 10 min, at which point 500 µL of 1% glucose was added. The pH at the interface between the bacteria and the material was monitored continuously at 37°C for 90 min, using the pH electrode attached to a chart recorder (LR 4220, Yokogawa Electric Corporation, Tokyo, Japan) in an incubator (Merck KGaA, Darmstadt, Germany). The tests were repeated three times for each material.

Statistical analysis

The differences in pH between the control and Barrier Coat groups at 90 min after glucose addition were evaluated by Student's *t* test at a significance level of 0.05.

RESULTS

Micro-CT analysis

Typical three-dimensional images of the experimental group before and after application of the material are shown in Figs. 1a, b. Figure 2a shows cross-sectional images of specimens following application of PRG Barrier Coat. Cured PRG Barrier Coat presented as a relatively opaque layer with scattered dense agglomerates (Figs. 1b, 2a). The cross-sectional image revealed that the thickness of the coating material was approximately

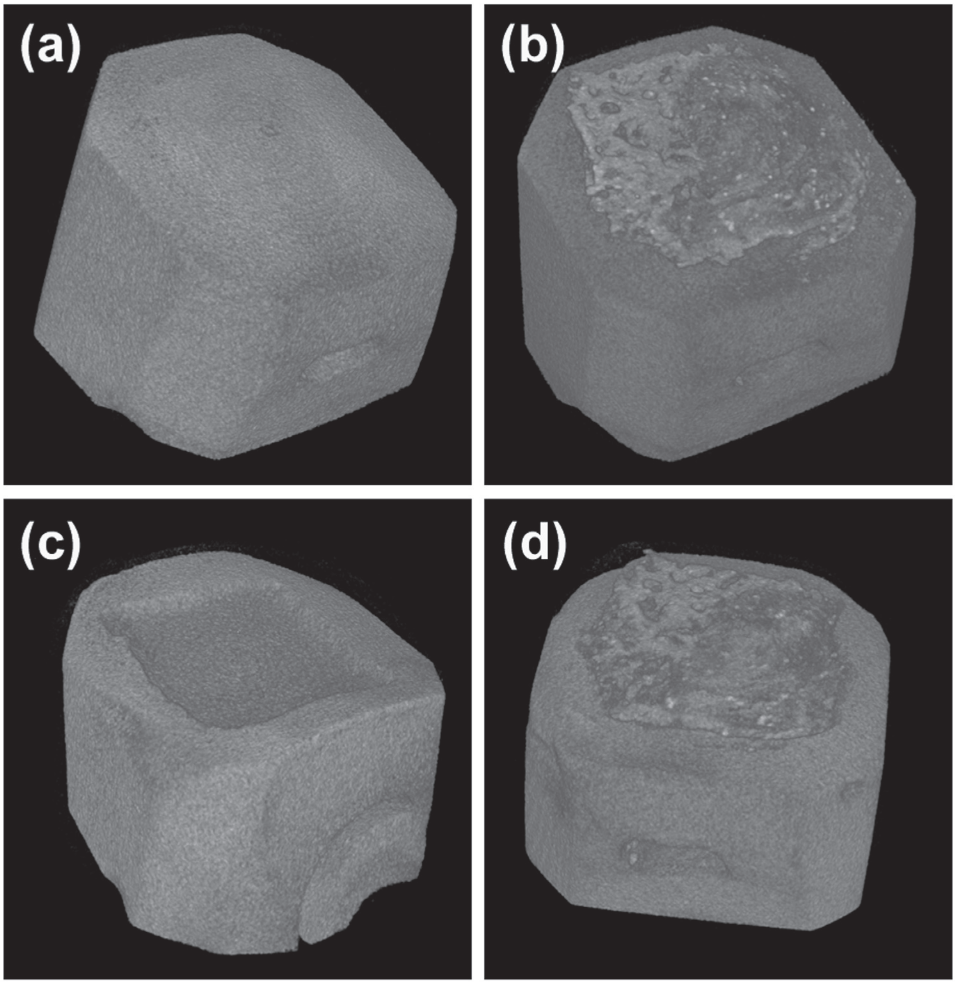

Fig. 1 Typical three-dimensional images reconstructed after micro-CT scanning.
a: before application of PRG Barrier Coat
b: after application of PRG Barrier Coat
c: after demineralization of non-coated control specimen
d: after demineralization of experimental specimen coated with PRG Barrier Coat

200 µm.

After demineralization for 3 d, a radiolucent concave-shaped superficial lesion with a depth of approximately 200 µm was observed in the specimens of the control group (Figs. 1c, 2b). In contrast, no sign of demineralization was observed from the three-dimensional and cross-sectional images of the root specimens coated with Barrier Coat (Figs. 1d, 2c), indicating that the resinous coating material based on S-PRG fillers completely protected the exposed root dentin from acid challenge. The same results were obtained for all of the three specimens tested.

SEM observation

Typical SEM images of the dentin-material interface are presented in Fig. 3. Coverage of the root dentin surface with a dense resinous layer can be observed. From the images with lower magnification, the coating thickness was confirmed to be around 200 µm. The exposed dentin was hermetically sealed with resinous material and no evident gap was observed at the interface.

Bacteria-induced pH fall at the interface

The pH fall induced by bacterial carbohydrate fermentation at the interface between *S. mutans* and the PRG Barrier Coat-coated or non-coated PMMA plate is shown in Fig. 4. The pH at the interface remained stable in the first 10 min, but subsequently started to fall. Compared with the control group, which exhibited a remarkable drop in pH after addition of glucose solution, the rate of pH decrease in specimens treated with PRG Barrier Coat was reduced. The pH at the interface between the bacteria and the material after 90 min of incubation was significantly higher in the PRG Barrier Coat group (5.13±0.17) than in the control group (4.61±0.14).

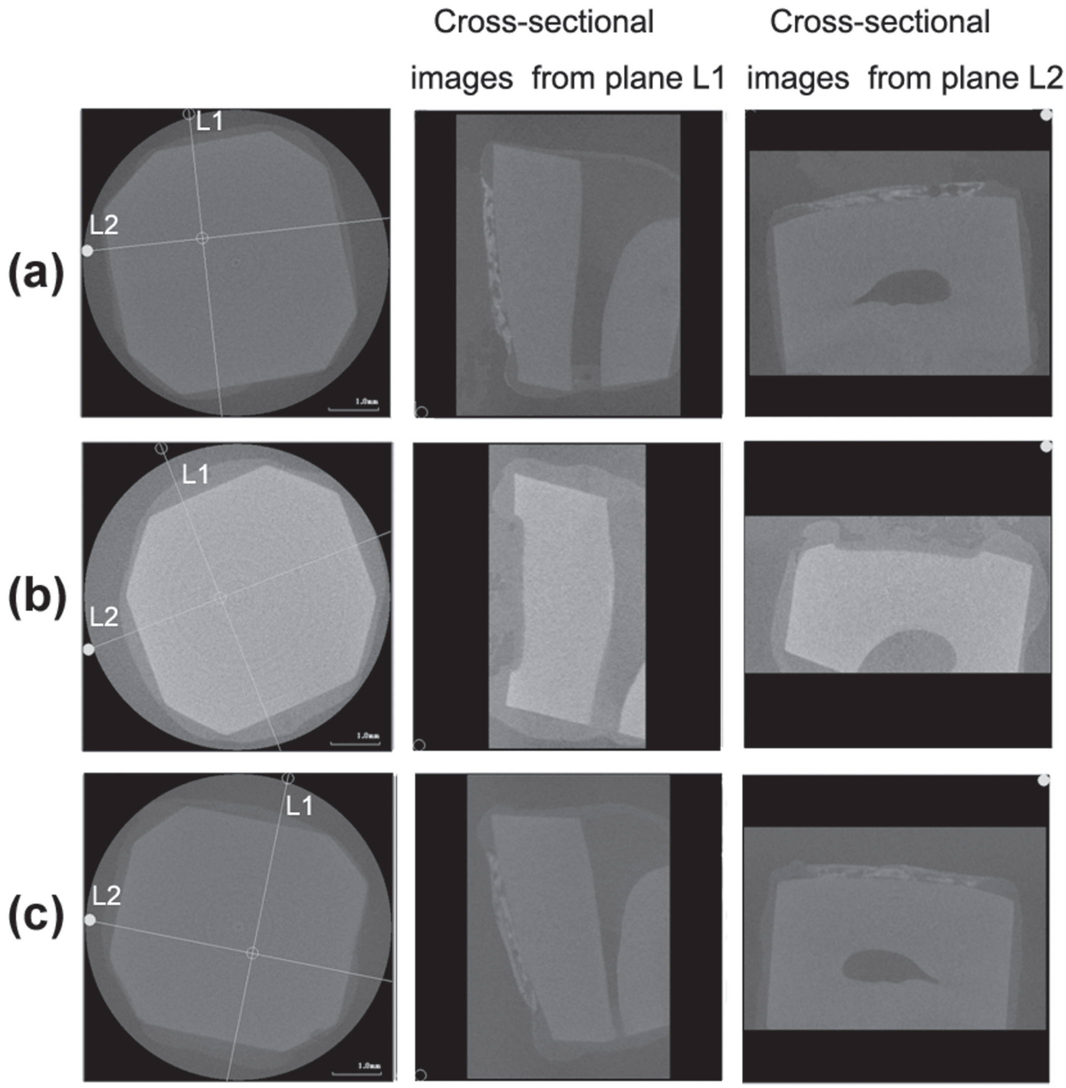

Fig. 2 Typical cross-sectional images generated from micro-CT scans.
The two lines, L1 and L2, represent the two perpendicular planes from which the cross-sectional images were obtained.
a: after application of PRG Barrier Coat
b: after demineralization of non-coated control specimen
c: after demineralization of experimental specimen coated with PRG Barrier Coat

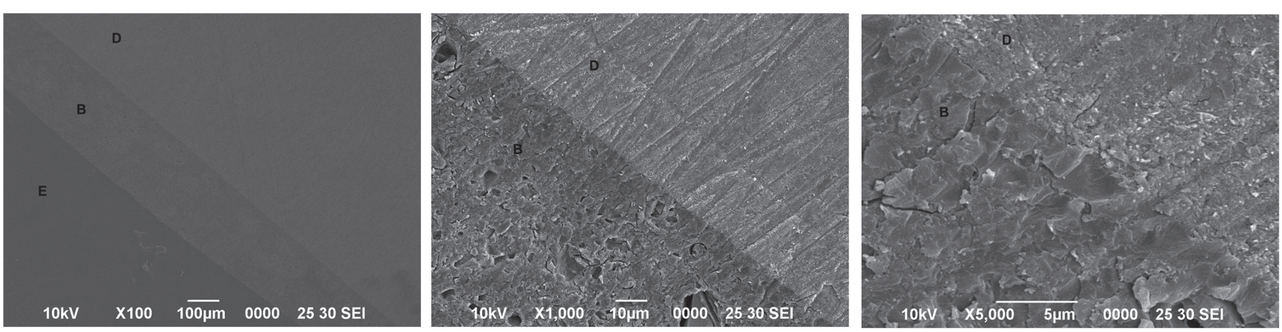

Fig. 3 SEM images of the interface between dentin and the PRG Barrier Coat layer (×100, ×1,000, ×5,000).
Key: B, PRG Barrier Coat; E, epoxy resin; D, dentin.

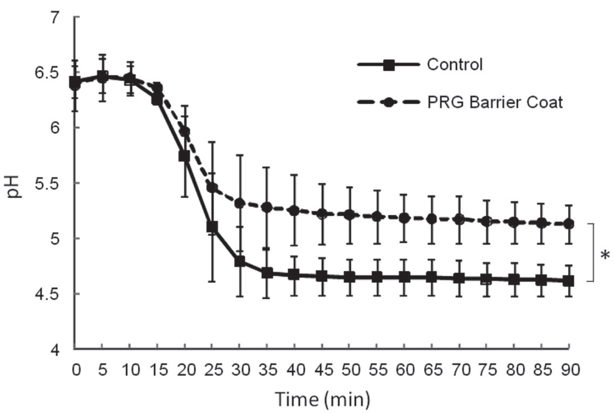

Fig. 4 Time-related pH changes at the interface between *S. mutans* and the PRG Barrier Coat-coated PMMA plate (PRG Barrier Coat) or non-coated PMMA plate (Control) after addition of glucose to stimulate acid production.
Data are mean±standard deviation of three independent experiments. *denotes a statistically significant ($p<0.01$, by Student's *t*-test) difference from the control at 90 min after glucose addition.

DISCUSSION

Exposure of root dentin after gingival recession is very common among the aged population because the thin cementum can be easily removed by intensive root planing or over-zealous toothbrushing[23]. Therefore, root specimens without cementum were used in the present study to investigate the possible protective effects of PRG Barrier Coat, a resinous material that releases multiple ions including F^-, Al^{3+}, B^-, and Sr^{2+}, against initiation of root caries. Demineralization of the tooth surface can be investigated through various methods including contact microradiography[27,28], polarized light microscopy[29], nanoindentation testing[24] and confocal laser microscopy[24,30], among which contact microradiography is accepted as the "golden standard" technique for the determination of mineral content and caries lesion depth. However, specimen preparation in all these methods is destructive and time consuming. In contrast to these conventional methods, micro-CT can provide three dimensional and cross-sectional images non-destructively, precisely and efficiently[31]. Moreover, by taking advantage of mineral reference phantoms, precise information of mineral density in teeth can be readily quantified[24,32]. In this study, micro-CT was adopted to study the possible protective effects of PRG Barrier Coat against acid challenge.

Adhesive systems, which can provide a physical barrier through the formation of a hybrid layer[33], have been advocated as an effective measure to protect exposed roots from acid attack. Self-etching adhesives are especially attractive clinically because of their low technique-sensitivity and easy application, which lead to reduced treatment time and thus less contamination. Despite some encouraging results demonstrating reduced severity of root surface lesions, coating with adhesives was unable to fully resist the demineralization induced by acid attack[23,27,28,30,34-37]. One reason for this phenomenon may be that the coating thickness of the adhesives was too thin to act as a substantive physical barrier against demineralization[27,28]. Kaneshiro *et al.* developed an experimental self-etching resin coating system by assembling a self-etching primer and an acrylate-based resin, and demonstrated that it can prevent acid demineralization on root surfaces by producing an appropriately thick coating with an integral hybridized layer[27]. Another problem with coating the root surface with adhesives is that modern one-step self-etching adhesives are highly permeable to water because of the incorporation of hydrophilic resin components into adhesive mixtures that lack additional hydrophobic resin coatings[38-40]. Furthermore, the permeability of adhesives coated onto root dentin can be further intensified under lactic acid attack[41]. Together, these evidences indicate an urgent need for the development of other simplified resinous materials that can effectively protect the exposed root surface from chemical and biological challenges.

The S-PRG filler-containing resinous coating material tested in this study dramatically inhibited demineralization in the acid challenge test. Several mechanisms may underlie the protective effects of PRG Barrier Coat. First, the hermetic sealing of the dentin surface with a relatively thick resinous layer, as supported by SEM observation, may act as a physical barrier to impede the ingress of acid buffer. Secondly, pH modulation by the S-PRG filler in acidic conditions may also contribute to its protective effects. It has been reported that S-PRG fillers, like other fluoride-releasing materials, could neutralize the storage media[20,42-44]. It is possible that the acidity of the buffer has been neutralized by the S-PRG fillers by the time it reaches dentin surface and thus has vastly diminished demineralization potential. The third explanation for the protective effect of PRG Barrier Coat against acid challenge may be that the acid resistance of the dentin surface underlying the coating material was enhanced after taking up multiple ions released from the material. It is well known that fluoride can improve the acid resistance of teeth by promoting the conversion of hydroxyapatite to fluoroapatite, and a recent study investigating a fluoride-releasing adhesive based on S-PRG fillers demonstrated that the adjacent dentin and enamel tissue took up fluoride from the material, resulting in a zone with enhanced resistance to acid attack[45,46]. Besides the formation of fluoroapatite, high concentrations of strontium released from S-PRG fillers[16,20,47] may further enhance acid resistance of teeth by converting hydroxyapatite to strontiumapatite[47-49]. Additionally, it has been reported that the eluates of resins filled with S-PRG fillers enhanced remarkably the formation of apatite on phosvitin-immobilized agarose beads in the presence of a mineralizing solution[16]. Therefore, the protective effects of PRG Barrier Coat

against chemical demineralization may also be related to the promotion of remineralization by the various ions released from the material.

The acidogenicity of cariogenic bacteria that results from carbohydrate fermentation underpins the initiation and development of root caries. Therefore, the inhibition of a bacteria-induced drop in pH at the bacteria:dentin interface is a prime target in providing protection against root caries. As revealed by our pH measurement tests, the S-PRG filler-containing coating resin significantly reduced the pH drop caused by bacteria after addition of glucose. This may be the result of fluoride release from the coating material. Fluoride can reduce acid production by *S. mutans* through a number of mechanisms[50], including inhibition of the glycolytic enzyme enolase[51,52] and the proton-extruding ATPase[53,54]. The reduced drop of pH on the surface of PRG Barrier Coat could also be related to interference with other intracellular or plaque-associated enzymes in *S. mutans*, such as acid phosphatase, pyrophosphatase, peroxidase and catalase by released fluoride[55]. S-PRG filler acts in a quasi-intelligent way such that its release of fluoride is acidity-dependent, and thus its protective effect is proportional to the threat being encountered[20,45,56,57]. In addition to its inhibitory effects on bacterial acid production, S-PRG filler-containing resins have been found to possess anti-plaque formation properties in the presence of saliva[58]. A "material film layer" has been observed on the surface of S-PRG filler-containing resins, and it is proposed that this film layer, which consists of Al^{3+}, Si^{4+}, Sr^{2+}, B^{-} and other ions released from S-PRG filler, can inhibit bacterial adhesion[59]. While the detailed mechanism is unclear, this combination of anti-acid production and anti-plaque effects would have obvious benefits in controlling caries attack.

In summary, the S-PRG filler-containing coating resin completely protected root dentin from acidic demineralization *in vitro* by forming a hermetic sealing layer with appropriate thickness. Moreover, this coating material was also capable of inhibiting a bacteria-induced drop in pH. These results indicate that coating root surfaces with the S-PRG filler-containing coating resin may be an effective measure in protecting exposed dentin against both chemical and biological challenges, and thus inhibit the initiation and development of root caries.

CONCLUSION

The present study investigated whether an S-PRG filler-containing coating resin could protect against root caries. It was found that the tested coating material formed a hermetic sealing layer on top of the exposed dentin surface, completely prevented acid buffer-induced demineralization, and inhibited the bacteria-induced drop in pH at the interface between the bacteria and the material. This protection may be related to the ability of the material to produce a physical barrier, modify pH and/or release various beneficial ions. In future studies, the effects of this material against biological challenge will also be examined using cariogenic bacteria culture techniques, and the wear resistance of this material and the durability of its protective effects will be further investigated.

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