Anti-demineralization characteristics of surface pre-reacted glass-ionomer (S-PRG) filler-containing varnishes

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This study investigated the anti-demineralization effects of surface pre-reacted glass-ionomer (S-PRG) filler-containing varnishes. Thirty-five bovine root specimens were divided into five treatment groups, with seven specimens each coated with 1) MI varnish (MIV), 2) F varnish (FV), 3) PRG varnish I (PV), 4) PRG varnish II (with sodium fluoride added, PVF), and 5) acid-resistant nail varnish (Control). A 3×1 mm area of the dentin surface adjacent to each varnish was demineralized for one week at 37°C. Integrated mineral loss (IML) of these lesions was determined by transverse microradiography, as was the amount of fluoride released by each material. IML was significantly lower in the PV and PVF groups than in the Control group, and was significantly lower in the PVF than in the MIV and FV groups. These findings indicated that S-PRG filler-containing varnishes, especially varnish containing sodium fluoride, had superior anti-demineralization effects on root dentin.

Keywords: Anti-demineralization, S-PRG filler, Root dentin, Transverse microradiography, Varnish

INTRODUCTION

Root caries are highly prevalent in middle-aged and older people1-4, making the prevention of root caries crucial in dentistry. Clinically, glass-ionomer cements and resin composites have been shown useful for restoring root cavities. However, because root caries lesions advance chronically and circularly at the gingival margins with extremely unclear borders, the removal of caries and restorative steps are often difficult, depending on the condition of areas of decay. Steps recommended for subjects at “high risk”, including those with active root caries lesions and those at risk of lesion progression, include brushing with toothpaste containing 5,000 ppm fluoride, frequent rinsing with solution containing 0.025–0.1% fluoride, and topical application of fluoride varnish two to four times a year or application of fluoride gel in trays5. Also, a combination method such as two daily tooth-brushing with a fluoride toothpaste (0.1%F) and swabbing with a 2% NaF solution for 2 min at every 2 months was reported6. Furthermore, a daily, self-applied 1% sodium fluoride gel in combination with restorative intervention was effective for root caries7.

Fluoride varnishes were developed primarily to prolong contact time between fluoride and teeth8. These varnishes, which have been shown to be toxicologically safe and easy to use, were found to prevent caries when re-applied regularly and combined with normal oral hygiene procedures8-10. A recently developed surface pre-reacted glass-ionomer (S-PRG) filler, which was shown to release aluminum (Al3+), borate (BO33−), sodium (Na+), silicate (SiO32−), strontium (Sr2+), and fluoride (F−) ions11, has been introduced into various dental materials. The materials constituting the filler provide excellent fluoride releasing and recharging abilities12-18. Moreover, resinous tooth-coating materials and denture base resins containing this filler were reported to have anti-demineralization effects when tested against bovine enamel14 and dentin15,16. Putative varnishes containing S-PRG filler, which can serve as reservoirs of fluoride and other ions, may substitute for conventional fluoride varnishes. The present study investigated the anti-demineralization activity of two S-PRG filler-containing varnishes on adjacent root dentin and compared the activity of these with conventional varnishes. The null hypothesis was that these S-PRG filler-containing varnishes have the same anti-demineralization effects on adjacent dentin as conventional varnishes.

MATERIALS AND METHODS

Preparation of experimental specimens

Dentin specimens were prepared as described15,17,18. The experimental procedures are outlined in Fig. 1. Briefly, lower central incisors from 2- to 3-year-old cattle were obtained from a slaughterhouse. Using a sectioning machine (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA), these teeth were separated into 5-mm-thick root cylinders, each of which was cut in half longitudinally using a diamond-coated wire sectioning machine (Well type 3242, Walter Ebner, Mannheim, Germany). Experimental surfaces, approximately 5×4 mm in size, were prepared by cutting 1 mm from the buccal and lingual root surfaces with the same machine. The surfaces were polished with #2,000 waterproof abrasive paper (Fuji Star, Sankyo Rikagaku, Saitama, Japan) and cleaned ultrasonically for 5 min with deionized water. Thirty-five bovine root dentin specimens with flat surfaces were prepared.
Table 1 Varnishes tested in this study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Varnish</th>
<th>Main ingredients (Manufacturer information)</th>
<th>Lot number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIV</td>
<td>MI varnish</td>
<td>5% sodium fluoride, CPP-ACP, surface-treated silica, silicon dioxide/ethyl alcohol, ethoxyethanol/polyvinyl acetate, rosin (hydrogenated)/L-menthol</td>
<td>1308012</td>
<td>GC America</td>
</tr>
<tr>
<td>FV</td>
<td>F varnish</td>
<td>5% sodium fluoride, anhydrous sodium dihydrogen phosphate, light anhydrous silicic acid/ethyl lactate/ester gum, rosin/saccharin, aluminum lake</td>
<td>206GA</td>
<td>Bee Brand Medico Dental</td>
</tr>
<tr>
<td>PV</td>
<td>PRG varnish I</td>
<td>S-PRG filler (mean dia. 3 μm, Filler contents: 40 wt%)/solvent/rosin/viscosity modifier</td>
<td>021414</td>
<td>Shofu</td>
</tr>
<tr>
<td>PVF</td>
<td>PRG varnish II</td>
<td>S-PRG filler (mean dia. 3 μm, Filler contents: 40 wt%), 5% sodium fluoride/solvent/rosin/viscosity modifier</td>
<td>021414F</td>
<td>Shofu</td>
</tr>
</tbody>
</table>

S-PRG filler: Surface pre-reacted glass-ionomer filler.

**Varnish application**

Twenty-eight pieces of masking tape, each with a 3×1 mm rectangular hole, were prepared, with one each affixed to the flat dentin surface of the specimens. The four types of varnish used in this study are shown in Table 1. Using a brush exclusive to each varnish, the four experimental varnishes were each applied to dentin, exposed by the 3×1 mm rectangular holes in the masking tape, of seven samples each. Because the masking tape was approximately 70-μm-thick, the thickness of each applied material was approximately 70 μm. Seven specimens each were coated with 1) MI varnish (MIV: 5% NaF+ CPP-ACP; GC America, Alsip, IL, USA), 2) F varnish (FV: 5% NaF; Bee Brand Medico Dental, Osaka, Japan), 3) PRG varnish I (PV: 40% S-PRG filler; Shofu, Kyoto, Japan), 4) PRG varnish II (PVF: 5% NaF+ 40% S-PRG filler; Shofu), and 5) acid-resistant nail varnish (Control). PV and PVF were novel varnishes, and others were available commercially. The remaining surface area was left exposed for demineralization.

**Demineralization**

Seven specimens of each of the five groups were placed at the bottom of a polypropylene container (5-059-01, AS ONE, Osaka, Japan), to which were added 40 mL 8% methylcellulose gel (Methocel MC, Sigma-Aldrich, St. Louis, MO, USA). After 24 h, 70 mL of acid buffer (50 mM acetic acid, 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, pH 5.0) were added to each container. All containers were maintained for one week at 37°C.

**Transverse microradiography (TMR) analyses**

Three 300-μm-thick sections were cut perpendicularly to the experimental surface of each sample using the diamond-coated-wire sectioning machine. The sections were placed on a Perspex holder in a droplet of water.
and covered with thin polyester sheets to avoid dentin shrinkage\textsuperscript{19}. Together with an aluminum step wedge of 13 steps, ranging from 0 to 300 μm in thickness, the sections were radiographed on a high-resolution glass film plate (High-Resolution Plate, Konica Minolta, Tokyo, Japan) with a nickel-filtered Cu-Kα source operated at 15 mA and 25 kV for 20 min (PW3830, Spectris, Surrey, UK). The radiographic images were analyzed using a microscope/video camera/microcomputer setup and software (TMR2000, Inspektor Research System, Amsterdam, The Netherlands)\textsuperscript{20}. The 300 μm-area adjacent to the material was analyzed. Parameters measured included integrated mineral loss (IML: vol%×μm), lesion depth (LD: μm), and mineral content \(\text{vs. LD}^{21,22}\).

Measurement of released fluoride
Twenty-four pieces of masking tape, approximately 70-μm thick, each with a round hole 6 mm in diameter were affixed to small plastic sheets. Each of the four experimental varnishes was applied to the sheets exposed by six of the round holes to a thickness of approximately 70 μm using an exclusive brush. Each plastic sheet coated with varnish was immersed in 5 mL of deionized water for seven days at 37°C, followed by the addition to each of 0.5 mL total ionic strength adjustment buffer (TISAB III, Thermo Electron, Beverly, MA, USA). Fluoride concentrations were measured with a combination fluoride electrode (Orion 9609BNWP ionplus Sure-Flow Fluoride, Thermo Fisher Scientific, Waltham, MA, USA) connected to a fluoride-ion meter (Orion STAR A214, Thermo Fisher Scientific).

Statistical analysis
IML and LD in the five groups and fluoride concentrations in four groups were compared by one-way ANOVA, followed by Tukey's post hoc comparison tests. All statistical analyses were performed using a statistical software package (SPSS-PC software version 10.1, SPSS Japan, Tokyo, Japan), with \(p<0.05\) defined as statistically significant.

RESULTS

Representative TMR images and profiles
Figure 2 shows TMR images (a) and mineral profiles (b) of representative specimens in each experimental group. The TMR image of the Control sample showed an unclear surface layer, and the representative mineral profile showed a low and indistinct peak near the surface and severe lesions. In contrast, the TMR images of the MIV, FV, PV and PVF samples had clearer surface layers than that of the Control sample, with the PV and PVF samples especially showing well-defined surface layers. The mineral profiles of the MIV, FV, PV and PVF samples showed distinct peaks, with a high volume percent of minerals in the near-surface region at depths of about 10 μm.

Average mineral profiles
The average mineral profiles of the five groups of samples are shown in Fig. 3. The surface mineral peak of the Control samples was extremely low, accompanied by severe lesions. In contrast, the surface mineral peak of the MIV coated samples was almost 25 volume percent, the peaks of the FV and PV samples were 30–40 volume percent and the peak of the PVF group was over 40 volume percent. In addition, the lesion bodies in the MIV and FV groups were 10–15 volume percent, whereas those in the PV and PVF groups were almost 20 volume.
percent. Mineral volumes in the PV and PVF groups were higher than those in the MIV and FV groups at depths of 0–100 μm.

**IML and LD**
Table 2 shows intergroup comparisons of mean IML and LD of the seven sections in the five experimental groups. IML was significantly lower in the PV (3,386±292) and PVF (2,855±419) groups than in the Control group (4,282±483) ($p<0.05$ each). Furthermore, IML was significantly lower in the PVF than those in the MIV (3,982±314) and FV (3,982±314) groups ($p<0.05$ each), but there were no significant differences between the PV and PVF groups ($p>0.05$). The LD in the PVF (243±36) group was significantly smaller than LDs in the FV (320±43) and PV (301±15) groups ($p<0.05$), but did not differ significantly from LDs in the Control (268±39) and MIV (285±43) groups ($p>0.05$).

**Fluoride release**
Mean concentration of released fluoride was significantly lower in the PV than in the MIV, FV and PVF groups ($p<0.05$, Table 3).

**DISCUSSION**
This study showed that IML was significantly lower in samples covered with PVF, one of the experimental S-PRG filler-containing varnishes, than in samples covered with the conventional varnishes MIV and FV ($p<0.05$). In contrast, IML in samples covered with PV as

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**Table 2**  Intergroup comparisons of integrated mineral loss (IML) and lesion depth (LD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>IML (vol%×μm)</th>
<th>LD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4,282 (483)a</td>
<td>268 (39)a,b</td>
</tr>
<tr>
<td>MIV</td>
<td>3,835 (474)a,b</td>
<td>285 (43)a,b</td>
</tr>
<tr>
<td>FV</td>
<td>3,982 (314)a,b</td>
<td>320 (43)a</td>
</tr>
<tr>
<td>PV</td>
<td>3,386 (292)a,b</td>
<td>301 (15)a</td>
</tr>
<tr>
<td>PVF</td>
<td>2,855 (419)c</td>
<td>243 (36)b</td>
</tr>
</tbody>
</table>

Mean (±SD), $n=7$
Values with the same superscript letters did not show significant differences between groups.
The IML of PV and PVF were significantly lower than that of Control ($p<0.05$). Furthermore, the IML of PVF was significantly lower than those of MIV and FV ($p<0.05$). There were no significant differences between PV and PVF ($p>0.05$).
The LD of PVF was smaller than those of FV and PV ($p<0.05$), however, was not different from those of Control and MIV ($p>0.05$).

**Table 3**  Measurement of mean released fluoride concentration for 7 days (ppm F)

<table>
<thead>
<tr>
<th>Groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MIV</td>
<td>6.0 (0.18)a</td>
</tr>
<tr>
<td>FV</td>
<td>6.1 (0.63)a</td>
</tr>
<tr>
<td>PV</td>
<td>1.5 (0.20)b</td>
</tr>
<tr>
<td>PVF</td>
<td>6.2 (0.085)a</td>
</tr>
</tbody>
</table>

Mean (±SD), $n=6$
Mean values in the same column having the same superscript are not significantly different ($p>0.05$).
another experimental varnish, did not differ significantly from samples covered with MIV and FV (p>0.05). The amount of fluoride released by MIV, FV and PV were significantly greater than the amount released by PV. Thus, the null hypothesis was rejected, in part.

The functions of ions released from S-PRG filler were summarized[20], that is, F− ion: fluoroapatite production, antibacterial effect, remineralization of demineralized lesions, Sr2+ ion: improvement of bone formation and mineralization, Al3+ ion: suppression of hypersensitivity, SiO32− ion: remineralization of tooth, BO33− ion: antibacterial effect, promotion of bone formation.

Although PV released a lower quantity of F− ion than MIV and FV, their IMLs did not differ significantly, suggesting that multi-ions released by the S-PRG filler played an important role in anti-demineralization. S-PRG fillers release F− and Sr2+ ions, which react with hydroxyapatite to form fluoroapatite, fluoridated apatite, and/or strontium apatite incorporated at the calcium (Ca) site in hydroxyapatite (HAP)[24]. In addition, BO33−, Sr2+, Na+, Al3+, SiO32− and F− ions released by these fillers have buffering capacity and protective effect on enamel demineralization[25]. Sr2+ ion released from glass-ionomer cement was shown to be involved in the remineralization process of caries dentin[26]. Also, the remineralization was affected by F− ion, when they were used in conjunction in enamel[27]. By the same mechanism, Sr2+ ion released from S-PRG filler may play roles in the acid resistance and remineralization in dentin[28]. These fillers also release SiO32− ion, and silica and hydroxyapatite nanoparticles have been reported to infiltrate into demineralized dentin, acting as seeds within the collagen matrix[29]. Although the amount of F− ion released by PVF was significantly higher than that released by PV, their IMLs did not differ significantly, indicating that the addition of fluoride to PRG varnish I was not essential for inhibition of dentin demineralization.

The LD of PVF coated lesions was significantly shallower than the LDs of lesions covered with FV and PV (p<0.05), but was not significantly different from those of lesions covered with Control varnish and MIV (p>0.05). LD may therefore depend on the pH of the solution rather than on F− ion concentration[30].

The TMR images of PV and PVF samples showed especially clearer surface layers than those of the other samples. Because Sr2+ ion was incorporated into the Ca site in HAP of dentin after immersion in the S-PRG filler eluate[20], Sr2+ ion was probably incorporated into these surface areas in PV and PVF samples. However, radiopacity of Sr2+ ion incorporated may influence the image of TMR. Further study will be required on this point.

The delivery of fluoride has been shown to be effective in preventing and reversing primary active root lesions[8,31,32]. Rinsing and toothbrushing with amine fluoride dentifrice twice a day significantly remineralized active soft and leathery primary root caries lesions[31]. Moreover, daily use of a fluoride mouthrinse significantly increased the number of reversed lesions[32], and dentifrice containing 5,000 ppm F was significantly better at remineralizing primary root caries than dentifrice containing 1,100 ppm F[33]. Professional application of fluoride varnish, a procedure common in many European countries, is regarded as a supplement for individuals at risk of caries[34]. The application of topical fluoride varnish to enamel application was effective in reversing white spot lesions after debonding and should be considered a routine caries prevention measure after orthodontic treatment[35].

The S-PRG filler-containing varnishes used in this study consisted of S-PRG filler, solvent, rosin and a viscosity modifier. In general dental varnishes, rosin is dissolved in organic solvents, with drying resulting in the formation of a coating on the tooth surface. High amounts of fluoride ion are released immediately following the application of conventional varnishes to root dentin surfaces, with the amount decreasing over time (data not shown). In contrast, fluoride ions released from S-PRG filler-containing varnishes are thought to be reabsorbed in the materials, similar to S-PRG filler-containing denture base resins[36], resulting in constant release of fluoride ions from these varnishes.

According to information supplied by its manufacturer, MI varnish contains casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), which is thought contributes to its anticariogenic and remineralization activities[37-40]. However, this investigation found that the IML of MIV was similar to those of FV and PV, and significantly higher than that of PVF. Further investigations should compare S-PRG fillers and CPP-ACP.

CONCLUSION

The S-PRG filler-containing varnish with 5% sodium fluoride could better inhibit demineralization of root dentin adjacent to the coated surface than could conventional varnishes.

ACKNOWLEDGMENTS

This research was financially supported by Shofu. The sponsor had no control over the interpretation, writing or publication of this work.

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