Original Article

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Effectiveness of different coated materials on root caries prevention under simulated tooth brushing: an *in vitro* study

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Objective: Sealing tooth root surfaces with coating material is expected to represent an effective approach to preventing root caries. The aim of this study is to investigate the effect of tooth brushing on the efficacy of different coating materials in root caries prevention.

Materials and Methods: The experiment was conducted on root dentin specimens under simultaneous demineralization conditions. Fifty specimens were used in this study. The specimens were divided into 5 groups: Group 1 (control group), no treatment; Groups 2-3, Clinpro White Varnish application with or without brushing; and Groups 4-5, PRG Coat Barrier application with or without brushing. A multispecies biofilm model was formed on specimens that were exposed to 1% sucrose for 10 days. Then, the % *cell viability*, bacterial density, % surface microhardness loss, and lesion depth were assessed using a confocal laser scanning microscope, microcomputed tomography, and Knoop microhardness tester, respectively.

Results: The *Kolmogorov–Smirnov test* revealed a *normal* distribution of all data. Differences within and between mean values for groups were statistically analyzed using one-way ANOVA and Dunnett's T3 post hoc test, respectively. No significant differences in bacterial density and biofilm thickness were found among the groups. A difference in the %surface microhardness loss was noted in the control group with relatively higher %cell viability compared with the other groups. The deepest carious lesion was found in the control group. The shallowest carious lesion was found in the PRG Coat Barrier-coated dentin-without brushing group. Brushing only increases the lesion depth on Clinpro White Varnish-coated dentin.

Conclusions: Applying either Clinpro White Varnish or PRG Coat Barrier has the benefits of reducing the depth of root caries and increasing the hardness of demineralized root dentin. Brushing reduces the efficacy of Clinpro White Varnish.

Keywords: root caries, prevention, fluoride varnish, PRG coating material, tooth brushing, biofilm formation

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Introduction

The world's elderly population is growing rapidly. Exposure of the tooth root surface is found more frequently as a result of aging [1]. The root surface is considered to be highly susceptible to bacterial adhesion and acid attack [2]. Bacterial species typically associated with root caries, such as mutans streptococci, *Lactobacillus* spp., and *Actinomyces* spp., can be detected [3].

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The acidity in dental plaque generated by sugar metabolism may cause demineralization, resulting in the development of root caries [4]. Additionally, other risks, such as oral, medical, behavioral and social factors, are reportedly associated with root caries development [5]. Preventive treatments seem to be beneficial for decreasing the progression and initiation of root caries [6].

During the last three decades, fluoride varnishes have been recognized as effective strategies for caries prevention. Fluoride plays an important role in inhibiting mineral loss and enhancing the mineral gain of calcium and phosphate in the form of fluorohydroxyapatite, which is more acid-resistant than hydroxyapatite. In addition, fluoride also interacts with bacterial enzymes and leads to reduced acid production, resulting in the prevention of demineralization [7]. The use of fluoride varnish for caries control primarily occurs through its topical effect on demineralization and remineralization mechanisms [8]. The application of fluoride varnishes has helped reduce the prevalence of root caries [9].

Currently, a coating material with the bioactive properties of pre-reacted glass ionomer (PRG) filler particles has been developed. This material enhances the anticaries properties of both antibacterial and remineralization processes [10,11]. The application of this coating material onto root surfaces is beneficial for caries prevention [11].

The effect of tooth brushing on coating materials has been reported [12,13]. Different coating materials may have differences in resistance of mechanical properties which lead to the effect of caries prevention.

It was very interesting to determine the effectiveness of either a fluoride varnish or a PRG coating material as a root coating surface to improve the acid resistance and antibacterial properties of coated root surfaces and to investigate the effect of tooth brushing on the effectiveness of these materials. The aims of the present study were to assess the effect of a fluoride varnish and a PRG coating material on multispecies biofilm formation, the microhardness of root dentin and artificial root caries formation. In addition, the effect of tooth brushing on the effectiveness of these materials was investigated.

Materials and Methods

Ethical approval for this study was obtained from the ethics committee in human research (Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board; MU-DT/PY-IRB) (COE. MU-DT/PY-IRB 2022/027.2305 and 2022/033.0706). The experiment was designed to evaluate the anticaries effect of a fluoride varnish (Clinpro White Varnish, 3M ESPE, Seefeld, Germany) and a PRG coating material (PRG Barrier Coat, Shofu, Kyoto, Japan) on root dentin under simultaneous demineralization and the effect of tooth brushing when studying the effectiveness of these materials. The multispecies biofilm model was formed on root dentin specimens coated with either Clinpro White Varnish or PRG Barrier Coat with or without tooth brushing to investigate the percentage of cell viability (%cell viability), bacterial density, biofilm thickness, carious lesion depth, change in mineral density and percentage of surface microhardness loss (%SHL) for carious lesions (Figure 1).

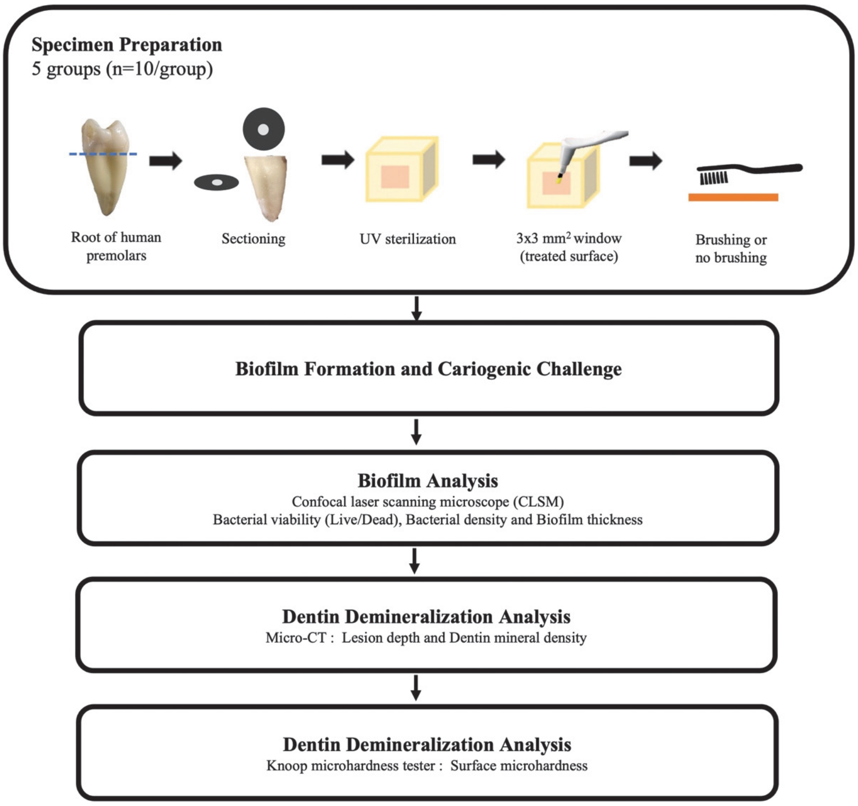

Figure 1 Schematic diagram illustrating the experimental design

Specimen preparation

Fifty premolars extracted from *orthodontic reasons* have stored in 0.1% thymol solution were employed. Flat root dentin specimens were prepared from the cervical to middle of the root by cutting the roots with a low-speed diamond saw (Isomet^{TM}; Buehler, Evanston, IL, USA) to obtain a 6 mm x 6 mm rectangular specimen with 1 mm thickness. The depth of the prepared root dentin was located at the middle of the root dentin. The specimens were polished with 600-, 800-, 1,000-, 1,200-, 2,000-, 4,000- and 5000-grit silicon carbide papers (Buehler, Buehler Ltd, Lake Bluff, IL, USA) with 50 strokes for each grit by finger pressure and polished with diamond paste from 6 microns down to 0.25 microns (DP-Paste, Struers A/S, Copenhagen, Denmark). The specimens were cleaned in an ultrasonic distilled water bath for 10 minutes to remove debris. The initial surface microhardness of the specimens was determined using a Knoop diamond indenter (Future-Tech Corp *FM-700*, Tokyo, Japan) with a 5-g load for 5 seconds. Three indentations were randomly created per sample. The average of three hardness values was calculated and used for further calculations.

All the polished specimens were then covered with nail varnish (Revlon Nail Enamel, Revlon, NY, USA) by leaving a 3 x 3 mm window of exposed root dentin to determine the carious lesion depths. The covered surface served as a reference surface. All the specimens were sterilized in a UV chamber for 90 minutes per side and further divided into 5 groups of 10 specimens.

One group was preserved as a control group without any treatment, and the remaining four groups were used as experimental groups, which were treated with either Clinpro White Varnish for 2 groups or PRG Barrier Coat for

2 groups according to the manufacturer's instructions (Table 1). To apply Clinpro White Varnish, a thin layer of varnish was applied onto the root surface, and the coated surface was left to dry for 1 minute. For the PRG Barrier Coat, 1 drop of activator was mixed with 1 drop of base. The mixture was then applied onto the root surface and left for 3 seconds without any disturbance. The coated surface was light-cured for 10 seconds with a blue visible light curing unit (LED Bluephase; Ivoclar Vivadent, Schaan, Liechtenstein). Coated specimens were left in 100% relative humidity environments for 24 hours. The uncoated specimens in the control group were subjected to demineralize without brushing, while other coated specimens were subjected to demineralize with and without brushing.

Half of the experimental groups from each coating material group were subjected to tooth brushing with a superspiral original head toothbrush (Systema, Lion Corporation Ltd., Bangkok, Thailand) and Colgate Total Charcoal Deep Clean toothpaste (Colgate-Palmolive, Chonburi, Thailand) with a load of 200 g at a frequency of 100-120 strokes per minute for 400 strokes using a brushing machine (TBS-V8, King Mongkut's Institute of Technology, Bangkok, Thailand) to represent 10 days of brushing twice per day [14].

Multispecies biofilm formation and Cariogenic challenge

Saliva samples were collected from 3 healthy donors who had no medical problems and no medicine intake within the past 1 month. The saliva was centrifuged at 4000x g for 15 minutes at 4 °C, diluted in phosphate buffer solution (PBS) at a ratio of 1:10 and sterilized using a 0.2-µm pore size membrane filter. Filtered saliva was stored at 4 °C until use. Then, the prepared specimen surfaces were covered with sterile-filtered human saliva for 16 hours at 37 °C to induce pellicle formation. The specimens were then transferred to the biofilm-forming model.

Three bacterial strains, including the cariogenic bacteria, *Streptococcus mutans* (ATCC 25175), *Lactobacillus rhamnosus* (ATCC 7469) and *Actinomyces naeslundii* (ATCC 12104), were used to create multispecies biofilms. The bacteria were cultured in brain heart infusion (BHI) agar in a 5% CO_2 chamber at 37 °C for 48 hours and transferred to BHI broth to cultivate until the expected mid-log phase of the growth curve was reached. The culture times to each the mid-log phase for each bacterial species were evaluated during our pilot study. Twelve hours for *Streptococcus mutans*, eighteen hours for *Lactobacillus rhamnosus*, and eighteen hours for

Table 1 Materials details according to manufacturer's data

Root surface coating agents	Manufacturer	Batch no.	Active ingredients shown in the label	Application
Clinpro™ White Varnish	3M ESPE, Seefeld, Germany	NC65882	Sodium fluoride (NaF)	Apply varnish evenly in a thin layer over treatment areas with sweeping, horizontal brush strokes.
PRG-barrier coat bioactive varnish	Shofu, Kyoto, Japan	081901	Bioactive S-PRG (Surface Pre-Reacted Glass ionomer) filler	1. Add 1 drop of activator to base, and mix 2. Apply on clean tooth surface, and leave undisturbed for 3 seconds 3. Light cure for 10 seconds

Actinomyces naeslundii were investigated and used. After reaching the mid-log phase, the three bacterial suspensions were diluted to an optical density of 1 x 10^8 CFU/ml or 0.5 McFarland. Each prepared 0.5 McFarland standard for each bacterial species was further mixed into a multispecies solution at a 1:1:1 ratio (by volume) to obtain an inoculum for multispecies biofilm formation.

The saliva-coated specimens were covered with 600 µl of bacterial suspension and placed on a shaker set at 150 rpm inside the 5% CO_2 incubator at 37 °C for 24 hours to allow for bacterial adhesion to the specimen surfaces. Then, the specimens were washed with 600 µl of PBS twice and transferred to a new 24-well plate (Figure 2).

The specimens covered with multispecies biofilm were subjected to a cariogenic challenge with a daily cycle involving the provision of different media for 10 days. To simulate the supply of nutrition, the specimens were provided with 600 µl of BHI medium supplemented with 1% sucrose for 30 minutes followed by artificial saliva for 4 hours [15]. Then, the specimens were rinsed in PBS twice before being returned to each medium. This cycle was repeated three times (at 8:00 am, 12:30 pm, and 5:00 pm) per day before simulating a night of rest without media provision. Then, the specimens were collected for further analysis [16] (Figure 3) using a confocal laser scanning microscope, microcomputed tomography and Knoop microhardness tester, respectively. To prevent the dehydration of specimens among testing process, all specimens were kept in 100% relative humidity.

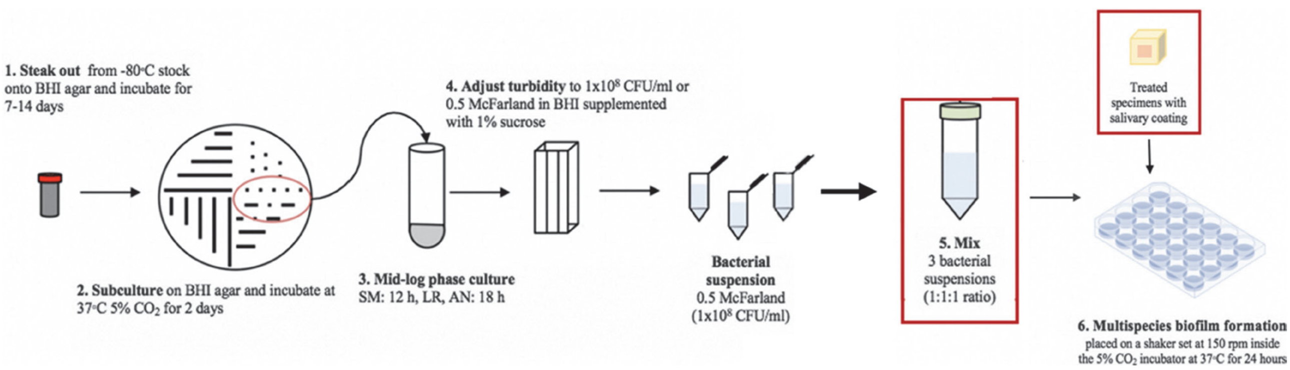

Figure 2 Schematic diagram illustrating the workflow of establishing the multispecies biofilm formation model

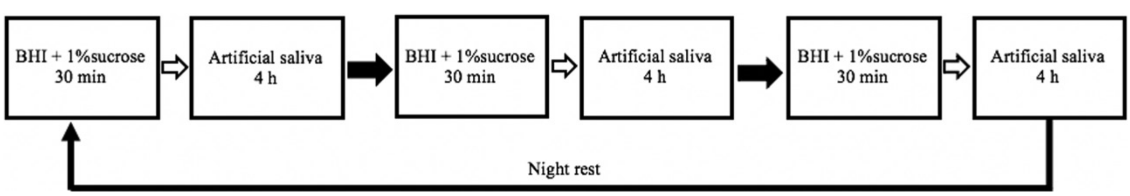

Figure 3 Artificial mouth model setting for the cariogenic biofilm challenge

Biofilm analysis

Confocal laser scanning microscopy (Leica DMi8, Leica Microsystems, Singapore) was used to assess the %cell viability (live/dead ratio: %cell viability), bacterial density (biovolume: $\mu m^3/\mu m^2$) and biofilm thickness (biomass thickness: µm). The specimens were stained using a Live/Dead Bac Light™ Bacterial Viability kit (Molecular Probes, Eugene, USA), which is composed of two fluorescent dyes: SYTO9 (a green stain for live bacteria) and isopropidium iodide (a red stain for dead bacteria). For staining, 3 µ*l of each dye was mixed into a tube containing 1 ml of filtered distilled water.* The specimens were rinsed in PBS twice before dying. *The mixed dye was placed onto a specimen in which a biofilm was formed and allowed to set for 20 minutes under light protection. Then,* the specimens were observed under confocal laser scanning microscopy using optical lenses with a magnification of 20X. The series of images obtained at 1-µm intervals in the z-section were then reconstructed into a 3-dimensional model using Leica LAS X software (Leica DMi8, Leica Microsystems, Singapore). An excitation wavelength of 488 nm was used, and the emitted light was collected between 500 and 560 nm. At least three representative optical fields were examined for each specimen. The bacterial density over the studied area *was calculated using the color segmentation method. The live and dead bacterial numbers* in the biofilm were calculated from the total number of green and red pixels using Comstat 2.1 software (Technical University of Denmark, Kongens Lyngby, Denmark) [17]. In addition, the average biofilm thickness over the observed area was calculated as a representative of biofilm thickness.

Dentin demineralization analysis

An X-ray micro-CT system (Bruker micro-CT SkyScan1173; Bruker, Antwerp, Belgium) was used to assess the changes in mineral density (MD) and the depth of the lesion by determining the relative mineral loss values of root dentin. [18] Each specimen was mounted onto a computer-controlled turntable stage with the treated dentin surface perpendicular to the X-ray beam. The tube voltage was 70 kV at a current of 114 µA. The distance between the X-ray source and the specimen was 51.9 mm, and that between the X-ray source and detector was 364 mm. Each specimen was rotated 360° in steps of 0.4°. The Bruker-MicroCT mineral phantoms and specimens were scanned to obtain an MD calibration. The MD calibration phantoms, namely, two calcium hydroxyapatite rods (0.25 and 0.75 $g.cm^{-3}$) with a diameter of 8 mm each, were scanned using the same setup and parameters as those used for the tooth specimens. Each specimen was scanned with micro-CT over the experimental period of 10 days of demineralization.

A 3D image was reconstructed from 505 two-dimensional (2D) images in 16-bit TIFF format with a resolution of 2,240 x 2,240 pixels and a voxel size of 7.0 mm using Bruker's NRecon software (Bruker, Antwerp, Belgium). Regions of interest of the demineralized lesions were selected. The deepest area of carious lesion depths was determined using Bruker's DataViewer software (Bruker, Antwerp, Belgium). Grayscale (CT) values were converted into MD values ($g.cm^{-3}$) with a linear attenuation coefficient curve based on the grayscale values obtained from the phantoms (linear regression, $R^2 > 0.9998$) using Bruker's CTAn software (Bruker, Antwerp, Belgium). The depth of the carious lesion (µm) and the dentin mineral density (g/cm^3) were determined and recorded.

Surface microhardness loss was used as an indicator of dentin demineralization. The final surface microhardness of the specimens was determined for three indentations using the Knoop diamond indenter at a 5-g load for 5 seconds. The specimens were cleaned with a soft paper wiper to remove the biofilms, and then the surface microhardness was determined. The indentations were made in the window area of the specimen. For testing, the window area was divided into three parts. One indentation was made at the center of each divided part. The average of three hardness values was calculated and used as the final surface hardness and used for further calculation. The difference between the average initial and final surface hardness was calculated to determine the %SHL [19].

Statistical analysis

The data of fifty specimens were calculated. The %cell viability, bacterial density, biofilm thickness, initial hardness, final hardness, %SHL, dentin mineral density at the demineralized lesion and depth of the lesion were expressed as the means and standard deviations. The *Kolmogorov–Smirnov test* revealed a *normal* distribution of all data. Differences within and between mean values for groups were statistically analyzed using one-way ANOVA and Dunnett's T3 post hoc test, respectively. All the statistical tests were performed with PASW Statistic 18 (IBM, Armonk, NY, USA). The significance level of all the tests was set at $p=0.05$.

Results

The means and standard deviations for the %cell viability, bacterial density, biofilm thickness, initial hardness, final hardness, %SHL, dentin mineral density at demineralized lesion and depths of lesions are presented in Table 2.

No significant differences in initial hardness were found among the groups ($p=0.24$). The Knoop hardness of the control group, varnish-coated dentin with/without tooth brushing groups and PRG-coated dentin with/without tooth brushing groups were shown in Table 2.

Table 2 Means and standard deviations for the percentage of cell viability, bacterial density, biofilm thickness, initial hardness, final hardness, percentage of surface hardness loss, dentin mineral density at demineralized lesion and depth of lesion.

		Means ± standard deviations							
		Initial hardness (N/mm^2)	Final hardness (N/mm^2)	Surface hardness loss (%)	Cell viability (%)	Bacterial density (µm^3/ µm^2)	Biofilm thickness (µm)	Dentin mineral density (g/cm^3)	Depth of lesion (µm)
Control		62.28 ± 2.67^a	15.59 ± 3.01^c	75.02 ± 4.47^a	95.25 ± 8.32^a	9.47 ± 4.71^a	18.45 ± 11.29^a	0.34 ± 0.08^b	53.20 ± 8.85^a
Clinpro White Varnish	Brushing	59.06 ± 5.63^a	31.23 ± 1.18^b	37.60 ± 9.16^b	86.48 ± 6.87^{a,b}	10.67 ± 3.52^a	23.46 ± 14.64^a	0.57 ± 0.20^{a,b}	38.50 ± 8.25^b
	No brushing	60.51 ± 2.73^a	36.56 ± 4.33^a	48.24 ± 4.05^b	76.58 ± 5.89^b	9.70 ± 4.56^a	24.30 ± 8.20^a	0.95 ± 0.41^a	26.60 ± 6.43^c
PRG Barrier Coat	Brushing	61.80 ± 1.54^a	33.41 ± 3.20^{a,b}	45.90 ± 5.35^b	83.24 ± 10.00^{a,b}	11.16 ± 2.04^a	21.97 ± 3.74^a	0.84 ± 0.21^a	22.40 ± 3.50^c
	No brushing	59.49 ± 4.45^a	35.10 ± 4.69^a	40.66 ± 9.47^b	67.07 ± 16.85^b	11.84 ± 2.61^a	21.37 ± 3.72^a	0.92 ± 0.35^a	21.00 ± 4.67^c
p value of ANOVA		0.24	0.00	0.00	0.00	0.57	0.68	0.00	0.00

Means and standard deviations with the same superscript indicate no statistically significant difference among columns.

Significant differences in the Knoop hardness were found among the groups (*p=0.00*) when the hardness was determined after demineralization. The varnish-coated dentin with tooth brushing group exhibited greater hardness values (31.23 ± 1.18 N/mm^2) than the control group (15.59 ± 3.01 N/mm^2) (*p=0.00*), but no significant difference in hardness was noted for the PRG-coated dentin with tooth brushing group (33.41 ± 3.20 N/mm^2) (*p=0.42*). No significant differences in hardness were found between the PRG-coated dentin with tooth brushing and without tooth brushing groups (*p=0.98*). Therefore, all experimental groups demonstrated higher hardness after demineralization than the control group.

The analysis of surface hardness loss for root dentin after demineralization revealed significant differences among groups (*p=0.00*). A significant loss of mineral content in root dentin without coating was observed in the control group (75.02 ± 4.47%) compared with the other groups (*p=0.00*). Additionally, statistically significant differences were not observed among the four experimental groups (*p*≥0.05).

Confocal laser microscopy revealed a significant difference in the %cell viability (*p*=0.000) with no significant difference in bacterial density (*p=0.57*) and biofilm thickness (*p=0.68*) among groups. Higher %cell viability was found in the control group (95.25 ± 8.32%) than in the varnish-coated dentin (76.58 ± 5.89) and PRG-coated dentin (67.07 ± 16.85) without tooth brushing groups (*p=0.00*). Representative 2D and 3D images of the %cell viability and bacterial density under the confocal laser microscope are shown in Figures 4 and 5, respectively. Differences in live cells, dead cells and combination cells were noted in the 2D images. A globular appearance was detected for the biofilms in the varnish-coated dentin groups, both with and without brushing. Globules were found prominently in the varnish-coated dentin group without brushing. In the PRG-coated dentin treatment, a relatively flat biofilm that was denser was observed in the PRG-coated dentin without brushing group. Regarding the 3D images, scratch lines from brushing the surfaces of the varnish-coated dentin with tooth brushing group were found, while they were not clearly noted for PRG-coated dentin with brushing.

Based on micro-CT analysis, significant differences in dentin mineral density and the depth of carious lesions were found among groups with significant values of *p=0.00* and *p=0.00*,

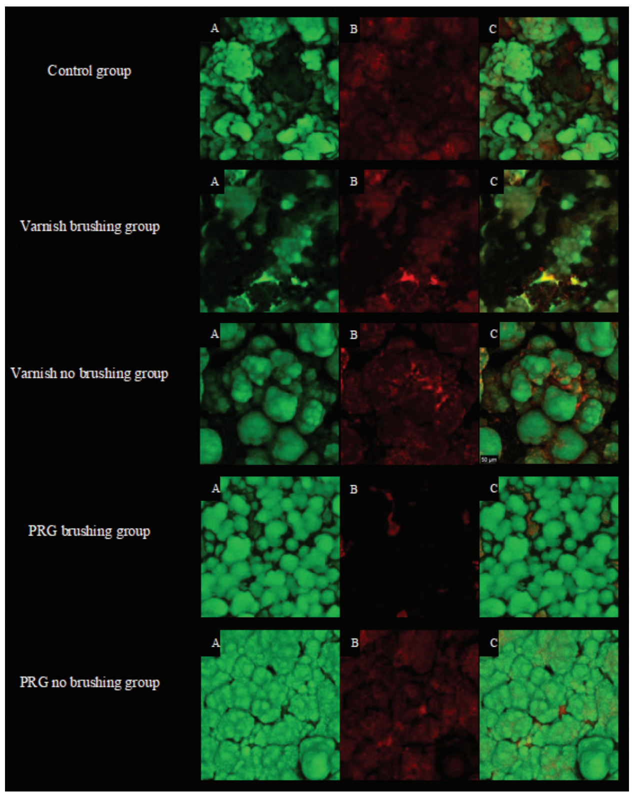

Figure 4 Representative 2D images of the % cell viability and bacterial density obtained by confocal laser microscopy. A; Live cells are shown in green, B; dead cells are shown in red, and C; the combination of live and dead cells.

respectively. Increased mineral density was found in the varnish-coated dentin without tooth brushing group (0.95 ± 0.41 g/cm^3) compared with the control group (0.34 ± 0.08 g/cm^3) ($p=0.01$). However, no statistically significant differences were found between the varnish-coated dentin with tooth brushing (0.57 ± 0.20 g/cm^3) group and the control group ($p=0.06$). No statistically significant differences in mineral density among groups coated with both fluoride varnish and PRG with either brushing or no brushing were found ($p \geq 0.05$). The deepest depth of carious lesions was noted in the control group (53.20 ± 8.85 μm), which showed a significant difference compared with the other experimental groups. Meanwhile, the varnish-coated dentin with tooth brushing group (38.50 ± 8.25 μm) was also found a significant difference among groups, however, the depth of lesion was shallower than the control group. The shallowest depth of carious lesions was noted in the PRG-coated dentin without tooth brushing group. Therefore, no significant differences were found among the PRG-coated dentin without tooth brushing group, the PRG-coated dentin with tooth brushing group and the varnish-coated dentin without tooth brushing group ($p \geq 0.05$). Representative micro-CT images of each group in coronal and transaxial views are shown in Figures 5 and 6, respectively. Clearly, homogeneous cavitation in the artificial caries was observed in the control group with the deepest lesion depth. Shallow cavitation of artificial caries was observed in the other experimental groups: the varnish-coated dentin with tooth brushing, varnish-coated dentin without tooth brushing, PRG-coated dentin with tooth brushing and PRG-coated dentin without tooth brushing groups. In the PRG-coated dentin without tooth brushing group, the shallowest cavitation was observed with less homogeneous cavitation than the control. Nonhomogeneous cavitation of artificial caries was observed in the varnish-coated dentin groups both with and without tooth brushing. The pattern of artificial caries might be related to the pattern of brushing. In addition, for PRG-coated dentin groups, surfaces coated with the bioactive varnish were observed both with and without brushing with different varnish thicknesses. A relatively thicker layer of varnish was found in the group without brushing. The bioactive varnish was indicated by the white opaque line or area caused by metal ions contained in the PRG-barrier coat bioactive varnish (Figures 6-7 and Table 2).

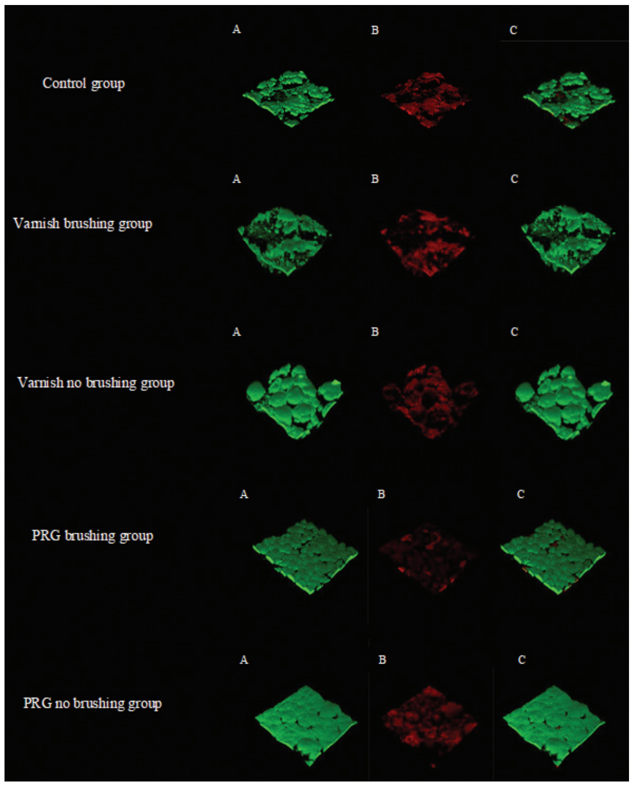

Figure 5 Representative 3D images of the % cell viability, bacterial density and biofilm thickness obtained by confocal laser microscopy. All z-stack slices were reconstructed into 3D images. A; Live cells are shown in green, B; dead cells are shown in red, and C; the combination of live and dead cells.

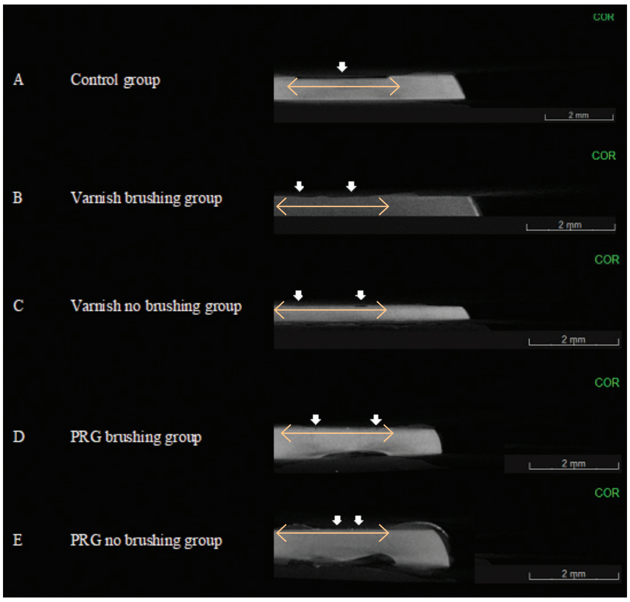

Figure 6 Micro-CT images in coronal view showing the carious lesion depth of each group (white arrow) and the demineralized area (yellow arrow). A, Control group; B, Varnish brushing group; C, Varnish no brushing group; D, PRG brushing group; and E, PRG no brushing group.

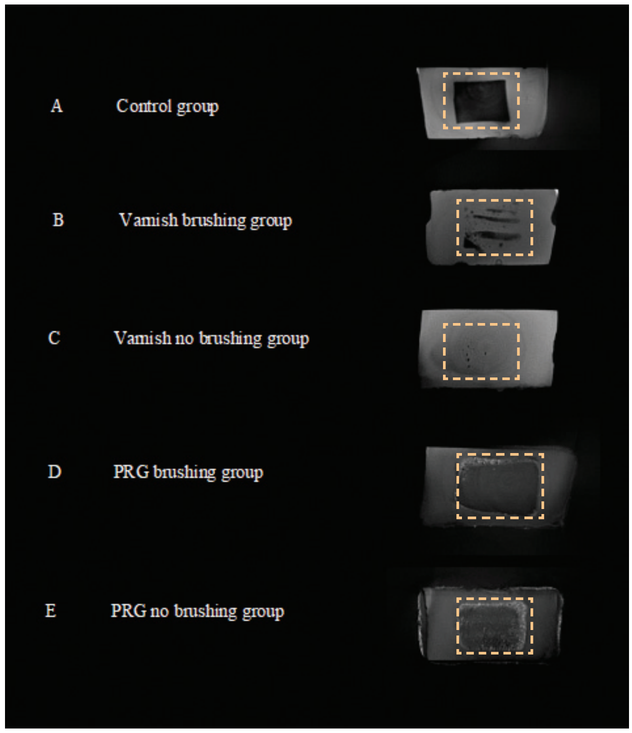

Figure 7 Micro-CT images in transaxial view showing the carious lesion pattern of each group (the area inside the yellow dotted line indicated demineralized area). A, Control group; B, Varnish brushing group; C, Varnish no brushing group; D, PRG brushing group; and E, PRG no brushing group.

Figure 6. Micro-CT images in coronal view showing the carious lesion depth of each group (white arrow) and the demineralized area (yellow arrow). A, Control group; B, Varnish brushing group; C, Varnish no brushing group; D, PRG brushing group; and E, PRG no brushing group.

Discussion

The effectiveness of applying biannual fluoride varnish to non-cavitated carious lesions has been reported in children [20, 21] and young adults [22]. These publications reported the effectiveness of Clinpro White Varnish for caries prevention, especially on enamel. The benefits of fluoride varnishes include their simple application and prolonged contact with the demineralized surface of the enamel, which make the varnish more effective [23,24]. A benefit of fluoride varnish for root caries prevention has also been reported [9]. A PRG filler that releases aluminum (Al3+), borate (BO33−), sodium (Na+), silicate (SiO32−), strontium (Sr2+), and fluoride (F−) ions has been developed [25]. The incorporation of this filler into resinous coating materials provides fluoride releasing abilities and anti-demineralization effects on bovine enamel [26], dentin [27], and root dentin [28].

This study was intended to investigate the effectiveness of Clinpro White Varnish and PRG Barrier Coat in root caries prevention. The Clinpro White Varnish and the PRG Barrier Coat were selected for use in this study because of their compositions. The compositions of Clinpro White Varnish include pentaerythritol glycerol ester of colophony resin, n-hexane, ethyl alcohol, sodium fluoride, water, flavors, thickener and tricalcium phosphate. The release of calcium and phosphate ions from this varnish not only promotes the deposition of minerals on the surface of the enamel but also potentiates the remineralization of the subsurface lesion body [29,30]. The PRG barrier coat is composed of 2 parts: a base and an active part. The components of the base are surface pre-reacted glass-ionomer (S-PRG) glass fillers, water, methacrylic acid monomer and others, whereas the compositions of the active part are phosphoric acid monomer, bis-MPEPP (2,2'-bis (4-methacryloxy polyethoxyphenyl) propane), carboxylic acid monomer, TEGDMA (triethylene glycol dimethacrylate), photoinitiator, methacrylic acid monomer, polybasic carboxylic acid and others. The release of various ions from the PRG filler in this material not only promotes the remineralization and deposition of minerals onto the tooth surface but also has buffering capacity and antibacterial effects [26, 28, 31, 32]. These positive results might be beneficial for root caries prevention.

The reaction of fluoride varnish to prevent remineralization is dependent on its soluble fluoride concentration in the short term and on the dissolution of the insoluble fluoride present in the varnish matrix in the long term [33]. The insoluble fluoride in the varnish might play an important role in slowing this process compared with the soluble fluoride concentration, which is responsible for chemically forming fluoride calcium-like reservoirs on the enamel over a short period of time [33]. However, lower concentrations of insoluble and soluble fluoride were observed for Clinpro White Varnish [34]. In addition, the presence of modified tricalcium phosphate could potentiate remineralization, especially on enamel [35, 36].

The cumulative amounts of ions released from Clinpro White Varnish at 24 hours were 3.2 mmol/g for calcium ions, 0.8 mmol/g for phosphate ions and 74 mmol/g for fluoride ions [37]. Therefore, this amount of fluoride ions was relatively low compared with the amount contained in Clinpro White Varnish (2.26 wt% fluoride). The fluoride percentage released from Clinpro White Varnish was only 6% after 24 hours [37]. In addition, fluoride varnish has a short lifespan in the oral environment, because it may be removed by the action of the cheeks and tongue, salivary flow, mastication and oral hygiene procedures. Additionally, varnishes should release their ions within a relatively short time period before the varnish is lost. It has been estimated that varnishes only remain in situ for up to 24 hours [38].

The presence of fluoride ions improves the acidic dissolution resistance of the enamel and protects the enamel surface. Lower concentrations of fluoride enhance remineralization on demineralized enamel and the formation of fluorapatites, which are less susceptible to acids. A higher fluoride concentration leads to calcium fluoride formation, which is a reservoir of further remineralization [39-43]. For root dentin, fluoride from fluoride varnish has the potential to remineralize root caries and increase the hardness of demineralized root dentin [44]. The effect of fluoride release from Clinpro White Varnish remineralizes root caries, leading to an increase in the hardness of demineralized root dentin in this study.

For PRG Coat Barrier, the potentials of the ions released from the S-PRG filler have been proposed as follows: F^- ion: fluorapatite formation, antibacterial effect, remineralization of demineralized lesions; Sr^{2+} ion: improvement of bone formation and mineralization; Al^{3+} ion: suppression of hypersensitivity; SiO_3^{2-} ion: remineralization; and BO_3^{3-} ion: antibacterial effect, promotion of bone formation [45-46]. In addition, the BO_3^{3-}, Sr^{2+}, Na^{+}, Al^{3+}, SiO_3^{2-} and F^{-} ions released by the S-PRG fillers have a buffering capacity and protective effect against demineralization [47]. This multi-ion release by the S-PRG filler may play an important role in anti-demineralization and caries prevention by inhibiting biofilm formation [48]. The effect of multiple ions released from PRG Coat Barrier to inhibit biofilm formation and remineralize root caries, enhancing the increase in the hardness of demineralized root dentin, was also found in this study. Therefore, no significant differences in %surface hardness loss between the application of Clinpro White Varnish and PRG Coat Barrier for both brushing and no brushing conditions were found. This result might imply that there were no differences in the effectiveness of Clinpro White Varnish and PRG Coat Barrier regarding the remineralization of root caries.

The effectiveness of Clinpro White Varnish and PRG Coat Barrier in preventing demineralization and remineralization might simultaneously reduce the depth of artificial root surface lesions and improve dentin mineralization density, as demonstrated in the present study under micro-CT. A clear benefit was found when the PRG coat barrier was applied onto the root dentin both with and without tooth brushing. Therefore, that benefit was clearly found when Clinpro White Varnish was applied to the root dentin only in the treatment without tooth brushing. Tooth brushing seems to reduce the effectiveness of Clinpro White Varnish. A deeper depth of lesion and a lower mineral density were found in the root dentin group coated with varnish and subjected to tooth brushing compared with the root dentin group coated with varnish without tooth brushing. Brushing after the application of Clinpro White Varnish may reduce the amount of varnish covering the root surfaces [49], resulting in a lower mineral density of root dentin. These findings might explain whether the carious lesions were significantly deeper in the group coated with Clinpro White Varnish with brushing compared with Clinpro White Varnish without brushing.

The effect of tooth brushing on the depth of the lesion and the mineral density found when Clinpro White Varnish was applied could not be observed with the application of PRG Coat Barrier. A benefit of resin-based coating materials to remain after the simulation of 10,000 cycles of tooth brush abrasion or approximately one year of clinical toothbrushing has been reported [12,13]. However, a significant reduction in fluoride varnish after brushing was addressed [49]. The relatively high wear resistance of resin-based coating material to tooth brushing might have a benefit over the varnish coating material. Since PRG Coat Barrier is a resin-based coating material, it may withstand brushing better than Clinpro White Varnish, leading to better results for caries prevention in terms of the depth of lesion and the mineral density compared with Clinpro White Varnish.

Regarding the live/dead ratio observation, the effect of both Clinpro White Varnish and PRG Coat Barrier on multispecies biofilm formation was observed. However, there was no effect of either coating material, with a lack of significant differences in biovolume and biofilm thickness. A higher concentration of fluoride is toxic to cariogenic bacteria, whereas lower levels of fluoride inhibit the enzymes of cariogenic streptococci [39]. Given the relatively low amount of fluoride in Clinpro White Varnish [37], the released fluoride might inhibit the enzymes of cariogenic streptococci, causing the lower live/dead ratio compared with the control group. Thus, the number of multi-ions from PRG Coat Barrier might have a bactericidal effect on multispecies biofilms by suppressing the growth and pathogenicity of bacteria [50], resulting in a lower live/dead ratio compared with the control group. The effect of the coating materials on the live/dead ratio of bacteria in multispecies biofilms was observed only without brushing. Brushing might reduce the effectiveness of the coating materials in harming the bacteria.

The results of this study demonstrated that caries prevention from both types of coating materials was reduced by tooth brushing, especially when Clinpro White Varnish was applied. Nevertheless, PRG Coat Barrier which is a resin-based coating material has been shown an additional benefit for caries prevention as it is able to act as a physical and chemical barrier that was better than Clinpro White Varnish. There were many publications that revealed the anticaries properties of fluoride-containing products depended on retaining fluoride on tooth structure or in saliva which was time dependence. To maintain the anticaries properties of these coating materials, the frequency of application should be considered [51].

Conclusion

PRG Coat Barrier demonstrated a benefit over Clinpro White Varnish for root caries prevention in terms of the depth of carious lesions and demonstrated comparable results to Clinpro White Varnish for root caries prevention in terms of the %surface hardness loss, %cell viability and dentin mineral density under tooth brushing conditions. Without tooth brushing, the effectiveness of both materials was not different.

Clinical relevance

Clinpro White Varnish may have a shorter lifespan than PRG Coat Barrier because it may be removed by brushing more than PRG Coat Barrier, potentially reducing the effectiveness of varnish for caries prevention when compared with PRG Coat Barrier. Therefore, the reapplication of coating materials may be necessary to maintain their caries preventive effects.

References

1. Hayes M, Burke F, Allen PF. Incidence, prevalence and global distribution of root caries. ***Monogr Oral Sci.*** 2017;26:1-8. doi: 10.1159/000479301.
2. Damé-Teixeira N, Parolo CCF, Maltz M. Specificities of caries on root surface. ***Monogr Oral Sci.*** 2017; 26:15-25. doi: 10.1159/000479303.
3. Zambon JJ, Kasprzak SA. The microbiology and histopathology of human root caries. ***Am J Dent.*** 1995 Dec;8(6):323-328. PMID: 8695011.
4. Burgess JO, Gallo JR. Treating root-surface caries. ***Dent Clin North Am.*** 2002 Apr;46(2):385-404, vii-viii. doi: 10.1016/s0011-8532(01)00003-9.
5. Steele JG, Sheiham A, Marcenes W, Fay N, Walls AW. Clinical and behavioural risk indicators for root caries in older people. ***Gerodontology.*** 2001 Dec;18(2):95-101. doi: 10.1111/j.1741-2358.2001.00095.x.
6. Slayton RL, Urquhart O, Araujo MWB, Fontana M, Guzmán-Armstrong S, Nascimento MM, *et al.* Evidence-based clinical practice guideline on nonrestorative treatments for carious lesions: a report from the American Dental Association. ***J Am Dent Assoc.*** 2018 Oct;149(10):837-849.e19. doi: 10.1016/j.adaj.2018.07.002.
7. Buzalaf MAR, Pessan JP, Honório HM, Ten Cate JM. Mechanisms of action of fluoride for caries control. ***Monogr Oral Sci.*** 2011;22:97-114. doi: 10.1159/000325151.
8. Bonetti D, Clarkson JE. Fluoride varnish for caries prevention: efficacy and implementation. ***Caries Res.*** 2016;50 Suppl(1):45-49. doi: 10.1159/000444268.

9. Zhang J, Sardana D, Li KY, Leung KCM, Lo ECM. Topical Fluoride to Prevent Root Caries: Systematic Review with Network Meta-analysis. *J Dent Res.* 2020 May;99(5):506-513. doi: 10.1177/0022034520906384.
10. Yamamoto S, Sayed M, Takahashi M, Matin K, Hiraishi N, Nikaido T, *et al.* Effects of a surface prereacted glass-ionomer filler coating material on biofilm formation and inhibition of dentin demineralization. *Clin Oral Investig.* 2021 Feb;25(2):683-690. doi: 10.1007/s00784-020-03577-x.
11. Ma S, Imazato S, Chen JH, Mayanagi G, Takahashi N, Ishimoto T, *et al*. Effects of a coating resin containing S-PRG filler to prevent demineralization of root surfaces. *Dent Mater J.* 2012;31(6):909-915. doi: 10.4012/dmj.2012-061.
12. Gando I, Ariyoshi M, Ikeda M, Sadr A, Nikaido T, Tagami J. Resistance of dentin coating materials against abrasion by toothbrush. *Dent Mater J.* 2013;32(1):68-74. doi: 10.4012/dmj.2012-186.
13. Tian HY, Yu P, Yuan CY, Zhang W, Qiu YX, Li DH, *et al.* Durability of protective effect of resin-based coating material on root surface. *Beijing Da Xue Xue Bao Yi Xue Ban.* 2016 Oct 18;48(5):889-893. PMID: 27752176.
14. Wiegand A, Schneider S, Sener B, Roos M, Attin T. Stability against brushing abrasion and the erosion-protective effect of different fluoride compounds. *Caries Res.* 2014;48(2):154-162. doi: 10.1159/000353143.
15. Rirattanapong P, Vongsavan K, Suratit R, Tanaiutchawoot N, Charoenchokdilok V, Jeansuwannagorn S, *et al*. Effect of various forms of calcium in dental products on human enamel microhardness in vitro. *Southeast Asian J Trop Med Public Health.* 2012 Jul;43(4):1053-1058. PMID: 23077830.
16. Göstemeyer G, Woike H, Paris S, Schwendicke F, Schlafer S. Root caries preventive effect of varnishes containing fluoride or fluoride + chlorhexidine/cetylpyridinium chloride in vitro. *Microorganisms.* 2021 Apr; 9(4):737. doi: 10.3390/microorganisms9040737.
17. Komalsingsakul A, Srisatjaluk RL, Senawongse P. Effect of brushing on surface roughness, fluoride release, and biofilm formation with different tooth-colored materials. *J Dent Sci.* 2022 Jan;17(1):389-398. doi: 10.1016/j.jds.2021.08.013.
18. Hamba H, Nikaido T, Inoue G, Sadr A, Tagami J. Effects of CPP-ACP with sodium fluoride on inhibition of bovine enamel demineralization: a quantitative assessment using micro-computed tomography. *J Dent.* 2011 Jun;39(6):405-413. doi: 10.1016/j.jdent.2011.03.005.
19. Amaechi BT, Tenuta LMA, Ricomini Filho AP, Cury JA. Protocols to study dental caries in vitro: microbial caries models. *Methods Mol Biol.* 2019;1922:357-368. doi: 10.1007/978-1-4939-9012-2_32.
20. Pitchika V, Kokel CJ, Andreeva J, Crispin A, Hickel R, Kühnisch J, *et al.* Effectiveness of a new fluoride varnish for caries prevention in pre-school children. *J Clin Pediatr Dent.* 2013;38(1):7-12. doi: 10.17796/jcpd.38.1.8189243pt1m6t716.
21. Poza-Pascual A, Serna-Muñoz C, Pérez-Silva A, Martínez-Beneyto Y, Cabello I, Ortiz-Ruiz AJ. Effects of fluoride and calcium phosphate-based varnishes in children at high risk of tooth decay: a randomized clinical trial. *Int J Environ Res Public Health.* 2021 Sep;18(19), 10049. doi: 10.3390/ijerph181910049.
22. Razmakhnina EM, Kiseleva EA. Rationale for caries prevention in young adults based on caries resistance rates. *Stomatologiia (Mosk).* 2018;97(2):34-36. doi: 10.17116/stomat201897234-36.
23. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2002;(3):CD002279. doi: 10.1002/14651858.CD002279. Update in: *Cochrane Database Syst Rev.* 2013;7:CD002279. PMID: 12137653.
24. Seppä L. Fluoride varnishes in caries prevention. *Med Princ Pract.* 2004;13(6):307-11. doi: 10.1159/000080466.
25. Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. *Dent Mater J.* 2010 Aug; 29: 392-397. doi: 10.4012/dmj.2010-015.
26. Kawasaki K, Kambara M. Effects of ion-releasing tooth- coating material on demineralization of bovine tooth enamel. *Int J Dent.* 2014; 2014: 463149. doi: 10.1155/2014/463149.

27. Shiiya T, Mukai Y, Tomiyama K, Teranaka T. Anti-demineralization effect of a novel fluoride-releasing varnish on dentin. ***Am J Dent.*** 2012 Dec; 25(6): 347-350. PMID: 23409625.
28. Shiiya T, Kataoka A, Tomiyama K, Fujino F, Mukai Y. Anti-demineralization characteristics of surface pre-reacted glass-ionomer (S-PRG) filler-containing varnishes. ***Dent Mater J.*** 2021 Mar;40(2):416-421. doi: 10.4012/dmj.2019-396.
29. Mohd Said SN, Ekambaram M, Yiu CK. Effect of different fluoride varnishes on remineralization of artificial enamel carious lesions. ***Int J Paediatr Dent.*** 2017 May;27(3):163-173. doi: 10.1111/ipd.12243.
30. Silverstone LM. Remineralization of human enamel in vitro. ***Proc R Soc Med.*** 1972 Oct;65(10):906-908. PMID: 5085103.
31. Kaga N, Nagano-Takebe F, Nezu T, Matsuura T, Endo K, Kaga M. Protective effects of GIC and S-PRG filler restoratives on demineralization of bovine enamel in lactic acid solution. ***Materials.*** 2020 May; 13(9):2140. doi: 10.3390/ma13092140.
32. Yamamoto S, Sayed M, Takahashi M, Matin K, Hiraishi N, Nikaido T, *et al.* Effects of a surface prereacted glass-ionomer filler coating material on biofilm formation and inhibition of dentin demineralization. ***Clin Oral Investig.*** 2021 Feb; 25(2):683-690. doi: 10.1007/s00784-020-03577-x.
33. Manarelli MM, Delbem AC, Lima TM, Castilho FC, Pessan JP. In vitro remineralizing effect of fluoride varnishes containing sodium trimetaphosphate. ***Caries Res.*** 2014;48(4):299-305. doi: 10.1159/000356308.
34. Godoi FA, Carlos NR, Bridi EC, Amaral F, França FMG, Turssi CP, *et al.* Remineralizing effect of commercial fluoride varnishes on artificial enamel lesions. ***Braz Oral Res.*** 2019 May;33:e044. doi: 10.1590/1807-3107bor-2019.vol33.0044.
35. Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Surfactant-modified beta-TCP: structure, properties, and in vitro remineralization of subsurface enamel lesions. ***J Mater Sci Mater Med.*** 2010 Jul;21(7):2009-2020. doi: 10.1007/s10856-010-4064-y.
36. Balakrishnan A, Jonathan R, Benin P, Kuumar A. Evaluation to determine the caries remineralization potential of three dentifrices: an in vitro study. ***J Conserv Dent.*** 2013 Jul;16(4):375-379. doi: 10.4103/0972-0707.114347.
37. Cochrane NJ, Shen P, Yuan Y, Reynolds EC. Ion release from calcium and fluoride containing dental varnishes. ***Aust Dent J.*** 2014 Mar;59(1): 100-105. doi: 10.1111/adj.12144.
38. Schemehorn BR, Wood GD, McHale W, Winston AE. Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources. ***J Clin Dent.*** 2011;22(2):51-54. PMID: 21702326.
39. Wasiluk A. Fluoride compounds in dental caries prophylaxis in children and adolescents – review of polish literature. ***Przegl Epidemiol.*** 2017;71(4): 603-611. PMID: 29417787.
40. Ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. ***Crit Rev Oral Biol Med.*** 1991;2(3):283-296. doi: 10.1177/10454411910020030101.
41. Wong L, Sissons C. A comparison of human dental plaque microcosm biofilms grown in an undefined medium and a chemically defined artificial saliva. ***Arch Oral Biol.*** 2001;46(6):477-86. doi: 10.1016/s0003-9969(01)00016-4. Erratum in: Arch Oral Biol 2001 Aug;46(8):779. Sissions CH [corrected to Sissons C]. PMID: 11311195.
42. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. ***Caries Res.*** 2005 Sep-Oct;39(5):371-379. doi: 10.1159/000086843.
43. Kim HN, Kim JB, Jeong SH. Remineralization effects when using different methods to apply fluoride varnish in vitro. ***J Dent Sci.*** 2018 Dec;13(4):360-366. doi: 10.1016/j.jds.2018.07.004.
44. Cai J, Burrow MF, Manton DJ, Hardiman R, Palamara JEA. Remineralising effects of fluoride varnishes containing calcium phosphate on artificial root caries lesions with adjunctive application of proanthocyanidin. ***Dent Mater.*** 2021 Jan;37(1):143-157. doi: 10.1016/j.dental.2020.10.021.
45. Yoneda M, Suzuki N, Hirofuji T. Antibacterial effect of surface pre-reacted glass ionomer filler and eluate—mini review. ***Pharm Anal Acta.*** 2015; 6: 349. doi:10.4172/2153-2435.100034.
46. Uo M, Wada T, Asakura K. Structural analysis of strontium in human teeth treated with surface pre-reacted glass-ionomer filler eluate by using extended X-ray absorption fine structure analysis. ***Dent Mater J.*** 2017 Mar; 36(2): 214-221. doi: 10.4012/dmj.2016-266.

47. Kaga M, Kakuda S, Ida Y, Toshima H, Hashimoto M, Endo K, *et al.* Inhibition of enamel demineralization by buffering effect of S-PRG filler-containing dental sealant. ***Eur J Oral Sci.*** 2014 Feb; 122: 78-83. doi: 10.1111/eos.12107.

48. Yamamoto S, Sayed M, Takahashi M, Matin K, Hiraishi N, Nikaido T, *et al.* Effects of a surface prereacted glass–ionomer filler coating material on biofilm formation and inhibition of dentin demineralization. ***Clin Oral Invest.*** 2021 Feb; 25(2), 683–690. doi: 10.1007/s00784-020-03577-x.

49. Hong L, Watkins CA, Ettinger RL, Wefel JS. Effect of topical fluoride and fluoride varnish on in vitro root surface lesions. ***Am J Dent.*** 2005 Jun;18(3):182-187. PMID: 16158810.

50. Kono Y, Tamura M, Cueno ME, Tonogi M, Imai K. S-PRG Filler Eluate Induces Oxidative Stress in Oral Microorganism: Suppression of Growth and Pathogenicity, and Possible Clinical Application. ***Antibiotics (Basel).*** 2021 Jul;10(7):816. doi: 10.3390/antibiotics10070816.

51. Cai J, Palamara J, Manton DJ, Burrow MF. Status and progress of treatment methods for root caries in the last decade: a literature review. ***Aust Dent J.*** 2018 Mar;63(1):34-54. doi: 10.1111/adj.12550.