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Effect of SI-R20401 to remineralize artificial incipient enamel lesions in primary teeth

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Abstract: We evaluated the effectiveness of the experimental surface pre-reacted glass-ionomer coating (SI-R20401) to remineralize artificial enamel lesions in primary teeth. For each of 12 sound primary molars, five regions were assigned, based on whether enamel was unground or ground and whether PRG coating was applied. The teeth were demineralized in 10% EDTA for 7 h and lactic acid solution for 3 days and immersed in artificial saliva (Group 1), demineralizing medium (Group 2) or deionized water (Group 3) for 1 month. DIAGNOdent reading, nanoindentation test and scanning electron microcopy/energy dispersive X-ray analysis were performed. Data were analyzed using analysis of variance and the Fisher protected least significance difference test at $\alpha = 0.05$. After immersion, a decrease in DIAGNOdent value for the demineralized enamel was observed only in the unground/non-PRG region of Group 1. In the ground/PRG region of Groups 1 and 3, the hardness and Young's modulus at the enamel surface were significantly higher than those at subsurface points. For unground enamel, Ca%, P%, and the Ca/P ratio at the enamel surface of the non-PRG region were significantly lower than those at subsurface points. In Group 2, scanning electron microcopy showed greater demineralization at the unground/non-PRG region compared with the unground/PRG region. Efficacy of SI-R20401 to remineralize the enamel lesions in primary teeth was partially observed, however, to arrest the lesion could not be demonstrated.
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Keywords: S-PRG coat; remineralization; primary tooth enamel; incipient enamel lesion; EDS analysis; SEM observation.

Introduction

Incipient enamel caries lesions are characterized by loss of minerals in the underlying lesion body, whereas the surface remains relatively highly mineralized (1). White-spot lesions are the first clinical sign of enamel demineralization and may be regarded as incipient enamel caries (1,2). These lesions may be active (with a rough, opaque enamel surface) or inactive (with a smooth and shiny enamel surface) (3,4). In childhood, early caries lesions (active noncavitated caries lesions) may be easily cavitated, yielding early carious lesions.

Incipient enamel carious lesions may be arrested or even remineralized (5,6). The common treatment strategy comprises application of fluoride, education in

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oral hygiene, and proper diet (7-10). Recent reports have advocated sealing or infiltration of incipient enamel caries lesions with low-viscosity light-curing resins (11,12).

Surface pre-reacted glass-ionomer (S-PRG) fillers are a new type of particles that can be incorporated into resinous materials. They are prepared by acid-base reaction between fluoroboroaluminosilicate glass and aqueous polyacrylic acid (13). A ligand exchange mechanism within the pre-reacted hydrogel endows S-PRG fillers with the ability to release and recharge with fluoride (14). In addition to fluoride, S-PRG fillers release Al, B, Na, Si, and Sr ions (13,15). Silicate and fluoride markedly induce remineralization of the dentin matrix (16). Strontium and fluoride also improve the acid resistance of teeth by converting hydroxyapatite into strontium apatite and fluorapatite (17). When they come into contact with water or acidic solutions, S-PRG fillers change the pH of the surrounding environment to a weakly alkaline range (18). Recently, a resinous coating material containing S-PRG fillers became available for use in caries prevention with the objectives of enhancing remineralization and reducing acidic attack by oral cariogenic bacteria (19).

Apart from remineralization of incipient enamel carious lesions in pediatric dentistry, arresting caries is useful for managing caries in uncooperative children and disabled patients, as well as teeth that are not fully erupted or with immature mineralization. Coating materials smooth the enamel surface and reduce bacteria retention along the surface of incipient caries lesions. However, little is known regarding the efficacy of S-PRG–filled coating material for remineralizing and/or arresting caries in primary tooth enamel. Thus, we examined the effects of an S-PRG-filled experimental coating material, SI-R20401, on changes in the microstructure of artificially demineralized incipient caries-like primary tooth enamel. The null hypothesis tested was that SI-R20401 application has no effect on the physical characteristics or elemental content of artificially demineralized primary tooth enamel.

Materials and Methods

Sample preparation

Twelve healthy primary molars that were extracted to expedite eruption of succedaneous teeth, or for orthodontic reasons, were used as substrates for the present study. Informed consent for tooth collection was obtained from parents and subjects, according to the regulations of Nagasaki University Dental School (Permission No. 26). The teeth were frozen in physiologic saline within 10 min after extraction.

As shown in Fig. 1, each tooth was divided into five regions according to whether the surface enamel was ground or unground and whether the S-PRG coating material (SI-R20401) was applied to that surface. For each tooth, the buccal enamel surface was selected as the unground enamel region, and the lingual surface—except for the central portion (control region)—was designated as the ground enamel region. The ground enamel region was designed to simulate a cavitated early carious lesion. For the ground regions, the surface enamel was ground with a water-cooled air turbine, using a diamond point (ISO #016, Shofu Inc, Kyoto, Japan).

Dental pulp was removed, and each tooth was ultrasonically cleaned in deionized water, after that the pulp chamber of each tooth was filled with white wax (Shofu Inc). The enamel of the proximal surfaces and central portion of lingual surfaces was coated with nail varnish. The teeth were demineralized in 10% EDTA (pH 7.2) for 7 h to accelerate the clear white lesion, and then in 0.1 M lactic-acid buffered solution (pH 4.75 demineralizing medium; 0.75 mM $CaCl_2/2H_2O$ and 0.45 mM KH_2PO_4) for 3 days. As shown as in Fig. 1, the buccal and lingual surfaces of each tooth were divided into a S-PRG-coated region (PRG) and an uncoated region (non-PRG) at the center of the tooth. S-PRG coating was not applied to the control region. For the PRG region, a brush was dipped in liquid and then in powder, and the liquid/powder mixture was rubbed on the enamel surface for 15 s. After 1 min, the surplus material on the enamel surface was removed with gauze. The main components of SI-R21104 powder are S-PRG fillers and ultrafine fillers; the SI-R21104 liquid is composed of polycarboxylic acid and water.

The teeth were divided into three groups (n = 4) according to the immersing solution; Group 1 (artificial saliva), Group 2 (demineralizing medium), and Group 3 (deionized water). Immersion in artificial saliva was designed to simulate the oral environment, immersion in demineralizing medium was designed to simulate an acidic oral condition with high caries activity, and immersion in deionized water was selected because it was unlikely to directly influence the components of the coating material. Artificial saliva, which has an electrolyte composition similar to that of human saliva, was prepared from 1.09 mmol/L $CaCl_2$, 0.68 mmol/L KH_2PO_4, 30 mmol/L KCl and 2.6 mmol/L NaF. The artificial saliva was buffered to pH 7.0 with 50 mmol/L *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). Each tooth was immersed in a glass bottle filled with 10 mL of solution at 24°C for 1 month. The solutions were changed every 48 h.

After 1-month immersion, the coating material was removed with a dental explorer. The specimens were sectioned perpendicular to the longitudinal axis of the tooth. Sectioning was performed using a low-speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) under copious water cooling. Two or three sectioned specimens were obtained from each tooth. After sectioning, the specimens were polished with wet 1200-grit silicon carbide papers (Marutoyo Co, Tokyo, Japan), and final polishing was performed using 0.3-µm aluminum oxide lapping films (Marutoyo Co). The polished specimens were stored in the containers at 4°C in 100% relative humidity until nanoindentation test.

Nanoindentation

One polished specimen from each tooth was used for nanoindentation evaluation with a nanoindentation tester (ENT-1100a; Elionix Co, Tokyo, Japan) at a chamber temperature of 26°C. Hardness (H) and Young's modulus (Y) were calculated according to previously reported procedures (20,21).

Indentations were made at 10-µm intervals from the enamel surface toward the pulp chamber, using a load of 300 mgf for 10 s. Data obtained from the first five indentations of each line were analyzed. Three lines of indentations were made along each region of a specimen. To ensure symmetrical indents and adequate spacing between indentations, the indentations were observed using a microscope with a charge-coupled device camera attached to the tester (700× magnification). After argon ion etching (ERA-8800 FE; Elionix Co) scanning electron microscopy (SEM) was used to examine the quality and morphology of indentations. Data derived from the two biomechanical parameters, H and Y, were normally distributed (Shapiro-Wilk test) and homoscedastic (modified Levene test). They were analyzed separately using two-way analysis of variance to examine the effect of nanoindentation locations (i.e., distance from the interface) and treatment conditions (i.e., the control, unground/non-PRG, unground/PRG, ground/non-PRG, and ground/PRG regions) for each group (Groups 1, 2, and 3) and the interaction of these two factors on the two biomechanical-parameters. For each parameter, post-hoc comparisons were performed using the Fisher protected least significance difference (PLSD) test with α = 0.05.

Scanning electron microcopy/energy dispersive X-ray analysis

For electron microcopy/energy dispersive X-ray (SEM/EDX) analysis, the elemental content of enamel was analyzed at 20 kV and 2,000× magnification. Analysis was conducted perpendicular to the tooth surface, at 5-µm intervals from the enamel surface, toward the pulp chamber. This method of analysis yielded seven measuring points: 0 (tooth surface), 1 (just beneath the surface), 2 (5 µm beneath the surface), 3 (10 µm beneath the surface), 4 (15 µm beneath the surface), 5 (20 µm beneath the surface), and 6 (25 µm beneath the surface). Measuring point 1 for SEM/EDX analysis and nanoindentation test was set at the same level and location from the surface. Three measuring lines were established for each test surface (Fig. 1). Analysis was performed with ZAF correction (atomic number, absorption, and fluorescence) based on standardless correction. The surfaces were carbon-coated for SEM/EDX analysis (GENESIS 2000; EDAX Inc, Tokyo, Japan), and gold-coated for SEM observation (JSM-6340F; JEOL, Tokyo, Japan).

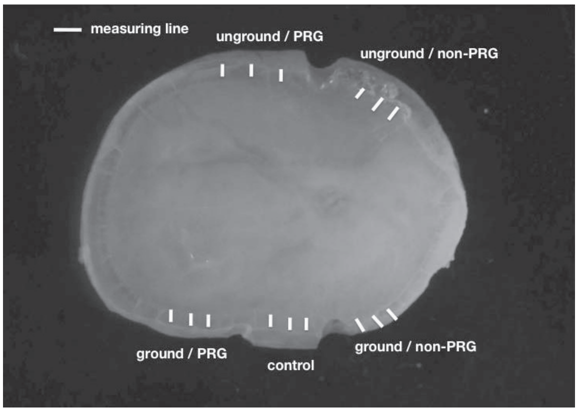

Fig. 1 Regions and measuring lines for nanoindentation test and scanning electron microcopy/energy dispersive X-ray analysis.

The data were statistically analyzed using one-way analysis of variance to examine the effect of location (i.e., measuring points 0-6) for each treatment condition (i.e., control, unground/non-PRG, unground/PRG, ground/non-PRG, and ground/ PRG) in each group (Groups 1, 2, and 3). Post-hoc comparisons were performed using the Fisher PLSD test with α = 0.05.

Results

After demineralization with 10% EDTA and 0.1 M lactic acid, all enamel regions that were not protected with nail varnish changed to a milky white color. Table 1 shows the mean DIAGNOdent readings for the different enamel surface regions. Before treatment and after demineralization, DIAGNOdent readings could not be obtained from regions coated with nail varnish. For all groups, DIAGNOdent readings after demineralization and after immersion were significantly higher than pretreatment readings in the respective media for all regions except the unground/non-PRG region of Group 1. In the unground/non-PRG region of Group 1, DIAGNOdent readings

Table 1 Mean (SD) DIAGNOdent values

Group (immersion medium)	measurement stage	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
Group 1	before treatment	0.05 (0.09) a, A, 1		0.15 (0.18) a, A, 1	
(artificial saliva)	after demineralization	5.10 (1.31) b, A, 1		4.25 (0.18) b, A, 1	
	after immersion	4.25 (0.60) b, A, 1	3.35 (1.25) A, 1	3.50 (0.60) c, A, 1	2.85 (0.19) A, 1
Group 2	before treatment	0.00 (0.00) a, A, 1		0.07 (0.10) a, A, 1	
(demineralizing medium)	after demineralization	4.73 (0.58) b, A, 1		4.87 (2.61) b, A, 1	
	after immersion	4.00 (1.56) b, A, 1	7.73 (2.95) B, 2	5.53 (2.02) b, A, B, 1	4.53 (1.72) A, 1
Group 3	before treatment	0.45 (0.37) a, A, 2		0.33 (0.27) a, A, 2	
(deionized water)	after demineralization	5.00 (2.23) b, A, 1		4.93 (1.83) b, A, 1	
	after immersion	5.67 (2.70) b, A, 1	6.13 (2.72) A, 2	3.47 (0.81) b, A, 1	4.60 (2.09) A, 1

Different alphabetic letters (a, b, c) in the same column and experimental group indicate a significant difference ($P < 0.05$).
Different capital letters (A, B, C) for surface conditions (ie, ground vs unground/non-PRG vs PRG) in the same experimental group indicate a significant difference ($P < 0.05$).
Different numerals (1, 2) among different groups in the same measurement stage indicate a significant difference ($P < 0.05$).

Table 2 Mean (SD) hardness values (unit: GPa)

measuring point	distance from surface (μm)	Group 1 (artificial saliva) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	3.25 (1.44) a, 1	2.24 (1.88) a, 1	1.96 (1.62) a, 1	1.06 (1.20) a, 2	2.38 (1.34) a, 1
2	10	1.78 (1.07) a, 1,2	3.12 (3.30) a, 1	0.75 (0.30) b, 2	1.80 (2.04) a, 1,2	2.87 (1.15) a, 1
3	20	2.39 (1.38) a, 1	2.60 (1.61) a, 1	0.74 (0.13) b, 2	2.04 (2.08) a, 1,2	2.90 (1.23) a, 1
4	30	2.77 (1.11) a, 1	3.57 (1.46) a, 1	0.68 (0.13) b, 2	2.09 (2.16) a, 1,2	2.66 (1.83) a, 1
5	40	2.46 (1.73) a, 1,2	4.21 (3.15) a , 1	0.98 (0.13) b , 2	2.39 (2.44) a , 2	2.22 (1.01) a, 2

measuring point	distance from surface (μm)	Group 2 (demineralizing medium) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	2.24 (1.69) a, 1	1.96 (1.00) a, 1	0.37 (0.13) b, 1	1.27 (0.41) a, 1	0.73 (0.06) b, 1
2	10	3.16 (0.01) a, 1	1.99 (0.61) a, 1	1.17 (0.85) a, b, 1	1.71 (0.03) a, 1	0.96 (0.04) a, 1
3	20	4.29 (0.38) a, 1	2.13 (0.55) a, 1,2	1.98 (1.36) a, 2	1.85 (0.13) a, 2	1.03 (0.06) a, 2
4	30	5.12 (1.32) a, 1	3.00 (0.79) a, 1,2	2.35 (0.50) a, 2	2.01 (0.11) a, 2	1.02 (0.09) a, 2
5	40	5.29 (0.85) a , 1	2.31 (0.25) a, 2	2.19 (0.41) a, 2	1.89 (0.25) a, 2	1.06 (0.08) a, 2

measuring point	distance from surface (μm)	Group 3 (deionized water) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	3.22 (0.45) c, 1	1.97 (0.19) a, 1	3.18 (0.12) a, 1	1.94 (1.43) a, 1	2.31 (0.26) a, 1
2	10	3.72 (0.27) c, 1	2.26 (0.16) a, 1	2.20 (0.31) b,c, 1	2.88 (0.91) a, 1	2.73 (0.65) a, 1
3	20	3.55 (0.37) c, 1	2.45 (1.27) a, 1	2.46 (0.10) b,c, 1	2.89 (0.40) a, 1	2.53 (0.30) a, 1
4	30	4.05 (0.28) b,c, 1	2.33 (0.73) a, 1	1.94 (0.51) c, 1	3.00 (1.20) a, 1	2.31 (0.14) a, 1
5	40	5.19 (0.13) a, 1	2.72 (0.17) a, 1	2.56 (0.21) b, 1	2.89 (0.91) a, 1	2.68 (0.48) a, 1

Different alphabetic letters (a, b, c) in the same column and experimental group indicate a significant difference ($P < 0.05$).
Different numerals (1, 2) in the same row indicate a significant difference ($P < 0.05$).

were significantly lower ($P < 0.05$) after immersion in artificial saliva.

Table 2 shows mean hardness (H) values. In the ground/PRG region of Groups 1 and 3, H at measuring point 1 was significantly higher than that at points 2-5 ($P < 0.05$). In the ground/PRG and unground/PRG regions of Group 2, H at point 1 was significantly lower than that at points 3-5 ($P < 0.05$).

Table 3 shows mean Young's modulus (Y) values. In the ground/PRG region of Groups 1 and 3, Y at measuring point 1 was significantly higher than that at points 2-5 ($P < 0.05$). In the ground/PRG and unground/non-PRG regions of Group 2, Y at point 1 was significantly lower than that at points 3-5 ($P < 0.05$). In the unground/PRG region in Group 2, Y at point 1 was significantly lower than that at points 2-5 ($P < 0.05$).

Table 3 Mean (SD) Young's modulus values (unit: GPa)

measuring point	distance from surface (μm)	Group 1 (artificial saliva) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	107.8 (68.0) a, 1	58.1 (36.5) a, 2	64.7 (55.2) a, 1,2	65.0 (27.7) a, 1,2	81.5 (41.9) a, 1,2
2	10	65.2 (33.5) a, 1,2	72.3 (61.2) a, 1,2	45.1 (35.2) b, 2	67.5 (45.3) a, 1,2	91.8 (37.8) a, 1
3	20	70.1 (34.8) a, 1	66.4 (34.5) a, 1	33.1 (16.7) b, 2	77.9 (50.4) a, 1,2	94.5 (35.5) a, 1
4	30	81.6 (36.6) a, 1	82.5 (32.4) a, 1	33.0 (18.3) b, 2	80.7 (63.5) a, 1	92.1 (49.5) a, 1
5	40	75.4 (50.6) a, 1,2	92.6 (63.7) a, 1	38.5 (13.9) b, 2	76.1 (55.7) a, 1,2	76.4 (34.0) a, 1,2

measuring point	distance from surface (μm)	Group 2 (demineralizing medium) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	56.7 (25.5) b, 1	40.6 (17.2) a, 1	21.7 (6.1) b, 1	34.7 (7.2) b, 1	16.1 (1.0) b, 1
2	10	75.6 (2.0) a,b, 1	42.9 (9.2) a, 1	44.0 (22.3) a,b, 1	42.6 (1.0) a, b, 1	20.5 (0.5) a, 1
3	20	94.4 (8.0) a,b, 1	44.3 (8.2) a, 1,2	58.7 (28.9) a, 1,2	44.8 (3.0) a, 1,2	22.0 (1.1) a, 2
4	30	111.3 (22.2) a, 1	58.4 (15.1) a, 1,2	71.4 (10.3) a, 1,2	47.9 (3.0) a, 1,2	21.8 (1.5) a, 2
5	40	110.0 (16.9) a,b, 1	47.9 (5.7) a, 1,2	75.8 (16.3) a, 1,2	45.4 (5.4) a, 1,2	22.4 (1.4) a, 2

measuring point	distance from surface (μm)	Group 3 (deionized water) Control	ground/ non-PRG	ground/ PRG	unground/ non-PRG	unground/ PRG
1	0	123.5 (2.1) b, 1	71.6 (6.5) a, 1	77.3 (3.8) a, 1	61.4 (29.1) a, 1	63.8 (4.9) a, 1
2	10	136.5 (9.2) a,b 1	88.6 (3.0) a, 1,2	56.5 (5.8) b, 2	84.2 (20.3) a, 1,2	70.0 (11.3) a, 2
3	20	136.5 (19.1) a,b, 1	93.2 (23.9) a, 1,2	61.4 (2.5) b, 1,2	81.1 (9.7) a, 2	66.7 (4.8) a, 1, 2
4	30	141.5 (9.2) a,b, 1	86.3 (16.5) a, 1,2	55.3 (11.5) b, 2	83.4 (15.6) a, 1,2	63.2 (4.4) a, 2
5	40	158.5 (0.7) a, 1	96.6 (11.3) a, 1,2	63.5 (3.9) b, 2	82.5 (9.2) a, 2	70.3 (8.3) a, 2

Different alphabetic letters (a, b, c) in the same column and experimental group indicate a significant difference ($P < 0.05$).
Different numerals (1, 2) in the same row indicate a significant difference ($P < 0.05$).

Table 4 Mean (SD) elemental content (mass %) of enamel in Group 1 (immersed in artificial saliva for 1 month)

Region	measuring point	distance from surface (μm)	C	O	Na	Mg	Al	P	Cl	Ca	Ca/P
Control	0		21.8 (2.2) a	18.0 (2.1) a	0.8 (0.3) b	0.8 (0.3) a	0.6 (0.2) a	18.7 (1.1) a	1.0 (0.3) a, b	38.3 (2.1) a, b	2.05 (0.12) a
	1	0 - 1	23.0 2.5) a	17.5 (2.0) a	1.0 (0.3) a, b	0.7 (0.3) a	0.6 (0.3) a	18.6 (0.9) a	0.9 (0.3) b	37.7 (1.3) b	2.03 (0.08) a
	2	5	21.7 (1.9) a	16.9 (2.5) a	0.9 (0.4) b	0.8 (0.3) a	0.6 (0.2) a	19.1 (1.0) a	1.1 (0.3) a	38.8 (1.8) a, b	2.04 (0.12) a
	3	10	22.2 (2.2) a	17.5 (1.7) a	1.1 (0.4) a, b	0.7 (0.2) a	0.7 (0.3) a	18.4 (0.7) a	1.0 (0.2) a, b	38.4 (2.0) a, b	2.09 (0.09) a
	4	15	22.1 (1.6) a	17.3 (1.8) a	1.0 (0.4) a, b	0.8 (0.2) a	0.6 (0.1) a	18.5 (0.8) a	1.0 (0.2) a, b	38.6 (2.0) a, b	3.42 (5.17) a
	5	20	22.7 (2.7) a	17.8 (2.9) a	0.9 (0.2) b	0.9 (0.2) a	0.6 (0.2) a	18.4 (1.2) a	1.1 (0.2) a	37.6 (1.3) b	2.00 (0.21) a
	6	25	22.1 (2.4) a	16.4 (1.9) a	1.2 (0.4) a	0.8 (0.4) a	0.7 (0.1) a	18.4 (0.9) a	1.1 (0.3) a	39.3 (1.7) a	2.14 (0.14) a
unground/ non-PRG	0		75.9 (2.0) a	22.5 (3.5) b	0.2 (0.2) b	0.2 (0.3) c	0.3 (0.4) c	0.4 (0.4) d	0.2 (0.3) c	0.3 (0.4) d	0.36 (0.47) d
	1	0 - 1	70.0 (6.4) b	23.8 (3.0) b	0.3 (0.3) b	0.2 (0.2) c	0.2 (0.2) c	2.0 (0.0) c	0.3 (0.4) c	3.1 (3.8) c	1.09 (0.90) c
	2	5	29.2 (5.2) c	27.6 (2.2) a	1.4 (0.3) a	0.8 (0.2) a	0.7 (0.2) a	14.3 (2.2) b	0.8 (0.2) b	25.1 (4.0) b	1.75 (0.11) b
	3	10	21.6 (2.6) d	17.9 (2.7) c	1.0 (0.2) c	0.9 (0.3) a	0.7 (0.2) a, b	18.9 (1.0) a	1.2 (0.2) a	37.9 (1.8) a	2.00 (0.07) a, b
	4	15	23.1 (2.0) d	16.4 (2.0) c	0.9 (0.3) c	0.8 (0.3) a, b	0.6 (0.2) a, b	18.7 (1.0) a	1.2 (0.3) a	38.2 (1.6) a	2.04 (0.11) a
	5	20	22.2 (2.2) d	16.4 (3.3) c	0.9 (0.5) c	0.6 (0.3) b	0.5 (0.3) b	18.7 (1.0) a	1.2 (0.3) a	39.4 (2.2) a	2.11 (0.09) a
	6	25	22.0 (1.8) d	16.5 (2.5) c	0.9 (0.4) c	0.8 (0.2) a, b	0.6 (0.2) a, b	18.8 (1.0) a	1.2 (0.2) a	39.3 (1.9) a	2.10 (0.12) a
unground/ PRG	0		60.5 (4.1) a	23.7 (1.9) b	0.5 (0.3) c	0.3 (0.2) c	0.2 (0.2) c	5.5 (0.9) c	0.18 (0.13) d	9.1 (1.6) d	1.66 (0.14) c
	1	0 - 1	30.6 (4.8) b	28.0 (3.1) a	1.1 (0.3) a	0.8 (0.2) a	0.7 (0.2) a	13.8 (2.1) b	0.82 (0.21) c	24.1 (4.5) c	1.74 (0.15) c
	2	5	23.3 (3.0) c	17.0 (2.6) c	1.0 (0.3) a	0.7 (0.3) a, b	0.6 (0.2) a, b	18.9 (1.1) a	1.20 (0.24) a, b	37.3 (3.2) b	2.00 (0.10) b
	3	10	23.9 (1.4) c	14.8 (1.7) d	0.8 (0.3) b	0.7 (0.2) b	0.7 (0.2) a	18.3 (0.9) a	1.19 (0.21) a, b	39.6 (1.1) a	2.17 (0.12) a
	4	15	23.6 (2.0) c	13.7 (1.5) d	1.0 (0.3) a, b	0.7 (0.2) a, b	0.6 (0.2) a, b	18.2 (0.9) a	1.24 (0.25) a	40.9 (1.9) a	2.24 (0.11) a
	5	20	23.8 (2.3) c	14.3 (1.5) d	1.0 (0.4) a, b	0.6 (0.2) b	0.6 (0.1) a, b	18.1 (0.7) a	1.08 (0.21) b	40.4 (1.8) a	2.23 (0.13) a
	6	25	23.3 (2.5) c	14.2 (2.0) d	0.8 (0.3) b	0.6 (0.3) a, b	0.5 (0.2) b	18.5 (0.8) a	1.14 (0.17) a, b	40.9 (2.1) a	2.21 (0.13) a
ground/ non-PRG	0		21.4 (2.0) b	19.3 (1.9) a	1.2 (0.4) a	0.7 (0.2) b	0.7 (0.2) a	18.5 (1.1) a, b	1.0 (0.3) a	37.2 (1.6) a	2.02 (0.17) b
	1	0 - 1	22.8 (2.4) a, b	17.8 (2.0) b	1.2 (0.2) a	0.9 (0.3) a, b	0.7 (0.2) a	18.5 (0.8) a, b	0.9 (0.2) a	37.4 (2.1) a	2.03 (0.12) b
	2	5	21.7 (2.3) a, b	18.2 (2.1) a, b	1.2 (0.3) a	1.0 (0.3) a	0.7 (0.1) a	18.7 (1.0) a	1.0 (0.2) a	37.6 (1.4) a	2.01 (0.10) b
	3	10	21.7 (1.8) a, b	18.6 (2.3) a, b	1.0 (0.2) a	0.8 (0.3) a, b	0.6 (0.3) a	18.1 (0.9) a, b	0.9 (0.3) a	38.2 (1.7) a	2.11 (0.12) b
	4	15	22.0 (2.3) a, b	17.7 (2.2) b	1.2 (0.3) a	0.9 (0.2) a, b	0.7 (0.2) a	18.7 (0.8) a	1.0 (0.2) a	37.8 (1.5) a	2.02 (0.08) b
	5	20	22.4 (1.8) a, b	17.5 (0.9) b	1.1 (0.5) a	0.8 (0.2) a, b	0.7 (0.2) a	18.4 (0.6) a, b	0.9 (0.2) a	38.3 (2.1) a	4.09 (5.61) a
	6	25	23.2 (3.3) a	17.5 (2.2) b	1.1 (0.4) a	0.8 (0.4) a, b	0.7 (0.2) a	17.9 (1.0) b	0.8 (0.3) a	37.9 (2.3) a	2.12 (0.12) b
ground/ PRG	0		22.0 (2.3) a, b	18.0 (1.7) a	1.0 (0.4) a	0.8 (0.3) a, b	0.6 (0.1) a	18.4 (1.1) a	1.00 (0.25) b	38.1 (1.8) a	2.08 (0.11) a
	1	0 - 1	21.8 (1.1) b	17.3 (2.2) a	1.1 (0.3) a	0.9 (0.3) a, b	0.7 (0.2) a	18.6 (0.8) a	1.21 (0.29) a	38.5 (1.5) a	2.07 (0.07) a
	2	5	22.2 (2.0) a, b	17.1 (2.3) a	1.1 (0.4) a	0.9 (0.1) a, b	0.6 (0.2) a	18.7 (1.0) a	1.17 (0.31) a, b	38.2 (1.4) a	2.05 (0.11) a
	3	10	21.3 (1.7) b	18.0 (2.5) a	1.2 (0.4) a	0.8 (0.3) b	0.6 (0.3) a	18.6 (1.0) a	1.12 (0.24) a, b	38.5 (2.0) a	2.07 (0.12) a
	4	15	22.8 (2.3) a	16.9 (2.8) a	1.0 (0.4) a	0.7 (0.2) b	0.7 (0.2) a	18.7 (0.7) a	1.10 (0.26) a, b	38.0 (1.5) a	2.04 (0.09) a
	5	20	22.2 (2.4) a, b	17.6 (2.3) a	1.0 (0.2) a	1.0 (0.2) a	0.6 (0.2) a	18.5 (0.7) a	1.03 (0.27) a, b	38.1 (1.3) a	2.06 (0.09) a
	6	25	23.4 (2.1) a	16.6 (2.4) a	1.0 (0.3) a	0.7 (0.2) a, b	0.6 (0.2) a	18.6 (0.7) a	1.00 (0.21) b	38.0 (1.4) a	2.01 (0.15) a

For each region, different alphabetic letters in the same column indicate a significant difference ($P < 0.05$).

Table 5 Mean (SD) elemental content (mass %) of enamel in Group 2 (immersed in demineralizing medium for 1 month)

Region	measuring point	distance from surface (μm)	C	O	Na	Mg	Al	P	Cl	Ca	Ca/P
Control	0		15.7 (2.5) a	24.9 (2.1) a	1.3 (0.3) b	1.1 (0.3) a	0.8 (0.2) a	19.3 (1.0) a, b	1.1 (0.2) a	36.0 (1.5) a	1.88 (0.09) a
	1	0 - 1	16.0 (1.9) a	24.1 (1.7) a	1.3 (0.3) a	1.0 (0.3) a	0.7 (0.2) a	19.2 (1.1) a, b	1.2 (0.3) a	36.5 (1.3) a	1.91 (0.11) a
	2	5	15.5 (2.1) a	24.5 (1.9) a	1.5 (0.3) a	1.0 (0.4) a	0.7 (0.2) a	19.5 (1.0) a, b	1.2 (0.2) a	36.1 (1.7) a	1.86 (0.10) a
	3	10	15.4 (2.4) a	25.4 (1.9) a	1.4 (0.3) a	0.9 (0.2) a	0.8 (0.2) a	19.3 (0.8) a, b	1.2 (0.3) a	35.5 (1.8) a	1.84 (0.09) a
	4	15	15.6 (2.5) a	25.2 (2.1) a	1.3 (0.4) a	1.0 (0.3) a	0.7 (0.2) a	19.6 (0.7) a, b	1.1 (0.2) a	35.6 (1.3) a	1.82 (0.07) a
	5	20	16.0 (1.5) a	24.9 (2.0) a	1.5 (0.5) a	0.9 (0.3) a	0.7 (0.2) a	19.0 (1.1) b	1.1 (0.3) a	36.0 (1.9) a	3.23 (5.24) a
	6	25	14.8 (1.8) a	24.9 (2.7) a	1.4 (0.4) a	1.1 (0.2) a	0.8 (0.3) a	19.7 (0.7) a	1.1 (0.2) a	36.2 (1.4) a	1.84 (0.08) a
unground/ non-PRG	0		29.2 (5.8) a	35.8 (5.4) a	1.53 (0.47) b, c	1.01 (0.26) a, b	1.01 (0.29) a, b	12.1 (2.3) d	0.7 (0.2) b	18.7 (3.8) d	1.55 (0.07) d
	1	0 - 1	22.7 (3.3) b	36.0 (2.4) a	1.76 (0.42) c	1.08 (0.30) a, b	1.06 (0.16) a	14.1 (1.5) c	0.8 (0.3) b	22.5 (2.5) c	1.60 (0.10) d
	2	5	19.1 (1.7) c	31.8 (2.7) b	1.87 (0.41) a	1.14 (0.26) a, b	1.17 (0.24) a	17.2 (1.1) b	1.1 (0.2) a	26.6 (2.1) b	1.55 (0.05) c
	3	10	19.4 (1.7) c	27.9 (3.1) c	1.54 (0.32) a, b	1.17 (0.39) a	1.04 (0.31) a	18.1 (1.4) a	1.2 (0.3) a	29.6 (1.8) a	1.64 (0.13) b
	4	15	19.3 (1.5) c	27.4 (2.3) c	1.52 (0.46) b	0.96 (0.26) b	0.84 (0.28) b, c	18.2 (0.7) a	1.1 (0.2) a	30.7 (2.2) a	1.69 (0.12) a, b
	5	20	19.4 (2.0) c	26.8 (1.9) c	1.35 (0.65) b	1.14 (0.28) a, b	0.81 (0.25) c	18.2 (1.0) a	1.1 (0.3) a	31.2 (2.0) a	1.72 (0.14) a
	6	25	18.7 (2.3) c	27.6 (2.6) c	1.60 (0.50) a, b	0.93 (0.25) b	0.89 (0.21) b, c	18.5 (0.9) a	1.0 (0.2) a	30.8 (1.5) a	1.67 (0.10) a, b
unground/ PRG	0		28.2 (3.4) a	37.7 (1.5) a	1.43 (0.39) b	1.03 (0.29) a, b	1.15 (0.43) a	11.7 (1.3) d	0.65 (0.11) d	18.1 (1.6) e	1.55 (0.09) a, b
	1	0 - 1	19.5 (2.2) b	37.8 (2.3) a	1.87 (0.43) a	1.14 (0.24) a	1.14 (0.26) a	15.1 (1.1) c	0.88 (0.19) c	22.6 (1.2) d	1.50 (0.08) b
	2	5	19.6 (2.5) b	33.2 (3.6) b	1.64 (0.27) a	1.17 (0.25) a, b	1.01 (0.26) a, b	16.3 (1.9) b	0.91 (0.12) b, c	26.2 (3.7) c	2.27 (2.63) a
	3	10	18.6 (2.0) b, c	26.3 (2.2) c	1.46 (0.41) b	1.01 (0.32) a, b	0.96 (0.22) a, b, c	18.8 (0.8) a	1.01 (0.24) a, b, c	32.0 (1.6) b	1.70 (0.07) a, b
	4	15	20.0 (2.3) b	24.0 (2.2) d	1.47 (0.49) b	1.08 (0.27) a, b	0.84 (0.32) b, c	18.5 (0.8) a	1.05 (0.21) a, b	33.1 (1.7) a	1.79 (0.09) a, b
	5	20	18.6 (2.3) b, c	25.0 (2.5) c, d	1.52 (0.38) b	1.03 (0.32) a, b	0.95 (0.25) a, b, c	19.1 (1.3) a	1.05 (0.24) a, b	32.8 (1.8) a	1.72 (0.12) a, b
	6	25	17.1 (2.9) c	26.2 (3.3) c	1.43 (0.31) b	0.95 (0.28) b	0.78 (0.10) c	18.8 (0.8) a	1.11 (0.26) a	33.6 (1.5) a	1.79 (0.13) a, b
ground/ non-PRG	0		21.2 (4.6) a, b	27.8 (3.6) b	1.95 (0.50) a	1.20 (0.34) a	1.00 (0.27) a	17.4 (1.4) a	0.77 (0.18) a	28.7 (1.1) a, b	1.65 (0.13) a
	1	0 - 1	20.0 (2.0) a, b	30.3 (2.4) a	1.72 (0.46) a	1.26 (0.35) a	0.96 (0.36) a	17.2 (1.2) a	0.80 (0.13) a	27.8 (1.4) a, b	1.62 (0.10) a
	2	5	21.6 (2.9) a	27.3 (2.2) b	1.62 (0.43) a	1.18 (0.37) a	0.86 (0.25) a	17.7 (0.9) a	0.79 (0.20) a	28.9 (1.3) a	2.30 (2.60) a
	3	10	21.5 (3.9) a, b	28.0 (2.9) b	1.76 (0.47) a	1.13 (0.34) a	0.92 (0.27) a	17.4 (1.4) a	0.78 (0.22) a	28.6 (2.3) a, b	1.65 (0.11) a
	4	15	20.9 (2.3) a, b	29.2 (2.1) b	1.66 (0.50) a	1.19 (0.34) a	1.02 (0.33) a	17.4 (0.9) a	0.73 (0.24) a	27.9 (1.9) a, b	1.61 (0.10) a
	5	20	19.9 (2.1) a, b	29.7 (3.0) a	1.92 (0.46) a	1.03 (0.25) a	0.89 (0.26) a	17.3 (0.8) a	0.78 (0.23) a	28.5 (1.8) a, b	1.65 (0.10) a
	6	25	19.4 (2.3) b	31.4 (2.1) a	1.72 (0.46) a	1.04 (0.29) a	0.88 (0.25) a	17.1 (1.2) b	0.87 (0.20) a	27.6 (2.0) b	1.62 (0.11) a
ground/ PRG	0		16.3 (2.1) b	27.8 (2.5) a, b	1.63(0.40) a, b	1.31 (0.40) a	0.84 (0.18) a	18.7 (0.8) a, b	0.87 (0.25) b	32.5 (1.9) a	1.74 (0.11) a
	1	0 - 1	16.4 (2.3) b	27.1 (2.2) a, b	1.73 (054) a, b	1.19 (0.27) a	0.96 (0.31) a	19.3 (0.9) a	0.93 (0.22) a	32.5 (1.8) a	2.35 (2.62) a
	2	5	16.7 (3.0) b	26.8 (2.3) b	1.56 (0.62) a, b	1.16 (0.27) a	0.93 (0.21) a	18.7 (1.1) a, b	0.88 (0.19) a	33.3 (1.5) a	1.78 (0.14) a
	3	10	16.3 (2.5) b	27.4 (2.4) a, b	1.89 (0.61) a	1.25 (0.23) a	0.88 (0.19) a	18.9 (0.9) a, b	0.96 (0.20) a	32.4 (1.5) a	1.72 (0.09) a
	4	15	15.6 (1.7) b	28.6 (1.8) a	1.64 (0.39) a, b	1.14 (0.34) a	0.92 (0.27) a	18.8 (0.8) a, b	0.95 (0.25) a	32.3 (2.0) a	1.72 (0.14) a
	5	20	18.4 (2.1) a	26.6 (1.9) b	1.53 (0.43) b	1.10 (0.26) a	0.87 (0.29) a	18.5 (1.0) b	0.94 (0.31) a	32.1 (2.1) a	1.74 (0.12) a
	6	25	16.8 (2.0) a, b	28.2 (3.2) a, b	1.72 (0.31) a, b	1.15 (0.41) a	0.78 (0.28) a	18.3 (1.3) b	0.92 (0.21) a	32.0 (2.5) a	1.75 (0.14) a

For each region, different alphabetic letters in the same column indicate a significant difference ($P < 0.05$).

Tables 4-6 show the mean elemental content of enamel in the five regions. Significant differences were identified in elemental content among the measuring points from the five regions in each group. In Group 1 (Table 4), compared with the points beneath the enamel surface, the C% and O% at measuring point 0, or points 0 and 1, was significantly higher. In contrast, the percentages of other elements were significantly lower in the unground/non-PRG and unground/PRG regions. In Group 2 (Table 5), compared with the points beneath the enamel surface, the C% at point 0, or points 0 and 1, and the O% at points 0, 1, and 2 were significantly higher. In contrast, the P%, Cl%, and Ca%, and Ca/P ratio at points 0 and 1, or points 0-2 were significantly lower for unground/non-PRG and unground/PRG regions, except for the Ca/P ratio of the unground/PRG region. In Group 3 (Table 6), compared with the points beneath the enamel surface, the C% and O% at points 0 and 1 were significantly higher; however, the P%, Cl%, Ca%, and the Ca/P ratio were significantly lower for unground/non-PRG regions. For the unground/PRG area, the C% at points 0 and 1 and the Ca/P ratio at point 0 were significantly higher than those at points beneath the enamel surface, however, the Mg% and P% at points 0 and 1 were significantly lower than the values for points beneath the enamel surface.

Table 7 shows the mean Ca/P ratio of the enamel surface in the different groups. There was no significant difference of the Ca/P ratio among measuring points for the control and ground/PRG regions in any experimental group. In all groups, the Ca/P ratio at the enamel surface (points 0 and 1) of the unground/non-PRG region was significantly lower than that at points exceeding 10 μm beneath the surface (points 3-6). In Group 1 (artificial saliva), the Ca/P ratio at points 0-2 of the unground/PRG region was significantly lower than that at points exceeding10 μm beneath the surface (points 3-6).

Figure 2 shows representative SEM images of unground enamel taken from different regions of each group after

Table 6 Mean (SD) elemental content (mass %) of enamel in Group 3 (immersed in deionized water for 1 month)

Region	measuring point	distance from surface (μm)	C	O	Na	Mg	Al	P	Cl	Ca	Ca/P
Control	0		27.5 (2.3) a	13.5 (2.7) a, b	0.9 (0.3) a, b	0.8 (0.3) a	0.62 (0.21) a	18.0 (1.0) a	0.97 (0.25) a	37.8 (2.1) a, b	2.11 (0.09) a
	1	0 - 1	28.4 (1.8) a	14.4 (1.5) a	0.7 (0.3) b	0.7 (0.3) a	0.46 (0.22) b	17.3 (0.6) b	0.95 (0.23) a	37.0 (1.3) b	2.14 (0.08) a
	2	5	28.0 (2.1) a	12.6 (2.6) b	0.7 (0.3) b	0.7 (0.3) a	0.55 (0.26) a, b	18.1 (0.6) a	1.01 (0.27) a	38.3 (2.3) a	2.11 (0.14) a
	3	10	28.4 (3.3) a	12.6 (2.5) b	0.8 (0.4) a, b	0.8 (0.4) a	0.54 (0.26) a, b	17.8 (0.9) a, b	0.90 (0.19) a	38.1 (1.8) a, b	2.14 (0.11) a
	4	15	28.2 (2.2) a	13.3 (2.0) a, b	1.0 (0.2) a	0.8 (0.2) a	0.66 (0.15) a	17.8 (0.7) a, b	1.02 (0.18) a	37.3 (1.9) a, b	2.10 (0.15) a
	5	20	28.7 (2.5) a	12.3 (2.2) b	0.7 (0.4) b	0.9 (0.3) a	0.59 (0.13) a, b	18.0 (1.1) a	0.90 (0.02) a	37.9 (1.6) a, b	2.11 (0.09) a
	6	25	28.3 (2.0) a	12.5 (1.3) b	0.8 (0.3) a, b	0.8 (0.2) a	0.62 (0.16) a	18.2 (0.6) a	0.97 (0.24) a	37.7 (1.4) a, b	2.07 (0.07) a
unground/ non-PRG	0		38.4 (8.5) a	27.3 (2.4) a	1.24 (0.35) a	0.78 (0.16) a, b	0.6 (0.1) a	11.3 (2.9) c	0.72 (0.19) c	19.7 (5.5) c	1.74 (0.12) d
	1	0 - 1	31.8 (4.0) b	21.0 (4.2) b	1.08 (0.52) a	0.92 (0.21) a	0.7 (0.2) a	15.6 (2.2) b	0.92 (0.31) b	28.0 (5.0) b	1.79 (0.10) c
	2	5	28.1 (2.6) c	13.7 (2.1) d	0.68 (0.41) c	0.71 (0.37) b	0.5 (0.3) a	17.9 (1.1) a	1.20 (0.35) a	37.2 (2.9) a	3.42 (5.16) b
	3	10	26.2 (2.4) c	16.6 (2.0) c	0.73 (0.43) b, c	0.70 (0.21) b	0.6 (0.2) a	17.7 (0.9) a	1.03 (0.23) a, b	36.4 (1.5) a	2.06 (0.12) a, b
	4	15	26.7 (1.7) c	15.3 (1.6) c, d	0.98 (0.42) a, b	0.75 (0.27) a, b	0.6 (0.1) a	17.9 (0.9) a	1.05 (0.20) a, b	36.7 (1.7) a	2.05 (0.14) a
	5	20	27.8 (1.8) c	14.9 (2.3) c, d	0.85 (0.30) b, c	0.78 (0.30) a, b	0.6 (0.2) a	17.4 (0.5) a	1.23 (0.29) a	36.4 (1.9) a	2.10 (0.12) a
	6	25	27.1 (3.0) c	14.1 (2.7) d	0.85 (0.43) b, c	0.69 (0.35) b	0.6 (0.3) a	17.5 (1.0) a	1.08 (0.34) a, b	38.0 (2.3) a	2.23 (0.25) a
unground/ PRG	0		43.6 (2.4) a	19.2 (3.0) b	0.87 (0.37) b	0.61 (0.18) b	0.52 (0.17) c	11.6 (1.6) e	0.71 (0.20) d	22.9 (3.1) c	1.99 (0.17) a
	1	0 - 1	35.8 (4.1) b	28.1 (1.9) a	1.18 (0.27) a	0.87 (0.12) a	0.67 (0.16) c	12.1 (1.1) d, e	0.74 (0.17) c, d	20.6 (1.5) d	1.71 (0.09) d
	2	5	33.0 (4.2) c	27.6 (1.5) a	1.41 (0.31) a	0.87 (0.23) a	1.08 (0.44) a	12.6 (1.5) c, d	0.77 (0.18) b. c, d	22.6 (2.2) c	1.80 (0.11) c
	3	10	33.3 (4.7) c	26.4 (2.9) a	1.32 (0.30) a	0.89 (0.16) a	1.00 (0.56) a, b	13.3 (1.2) c	0.83 (0.19) b, c, d	23.0 (1.9) c	1.73 (0.11) c, d
	4	15	29.1 (1.8) d	27.7 (2.6) a	1.44 (0.37) a,	0.92 (0.25) a	0.81 (0.17) b, c	14.3 (1.0) b	0.88 (0.16) b	24.8 (1.6) b	1.75 (0.12) c, d
	5	20	27.8 (2.0) d	26.5 (1.9) a	1.33 (0.23) a,	0.97 (0.25) a	0.71 (0.20) c	15.0 (0.8) b	0.86 (0.21) b, c	26.9 (1.8) b	1.80 (0.13) c
	6	25	27.9 (1.4) d	19.4 (2.2) b	1.01 (0.36) b	0.87 (0.22) a	0.81 (0.19) b, c	17.0 (0.9) a	1.03 (0.23) a	32.1 (1.6) a	1.90 (0.11) b
ground/ non-PRG	0		29.1 (3.2) a	12.4 (2.2) a, b	0.86 (0.45) a	0.74 (0.29) a	0.65 (0.31) a	17.2 (0.9) a	0.83 (0.23) a	38.2 (2.5) a, b	2.24 (0.12) a, b, c
	1	0 - 1	29.7 (2.9) a	13.0 (4.5) a	0.68 (0.37) a	0.63 (0.39) a	0.49 (0.27) a	17.0 (1.1) a	0.59 (0.35) b	37.8 (3.2) b	2.22 (0.17) b, c
	2	5	30.5 (2.2) a	10.8 (3.0) a, b, c	0.76 (0.33) a	0.71 (0.36) a	0.51 (0.21) a	17.6 (0.9) a	0.68 (0.29) a, b	38.5 (3.1) a, b	2.19 (0.11) c
	3	10	30.2 (2.9) a	9.9 (2.5) c	0.81 (0.43) a	0.65 (0.18) a	0.58 (0.19) a	17.1 (1.3) a	0.73 (0.18) a, b	40.0 (2.3) a	2.32 (0.21) a
	4	15	30.3 (2.7) a	11.3 (2.8) a, b, c	0.95 (0.33) a	0.58 (0.17) a	0.57 (0.22) a	17.4 (0.9) a	0.86 (0.12) a	38.1 (2.6) a, b	2.19 (0.12) b, c
	5	20	29.5 (2.4) a	10.3 (3.8) b, c	0.78 (0.46) a	0.60 (0.29) a	0.59 (0.21) a	17.7 (1.4) a	0.82 (0.26) a	39.7 (3.5) a, b	2.25 (0.16) a, b, c
	6	25	29.5 (3.1) a	11.2 (3.0) a, b, c	0.73 (0.38) a	0.64 (0.32) a	0.50 (0.30) a	17.0 (0.7) a	0.71 (0.23) a, b	39.8 (2.8) a, b	2.34 (0.14) a
ground/ PRG	0		29.0 (1.6) a, b	12.1 (2.0) b	0.93 (0.37) a	0.73 (0.22) a, b	0.60 (0.23) a	17.7 (0.7) a, b	0.89 (0.23) a	38.0 (1.3) a	2.15 (0.12) a
	1	0 - 1	28.9 (2.1) a, b	12.2 (2.4) b	0.95 (0.28) a	0.78 (0.16) a	0.58 (0.19) a	17.4 (0.7) a, b, c	0.96 (0.19) a	38.2 (1.9) a	2.20 (0.12) a
	2	5	27.4 (2.6) b	15.2 (3.8) a	0.83 (0.37) a	0.75 (0.38) a, b	0.57 (0.18) a	17.0 (0.9) c	0.87 (0.24) a	37.3 (2.4) a	3.53 (5.13) a
	3	10	28.2 (3.0) a, b	12.6 (2.9) b	0.88 (0.24) a	0.73 (0.14) a, b	0.61 (0.17) a	17.4 (0.7) a, b. c	0.95 (0.20) a	38.6 (2.9) a	2.22 (0.17) a
	4	15	29.7 (2.2) a	12.4 (2.2) b	0.77 (0.34) a	0.60 (0.28) b	0.52 (0.29) a	17.2 (0.8) b, c	0.88 (0.28) a	37.9 (2.2) a	3.54 (5.19) a
	5	20	28.8 (2.4) a, b	12.4 (2.5) b	0.89 (0.31) a	0.76 (0.29) a, b	0.64 (0.21) a	17.8 (0.6) a	0.87 (0.30) a	37.8 (1.7) a	4.79 (7.05) a
	6	25	29.6 (2.1) a	12.6 (1.7) b	0.87 (0.30) a	0.80 (0.80) a,	0.57 (0.14) a	17.3 (0.6) b, c	0.83 (0.21) a	37.4 (1.8) a	2.17 (0.15) a

For each region, different alphabetic letters in the same column indicate a significant difference ($P < 0.05$).

Table 7 Mean (SD) Ca/P ratios

group	region	Measuring point 0	1	2	3	4	5	6
Group 1	control	2.05 (0.12) a, A	2.03 (0.08) a, A	2.04 (0.12) a, A	2.09 (0.09) a, A	3.42 (5.17) a, A	2.00 (0.21) a, A	2.14 (0.14) a, A
	unground/ non-PRG	0.36 (0.47) c, D	1.09 (0.90) b, C	1.75 (0.11) a, B	2.00 (0.07) b, A, B	2.04 (0.11) b, A	2.11 (0.09) a, A	2.10 (0.12) b, A
	unground/PRG	1.66 (0.14) b, C	1.74 (0.15) a, C	2.00 (0.10) a, B	2.17 (0.12) a, A	2.24 (0.11) a, b, A	2.23 (0.13) a, A	2.21 (0.13) a, A
	ground/ non-PRG	2.02 (0.17) a, B	2.03 (0.12) a, B	2.01 (0.10) a, B	2.11 (0.12) a, B	2.02 (0.08) b, B	4.09 (5.61) a, A	2.12 (0.12) a, B
	ground/ PRG	2.08 (0.11) a, A	2.07 (0.07) a, A	2.05 (0.11) a, A	2.07 (0.12) b, A	2.04 (0.09) b, A	2.06 (0.09) a, A	2.01 (0.15) b, A
Group 2	control	1.88 (0.09) a, A	1.91 (0.11) b, A	1.86 (0.10) a, A	1.84 (0.09) a, A	1.82 (0.07) a, A	3.23 (5.24) a, A	1.84 (0.08) a, A
	unground/ non-PRG	1.55 (0.07) c, D	1.60 (0.10) b, D	1.55 (0.05) a, C	1.64 (0.13) b, B	1.69 (0.12) a, A, B	1.72 (0.14) a, A	1.67 (0.10) b, c, A, B
	unground/ PRG	1.55 (0.09) c, A, B	1.50 (0.08) b, B	2.27 (2.63) a, A	1.70 (0.07) b, A, B	1.79 (0.09) a, A, B	1.72 (0.12) a, A, B	1.79 (0.13) a, A, B
	ground/ non-PRG	1.65 (0.13) b, c, A	1.62 (0.10) b, A	2.30 (2.60) a, A	1.65 (0.11) b, A	1.61 (0.10) a, A	1.65 (0.10) a, A	1.62 (0.11) c, A
	ground/ PRG	1.74 (0.11) b, A	2.35 (2.62) a, A	1.78 (0.14) a, A	1.72 (0.09) b, A	1.72 (0.14) a, A	1.74 (0.12) a, A	1.75 (0.14) b, A
Group 3	control	2.11 (0.09) b, A	2.14 (0.08) a, A	2.11 (0.14) a, b, A	2.14 (0.11) b, A	2.10 (0.15) b, A	2.11 (0.09) a, A	2.07 (0.07) d, A
	unground/ non-PRG	1.74 (0.12) d, D	1.79 (0.10) a, C	3.42 (5.16) a, B	2.06 (0.12) c, A, B	2.05 (0.14) b, A	2.10 (0.12) a, A	2.23 (0.25) b, A
	unground/ PRG	1.99 (0.17) c, A	1.71 (0.09) a, D	1.80 (0.11) b, C	1.73 (0.11) d, C, D	1.75 (0.12) b, C, D	1.80 (0.13) a, C	1.90 (0.11) e, B
	ground/ non-PRG	2.24 (0.12) a, A, B	2.22 (0.17) a, B, C	2.19 (0.11) a, b, C	2.32 (0.21) a, A	2.19 (0.12) a, b, B, C	2.25 (0.16) a, A, B, C	2.34 (0.14) a, A
	ground/ PRG	2.15 (0.12) a, b, A	2.20 (0.12) a, A	3.53 (5.13) a, A	2.22 (0.17) b, A	3.54 (5.19) a, A	4.79 (7.05) a, A	2.17 (0.15) c, A

Different alphabetic letters (a, b, c) in the same column and experimental group indicate a significant difference ($P < 0.05$).
Different capital letters (A, B, C, D) in the same row indicate a significant difference ($P < 0.05$).

1-month of immersion in the different mediums. For the non-PRG and PRG regions, the enamel surface was found to be demineralized before PRG application and/ or 1-month immersion. Comparison of the degree of enamel demineralization between non-PRG and PRG regions revealed an approximately 20-μm-thick, highly

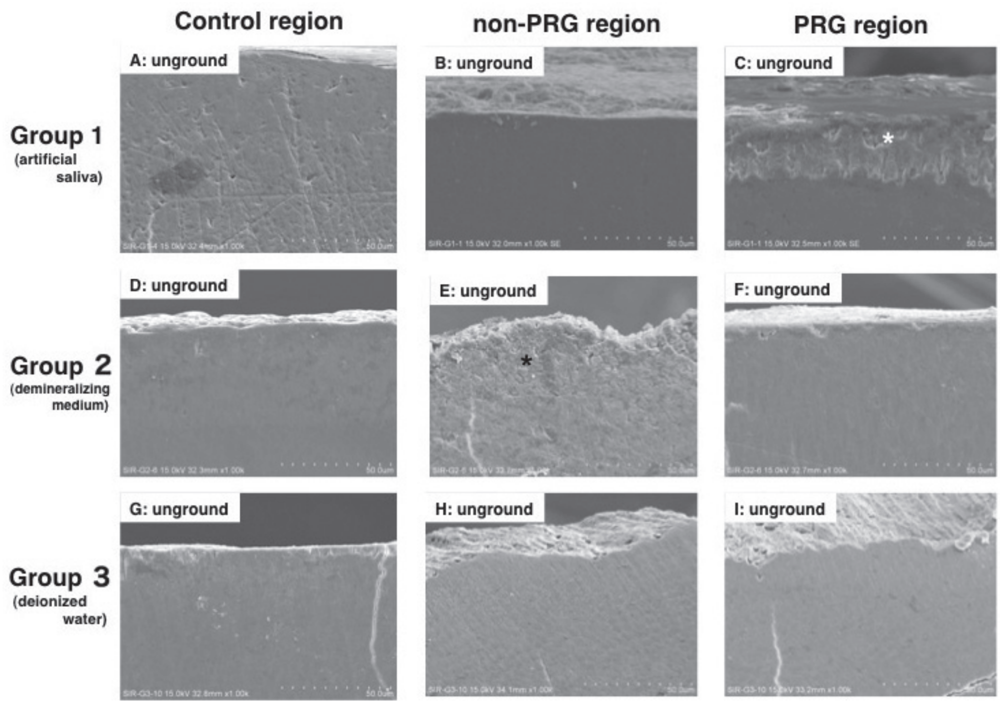

Fig. 2 Representative scanning electron microcopy images of unground enamel from different regions of each experimental group, after 1 month of immersion in different mediums. The enamel surface was demineralized in the non-PRG and PRG regions before PRG application. The asterisks show highly demineralized enamel lesions.
PRG: pre-reacted glass-ionomer

demineralized zone on the enamel surface of the PRG region of Group 1 (Fig. 2C). A 20-25-μm-thick demineralized zone, with clear enamel prism structures, was observed only in the non-PRG region of Group 2 (Fig. 2E). Microstructural differences of the demineralized zone were not observed between the non-PRG (Fig. 2H) and PRG regions (Fig. 2I) of Group 3.

Discussion

In the present study, the depth of artificially demineralized enamel showed large variation among the teeth and locations, ranging from <5 μm to >20 μm (Fig. 2). Tooth-to-tooth differences influenced the results of both nanoindentation test and EDX analysis (Tables 2-7).

The manufacturer-selected cut-off points for DIAGNOdent readings are 0-9 for sound or incipient caries, 10-17 for enamel caries, and 18-99 for dentine caries. A previous study reported that the cut-off points for DIAGNOdent readings were 0-4 for no caries or histologic enamel caries limited to the outer half of the enamel thickness, and 4.01-10 for histologic caries extending beyond the outer half of the enamel thickness (22). In the present study, mean DIAGNOdent readings after demineralization ranged from 4.25 to 5.10 and had large SDs (Table 1). These values are applicable to enamel caries limited to the outer half of enamel thickness, both in ground and unground regions. In the present study demineralization ranged from incipient enamel lesions to early enamel caries. Only in the unground/non-PRG region of Group 1, the DIAGNOdent reading after immersion was significantly lower than that after demineralization. These results suggest that artificial saliva induces remineralization.

A previous study reported that nanohardness and elastic modulus gradually decreased from the enamel surface toward the dentinoenamel junction; such variation correlates well with the decreasing trend in calcium content in this region (23). This phenomenon indicates that mineral content has a strong influence on nanohardness. A study of nanoindentation mapping of molar tooth enamel found great variation in the range of H and Y over different areas of an individual tooth (24). These variations corresponded to changes in chemistry, microstructure, and prism alignment. Nevertheless, the highest values for CaO and P_2O_5 content corresponded with the highest H and Y values at the enamel surface, while the lowest CaO and P_2O_5 values corresponded with the lowest H and Y values at the inner enamel region (24).

In the present study, only the H and Y values at measuring point 1 (enamel surface) of the ground/PRG region significantly differed from those at other points, i.e., beneath the enamel surface. H and Y values were significantly higher at the enamel surface than those beneath the surface for the ground/PRG region in Groups 1 (immersed in artificial saliva) and 3 (immersed in deionized water). However, those in the ground/PRG and unground/PRG regions of Group 2 (immersed in demineralizing medium) were significantly lower. Compared to unground regions, stronger demineralization occurred in ground regions. In the ground/PRG region, PRG coating and immersion in artificial saliva (Group 1), and PRG coating and immersion in deionized water (Group 3) contributed to recovery of H and Y values at the surface enamel. In Group 2, the S-PRG coating might have been dissolved by the demineralizing medium during the 1-month immersion. Hence, recovery of H and Y values in samples with S-PRG coating did not occur in either ground or unground regions.

In contrast to the results for H and Y, there were significant differences in elemental content between measuring points 0-1 (enamel surface) and points 2-6 (beneath the surface) for unground/non-PRG and unground/PRG regions in all groups (Tables 4-6). The crystalline qualities of calcium phosphate (apatite) are thought to be improved by increases in Ca% and Ca/P ratio (25). For all groups in the present study, the Ca/P ratio at points 0-1 (enamel surface) in the unground/non-PRG region was influenced by demineralization produced by EDTA and lactic acid; Ca/P ratio was significantly lower than that at subsurface points. In a comparison of Ca/P ratio between unground/non-PRG and unground/PRG regions, there was no significant difference at any point in Group 2 (samples immersed in demineralizing medium). In Group 2, the coating material might have been dissolved by the demineralizing medium, and thus regardless of PRG coating application, the efficacy of PRG might have influenced both for PRG-coated and PRG-uncoated regions. Hence, there was no significant difference between these two regions (Tables 5 and 7). However, the efficacy of PRG did not affect the H and Y values of these regions (Tables 2 and 3). The effect of acid demineralization on the efficacy of S-PRG coating material should be investigated in a future study with increasing number of specimens. In Group 1, the Ca/P ratios at points 0 and 1 for the unground/PRG region were significantly higher than that for the unground/non-PRG region, which may be due to the efficacy of PRG. In addition, the buffering effect of PRG (19) may help to arrest caries on primary enamel.

In the present study, the possibility of enamel remineralization by the SI-R20401 coating was partially observed. However, after 1-month immersion, release of F, B, Si, and Sr ions was not detected on S-PRG applied surfaces for any of the three mediums tested. Although a small number of these ions might have been released, the level of remineralization in the demineralized enamel lesion might below the level of detection. It is uncertain whether 1-month application of S-PRG coating or 1-month immersion of specimens in artificial saliva is adequate to arrest demineralization. Future studies should include recharge of ions to evaluate the effect of repeated SI-R20401 application and use longer periods of immersion in artificial saliva to investigate remineralization and the potential of the S-PRG coating to arrest caries.

Within the limitations of the present study, the null hypothesis that the S-PRG coating does not influence the physical qualities and elemental content of artificially demineralized primary tooth enamel must be rejected. The effect of S-PRG coating on remineralization of demineralized primary tooth enamel was partially observed. Unfortunately, the effect of SI-R20401 to arrest the demineralized primary tooth enamel could not be confirmed. Future studies should evaluate the stability of this coating material in the oral environment and its efficacy in remineralizing and arresting caries *in vivo*.

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