Effect of application of polishing paste containing S-PRG filler on prevention of tooth enamel demineralization

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Contents

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Summary

Cariogenic biofilm formation is an issue of aetiological importance in secondary caries, and is one of the main reasons for the failure of resin composite restorations. Therefore, a smooth and glossy surface is required in the restoration, which is achieved by finishing and polishing procedures completed with the use of appropriate equipment. Surface pre-reacted glass-ionomer (S-PRG) fillers are prepared using an acid-base reaction (with traditional glass ionomer cement) between fluoroaluminosilicate glass (base) and a polyacrylic acid in the presence of water; the preliminary product is a stable glass ionomer phase within the glass particles. A coating material which contains S-PRG filler has been reported to suppress demineralization of enamel and to inhibit plaque formation possibly by releasing fluoride, borate, strontium, silica, sodium, and aluminum ions. Considering the potential of S-PRG filler to prevent enamel lesions, the objective of this study was to use optical coherence tomography (OCT) to evaluate the ability of a polishing paste containing S-PRG filler to prevent acidic erosion on enamel substrates in composite restorations. And also, ultrasonic velocity measurement through the specimen was done.

Standardized cylindrical cavities (2 mm in diameter, 2 mm deep, with all margins in enamel) were prepared on the buccal surfaces of 18 extracted bovine teeth. The cavity walls were treated with self-etching primer for 20 s, a bonding agent was applied, and a LED curing unit was used and irradiated for 10 s. A light-cured resin composite was then condensed into the prepared cavity. After light-curing for 30 s, the restorations were finished with wet silicon carbide paper. These specimens were then randomly divided into three groups (n = 6): the unpolished “control” group, the “PRG” group polished with S-PRG paste, and the “DDP” group polished with diamond paste. Following polishing, the specimens were immersed in a 0.1 M lactic acid buffer solution for 10 min twice a day for 28 days. OCT signals were measured to obtain the peak signal intensity and width at $1/e^2$ at selected locations on the

enamel surface adjacent to the restoration. The specimens from each group were also observed using three-dimensional laser-scanning microscopy (LSM). In adittion, the propagation time of longitudinal ultrasonic velocities and the surface roughness (Ra) values were measured.

During the treatment period (from the baseline to Day 28), the maximum peak intensities in all groups increased significantly, but this tendency was less pronounced in the PRG group relative to the other groups (PRG: from −83.1 to −76.1 dB; control: from −83.0 to −39.6 dB; DDP: from −83.4 to −41.6 dB). While the maximum peak intensities for the control and DDP groups increased dramatically. In addition, the $1/e^2$ width in the control and DDP groups decreased over the treatment period (control: from 80.0 to 48.0 μm; DDP: from 78.0 to 52.7 μm); however, there was no significant change in $1/e^2$ width over time in the PRG group (from 78.0 to 79.0 μm). After 14 days of treatment, significantly higher $1/e^2$ values were obtained for the PRG group compared to those of the other groups.

The ultrasonic velocity of the control and DDP groups gradually decreased during the treatment period (control: from 3,303 to 3,134 m/s; DDP: from 3,299 to 3,222 m/s). However, the ultrasonic velocities of the PRG group increased significantly after day 14 of the treatment period and showed an upward trend thereafter (from 3,281 to 3,529 m/s). The PRG group showed significantly higher values than the other groups at all treatment periods from Day 14 to Day 28.

During the treatment period, the Ra values in all groups increased significantly, but this tendency was less pronounced in the PRG group relative to the other groups. Comparing across treatments, significantly smaller Ra values were obtained in the PRG group relative to the other groups on Day 1 and Days 14, 21, and 28. The LSM observations revealed different surface features with different groups. On Day 1 of treatment, smoother enamel surfaces were observed in the PRG and DDP groups when compared to the control group. Although roughened enamel surfaces were observed for all specimens on Days 14 and 28, the enamel surfaces of the PRG group were relatively smoother than those of the other groups.

Based on the results of this study, it was concluded that the polishing paste containing S-PRG filler appears to inhibit demineralization of the enamel around resin composite restorations. However, because demineralization of the enamel structure in the oral environment may be different to that in the conditions tested here, further *in vivo* researches will be required to prove the clinical effectiveness of the polishing paste containing S-PRG filler.

Introduction

Direct restorations of teeth are now widely employed to restore both anterior and posterior lesions because of recent improvements in the mechanical properties and adhesive performance of such restorations[1]. The final step in a direct restoration procedure involves finishing and polishing the resin composite surface to minimize gingival irritation, surface stains, and bacterial adhesion[2]. In particular, the surface of resin composites has been shown to be susceptible to bacterial adhesion, which is one of the stages of biofilm formation in the marginal areas of restorations[3]. Cariogenic biofilm formation is an issue of aetiological importance in secondary caries, and is one of the main reasons for the failure of resin composite restorations[4]. Therefore, a smooth and glossy surface is required in the restoration, which is achieved by finishing and polishing procedures completed with the use of appropriate equipment.

Although resin composite restorations are now viable restoration options, failed restoration replacements remain a major issue for dentists, who may spend a considerable amount of time replacing the restorations or repeating the procedure. Secondary caries are the most common late complication in resin composite restorations[5]. Such lesions are typically found adjacent to the restoration and were traditionally thought to be associated with the gap between the tooth and the restoration[6]. Gap size and mechanical loading have been shown to be associated with lesion severity in oral condition models; however, the results of such models do not correspond exactly to the results obtained from *in situ* studies using restorative materials[7]. Reducing the severity of lesion formation may lead to the longevity of the restorations; certain materials with remineralization potential could be used to achieve such effects.

Surface pre-reacted glass-ionomer (S-PRG) fillers are prepared using an acid-base reaction (with traditional glass ionomer cement) between fluoroaluminosilicate glass (base) and a polyacrylic acid in the presence of water; the preliminary product is a stable glass ionomer phase within the glass particles[8]. A coating material which contains S-PRG filler has been reported to suppress demineralization of enamel[9] and to inhibit plaque formation possibly by releasing fluoride, borate, strontium, silica, sodium, and aluminum ions[10]. Indeed, compared to fluoroaluminosilicate glass filler materials, resin composites containing S-PRG filler release many types of ion at higher rates; they also have a modulation effect on oral acidity that causes the pH of the surrounding environment to become weakly alkaline upon contact with water or acidic solutions[11].

In order to manage the demineralization process of enamel with minimal intervention, various diagnostic techniques have been introduced to identify early mineral changes[12]. For example, optical coherence tomography (OCT) has been applied to determine tooth mineralization with the advantage of being non-invasive and avoiding radiation-induced tissue damage[13, 14]. OCT observations can visualize the internal structure of demineralized lesions without destroying the specimen [15]. Tooth enamel is composed of hydroxyapatite crystal, which is known to exhibit birefringence; both de- and re-mineralization processes alter the birefringence of tooth enamel with an anisotropic refractive index[16]. The underlying principles of OCT clearly differentiate it from transverse microradiography, which has previously been thought of as the gold standard for detecting the mineral content status of tooth substrates[17].

Considering the potential of S-PRG filler to prevent enamel lesions, the objective of this study was to use OCT to evaluate the ability of a polishing paste containing S-PRG filler to prevent acidic erosion on enamel substrates in composite restorations. And also, ultrasonic velocity measurement through the specimen was done. The null hypothesis to be tested was that the use of polishing paste containing S-PRG filler would not result in protective effects against enamel erosion.

Materials and methods

Specimen preparation

Eighteen extracted bovine incisors were used in this study. Standardized cylindrical cavities (2 mm in diameter, 2 mm deep), for which all margins were in the enamel of the tooth, were prepared with a diamond point (202CR; ISO #021; Shofu, Kyoto, Japan) fixed within a high-speed handpiece (TwinPower Turbine P; J. Morita Mfg., Kyoto, Japan) with copious air-water spray. A super-fine-grained diamond point (SF202CR; ISO #02D; Shofu) was used for finishing each preparation, and a new bur was changed after every six preparations.

The cavity walls were treated with self-etching primer (Clearfil SE Bond 2; Kuraray Noritake Dental, Tokyo, Japan) for 20 s, a bonding agent was applied, and a LED curing unit (Pencure; J. Morita Mfg.) was used and irradiated for 10 s. A light-cured resin composite (Clearfil AP-X; shade A2, Kuraray Noritake Dental) was then condensed into the prepared cavity and a polyester transparent strip was placed over the sample. After light-curing for 30 s, the restorations were finished with wet silicon carbide (SiC) paper (Fuji Star Type DDC; Sankyo Rikagaku, Saitama, Japan) starting with #240-grit to #2,000-grit to standardize flat surface. The specimens were rinsed with tap water and air dried. Finally, the specimens were divided into three groups as below:

1) Control group: the specimens' surfaces were not polished.

2) PRG group: the specimens' surfaces were polished with a polishing paste containing S-PRG (PRG Compoglass; Shofu) using Super-Snap Buff Mini-Disks (Shofu) for 30 s.

3) DDP group: the specimens' surfaces were polished with a polishing paste containing diamond particles (DirectDia Paste; Shofu) using Super-Snap Buff Mini-Disks for 30 s.

All polishing procedures were performed using a micromotor handpiece (Torqtech

CA-DC; J. Morita Mfg.) with 5,000 rpm and a constant pressure of 0.5 N monitored by a digital balance (AT200; Mettler-Toledo, Greifensee, Switzerland). All restorations and polishing procedures were conducted by one operator. The information on the polishing pastes used are shown in Table 1.

For each group, the prepared specimens (n = 6) were immersed in an undersaturated 0.1 M lactic acid buffer solution (pH 4.75, 0.75 mM $CaCl_2 \cdot 2H_2O$, and 0.45 mM KH_2PO_4) for 10 min, and then were placed in artificial saliva (pH 7.0, 14.4 mM NaCl, 16.1 mM KCl, 0.3 mM $MgCl \cdot 6H_2O$, 2.0 mM K_2HPO_4, 1.0 mM $CaCl_2 \cdot 2H_2O$, and 0.10 g/100 mL sodium carboxymethyl cellulose)[9). These procedures were conducted twice daily (with a time interval of 10 h) over the 28-day test period. Between acid treatments, the specimens were stored in artificial saliva at 37°C.

OCT measurements

A time-domain OCT imaging system (J. Morita Tokyo Mfg., Saitama, Japan) was used in this study. The scanning probe of the time-domain OCT device was fixed 2 mm from the specimen surface. The scanning light beam was set perpendicular to the specimen surface, and changes in the specimen were determined based on the signal intensity values from the OCT images. The width between points, where the signal intensity decreased to a value of $1/e^2$ and corresponded precisely to the peak signal intensity of the scanned profile line, was calculated. A width of $1/e^2$ is equivalent to the distance between the two points where the intensity falls to 13.5% of the maximum value. Peak intensities and the widths at $1/e^2$ were obtained for six points around the restoration in each specimen, and these obtained data were averaged (mean values). Six specimens were examined in each treatment group. Measurements were taken at 23°C ± 1°C and 50% ± 5% relative humidity. Data were collected before the polishing procedures (baseline) and on days 1, 7, 14, 21, and 28 during the treatment period.

Ultrasonic velocity measurement

Ultrasonic velocity was measured using a system comprising a pulser-receiver (5900PR; Panametrics, Waltham, MA, USA), a longitudinal wave transducer (V112; Panametrics), and an oscilloscope (Waverunner LT584; LeCroy, Chestnut Ridge, NY, USA). Measurements were taken before the polishing procedures (baseline) and on days 1, 7, 14, 21, and 28 during the treatment period. The equipment was initially calibrated using a standard procedure with a stainless steel calibration blocks (2211M; Panametrics). The transducer was oriented perpendicular to the contact surface of each sample to obtain an echo signal. The ultrasonic waves propagated from the transducer to the tooth were transmitted through the tooth and detected by the transmitter on the opposite side. Measurements were taken at 23°C ± 1°C and 50% ± 5% relative humidity.

Statistical analysis

Statistical analyses were performed using a software (SigmaPlot 13; Systat Software, Chicago, IL, USA). First, the data for each group were tested for homogeneity of variance (Bartlett's test) and normal distribution (Kolmogorov-Smirnov test), and then analyzed using repeated-measures ANOVA followed by the Tukey-Kramer *post-hoc* multiple-comparisons test. Statistical significance was set at $p < 0.05$.

Laser-scanning microscopy measurements

For each group, specimens were treated with the same procedures described above, were also observed using three-dimensional laser-scanning microscopy (LSM) (VK-8700; Keyence, Osaka, Japan). The recorded image size was 81.5 × 71.5 μm^2, and the resolution was 1024 ×

768 pixels. Surface roughness (Ra) values were calculated and averaged from three different sites of the obtained image.

Results

Maximum peak intensities and widths at $1/e^2$ are shown in Tables 2 and 3. During the treatment period (from the baseline to Day 28), the maximum peak intensities in all groups increased significantly, but this tendency was less pronounced in the PRG group relative to the other groups (PRG: from −83.1 to −76.1 dB; control: from −83.0 to −39.6 dB; DDP: from −83.4 to −41.6 dB). While the maximum peak intensities for the control and DDP groups increased dramatically, the rate of decrease in the PRG group was 84.9% for Day 28 compared to that of Day 1. In addition, the $1/e^2$ width in the control and DDP groups decreased over the treatment period (control: from 80.0 to 48.0 μm; DDP: from 78.0 to 52.7 μm); however, there was no significant change in $1/e^2$ width over time in the PRG group (from 78.0 to 79.0 μm). After 14 days of treatment, significantly higher $1/e^2$ values were obtained for the PRG group compared to those of the other groups.

The average ultrasonic velocity of the specimens in each group is shown in Table 4. The ultrasonic velocity of the control and DDP groups gradually decreased during the treatment period (control: from 3,303 to 3,134 m/s; DDP: from 3,299 to 3,222 m/s). However, the ultrasonic velocities of the PRG group increased significantly after day 14 of the treatment period and showed an upward trend thereafter (from 3,281 to 3,529 m/s). The PRG group showed significantly higher values than the other groups at all treatment periods from Day 14 to Day 28.

Ra values of the specimens are listed in Table 5. During the treatment period, the Ra values in all groups increased significantly, but this tendency was less pronounced in the PRG group relative to the other groups (PRG: from 0.068 to 0.281 μm; control: from 0.069 to

0.795 μm; DDP: from 0.069 to 0.773 μm). Comparing across treatments, significantly smaller Ra values were obtained in the PRG group relative to the other groups on Day 1 and Days 14, 21, and 28.

Representative LSM images of the enamel surfaces are shown in Fig. 1. On Day 1 of treatment, smoother enamel surfaces were observed in the PRG and DDP groups when compared to the control group. Although roughened enamel surfaces were observed for all specimens on Days 14 and 28, the enamel surfaces of the PRG group were relatively smoother than those of the other groups.

Discussion

In the present study, the ability of polishing paste containing S-PRG filler to prevent acidic attack on the enamel substrate around the margin of resin composite restorations was assessed. To this end, bovine teeth rather than human teeth were used; although human teeth are the ideal specimens in *in vitro* studies, bovine teeth are commonly used as human-teeth substitutes. While the comparative properties of human and bovine tooth tissues remain under consideration, current consensus recommends the continued use of bovine enamel as a substitute for human enamel[18)]. One of the advantages of bovine teeth are their large size, which makes fabricating test specimens much simpler. Previous studies have compared the use of bovine and human teeth for research purposes. For example, one review concluded that bovine teeth can be considered as a suitable substitute for human teeth during *in vitro* bond strength tests of adhesive systems[19)]. Another study compared the acid-mediated dissolution of human and bovine enamel *in vitro* under conditions representative of erosion, and it was concluded that bovine enamel can be used as a substitute for human enamel in erosion studies over moderate exposure periods[20)]. Nevertheless, when interpreting the results of studies with bovine teeth, the differences in their chemical and physical properties relative to human teeth must be taken into account.

In this study, the inhibitory effect of polishing paste containing S-PRG filler on enamel demineralization when the restored specimens were immersed in a pH 4.75 lactic acid buffer solution was examine. Acid production by oral bacteria dissolves the mineral phases of the enamel surface and facilitates the formation of tooth caries; lactic acid is the major acid that causes caries, hence our choice of buffer solution, followed by acetic and propionic acids[21)]. Given the concept of "critical pH," the most important method for relating changes in pH to caries activity across treatment groups is to classify values according to a hypothetical critical decalcification pH level of 5.0[22)].

When light is incident on a tooth substrate, four phenomena occur: interaction between the tooth and light flux, specular reflection at the surface, diffuse light reflection at the surface, and absorption and scattering of the flux within the tooth[23)]. Therefore, accurate determination of optical constants from OCT image data is vital because these optical constants describe the optical field-sample interaction[24)]. Intensity is generated by two mechanisms in OCT images: (1) the intrinsic birefringence of the enamel rotates the phase angle of the incident light between two orthogonal axes as the light propagates through the enamel without changing the degree of polarization and (2) depolarization scrambling from scattering reduces the degree of polarization. The second mechanism is used to measure the severity of demineralization[25)]. Therefore, areas of demineralization show increased reflectivity in OCT images, which results in higher peak intensities. This observation is contingent on the inverse relationship between the intensity value of light backscattering and the mineral content within the enamel surface[26)]. Reflectivity from the lesion area can be directly determined and then used as a measure of lesion severity[27)]: shallow lesions show a loss of penetration depth in OCT images, which is correlated with mineral loss and can be detected using LSM. In the present study, following 21 days of treatment, both the control and

DDP groups showed a strong reflection on the enamel surface due to the strong depolarization caused by light scattering at the acid-roughened enamel surface.

Previous studies proposed that the depth at which the peak signal intensity decreases by $1/e^2$ can be used to determine the cutoff signal intensity[28)]. In this study, the $1/e^2$ values in the DDP and control groups decreased as the treatment period progressed, but no change in $1/e^2$ was observed in the PRG group. Furthermore, lower signal intensity with a wider $1/e^2$ value was recorded in the PRG group relative to the other groups on Day 28. These results showed that in PRG group the OCT signal was generated by light that traveled over a longer pathway than that which was observed in the DDP and control groups. It is possible that the presence of ions from the PRG filler in the polishing paste may have strengthened the enamel surface and protected it from acid attack[29)], ultimately leading to changes in the optical properties of PRG specimens[26)]. The LSM observations of the PRG group revealed the relatively smoother enamel surfaces of specimens, and these results were in agreement with the data collected from specific measurements. In addtion, changes in ultrasonic velocity are related to the degree of mineralization. In the demineralization process, the acid reduces the solidity of the enamel, resulting in a reduction of the ultrasonic velocity. In contrast, in the remineralization process, the increase in ultrasonic velocity is directly proportional to the volume concentration of minerals of the enamel[30)]. Therefore, it was suggest that when S-PRG filler is incorporated into polishing paste formulations, the ions released from the glass-ionomer phase contribute to the suppression of tooth surface demineralization.

In the present study, the S-PRG filler acted as fluoride-releasing material; it released aluminum, borate, fluoride, sodium, silica, and strontium ions, among which strontium and boron ions are known to inhibit the growth of oral bacteria[31)]. In addition, Uo *et al.*[32)] reported that the local structure of strontium in enamel after immersion in S-PRG filler eluate was similar to that of strontium-containing hydroxyapatite. The combination of strontium and fluoride ions improves the crystallinity of carbonated hydroxyapatite[33]. Therefore, fluoride and strontium ions released from the S-PRG filler in the polishing paste used here may have contributed to improving the remineralization of the enamel substrate and protecting it from acid erosion[34, 35].

In a previous study, the clinical performance of refurbished resin composite restorations was compared to that of untreated restorations, and it was concluded that no significant differences existed in the survival curves of the refurbished and untreated groups[36]. The main reasons for this observation were secondary caries and fracture of the tooth, neither of which are failures of the materials themselves but are rather related to the operator's and patient's characteristics. In contrast, it was indicated that by using polishing paste containing S-PRG filler not only can the luster and marginal adaptation of defective resin composite restorations be improved but also the enamel margins become protected against acidic attack.

Conclusions

1. Although the signal intensity measured using OCT significantly increased in all groups over the treatment period, widths at $1/e^2$ did not change significantly in the PRG group.
2. The ultrasonic velocity of PRG group showed significantly higher values than the other groups at all treatment periods from Days 14 to Day 28.
3. The enamel surfaces of the PRG group were relatively smoother than those of the other groups on Day 14 and 28.

From the results of the present study, it was concluded that polishing teeth with paste containing S-PRG could effectively prevent enamel demineralization. Further *in vivo* researches will be required to prove the clinical effectiveness of the polishing paste containing S-PRG filler.

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Tables and figures

Table 1 Materials used in this study and their main components

Polishing paste	Code	Main components	Manufacturer	Lot No.
PRG Compogloss	PRG	Glycerin, diamond powder (1 µm), aluminum oxide, S-PRG filler, purified water, viscosity agent	Shofu, Kyoto, Japan	91701
DirectDia paste	DDP	Glycerin, diamond powder (3 µm), viscosity agent, pH regulator, colorant	Shofu	1217064

S-PRG: surface pre-reacted glass-ionomer

Table 2 Maximum peak intensities of the cutting enamel of specimens under three treatments

	Treatment period (days)					
	Baseline	1	7	14	21	28
Control group	-83.0 (8.7)^{aA}	-83.0 (8.7)^{aA}	-79.5 (3.7)^{aA}	-71.2 (4.6)^{bA}	-50.7 (3.8)^{cA}	-39.6 (2.6)^{dA}
PRG group	-83.1 (7.6)^{abA}	-89.6 (6.2)^{aA}	-85.3 (4.5)^{abB}	-81.3 (3.4)^{bcB}	-79.6 (3.8)^{bcB}	-76.1 (3.7)^{cB}
DDP group	-83.4 (7.5)^{aA}	-86.1 (8.0)^{aA}	-82.8 (3.5)^{aAB}	-74.0 (2.9)^{bA}	-55.6 (6.2)^{cA}	-41.6 (4.1)^{dA}

Control group: unpolished; PRG group: polished with PRG; DDP group: polished with DDP

unit: dB (values in parenthesis indicate standard deviations); n = 6 teeth per group.
Within groups, means with the same lowercase letter are not significantly different ($p > 0.05$).
Between groups and within storage times, means with the same uppercase letter are not significantly different ($p > 0.05$).

Table 3 1/e^2 width of the cutting enamel of specimens under three treatments

	Treatment period (days)					
	Baseline	1	7	14	21	28
Control group	80.0 (3.7)^{aA}	80.0 (3.7)^{aA}	77.5 (5.9)^{abA}	72.5 (4.8)^{bA}	61.7 (3.3)^{cA}	48.0 (7.5)^{dA}
PRG group	78.0 (5.2)^{aA}	83.6 (3.8)^{aA}	82.3 (7.9)^{aA}	79.7 (6.6)^{aB}	79.2 (4.2)^{aB}	79.0 (5.0)^{aB}
DDP group	78.0 (4.7)^{abA}	82.3 (3.7)^{aA}	79.0 (7.1)^{abA}	73.7 (3.4)^{bA}	65.0 (3.7)^{cA}	52.7 (6.9)^{dA}

Control group: unpolished; PRG group: polished with PRG; DDP group: polished with DDP

unit: µm (values in parenthesis indicate standard deviations); n = 6 teeth per group.
Within groups, means with the same lowercase letter are not significantly different ($p > 0.05$).
Between groups and within storage times, means with the same uppercase letter are not significantly different ($p > 0.05$).

Table 4 Ultrasonic velocity of the cutting enamel of specimens under three treatments

	Treatment period (days)					
	Baseline	1	7	14	21	28
Control group	3,303 (48)^{aA}	3,286 (42)^{aA}	3,229 (39)^{abA}	3,190 (51)^{bcA}	3,165 (43)^{bcA}	3,134 (41)^{cA}
PRG group	3,281 (63)^{aA}	3,309 (66)^{aA}	3,358 (64)^{abB}	3,459 (82)^{bB}	3,482 (83)^{bB}	3,529 (86)^{bB}
DDP group	3,299 (60)^{aA}	3,295 (59)^{aA}	3,284 (65)^{aAB}	3,264 (53)^{aA}	3,235 (47)^{aA}	3,222 (51)^{aA}

Control group: unpolished; PRG group: polished with PRG; DDP group: polished with DDP

unit: m/s (values in parenthesis indicate standard deviations); n = 6 teeth per group.
Within groups, means with the same lowercase letter are not significantly different ($p > 0.05$).
Between groups and within storage times, means with the same uppercase letter are not significantly different ($p > 0.05$).

Table 5 Surface roughness of the cutting enamel of specimens under three treatments

	Treatment period (days)					
	Baseline	1	7	14	21	28
Control group	0.069 (0.008)^{aA}	0.069 (0.008)^{aA}	0.198 (0.032)^{bA}	0.350 (0.036)^{cA}	0.498 (0.048)^{dA}	0.795 (0.050)^{eA}
PRG group	0.068 (0.006)^{aA}	0.020 (0.002)^{bB}	0.141 (0.013)^{cB}	0.184 (0.011)^{dB}	0.244 (0.015)^{eB}	0.281 (0.011)^{fB}
DDP group	0.069 (0.006)^{aA}	0.037 (0.005)^{aC}	0.150 (0.020)^{bB}	0.273 (0.018)^{cC}	0.495 (0.034)^{dA}	0.773 (0.058)^{eA}

Control group: unpolished; PRG group: polished with PRG; DDP group: polished with DDP

unit: µm (values in parenthesis indicate standard deviations); n = 6 teeth per group.
Within groups, means with the same lowercase letter are not significantly different ($p > 0.05$).
Between groups and within storage times, means with the same uppercase letter are not significantly different ($p > 0.05$).

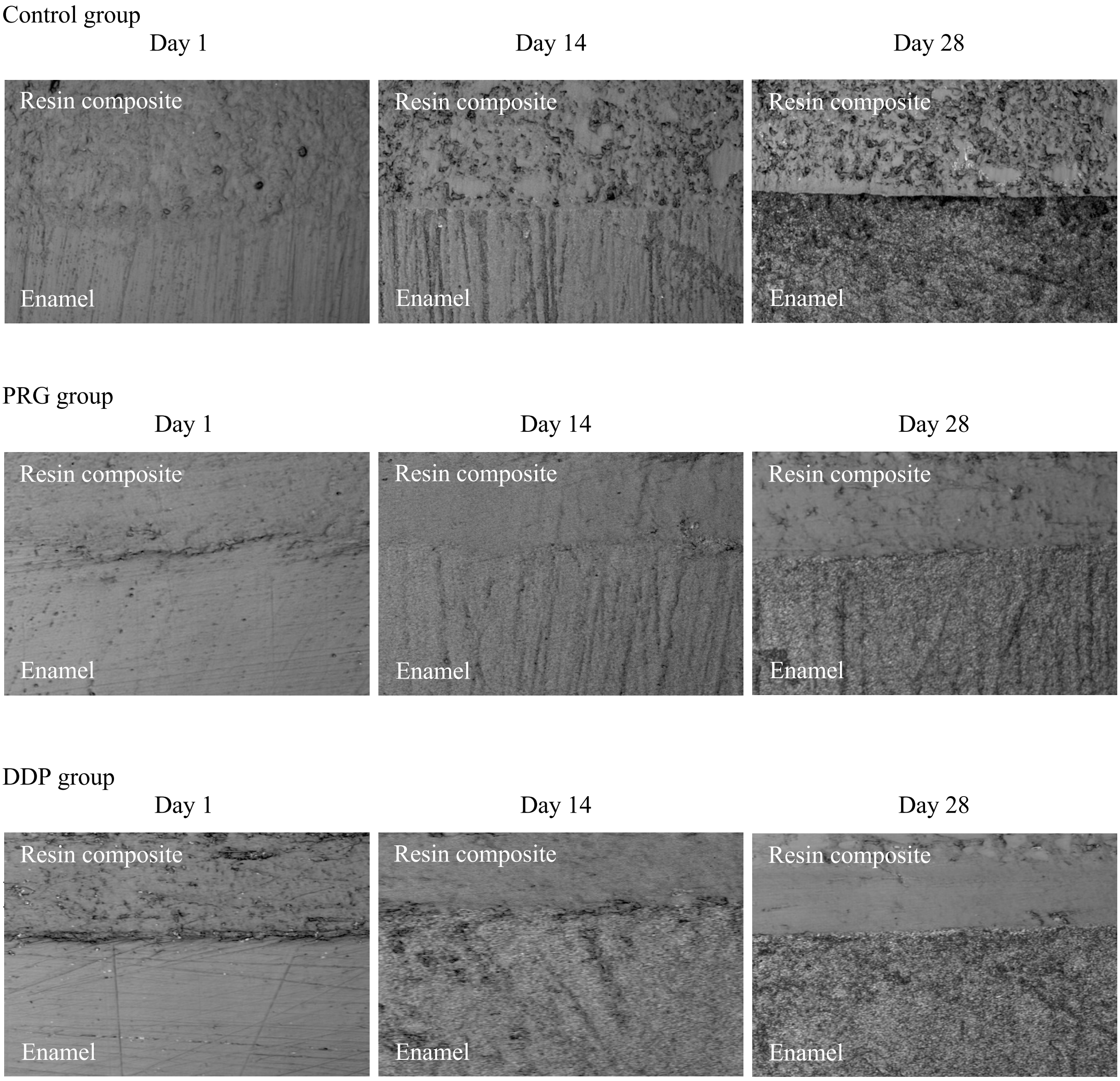

Fig. 1 LSM images of the resin composite/enamel interface of specimen surfaces subjected to the three experimental treatments.

Control group: unpolished; PRG group: polished with surface pre-reacted glass-ionomer (PRG Compogloss) paste; DDP group: polished with diamond-containing (DirectDia) paste.