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Protective effects of glass ionomer cement and surface pre-reacted glass ionomer filler restoratives on demineralization of bovine enamel in lactic acid solution

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Abstract: We investigated the protective effects of glass ionomer cement (GIC) and surface pre-reacted glass ionomer (S-PRG) fillers used as dental restorative materials on the demineralization of bovine enamel during the incubation in a lactic acid solution with pH = 4.0 conducted for 6 d at a temperature of 37 °C. Scanning electron microscopy images of the specimen surfaces from the group containing only enamel blocks revealed the presence of apparent enamel prism peripheries after 6 d of treatment. The mineral was etched from the enamel surface, and a large number of Ca ions were detected in solution. In contrast, the enamel blocks incubated together with discs of GIC or S-PRG fillers did not exhibit demineralized surfaces, owing to the neutralization of the acid solution by the fillers within the first 3 h of the incubation process. The observed pH neutralization effect was attributed to the release of different ions from the fillers and could be utilized for suppressing the progression of caries.

Keywords: pH neutralization; ion release; enamel demineralization; glass ionomer cement; surface pre-reacted glass ionomer filler

1. Introduction

Various bioactive materials have been explored for possible uses in dental applications, owing to the recent developments of novel dental materials and procedures. In particular, the effects of these bioactive materials on the stability and longevity of dental restorations as well as on the oral pathogens inducing dental caries were considered [1]. Dental caries, one of the most widely spread preventable chronic diseases [2], is caused by the organic acids produced as byproducts of the metabolism of fermentable carbohydrates by oral bacteria, which disturb the calcium phosphate balance of the enamel and dentine in a low-pH intraoral environment [3]. Active caries development generally occurs at pH < 4.9 under the action of lactic acid. However, this process stops when the pH of the medium increases to 5.7 [4]. Previously, chemical modifications of the restoration material surface that might prevent bacteria adherence and suppress plaque and biofilm formation have been proposed. For this purpose, novel biomaterials that enable slow release of chemicals or ions lethal to bacterial cells must be developed [5]. The concept of "minimum intervention dentistry" formulated in 2000 emphasized that caries treatments should aim at both preserving the tooth structure through noninvasive procedures and detecting suspicious early lesions [6]. The currently adopted diagnosis and treatment policy primarily focuses on self-healing through remineralization based on the use of

bioactive materials before employing a restorative approach that requires the removal of tooth tissue [7].

The main purpose of developing bioactive and antibacterial materials is to prevent damage of hard tissues. Glass ionomer cements (GICs) have been widely used for the conservative treatment of enamel and dentin because of their beneficial properties such as strong chemical bonding to the tooth substrate and release of fluoride ions [8]. Furthermore, while conventional GICs release the ions of their constituent materials, light-cured GICs produce significant amounts of Al, Ca, F, Na, P, Si, and Sr ions in neutral or acidic solutions over time and can effectively neutralize lactic acid solutions [9,10] to prevent demineralization of the areas adjacent to the tooth surface and suppress the formation of initial caries.

A few years ago, a new zinc-containing GIC characterized by a higher flexural strength as compared with those of conventional GICs was developed [11]. However, after two years of clinical trials, it was found to be unsuitable for cavity preparation [12]. Meanwhile, surface pre-reacted glass ionomer (S-PRG) fillers were introduced as bioactive materials that were able to retain their basic properties by forming a stable glass ionomer phase on the treated surface through the acid–base reaction between the fluoro-boro-alumino-silicate glass and polyacrylic acid [13]. These fillers represent a novel class of particle materials that can be incorporated into resin matrices to induce the release of different types of ions and thus produce a strong bioactive effect. The results of in-vitro studies demonstrated the effectiveness of such materials in inhibiting the demineralization of the tooth substrate [14–16] and preventing the initiation of caries. In addition, an endodontic root canal sealer containing an S-PRG filler (i.e., S-PRG sealer) exhibited good antibacterial and anti-inflammatory properties. Finally, the implantation of S-PRG sealer into the subcutaneous tissues of rats noticeably decreased their inflammatory responses [17].

The primary objective of this study was to examine the inhibitory effects of GIC restoratives and coating materials containing S-PRG fillers on enamel demineralization to evaluate their anticaries properties. The null hypothesis was that the GIC and S-PRG fillers would not inhibit the demineralization of bovine enamel specimens incubated in a lactic acid solution with pH = 4.0.

Table 1. Materials used in this study.

Material/(Code)	Manufacturer	Composition/filler	Lot No.
GlasIonomer FX ULTRA /(FXU)	Shofu Inc.	Powder: fluoroaluminosilicate glass, polyacrylic acid Liquid: polyacrylic acid, polybasic carboxylic acid, distilled water	P: 061049 L: 061034
Fuji IX GP Extra /(FIXE)	GC Corp.	Powder: fluoroaluminosilicate glass, pigments, fluorescent material Liquid: acrylic acid-tricarboxylic acid co-polymer, tartaric acid, distilled water	P: 1108171 L: 1108171
CAREDYNE RESTORE /(FIXE)	GC Corp.	Powder: fluoroaluminosilicate glass, fluorozincsilicate glass Liquid: acrylic acid-tricarboxylic acid co-polymer, polyacrylic acid, distilled water	P: 1812121 L: 1812071
PRG Barrier Coat /(BC)	Shofu Inc.	Base: S-PRG glass fillers, distilled water methacrylic acid monomer, others Active: Phosphoric acid monomer, bis-MPEPP, carboxylic acid monomer, TEGDMA, photoinitiator, methacrylic acid monomer, polybasic carboxylic acid, others	P: 031503 L: 111711
S-PRG: surface pre-reacted glass ionomer, bis-MPEPP: 2,2'-bis (4-methacryloxy polyethoxyphenyl) propane, TEGDMA: triethylene glycol dimethacrylate			

2. Materials and Methods

2.1. Preparation of bovine enamel blocks

Figure 1 shows the schematic of the utilized experimental procedure. The flattened enamel surface on the buccal aspect of the crown part of a bovine upper central incisor was exposed longitudinally with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) using water as a coolant. The exposed enamel surface was polished with waterproof 600-, 1,200-, and 2,000-grit silicon carbide abrasive papers (Sankyo Rikagaku Co., Ltd., Saitama, Japan) under running water to increase its smoothness and then cut into slabs with thicknesses of approximately 1.0 mm. The dentin side was painted with nail varnish, and the slabs were cut into enamel blocks with dimensions of approximately 2 mm × 2 mm using a diamond saw, which were subsequently cleaned for 20 s in distilled water with an ultrasonic cleaner. Four enamel blocks were obtained from each bovine crown (Figure 1a).
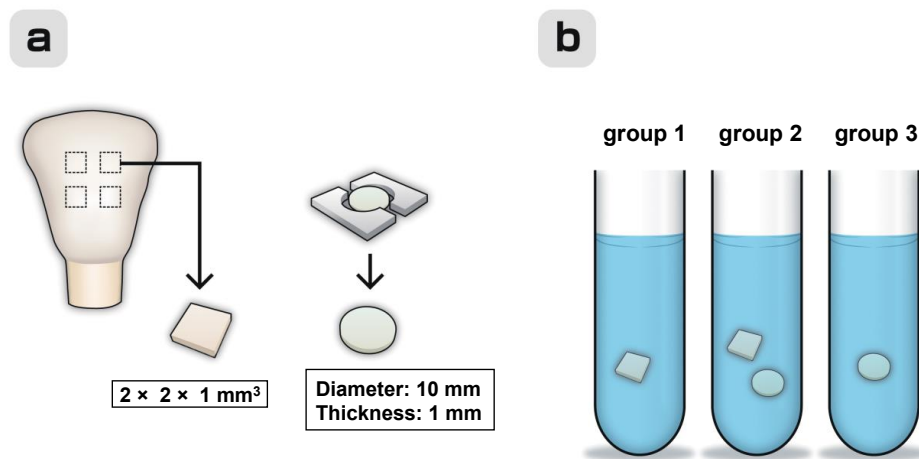
Figure 1. Schematic of the experimental procedure. (**a**) Preparation of enamel blocks from bovine crowns and restorative discs. (**b**) Test specimens classified into the following groups: enamel blocks only (group 1), restorative discs with enamel blocks (group 2), and restorative discs without enamel blocks (group 3). All specimens were immersed in 5 mL of the lactic acid solution with pH = 4.0.

2.2. Preparation of restorative discs and arrangement of specimens into test groups

The test specimens were classified into the following three groups (Figure 1b): enamel blocks only (group 1), restorative discs with enamel blocks (group 2), and restorative discs without enamel blocks (group 3) (n = 6 for each group). The formulations and chemical compositions of all the commercially available materials used in this study are listed in Table 1 (this information was provided by the respective manufacturers). Three high-viscosity GICs were mixed as per their manufacturer's instructions at a temperature of 23±2 °C. The freshly mixed cement was placed in a stainless-steel split mold to form disc-shaped specimens with internal diameters of 10 mm and thicknesses of 1 mm. The cement was covered with a polyethylene sheet and compressed using a metal plate. The resulting mold was stored in an incubator for 1 h at 37.0 °C, after which the polyethylene sheet was removed. The obtained disc-shaped GIC specimens were stored in an environment with a 100% relative humidity and temperature of 37 °C for 24 h before immersion in 5 mL of the lactic acid solution at 37 °C for different periods. To produce discs containing S-PRG coating material (PRG Barrier Coat), four ampoules of the base and active components were mixed using a microbrush. The resultant mixture was placed in a stainless-steel split mold and illuminated with a light-curing unit (BlueShot, Shofu Inc., Kyoto, Japan) for 60 s from both sides. Acrylic resin discs of the same size were used as controls.
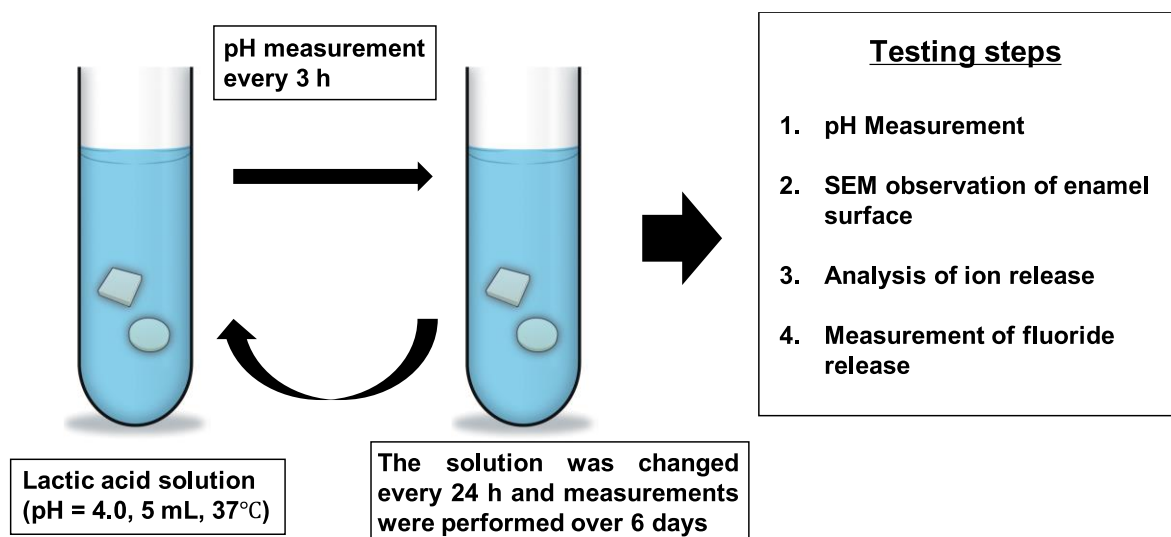
Figure 2. Description of the experimental methodology utilized for comparing the effects of GIC and S-PRG fillers on the demineralization of bovine enamel during incubation in the lactic acid solution with pH = 4.0 for 6 d at 37 °C.

2.3. pH measurements, enamel demineralization, and SEM observations

The utilized experimental methodology is described in Figure 2. DL-lactic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was diluted with distilled water to adjust its pH to 4.0. Next, 5 mL of the resulting solution was pipetted into 50-mL glass conical tubes (Corning, NY, USA). Specimens from each group were immersed in these tubes and incubated at 37 °C. The pH of each solution was measured every 3 h within a 24-h period. Afterwards, the specimens were transferred to tubes containing fresh lactic acid solutions and incubated for another 24 h at 37 °C. This process was repeated over a period of 6 d. To conduct pH measurements, a pH electrode (Orion 8102BNUWP) connected to a pH/ion meter (Orion 2115010 Dual Star pH/ion meter) was placed at the center of the conical tube. After 6 d of incubation, the enamel blocks were removed from the tubes and examined by scanning electron microscopy (SEM). For this purpose, they were rinsed with distilled water, dehydrated in a graded series of ethanol solutions (60–100%), sputter-coated with Au, and observed under a scanning electron microscope (JEOL JCM-600 Plus NeoScope, JEOL, Tokyo) at an accelerating voltage of 5 kV.

2.4. Fluoride ion release measurements

After the pH measurements (performed every 24 h), 1 mL of each solution was pipetted into the wells of a 24-well dish. Subsequently, TISAB III buffer (Thermo Fisher Scientific, Waltham, MA, USA) was added to the wells in the amount of 10 vol.% to provide a background with constant ionic strength. The solution mixture in the wells was stirred gently for 5 min and then subjected to fluoride ion release measurements. The amounts of fluoride ions produced by various specimens were determined using a fluoride ion-selective electrode (Orion 9609 BNWP, Thermo Fisher Scientific, Waltham, MA, USA) attached to an ion analyzer (Orion 2115010 Dual Star pH/ion meter, Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Analysis of released ions

After the completion of the pH and fluoride ion measurements, the solutions collected after 1 and 6 d of incubation were subjected to an ion analysis procedure. Al, B, Ca, Na, P, Si, Sr, and Zn ion release profiles were obtained by inductively coupled plasma-optical emission spectroscopy (ICP–OES) using an Optima 5300 DV system (Perkin-Elmer, MA, USA). ICP–OES calibration standards were prepared from their corresponding stock solutions on a gravimetric basis. Three

target calibration standards were obtained for each ion type, while deionized water and the lactic acid solution (pH = 4.0) were used as controls.

2.6. Statistical analysis

All data were presented as mean ± standard deviation (n = 6). The obtained data sets (pH values and Ca and P ion release profiles) were compared by performing one-way analysis of variance (ANOVA) and the Tukey-Kramer post-hoc test; values with $p < 0.05$ were considered statistically significant. The differences between the Al, B, Na, Si, Sr, and Zn release profiles recorded after 1 and 6 d of incubation were analyzed by conducting Student's t-test. The statistical differences between the results of the fluoride ion release measurements were assessed through one-way ANOVA and Tukey's multiple comparison post-hoc test; values with $p < 0.05$ were considered statistically significant. All statistical analyses were performed using GraphPad Prism software, version 8.1.2 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. pH variations

Figure 3 shows the results of the conducted pH measurements. For the solutions containing only enamel blocks (group 1), the initial pH increased slowly from 4.0 to 4.1 after 3 h and ultimately to 5.2 after 24 h of treatment. For the solutions containing GIC and S-PRG discs as well as enamel blocks (group 2), the pH steadily increased to more than 4.9 after 3 h, then to 5.2 after 6 h, and finally to 5.7 after 12 h of incubation (Figure 3a). Between days 1 and 6 of measurements, the pH of group 1 was approximately 5.1, while that of group 2 was 5.9–6.8 (Figure 3b). Figure 3c shows the changes in the pH of the solutions containing only discs of the studied restorative materials (the no-enamel group or group 3) observed during the first 24 h of incubation, while Figure 3d displays the changes in pH detected over the period between 24 h and 6 d. These graphs indicate that the pH variations of group 2 were much larger than those of group 1 at all measurement times ($p < 0.01$). In addition, the pH change for group 3 was more significant than that for the control (acrylic disc) at all measurement times ($p < 0.01$). Finally, the variations in the pH values of groups 2 and 3 exhibited the same trends at all measurement times.
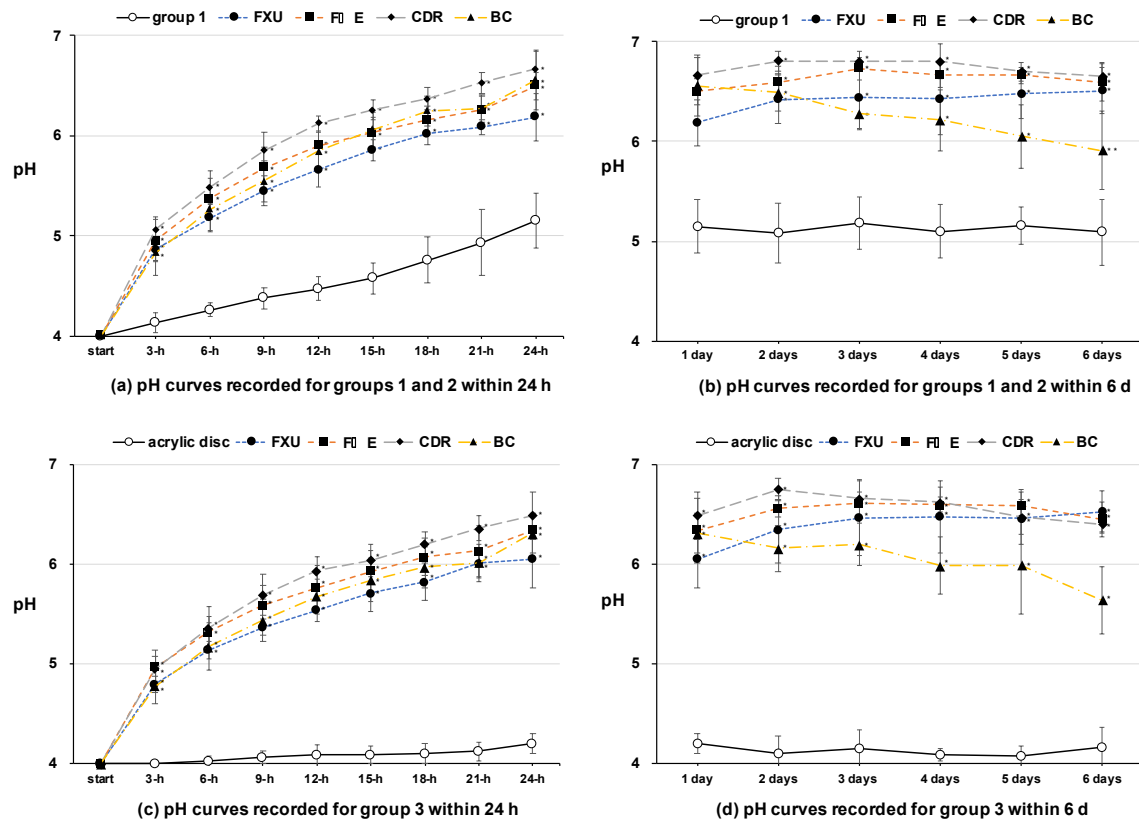
Figure 3. Variations of the pH values observed for different specimen groups. pH curves recorded for groups 1 and 2 after incubation for (**a**) up to 24 h and (**b**) over a period from 24 h to 6 d. pH curves recorded for group 3 after incubation for (**c**) up to 24 h and (**d**) over a period from 24 h to 6 d. Data were statistically analyzed by performing one-way ANOVA with Dunnett's multiple comparisons test ((a), (b): control = group 1; (c), (d): control = acrylic disc; n = 6, mean ± SD). *p < 0.01, **p < 0.05.

3.2. SEM observations

Figure 4 shows representative SEM images of the enamel block surfaces from different specimen groups obtained after 6 d of incubation. Before incubation, the enamel blocks exhibited smooth and polished surfaces with visible polishing lines and without prism outlines (Figure 4a). However, in the case of group 1 containing only enamel blocks, enamel rods appeared on the block surfaces due to demineralization (Figures 4b and c). Meanwhile, no morphological changes were observed for the enamel specimens incubated with FXU, FIXE, CDR, and BC disks (group 2), and their surfaces were similar to the polished surfaces of the control specimens (Figures 4d–h).
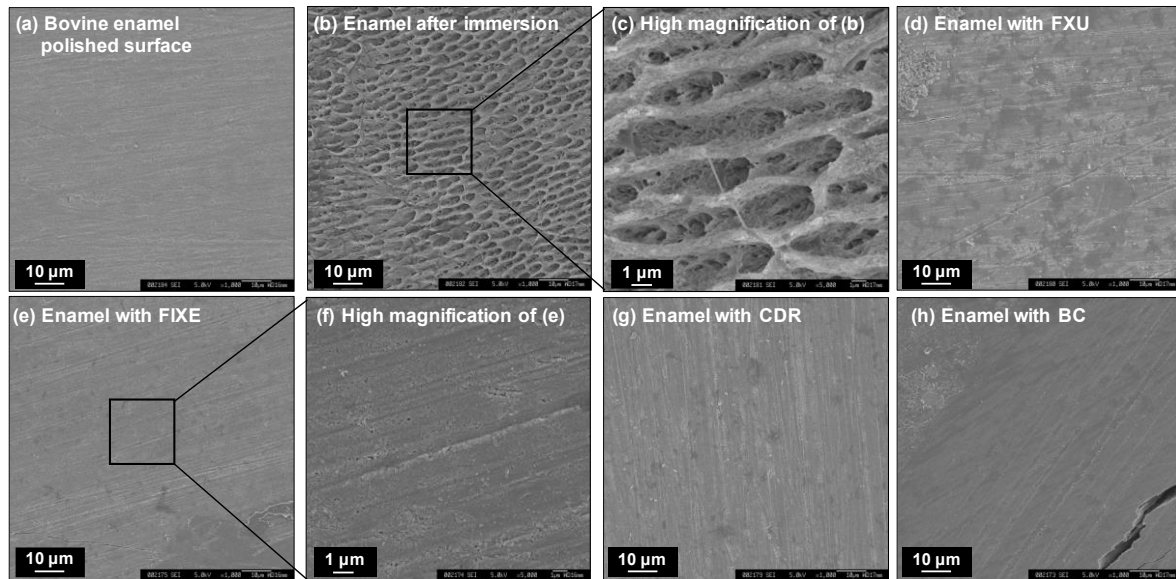
Figure 4. Representative SEM images of the enamel surfaces obtained after 6 d of incubation. (**a**) Smooth and polished surface of the bovine enamel block. (**b**) Demineralized enamel after the 6-d incubation in the lactic acid solution with pH = 4.0 (group 1). (**c**) High-magnification image of the sample surface depicted in panel (b) (×5000). Non-damaged surfaces of the enamel blocks incubated with (**d**) FXU, (**e**) FIXE, (**g**) CDR, and (**h**) BC disks (group 2). (**f**) High-magnification image of the sample surface depicted in panel (e) (×5000).

3.3. Release profiles of Ca and P ions

Figure 5 shows the obtained Ca and P ion release profiles. The highest amounts of released Ca ions were observed for the solutions containing only enamel blocks (group 1); they were equal to 6.3 µg/mL after 1 d and 6.1 µg/mL after 6 d of incubation ($p < 0.01$). The amounts of Ca ions released from CDR were higher than those released from FXU, FIXE, and BC (groups 2 and 3) ($p < 0.05$). For instance, their magnitudes determined for the solutions containing CDR discs and enamel blocks (group 2) were equal to 0.8 µg/mL after 1 d and 0.4 µg/mL after 6 d. Meanwhile, for the solutions containing CDR discs without enamel blocks (group 3), the concentration of released Ca ions was 0.6 µg/mL after 1 d and 0.4 µg/mL after 6 d of incubation. For the solutions containing FXU, FIXE, and BC disks with and without enamel blocks, the amounts of released Ca ions were below 0.1 µg/mL after both 1 and 6 d of treatment. Thus, the contents of Ca ions released by the enamel blocks during the incubation with the restorative materials (group 2) were very low as compared with the values obtained for group 1 ($p < 0.05$). Their magnitudes determined for group 3 were also very small.

The highest amounts of detected P ions were obtained for the solutions containing only enamel block (group 1); their values were equal to 10.5 µg/mL after 1 d and 10.2 µg/mL after 6 d ($p < 0.01$). The discs in group 2 released ten times more P ions as compared with those released in group 1; their contents after 1 d of incubation were 1.6 µg/mL for FXU, 1.4 µg/mL for FIXE, 0.6 µg/mL for CDR, and 0.3 µg/mL for BC ($p < 0.05$) (all these values decreased after 6 d of treatment). Thus, the amount of P ions released by BC (group 3) after 1 d of incubation was the lowest one. The relatively small P contents obtained for group 2 can be attributed to the dissolution of various components of the demineralized enamel. The trends observed for the amounts of P ions released in groups 2 and 3 were very similar; however, the concentrations of P ions released in group 3 were smaller than those released in group 2 by more than a factor of 2. Finally, the average amount of released P ions noticeably decreased over the period between days 1 and 6.
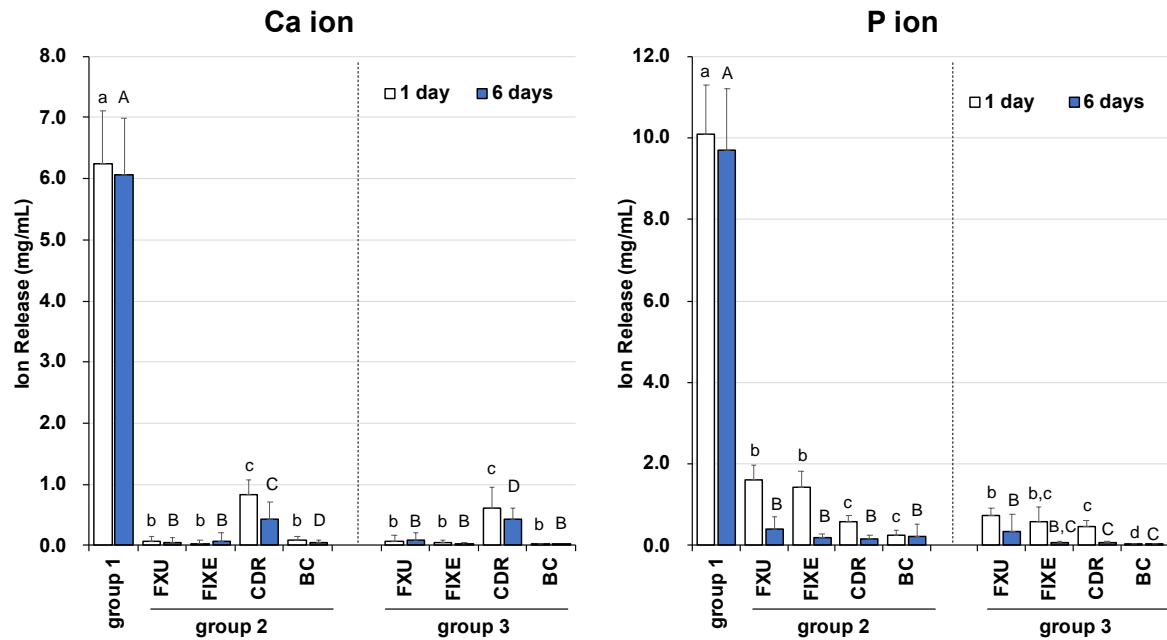
Figure 5. Ca and P ion release profiles. The solutions containing enamel blocks (group 1) exhibit high Ca and P concentrations. The solutions containing CDR discs with and without enamel blocks (groups 2 and 3, respectively) have higher Ca concentrations as compared with those of the solutions containing the other discs. Statistical differences were assessed by performing one-way ANOVA with Tukey's multiple comparison post-hoc test (after 1 and 6 d of incubation). Values with $p < 0.05$ were considered statistically significant. The bars with same letters are not significantly different ($p > 0.05$).

3.4. Release profiles of Al, B, Na, Si, Sr, and Zn ions

Figure 6 shows the Al, B, Na, Si, Sr, and Zn ion release profiles obtained for the solutions containing FXU, FIXE, CDR, and BC disks with and without enamel blocks after 1 and 6 d of incubation. The Al, Si, Na, and Sr ion release profiles of all the tested materials and their changes observed with increasing exposure time look very similar. The amounts of ions released by FXU, FIXE, and CDR after both 1 d and 6 d of incubation can be arranged in the order of Na > Al > Si > Sr. More importantly, the concentrations of Zn ions released in the CDR solutions (group 2) determined clear release and were equal to 1.4 µg/mL after 1 d and 1.1 µg/mL after 6 d. BC exhibited the highest Na and B release rates (followed by those of Si and Sr ions), which decreased significantly between days 1 and 6 ($p < 0.01$). Finally, the number of released Al ions was very small, and their concentration decreased significantly over the period from day 1 to day 6 ($p < 0.05$).
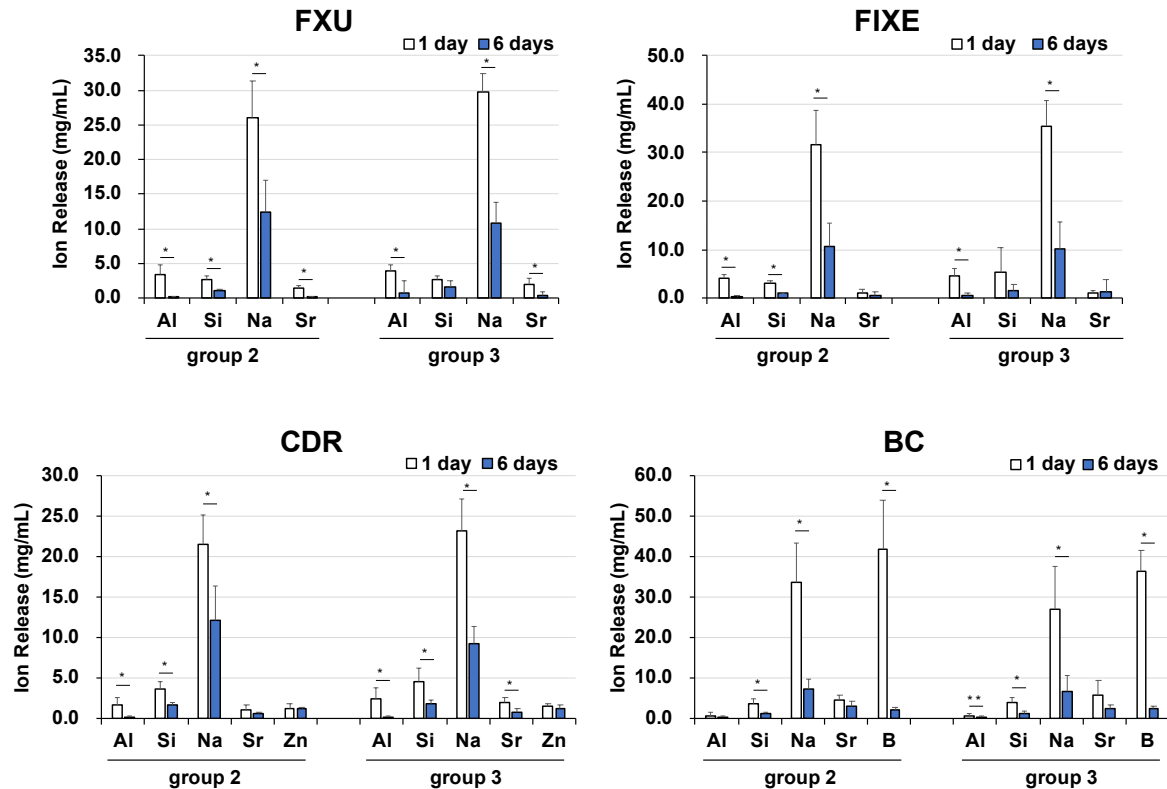
Figure 6. Al, B, Na, Si, Sr, and Zn ion release profiles. Concentrations of Al, B, F, Na, Si, and Sr ions in the solutions containing FXU, FIXE, CDR, and BC discs with and without enamel blocks (groups 2 and 3, respectively) determined after 1 and 6 d of incubation. The differences in values obtained after 1 and 6 d were analyzed by conducting Student's t-test. *$p < 0.01$, **$p < 0.05$.

3.5. Fluoride ion release

Figure 7 shows the fluoride release profiles obtained for specimen groups 2 and 3. The highest amount of fluoride ions released after 1 d was observed for FIXE ($p < 0.05$) followed by CDR and FXU. However, their magnitudes sharply decreased after 3 d of incubation and then remained constant till day 6. Further, the concentrations of fluoride ions released in the FXU and CDR solutions were not significantly different over the entire measurement period ($p > 0.05$). The fluoride content released in the BC solution was approximately half of those released in the GIC solutions regardless of the measurement time, and their differences were significant ($p < 0.05$). Finally, the concentrations of fluoride ions released in groups 2 and 3 exhibited similar trends.
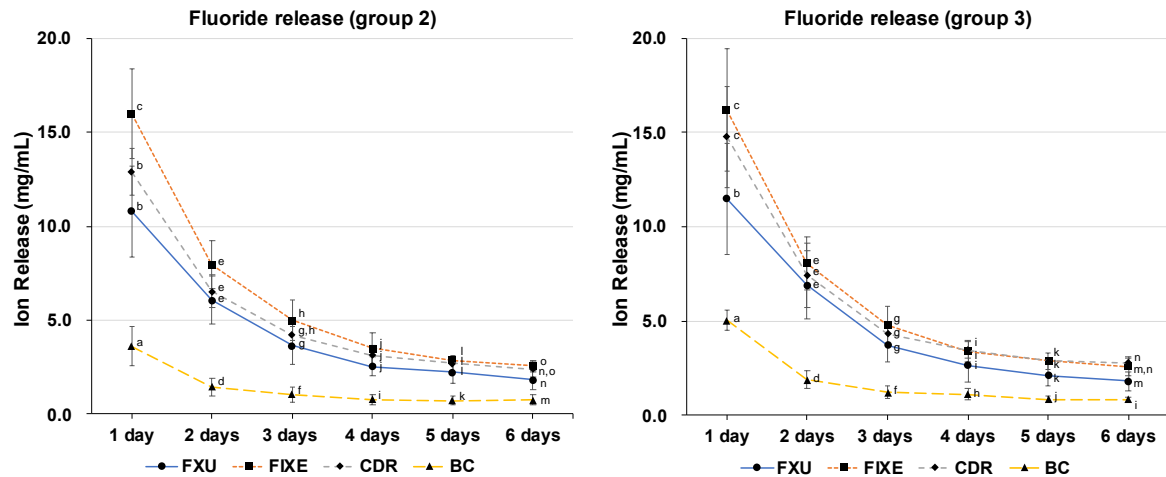
Figure 7. Fluoride ion release profiles. The concentrations of released fluoride ions decreased over time for all GICs in groups 2 and 3. The fluoride content in the BC solution was significantly lower than those in the GIC solutions (p < 0.05). Statistical differences between various materials were assessed by performing one-way ANOVA with Tukey's multiple comparison post-hoc test. Values with p < 0.05 were considered statistically significant. The bars with same letters are not significantly different (p > 0.05).

4. Discussion

In the present study, we investigated the protective effects of GIC and S-PRG restorative materials on the enamel surface immersed in lactic acid. The oral environment was simulated by adjusting the pH values of the test solutions to 4.0 each day. We found that the solutions containing only enamel blocks increased their pH values to approximately 5.1 after 24 h. The rods of the enamel blocks were visible in the obtained SEM images, owing to the loss of mineral tissue caused by the exposure to a low-pH environment. Further, a very strong correlation between the rate of release of Ca ions and degree of enamel demineralization was observed, and the dissolved Ca ions effectively neutralized the acid solution. However, the pH values of the solutions containing discs of restorative materials increased rapidly from 4.0 to more than 4.9 within 3 h; as a result, the demineralization of the enamel blocks did not occur. SEM observations confirmed the absence of morphological changes of these blocks even after their exposure to a low-pH environment for 6 d. The Al, B, F, Na, Si, Sr, and Zn ions released from the GIC and S-PRG discs neutralized the reaction solution quickly and inhibited enamel demineralization at the early stage of the incubation process. Therefore, the null hypothesis stating that the GIC- and S-PRG-containing restorative materials would not inhibit the demineralization of enamel was rejected based on the obtained results. The observed effects of these restoratives on pH neutralization indicated that such demineralization indeed occurred during the short-term exposure to low-pH conditions.

The Al, Ca, F, Na, Si, Sr, and Zn ions released from the GIC- and S-PRG-containing restorative materials inhibited the demineralization process through neutralization. The glasses present in GICs are composed of calcium fluoro-alumino-silicates; hence, Al and Si are their primary network-forming elements. When the glass and acid components of GICs are mixed, an acid–base setting reaction is initiated, owing to the cross-linking of the carboxylic acid groups present in the aqueous solution of polyacrylic acids with the Al and Ca ions released from the glass powder [18]. The GICs continue to harden over time, leading to the formation of silicate or phosphate networks [19]. Because the electronic structure of Sr is very similar to that Ca, several Ca-containing glasses have been replaced with Sr-containing ones in some GIC products to enhance their radiopacity [20]. In this study, the release of Ca was observed only for CDR, a recently developed Ca-containing product for remineralizing and strengthening the tooth substrate. Further, a small amount of Ca ions was released in group 3. However, except for CDR, the results obtained for group 3 were unexpected because the FXU, FIXE, and BC disks did not contain Ca element. Thus, the Ca ions released from

these materials likely originated from some contaminants present in the incubation media or traces remained after the ICP tests. In any case, their contents were negligibly low. Analyses of the elemental distributions on the surfaces of FX-II and Fuji IX cements conducted by energy-dispersive X-ray spectroscopy and X-ray photoelectron spectroscopy confirmed the presence of Ca in their glass cores. However, it was also found that when the cement matrix was immersed into a $CaCl_2$ solution, it absorbed Ca ions from it. These ions formed chemical bonds with the carboxylic acid groups of the cement matrix to produce a polyacid salt matrix that increased surface hardness [21]. Moreover, P ions were also likely present in the reaction solution (their effects on the release of ions from the surfaces of glass particles as well as on the working and setting times of GICs have been studied previously [22]). In this work, the small amount of P ions released by the disc-like specimens in group 2 was related to the dissolution of some components of the demineralized enamel. Further, the P release profiles of groups 2 and 3 were similar. However, the concentration of P ions released in group 3 was very low as that group consisted of only discs of the investigated restoratives. In general, the differences in the amounts of ions released by various cements can be attributed to the differences in their chemical compositions.

Both F and Sr ions play important roles in enamel restoration. Fluoride ions promote the formation of hard tissue by precipitating a calcium-fluoride-like (fluoro-apatite) layer onto the tooth surface, thus reinforcing the tooth structure [23]. In addition, they enhance the remineralization of partially demineralized enamel during the early stages of caries formation in the presence of Ca and PO_4^{3-} ions from the saliva. The fluoridated enamel is more acid-resistant than the native enamel [24]; hence, fluoride-releasing materials may serve as reservoirs that increase the fluoride levels in the saliva, plaque, and dental hard tissues. In addition, fluoride helps increase the longevity of restorations by increasing the size of the acid-resistant zone adjacent to the cavity wall, thereby preventing further losses of mineral ions [5]. Meanwhile, Sr ions reinforce the tooth structure by converting hydroxyl apatite into Sr apatite, which results in the formation of an acid-resistant layer on the tooth surface [25]. They also promote remineralization and antibacterial activity while exhibiting a synergistic effect in the presence of F ions [26,27].

The structure of S-PRG fillers with a stable glass ionomer phase allows the release of Al, B, F, Na, Si, and Sr ions in resinous sealants [28]. In a previous study on coating materials [14], we found that the concentration of Na ions in a test solution was the highest one followed by those of B, Si, Sr, F, and Al ions. The concentration of each ion reached maximum after 24 h and then decreased over time. Although the amounts of released ions decreased gradually, enamel demineralization was inhibited in this process. Thus, the release of even small ion concentrations can produce a strong inhibitory effect on the demineralization process. The above-listed ions can effectively neutralize acidic plaque and promote anticaries activity in clinical settings. Moreover, the anticariogenic activity of F ions increases when the latter are combined with Al, Na, Si, or Sr ions. For this reason, the S-PRG discs used in the present study functioned as buffers that made the test solutions less cariogenic by reducing their acidity during the incubation period.

The obtained experimental results confirmed that the GIC- and S-PRG-containing restorative materials increased the pH of the acidic environment to the neutral level and reduced the likelihood of tooth caries through the localized protection of the tooth surface from acid attacks. Note that B ions were detected only in the BC solutions, while Ca and Zn ions were released only in the CDR ones. The fluoride leaching from GICs produces an antibacterial effect and helps prevent the formation of secondary caries [8]. Furthermore, the antibacterial effect of the B and F ions released by S-PRG fillers in resin composites inhibits the metabolism of *Streptococcus mutans* and limits the acid production [29]. Thus, to prevent the formation of dental biofilms and caries, it is necessary to develop bioactive dental restorative materials with enhanced antibacterial characteristics.

5. Conclusions

It was found that GIC- and S-PRG-containing restoratives could serve as buffers for lactic acid solutions that rapidly increased their pH values to the neutral level by releasing various ions, which inhibited enamel demineralization. The bioactive properties of these materials make them suitable

for the clinical restoration of early carious lesions in a biomimetic, atraumatic, and non-cavitated manner.

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