Effects of Surface Prereacted Glass on Saliva-Derived Polymicrobial Biofilms in an Active Attachment Biofilm Model

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Abstract
Bioactive restorative materials are being developed to either influence the de/remineralization balance of the dental hard tissues locally or to release components that interact with the oral microbiota. Surface prereacted glass (S-PRG, Shofu, Japan) is a material that may influence both processes. S-PRG releases fluoride, which can interact with the de/remineralization process, and a range of other compounds that may influence the oral microbiota. In the current study, several experiments were performed to investigate the potential of S-PRG to influence both the growth and lactic acid production of saliva-derived polymicrobial biofilms. Biofilm formation was studied using the Amsterdam Active Attachment model. An eluate of the S-PRG particles was tested by adding it to the growth medium or by exposing the biofilms to it for 1 h. The effect of S-PRG particles was tested by adding the particles to the growth medium. The current experiments showed that the presence of S-PRG eluate in the medium influenced biofilm growth and lactic acid production even at low concentrations. The composition of the biofilms changed in the presence of S-PRG eluate, even at concentrations of S-PRG eluate at which biofilm viability was not affected. Treatment of developing biofilms with S-PRG eluate did neither show an effect on biofilm viability nor on lactic acid production. The addition of S-PRG particles to the growth medium resulted in both a lower biofilm viability and lower lactic acid production, indicating that the release of ions from the particles was fast enough to influence biofilm formation. From the current experiments, it can be concluded that S-PRG has the potential to influence biofilm growth, but the presence of the released ions during biofilm formation is required to show an effect.

Introduction
Dental caries and periodontitis are two major causes of tooth loss. Several strategies have been developed to reduce tooth loss, either by shifting the de/remineralization balance toward remineralization (for caries) or by...
reducing the bacterial load (for both caries and periodontitis). A newer strategy is to use bioactive restorative materials that contribute to the before mentioned strategies. These materials can either influence de/remineralization locally by releasing components (like fluoride) that shift the de/remineral balance toward remineralization or even by releasing components that interact with the oral microbiota. One of these bioactive materials is surface prereacted glass (S-PRG, Shofu Inc., Kyoto, Japan). The S-PRG particles are formed by an acid–base reaction between fluoroaluminosilicate glass and polyacrylic acid. S-PRG is used as filler in composite material and is known to release several types of ions, including Al, B, Na, Si, Sr, and F [Fujimoto et al., 2010; Miki et al., 2016; Yassen et al., 2016]. The effects of the S-PRG filler and its released ions on dental hard tissues have been studied extensively [Mukai et al., 2009; Shiiya et al., 2012, 2014, 2016; Shimazu et al., 2012; Amaechi et al., 2017], showing positive effects on the de/remineralization balance of dental hard tissues. An increasing number of studies show that S-PRG and the released ions from S-PRG have an effect on bacterial species and biofilm formation. For example, Hotta et al. [2014] demonstrated that S. sanguinis and S. mutans showed reduced adherence to S-PRG blocks compared to an unfilled resin, while Saku et al. [2010] found reduced adherence of S. mutans to S-PRG-containing BeautifilII resin during a 2-h adherence assay. The effect was larger when the resin was coated with saliva. In the same study, the S-PRG-containing resin accumulated less bacteria during an 8 h in situ test. In addition, Miki et al. [2016] showed a reduction in the number of S. mutans growing in an 18 h period on S-PRG-containing resin composites. The effect of S-PRG-containing materials on bacterial attachment or growth is attributed to the release of ions from this material that exhibit antibacterial properties. Both the growth rate of a number of oral bacteria and their biofilm formation was reduced is the presence of S-PRG eluate [Shimazu et al., 2016].

In general, studies so far have looked at short-term effects of S-PRG, that is, the initial attachment of bacteria or relatively short periods of biofilm formation. Since mainly the effect of S-PRG on single-species biofilms has been studied [Yoneda et al., 2012; Suzuki et al., 2014; Shimazu et al., 2016; Nomura et al., 2018, 2021], the effect of S-PRG on a multispecies biofilm remains unknown. The Amsterdam Active Attachment model [Exterkate et al., 2010] has been developed to grow polymicrobial, saliva-derived biofilms over prolonged periods of time. This model allows for the development of 24 individual biofilms on a substratum of choice, and the design allows for proper control of treatments in terms of concentration and exposure times. The current study was designed to evaluate whether S-PRG influences the formation and metabolic activity by measuring lactic acid production of saliva-derived biofilms in an active attachment biofilm model using hydroxyapatite discs as substratum.

**Materials and Methods**

**Surface Prereacted Glass**

S-PRG was tested as eluent or as solid particles. The eluent was prepared by mixing S-PRG particles with high-purity water in a 1:1 (weight:volume) ratio. After 24 h, the particles were spun down, and the supernatant was used. The eluent was provided by Shofu, filter sterilized and stored at room temperature until used. The undiluted eluent is referred to as 100%. The S-PRG particles with a diameter of 0.8 or 3 µm, also provided by Shofu, were added to the growth medium just prior to use.

**Biofilm Model**

Biofilms were grown in the Amsterdam Active Attachment model [Exterkate et al., 2010]. In short, the model consists of a custom-made stainless steel lid with 24 clamps that fitted on top of standard 24-well plates (Greiner BioOne, The Netherlands). Dense hydroxyapatite discs (99 mm, appr. 2 mm thick, Himed, USA) were used as substratum to grow the biofilms on.

**Saliva Collection**

Saliva was collected on ice from a single donor, who refrained from oral hygiene for 24 h, and diluted 2-fold with 60% sterile glycerol to protect the bacteria from cryo damage. Saliva was aliquoted and stored at −80°C until used. The Medical Ethical Committee of the VU-Medical Center (VU-METc) has decided on July 25th, 2011, that the one-time collection of saliva from volunteers is not subjected to Medical Research Involving Human Subjects Act (Wet WMO).

**Growth of Biofilms**

The inoculum for the polymicrobial biofilm consisted of 50-fold diluted saliva in semi-defined buffered medium consisting of 2.5 g/L Mucin (Sigma M-2378), 2.0 g/L Bacto Peptone (Difco 0118-01-8), 2.0 g/L Trypticase Peptone (BBL 211921), 1.0 g/L Yeast Extract (Bacto 212750), 0.35 g/L NaCl, 0.2 g/L KCl, 0.2 g/L CaCl₂, 0.1 g/L cysteine hydrochloride, 0.001 g/L Hemin (Sigma H-1652), 0.0002 g/L vitamin K₁ [McBain et al., 2005], supplemented with 0.2% sucrose and 50 mmol/L Pipes at pH 7.0.

Biofilms were produced by adding 1.5 mL of the inoculum to each well. The model was subsequently incubated anaerobically (10% CO₂, 10% H₂, and 80% N₂) for 8 h at 37°C. After this initial inoculation period, the lid was transferred to a new plate containing fresh medium (without bacteria) and incubated for another 16 h. In every experiment, biofilms were grown in an 8/16 h schedule until the biofilms were harvested. For a 48 h experiment, the growth medium was refreshed at 8, 24, and 32 h.
Fig. 1. Effect of repeated treatment of developing biofilms with S-PRG eluent on biofilm viability (a) and lactic acid production (b) versus time points (h) of the treatments. Groups (n = 6) were not statistically different from each other.
At the end of each experiment, after 48 h, biofilms were incubated for 3 h with 0.2% glucose in Buffered Peptone Water [Exterkate et al., 2010] to determine the lactic acid production capacity of the biofilms. After this assay, the biofilms were harvested by sonication, diluted and plated on tryptic soy agar plates supplemented with 5% sheep blood. Plates were incubated for at least 48 h, and the number of colony-forming units (CFU) per biofilm was determined. When required, a 1.4-mL aliquot of the samples was stored at –80°C for subsequent sequencing to determine the biofilm composition.

**Next-Generation Sequencing**

After harvesting the biofilms, a 1.4-mL aliquot was used for DNA isolation. The samples were thawed, centrifuged at 13,000 rpm for 5 min, and supernatant was discarded. The biofilm pellet was resuspended in 200 µL Tris-EDTA buffer for DNA isolation and subsequent sequencing as described previously [Volgenant et al., 2017]. Briefly, after purification, the bacterial DNA concentration was determined by qPCR, and the V4 hypervariable region of the 16S rRNA gene was amplified. The generated amplicons were pooled in equimolarly, purified from agarose gels (Qiagen, Roermond, The Netherlands), and sequenced (2 × 251 nt) using the Illumina MiSeq platform and Illumina MiSeq reagent kit V2 (Illumina Inc., San Diego, CA, USA) at the VUmc Cancer Center, Amsterdam, The Netherlands. The flow cell was loaded with 6.5 pmol DNA containing 50% PhiX. The sequence data were processed as described previously [Koopman et al., 2016]. However, 25 mismatches (still 10%) were allowed in the overlap region of the paired reads, since the read lengths were 2 × 251 nt. The OTU table was subsampled at a depth of 8,800.

**Experiments with S-PRG Eluent**

S-PRG eluent was tested in two different ways. The eluent was either used as a 60-min treatment on developing biofilms or added to the growth medium in a range of concentrations. Developing biofilms were treated with the 100% S-PRG eluent (1.6 mL/well) just prior to a medium refreshment. After the treatment, the biofilms were rinsed three times with 1.7 mL sterile cysteine peptone water and subsequently placed on top of a new plate containing fresh medium. The experimental groups differed in the onset of the S-PRG treatments: treatments were started at either 8, 24, or 32 h of biofilm formation and were conducted at every medium change thereafter and at the final treatment at 48 h of biofilm formation, just prior to the lactic acid production assay and harvesting of the biofilm. As control, biofilms were exposed to sterile water instead of eluent. This schedule resulted in biofilms left untreated or being treated twice, three times, or four times (n = 6).

In an alternative experiment, S-PRG eluent was added to the growth medium in a series of concentrations, expressed as percentage of the original eluent. This design resulted in continuous presence of (diluted) S-PRG eluent (n = 4). In order to obtain the same final concentration of the constituents in the growth medium, the medium was prepared two-fold concentrated. The final medium was a mixture of this 2-fold concentrated growth medium, S-PRG eluent, and sterile ultrapure water. The mixture of S-PRG eluent and water always made up 50% of the final volume.

**Experiments with S-PRG Particles**

S-PRG particles were only tested as an addition to the growth medium. When S-PRG particles are exposed to a liquid, they will release ions that may influence biofilm growth. The current experiments were designed to study if the release of ions is fast enough to influence biofilm growth. S-PRG particles with a diameter of 0.8 μm or 3 μm were added in a 1:50 or 1:250 particle to medium ratio (wt/vol). The biofilms were grown with three different inoculation ratios (vol/vol) of saliva versus growth medium: 1:50, 1:500, and 1:5,000 (n = 4). The rationale behind the latter experiment was that when fewer bacterial cells are present in the inoculation medium, S-PRG may have a larger effect on biofilm formation.

In an additional experiment, the effect of adding S-PRG particles on an already established biofilm was evaluated. Biofilms were allowed to grow undisturbed during 24 h and the next 24 h period, in the presence of S-PRG particles (1:50 or 1:250 ratio) (n = 4).

**Statistics**

Differences in CFU counts and lactic acid production were tested for significance using ANOVA and post hoc Duncan’s multiple range test using SPSS (IBM SPSS statistics version 27).

**Results**

First, saliva-derived biofilms were repeatedly treated with S-PRG eluent during their development. Even when the first 1 h treatment was performed after only 8 h of biofilm formation, resulting in a total of four treatments, no effect on biofilm viability could be observed (Fig. 1a). In addition, the potential of these biofilms to produce lactic acid during a 3 h incubation with 0.2% glucose did not decrease (Fig. 1b). These findings were in contrast to previous studies [Yoneda et al., 2012; Suzuki et al., 2014; Shimazu et al., 2016; Nomura et al., 2018, 2021] that evaluated the effect of S-PRG on biofilm formation and bacterial growth. However, only one other study evaluated the effect of short-term exposure of oral biofilms to S-PRG eluate and found a comparable lack of efficiency [Kim et al., 2020].

To further study the potential of S-PRG to influence biofilm growth and behavior, additional experiments were carried out in which S-PRG was present throughout the experiment. When S-PRG eluent was added to the growth medium, biofilm formation was reduced increasingly with increasing concentration of S-PRG in the growth medium, however, only at 12.5% and 50%, this reduction was statistically significant (Fig. 2a). Lactic acid production was reduced with increasing S-PRG levels and already reached statistical significance at the lowest concentration of S-PRG tested (1.6%) present in the medium (Fig. 2b).
Fig. 2. Effect of presence of diluted S-PRG eluent during growth on biofilm viability (a) and lactic acid production (b). Bars show the average and standard deviation of the mean. Groups (n = 4) with the same letter are not statistically significantly different to each other.
The principal component analysis of the microbial profiles of the biofilms grown in the presence of S-PRG eluent revealed that changes in the microbial composition already occurred at the lowest concentration of S-PRG eluent tested (1.6%, Fig. 3a). The PCA ordination plot shows that the groups with S-PRG present during biofilm formation shifted away from the control groups. Obviously, the effect was largest at the 12.5% and 50% levels. When these two concentrations were left out of the analysis, the composition at 1.6% was already different from the controls (Fig. 3b). From these first experiments, it became clear that the presence of diluted S-PRG eluent during biofilm formation did have an effect on biofilm growth, lactic acid production, and bacterial composition.

As the eluent was produced by exposing S-PRG particles to water for 24 h, the question remained if the release of ions from the S-PRG particles would be fast enough to influence biofilm growth and metabolism. Therefore, the effect of the presence of S-PRG particles in the growth medium on biofilm growth and metabolism was also evaluated.

The addition of S-PRG (ratio 1:50, either as 0.8 or 3 μm particles) to the growth medium resulted in a reduction of biofilm formation (Fig. 4b) and lactic acid formation irrespective of particle size or inoculation ratio (Fig. 4a). Even at a 1:250 ratio, both biofilm formation and lactic acid production were still reduced (Fig. 4a, b). Also, when biofilms were allowed to grow in the absence of S-PRG particles for 24 h and subsequently 24 h in the presence of S-PRG particles, biofilm formation and lactic acid production were reduced (Fig. 5).

**Discussion**

Two main parameters were measured in the current set of experiments: the number of CFU measured at the end of the experiment as a measure of biofilm viability and the lactic acid production as measure of metabolic activity. The combined data show that ions released from S-PRG influenced biofilm formation, even when present at low levels.

In the first experiment using S-PRG eluent, 1-h treatments of saliva-derived biofilms with the eluate showed no effect on biofilm viability or lactic acid production. This is an indication that S-PRG eluent has no bactericidal effect on biofilms as both lactic acid production and biofilm viability were measured immediately after the last treatment with S-PRG eluent. Repeated treatments did not result in an increased efficacy, which may indicate that the retention of the eluent in the biofilm is low or that the rinsing steps (which are part of the treatment regime) effectively removed the eluent from the system. These findings are in line with results from Kim et al. [2020] who found that twice-daily treatments with S-PRG eluate had no effect on biofilm viability. However, as these findings are in contrast to other publications [Yoneda et al., 2012; Suzuki et al., 2014; Shimazu et al., 2016; Nomura et al., 2018, 2021] in which S-PRG eluate was tested while continuously present, S-PRG eluent was added to the growth medium in subsequent experiments.

The addition of S-PRG eluent to the growth medium resulted in reduced biofilm formation at the higher concentrations of 12.5% and 50%. However, even at the lowest S-PRG concentration (1.6%), lactic acid production was already strongly reduced (38%). It should be noted that lactic acid production was determined in the absence of S-PRG eluent at the end of the experiment, just prior to harvesting the biofilms. Next-generation sequencing was used to determine the composition of the formed biofilms. From the PCA plot (Fig. 3), it is clear that at the higher concentrations of 12.5% and 50%, the composition differed from the untreated biofilms. While these high concentrations’ biofilm growth was also hampered, resulting in much lower CFU counts (Fig. 2); even at the lower S-PRG concentrations, a shift in bacterial composition was observed. This shift occurred without a noticeable effect on the overall viability. PCA analysis showed that the same OTUs were responsible for the shift that was observed at the lower concentration range (1.5–6.3%) compared to the full range of concentrations, in which the higher concentrations (12.5%, 50%) resulted in the largest differences.

**Fig. 3.** Principal component analysis of the biofilm microbiota profiles formed in the presence of different amounts of S-PRG eluent. At 12.5% and 50% of S-PRG eluent, the composition was clearly different from the other groups (a). PCA with the lower S-PRG concentrations only shows the differences in these groups: also at lower levels (with no effect on biofilm viability), differences in composition can be observed (b).

**Fig. 4.** Effect of adding S-PRG particles to the growth medium on biofilm viability (a) and lactic acid production (b). Biofilm formation was started with different inoculation ratios of saliva versus growth medium. Bars show the average and standard deviation of the mean. Groups (n = 4) with the same letter are not statistically significant different. (For figure see next page.)
Fig. 5. Biofilm viability (a) and lactic acid production (b) of biofilms that were grown 24 h in the absence of S-PRG particles and subsequently 24 h in the presence of S-PRG particles. Biofilm formation was started at two different inoculation ratios (1:50 and 1:5,000). S-PRG particles (0.8 μm) were added after 24 h at two different ratios (1:50 and 1:250). Bars show the average and standard deviation of the mean. Groups (n = 4) with the same letters are not statistically significantly different.
The presence of S-PRG already affected biofilm growth, in terms of composition, at levels that did not result in reduced CFU counts. S-PRG does not kill bacteria (Fig. 1), but apparently, it does influence bacterial growth with different efficacy for different species, resulting in small shifts in composition at lower concentrations and resulting in reduced overall growth at higher concentrations.

The inhibition of biofilm growth by already eluted ions is in line with the findings of Miki et al. [2016], who showed that 18 h growth of S. mutans was reduced in the presence of borate, fluoride, aluminum, and silicate at levels that were released from S-PRG-containing resin composites. However, the reduction of biofilm growth was larger in the current set of experiments compared to the findings of Miki et al. [2016].

When the S-PRG particles were added to the growth medium just prior to the start of the biofilm growth, biofilm viability and lactic acid production were reduced (Fig. 4). Ions eluted from the S-PRG particles did not reduce biofilm viability when applied as a treatment (Fig. 1), but growing the biofilms in the presence of the S-PRG eluate did reduce biofilm formation (Fig. 2). Thus, for the S-PRG particles to reduce biofilm growth, the release has to be sufficiently fast to inhibit further biofilm growth and lactic acid production. The data clearly showed that biofilm formation and lactic acid production were reduced in the presence of S-PRG particles, indicating that the release of ions from the S-PRG particles is indeed sufficiently fast. The effect of the inoculation ratio, that is, the number of bacterial cells present at the start of the biofilm formation, on CFU counts is limited but yet differences do reach significance. Lactic acid production is strongly reduced when biofilms were grown in the presence of S-PRG particles, irrespective of the ratio or size of the particles (Fig. 4b). Since the lactic acid production assay was performed in the absence of the particles, this is an indication that biofilm properties were changed compared to the control groups.

It is the inhibition of growth that results in the differences between biofilms grown in the presence of eluent or particles and their controls. This was also confirmed in the experiment where biofilms were allowed to grow in the absence of S-PRG for 24 h and then in the presence of S-PRG particles in the remaining 24 h. The addition of S-PRG to an already established biofilm resulted in slightly lower biofilm viability (appr. 50% reduction) but in an almost complete inhibition of lactic acid production. The reduction in viability when S-PRG particles were added after 24 h was lower compared to when S-PRG particles were added at the onset of biofilm formation.

There are only a limited number of articles that investigated the effect of S-PRG on bacterial viability during a longer period (days of more). Hahnel et al. [2014] showed no difference of S. mutans biofilm formation on resin-based composites (RBCs) containing a range of concentrations of S-PRG filler in a drip flow reactor. There was a small, concentration-dependent difference after 48 h, but after 120 h, no differences could be observed. Yoshihara et al. [2017] showed that there was no difference in growth of S. mutans in 24-well plates in the presence of S-PRG-filled RBC, a conventional RBC or growth in the absence of an RBC in a 30-h experiment. Both authors used single species, S mutans, to study the effect of S-PRG.

In conclusion, the current set of experiments showed that S-PRG can influence biofilm formation, metabolic activity, and biofilm composition, while it does not effectively kill bacteria in biofilms. However, S-PRG can only reduce biofilm growth and change the composition of the biofilms if the components eluted from S-PRG are present on-site. Whether S-PRG has clinical potential as a biofilm modulator requires additional experiments aimed at establishing its efficacy in intra-oral models. The addition of compounds that enhance the retention of the released ions might positively affect the efficacy of S-PRG in biofilm control.

Statement of Ethics

The current in vitro study was not subject to ethical approval. For the collection of saliva from a single donor, the Medical Ethical Committee of the VU-Medical Center (METC-VUMC) granted an exemption from ethics approval. The saliva donor gave written informed consent as required by the METC-VUMC.

Conflict of Interest Statement

Rob A.M. Exterkate and Wim Crielaard received funding from Shofu Inc. to perform the studies in this manuscript. Both authors have no personal relationship with Shofu Inc.

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Author Contributions

Rob A.M. Exterkate designed the studies, performed the experiments, and wrote the manuscript. Wim Crielard designed the studies and wrote the manuscript. Bernd W. Brandt analyzed the data and wrote the manuscript.

References

Amaechi BT, Key MC, Balu S, Okoye LO, Gakunga PT. Evaluation of the caries-preventive effect of toothpaste containing surface-reacted glass-ionomer filler. J Investig Clin Dent. 2017;8(4):e12249.

Exterkate RAM, Crielard W, Ten Cate JM. Different response to amine fluoride by Streptococcus mutans and polymicrobial biofilms in a novel high-throughput active attachment model. Caries Res. 2010;44(4):372–9.

Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. Dental Mater J. 2010;29(4):392–7.

Hahlne S, Warl RL, Schneider-Feyrer S, Giessibl FI, Brambilla E, Cazzaniga G, et al. Streptococcus mutans biofilm formation and release of fluoride from experimental resin-based composites depending on surface treatment and S-PRG filler particle fraction. J Adhes Dent. 2014;16(4):313–21.

Hotta M, Morikawa T, Tamura D, Kusakabe S. Adherence of Streptococcus sanguinis and Streptococcus mutans to saliva-coated S-PRG resin blocks. Dent Mater J. 2014;33(2):261–7.

Kim HJ, Cho MY, Lee ES, Jung HI, Kim BI. Effects of short-time exposure of surface pre-reacted glass-ionomer eluate on dental microcosm biofilm. Sci Rep. 2020;10(1):14425.

Koopman JE, Buijs MJ, Brandt BW, Keijser BJF, Crielard W, Zaura E. Nitrate and the origin of saliva influence composition and short chain fatty acid production of oral microcosms. Microb Ecol. 2016;72(2):479–92.

McBain AJ, Sissons C, Ledger RG, Sreenivasan PK, De Vizio W, Gilbert P. Development and characterization of a simple perfused oral microcosm. J Appl Microbiol. 2005;98(3):624–34.

Miki S, Kitagawa H, Kitagawa R, Kiba W, Hayashi M, Imazato S. Antibacterial activity of resin composites containing surface pre-reacted glass-ionomer (S-PRG) filler. Dent Mater. 2016;32(9):1095–102.

Mukai Y, Kamijo K, Fujino F, Hirata Y, Teranaka T, ten Cate JM. Effect of denture base resin with prereacted glass-ionomer filler on dentin demineralization. Eur J Oral Sci. 2009;117(6):750–4.

Nomura R, Morita Y, Matayoshi S, Nakano K. Inhibitory effect of surface pre-reacted glass-ionomer (S-PRG) eluate against adhesion and colonization by Streptococcus mutans. Sci Rep. 2018;8(1):5056.

Nomura R, Kitamura T, Matayoshi S, Ohata J, Sueno I, Iwashita N, et al. Inhibitory effect of a gel paste containing surface pre-reacted glass-ionomer (S-PRG) filler on the cariogenicity of Streptococcus mutans. Sci Rep. 2021;11(1):23495.

Saku S, Kotake H, Scougall-Vilchis RJ, Ohashi S, Hotta M, Horiiuchi S, et al. Antibacterial activity of composite resin with glass-ionomer filler particles. Dent Mater J. 2010;29(2):193–8.

Shiuya T, Mukai Y, Ten Cate JM, Teranaka T. The caries-reducing benefit of fluoride-release from dental restorative materials continues after fluoride-release has ended. Acta Odontol Scand. 2012;70(1):15–20.

Shiuya T, Tomiyama K, Iizuka J, Hasegawa H, Kuramochi E, Fujino F, et al. Effect of the coating material on root dentin remineralization in vitro. Am J Dent. 2014;27(5):258–62.

Shiuya T, Tomiyama K, Iizuka J, Hasegawa H, Kuramochi E, Fujino F, et al. Effects of resin-based temporary filling materials against dentin demineralization. Dent Mater J. 2016;35(1):70–5.

Shimazu K, Ogata K, Karibe H. Caries-preventive effect of fissure sealant containing surface reaction-type pre-reacted glass ionomer filler and bonded by self-etching primer. J Clin Pediatr Dent. 2012;36(4):343–7.

Shimazu K, Oguchi R, Takahashi Y, Konishi K, Karibe H. Effects of surface reaction-type pre-reacted glass ionomer on oral biofilm formation of Streptococcus gordonii. Odontology. 2016;104(3):310–7.

Suzuki N, Yoneda M, Haruna K, Masuo Y, Nishihara T, Nakanishi K, et al. Effects of S-PRG eluate on oral biofilm and oral malodor. Arch Oral Biol. 2014;59(4):407–13.

Volgenant CMC, Zaura E, Brandt BW, Buijs MJ, Tellez M, Malik G, et al. Red fluorescence of dental plaque in children: a cross-sectional study. J Dent. 2017;58:40–7.

Yassen GH, Huang R, Al-Zain A, Yoshida T, Gregory RL, Platt JA. Evaluation of selected properties of a new root repair cement containing surface pre-reacted glass ionomer fillers. Clin Oral Investig. 2016;20(8):2139–48.

Yoneda M, Suzuki N, Masuo Y, Fujimoto A, Iha K, Yamada K, et al. Effect of S-PRG eluate on biofilm formation and enzyme activity of oral bacteria. Int J Dent. 2012;2012:814913.

Yoshihara K, Nagoaka N, Maruo Y, Sano H, Yoshida Y, Van Meerbeek B. Bacterial adhesion not inhibited by ion-releasing bioactive glass filler. Dent Mater. 2017;33(6):723–34.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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