Impact of surface pre-reacted glass ionomer filler eluate on lipase gene expression in Candida albicans: An in vitro study

Watcharapong TONP拉萨NBTHON1,2, Masanao INOKOSHI1, Muneaki TAMURA3, Keita HATAN 1 and Shunsuke MINAKUCHI1

1 Department of Gerodontology and Oral Rehabilitation, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo, Tokyo 113-8549, Japan
2 Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, 99 Village No.9, Phitsanulok-Nakhon Sawan road, Tha Pho, Mueang Phitsanulok District, Phitsanulok 65000, Thailand
3 Department of Microbiology and Immunology, Nihon University School of Dentistry, 1-8-13 Kanda Surugadai, Chiyoda, Tokyo 101-8310, Japan

Corresponding author, Masanao INOKOSHI, E-mail: m.inokoshi.gerd@tmd.ac.jp

Although a surface pre-reacted glass ionomer (S-PRG) exerts a suppressive effect on Candida albicans (C. albicans) activity and growth, its influence on the expression of the lipase gene (LIP) family including LIP1–LIP10, an indicator of clinical infection, has not yet been investigated. Therefore, in this study, we evaluated the effect of S-PRG filler eluates on LIP expression in C. albicans using real-time reverse-transcription polymerase chain reaction. Candida albicans was treated with an S-PRG filler diluted at ratios of 1:32 and 1:64 for 24 h at 37°C. The diluted S-PRG filler eluates (1:32) suppressed lipase activity in C. albicans by downregulating LIP5 (0.54±0.25 relative to that of the control) and LIP8 (0.35±0.074) expression after 24 h, which corresponded with decreased lipase activity. At a dilution factor of 1:64, there was no significant difference in LIP expression. Thus, the S-PRG filler eluate has potential to suppress fungal activity by downregulating LIP expression.

Keywords: Candida albicans, Lipase, LIP gene, Surface pre-reacted glass ionomer filler, Oral candidiasis

INTRODUCTION

Candida albicans (C. albicans) is a common opportunist pathogenic yeast in the human gastrointestinal tract1). The oral mucosa, saliva, and immune system in the oral cavity represent natural defense mechanisms and maintain homeostasis2). However, these systems can be disrupted by blood dyscrasias and malignancy, immunological disorders, endocrine disorders, salivary changes, or medication; this leads to an imbalance in the oral microbiome and promotes oral infection3). Furthermore, the use of dentures is considered a clinical factor that promotes an optimal environment for fungal growth by preserving pathogenic cells and blocking the flow of saliva4). A previous study indicated that the prevalence of denture stomatitis, which is mainly caused by C. albicans, is up to 71% in patients who wear dentures5). Therefore, individuals suffering from systemic diseases and/or those wearing dentures, especially elderly patients, could be susceptible to oral candidiasis.

Candida albicans infection occurs in three main stages, cell adhesion, invasion, and biofilm formation6). Invasion of the host tissues is a crucial pathogenic step. Lipase is a pathogenic enzyme, and it is produced and released into the extracellular space to promote this stage5). This enzyme is encoded by the lipase gene (LIP) family members, including LIP1–LIP10; it was discovered and classified by Hube et al. by screening open reading frames (ORFs) and confirmed using Southern blotting5). Expression of the LIP family members has been reported in systemic C. albicans infections (kidney and liver) in a mouse model6). Upregulation of the expression of LIP family members, especially LIP4, LIP5, and LIP8, has been detected in C. albicans isolated from humans with oral candidiasis6). In addition, LIP8 has been confirmed to be a virulence factor of C. albicans7). Hence, there could be a correlation between gene expression and pathogenic behavior.

Recently, dental research has focused on the applications of surface pre-reacted glass ionomer (S-PRG) fillers. These fillers can release multiple ions, including Al3+, BO33−, F−, Na+, SiO43−, and Sr2+8) and can provide advanced therapeutic functions in dentistry, including promoting the remineralization of enamel and dentin9), preventing demineralization10), maintaining the homeostasis of neutral acidity11), exerting antibacterial effects12), promoting antifungal adhesion13,14), and preventing biofilm formation15). Several prototype materials have been developed based on this multifunctional concept15,17). Regarding its antifungal properties, Tsutsumi et al. reported that an eluate of the S-PRG filler can suppress fungal adhesion, fungal growth, and biofilm formation, preventing pathological transformation (yeast to hyphal form), downregulate secreted aspartyl proteinase gene expression, and induce oxidative stress, possibly leading to apoptosis in C. albicans17). Tsutsumi et al. hypothesized that the therapeutic effects of S-PRG fillers are related to the release of ions15). Moreover, a suppressive effect of the S-PRG filler on fungal adhesion, in a concentration-dependent manner, has been reported13,14).

Although several beneficial effects of S-PRG fillers against C. albicans have been identified, their effect on
LIP expression has not yet been investigated. Therefore, we aimed to determine the effect of an eluate derived from the S-PRG filler on the expression of LIP family members, including LIP1–LIP10, in C. albicans using real-time reverse-transcription polymerase chain reaction (RT-PCR). The null hypothesis was that there would be no difference in LIP expression between C. albicans treated with and without the S-PRG filler eluate.

**MATERIALS AND METHODS**

*Candida albicans* culture
*Candida albicans* (ATCC18804) was cultured for 12 h in Sabouraud glucose medium at a constant temperature of 37°C for 12 h. The cells were harvested from a culture plate and suspended in normal saline. The yeast suspension was centrifuged and washed twice with 0.87% NaCl solution. The suspension was adjusted to a cell density of 1×10⁷ cells/mL using a spectrophotometer (ODcalibrator)(U1100; Hitachi, Tokyo, Japan) at an optical density of 550 nm.

**Preparation of S-PRG filler eluate**
The S-PRG filler with a particle size of 1 µm (Shofu, Kyoto, Japan) was immersed in distilled water at a concentration of 1,000 g/L for 24 h, and then, the liquid was filtered, collected, and diluted. According to Tamura et al., the maximum dilution factors that have little effect on growth are 1:16 and 1:32⁵⁰. Therefore, these concentrations were tested in this study. Distilled water was used as a control.

**LIP expression analysis**
1. **Treatment of***C. albicans*** with the S-PRG filler eluate
   Culture media were prepared by adding S-PRG eluate at dilution factors of 1:16 or 1:32 to 2× yeast extract peptone dextrose broth. The final dilution ratios of the S-PRG filler elutes were 1:32 and 1:64. Subsequently, the prepared medium of the experimental treatment and control (distilled water) group was inoculated with the fungal cells prepared as in sections 2.1–2.3. The fungal cells were prepared as in sections 2.1–2.3. They were then treated with S-PRG eluate at a final dilution of 1:32 or 1:64. Distilled water was applied in the control group. Lipase activity was measured based on rapid colorimetric determination (QuantiChrom™ Lipase Assay Kit (DLPS-100), Abcam, Tokyo, Japan) according to the manufacturer’s instructions. After collecting the data (n=6/group), the gene expression, in terms of the cycle threshold value, was calculated as a ratio by comparing it with the level of the control, as described previously⁵⁰. The PCR primers used in this study are listed in Table 1.

2. **RNA extraction**
   After 24 h of culture, C. albicans cells were harvested from the plate. The pellet of cultured cells was obtained using centrifugation (10,000 rpm, 5 min, 4°C). Subsequently, zymolyase solution (Zymolyase; Nacalai Tesque, Kyoto, Japan) was added to the fungal pellet, followed by vortexing and incubation at 37°C for 20 min to lyse the cells. The MagExtractor-RNA kit (Toyobo, Osaka, Japan) was used to perform RNA extraction by adding 700 µL of lysis and binding buffer to the cell lysate (containing 2-ME), followed by vortexing and incubation at room temperature (25–28°C) for 15 min.

3. **RT-PCR**
   Magnetic beads were added to 700 µL of the pretreated solution in a tube. Subsequently, the solution was vortexed and kept still for 1 min. The tube was placed in a magnetic stand, and the solution was pipetted out. Cleaning solution I (600 µL) was added to the tube following vortexing for 10 s. Subsequently, the tube was placed in a magnetic stand, and the solution was pipetted out. Cleaning solution II (800 µL) was added following vortexing for 2 s. Subsequently, the tube was placed in a magnetic stand, and the solution was pipetted out; two cycles of cleaning solution II addition were performed. The eluate (40 µL) was vortexed and heated at 65°C for 2 min. The supernatant was stored as a stock RNA sample, and the absorbance at 260 nm was measured. Purified RNA was converted to complementary DNA using the ReverTra Ace™ qPCR RT Kit (Toyobo). RNA was denatured by incubating the RNA sample at 65°C for 5 min and then placed on ice. The reaction solution was prepared by mixing 0.1 µg of the RNA solution, 2 µL of 5× RT buffer, 0.5 µL of RT enzyme mix, and 0.5 µL of Primer mix. Nucleus-free water was added to a total volume of 10 µL. The mixed solution was incubated at 37°C for 15 min, followed by heat treatment at 98°C for 5 min. The solution was kept on ice or at −20°C, and absorbance was measured at 260 nm before performing RT-PCR. DNA samples (2 µL) were added to 96-well plates. RT-PCR was performed using a TaKaRa PCR Thermal Cycler Dice (Takara Bio, Shiga, Japan) according to the manufacturer’s instructions. The sample and working reagent were prepared following a modified protocol of the manufacturer. The testing solution was prepared by mixing 75 µL of H₂O and 75 µL of the calibrator in a 96-well plate. Subsequently, 5 µL of the sample was pipetted into a new well and mixed with 70 µL of testing solution using a multichannel pipettor at 37°C. The optical density of the sample at 412 nm (OD₁₀ min) was measured using a plate reader at 10 min (OD₁₀ min) and at 20 min (OD₅₀ min). Lipase activity was calculated using equation 1 provided as follows. Because the protocol was applied to a half volume, the constant value was identified by calculating the ratio of the ODcalibrator–ODH₂O with 150 µL and 75 µL. Consequently, this constant value was applied to calibrate the results. The sample size was 6 per group.

\[
\text{Activity} = \frac{\text{OD}_{20 \text{ min}} - \text{OD}_{10 \text{ min}}}{\text{OD}_{\text{calibrator}} - \text{OD}_{\text{H2O}}} \times 735 \text{ (unit/L)}
\]  

Where OD₂₀ min and OD₁₀ min are the OD₄₁₂ nm values of
Table 1  PCR primers for the LIP family members based on Stehr et al. (2004)6

| Gene   | Primer name | Sequence               | Annealing temperature (ºC) |
|--------|-------------|------------------------|-----------------------------|
| LIP1   | F           | ACAAATTCACTGGGATCAAGAG | 55                          |
|        | R           | ATAAAGTGACATGGACGTTACTG|                             |
| LIP2   | F           | TTTCCGACCTTTGTGTTCCAG  | 55                          |
|        | R           | ATATACTGCTTACAAGACCAAG|                             |
| LIP3   | F           | AGCTTTTACAACAGGGGACTC  | 59                          |
|        | R           | ACCAGGTCCCAATTGAGG     |                             |
| LIP4   | F           | TGATCAATTATATGGTAAGC   | 61                          |
|        | R           | TCTTTTTGGTGGAGTTATATTC |                             |
| LIP5   | F           | ACGGTGTGTCTCAACTATTCGG | 61                          |
|        | R           | ATGGGAACACACATCCATGCTG |                             |
| LIP6   | F           | TTAACCTGGTGGCAGGCT    | 59                          |
|        | R           | TCGATGCCCCTGGTGGAAC    |                             |
| LIP7   | F           | TGGAGTTTTATTCCATTTGCA  | 61                          |
|        | R           | ACGGAAGTACTGACTAGAATG  |                             |
| LIP8   | F           | AGAGTGATACAGACAAAAATC  |                             |
|        | R           | AAGACCATTACAGCATCAGTG  |                             |
| LIP9   | F           | TTTAAAGATATGTGGGAGCTAG |                             |
|        | R           | TAGGACCAAAGCCTTGTG     | 63                          |
| LIP10  | F           | TTAAGCTCAGTGCTAGATCTAC |                             |
|        | R           | TCCCCGATCTAAGTACCCACC  | 59                          |

LIP, lipase; F, forward primer; R, reverse primer; PCR, polymerase chain reaction

Table 2  Comparison of the expression of LIP family members after 24 h of treatment with S-PRG filler eluates

| Gene | Ratio of LIP gene expression (mean±s.d.) |
|------|-----------------------------------------|
|      | Control                                 | S-PRG (1:64) | S-PRG (1:32) |
| LIP1 | 1ab                                     | 0.660±0.179a | 1.366±0.477a |
| LIP2 | 1ab                                     | 0.726±0.183a | 1.546±0.705a |
| LIP3 | 1a                                      | 1.098±0.690a | 0.599±0.247a |
| LIP4 | 1a                                      | 0.733±0.632a | 0.852±0.353a |
| LIP5 | 1a                                      | 0.777±0.355ab| 0.541±0.252a |
| LIP6 | 1a                                      | 0.899±0.512a | 0.535±0.168a |
| LIP7 | 1a                                      | 0.906±0.609a | 1.620±1.883a |
| LIP8 | 1a                                      | 1.286±0.715a | 0.353±0.074a |
| LIP9 | 1a                                      | 0.652±0.341a | 0.650±0.330a |
| LIP10| 1a                                      | 1.376±0.903a | 0.600±0.273a |

The data were statistically compared using a one-way ANOVA with Tukey’s method. Different superscript letters in the same row indicate significant differences. If the comparing group shares at least one superscript letter, it means that there was no significant difference between the groups. LIP, lipase gene; S-PRG, surface pre-reacted glass ionomer; s.d., standard deviation
the sample at 20 min and 10 min, respectively, and OD_calibrator and OD_H2O are the OD412 nm values of the calibrator and water at 20 min. The number 735 is the equivalent activity (unit/L) of the calibrator under the assay conditions.

Statistical analysis
The data were statistically compared using one-way analysis of variance (ANOVA) with Tukey’s method using the R software package (R Foundation for Statistical Computing, Vienna, Austria). A statistical significance level of 95% ($p < 0.05$) was applied.

RESULTS
The expression levels of the LIP family members after treatment with the S-PRG filler eluate are presented in Table 2. An analysis based on one-way ANOVA with Tukey’s method showed that the S-PRG filler eluates suppressed the gene expression of LIP5 (0.54±0.25) and LIP8 (0.35±0.074) at a dilution of 1:32, compared to the control group after 24 h of incubation. In contrast, this suppressive effect on activity was not observed for other genes. At a dilution factor of 1:64, there was no significant difference in lipase activity compared with that in the control group after treatment with S-PRG filler eluates. A dilution ratio of 1:32 (75.08±12.57 unit/L) was also confirmed to have a statistically significant suppressive effect on lipase activity compared to that in the control group (94.08±9.50 unit/L, $p < 0.05$), as presented in Fig 1. However, there was no significant difference in lipase activity between treatment with a dilution factor of 1:64 (78.70±14.05 unit/L, $p > 0.05$) and the control group.

DISCUSSION
The effect of S-PRG fillers on the expression of LIP family members, including LIP1–LIP10, had not yet been investigated. Therefore, we evaluated the effect of S-PRG filler eluates on LIP expression in C. albicans. We found that a 1:32 dilution of S-PRG eluates downregulated the expression of only LIP5 and LIP8 after 24 h of incubation, corresponding to lower lipase activity. However, the 1:64 dilution of the S-PRG eluate did not exert a suppressive effect on any LIP genes. Therefore, the null hypothesis was partially rejected.

Based on a previous study, a fungicidal effect was hypothesized to be one of mechanisms through which the S-PRG filler exerts suppressive effects against C. albicans via fluoride ions, which can disrupt the fungal membrane, and sodium ions, which can create high osmotic pressure leading to cell lysis[10]. A recent study on ion release from the S-PRG filler containing a tissue conditioner (approximate values: $\text{Al}^{3+}=0.6$ ppm, $\text{BO}_3^{3−}=5.4$ ppm, $\text{SiO}_2^{1−}=8.8$ ppm, and $\text{F}^{−}=6.0$ ppm) showed no fungicidal effect based on fluorescence staining[11]. Li and Breaker demonstrated that fluoride, even at high concentrations of up to 300 mM (approximately 5,700 ppm), cannot inhibit C. albicans growth in liquid culture for >48 h[12]. Kaloriti et al. reported that NaCl at a concentration of 1 M (approximately 22,990 ppm of Na+) cannot kill C. albicans after treatment for 4 h[13]. However, low concentrations might affect fungal activity. Boric acid has been reported to interfere with cytoskeletal integration in C. albicans at the hyphal tip[14]. Based on this evidence, we believe that a sublethal dose of the S-PRG filler might have a fungistatic effect on C. albicans, although it interferes with cellular activity. In this study, the eluate at a dilution ratio of 1:32 downregulated LIP5 and LIP8 expression. According to a previous study[15], the estimated concentrations of ions released, which reflect the effect on LIP expression, were approximately 1.80 ppm [Al$^{3+}$], 272.48 [BO$^{3−}$], 16.69 [Na$^{+}$], 1.63 [SiO$_2^{1−}$], 7.00 [Sr$^{2+}$], and 4.06 [F$^{−}$].

The diluted concentrations tested in this study were selected based on the effects of the S-PRG eluate on fungal cell growth reported previously[16]. We speculated that the minimal inhibitory concentration of the S-PRG filler eluate could be within the final dilution factor range of 1:32 to 1:64 (corresponding to 1:16 and 1:32, respectively, in the previous report[17]). In addition, a high concentration of the S-PRG filler eluate was not tested in this study because many studies have reported relatively lower ion-release ability of the S-PRG filler containing dental materials compared to that with a pure eluate.

![Fig. 1 Lipase activity in Candida albicans after treatment with S-PRG eluate at a final dilution factor of 1:32 or 1:64. Distilled water was used as a control group. The data were statistically compared using a one-way ANOVA with Tukey’s method. The mean values and standard deviations are shown. Different superscript letters above the bar plot indicate significant differences. If the comparing group shares at least one superscript letter, it means that there was no significant difference between the groups. S-PRG, surface pre-reacted glass ionomer.](image_url)
of the S-PRG filler14,15,22,23, which might not correlate with clinical situations. Tonprasong et al. hypothesized that the solubility and chemical composition of each material matrix might affect the ion-releasing ability of the eluate15.

The correlation between LIP expression and the pathogenic behavior of C. albicans has been described in previous studies. Upregulation of the expression of LIP1-LIP9, and especially LIP4, LIP5, and LIP8, in C. albicans has been detected during infection16. Gäcser et al. studied the effects of mutations in LIP8 in C. albicans. They found that the deletion or addition of LIP8 affects colony morphology and proliferation7. In the present study, we confirmed that diluted S-PRG filler eluates (1:32) showed a tendency to suppress the expression of lipase in C. albicans by downregulating LIP5 and LIP8 expression, which could reduce the pathogenicity of C. albicans. However, the mechanism of action remains unclear. Tamura et al. reported that the S-PRG eluate can induce oxidative stress in fungal cells by suppressing hydrogen peroxide and catalase production17. We hypothesized that oxidative stress might induce atypical functions of the LIP genes. Based on ORF identification and amino acid analysis, LIP5 and LIP8 are similar in many regions, with up to 90% and 80% similarity, respectively16. It is possible that the S-PRG eluate might react with a specific site related to LIP5 and LIP8 structures.

Recently, a prototype of an S-PRG filler containing dental material was developed for various applications, including the prevention of microbial adhesion15,16 and biofilm formation15, the promotion of remineralization18, acid neutralization, and the prevention of caries11. The findings of the present study indicate that C. albicans growth can be inhibited by applying dental materials that directly contact the oral mucosa, such as denture base resins, denture adhesives, and tissue conditioners. However, the biosafety of S-PRG fillers needs to be investigated in clinical trials. Diluted eluates of S-PRG fillers or single-ion models have been tested for cytotoxicity in many human cells, such as cementoblasts20, osteoblasts25,26, dental pulp cells27,28, and mesenchymal stem cells29. However, the optimal concentration used in these studies cannot be estimated for our expected material containing the S-PRG filler because the tested cells are less related to the cells in the oral mucosa. Kashiwagi et al. identified the optimal dilution of the S-PRG filler eluate for human gingival fibroblasts (HGFs). After 72 h of culture, the S-PRG filler eluate diluted 1:100 or more combined with ≥2% fetal bovine serum showed no negative effect on HGFs, whereas a higher concentration of S-PRG filler was found to negatively affect cell growth29. Even though the safe dose has been determined, this cannot be considered non-toxic in our setting because of the relatively long period of the experimental setting. Rossoni et al. tested the systemic toxicity of various concentrations of S-PRG eluate (5–50% v/v) via direct injection into the hemolymph of Galleria mellonella (greater wax moth) larvae. They found that the survival rate of larvae does not decrease under any condition30. Owing to the lack of evidence, the cytotoxicity of the S-PRG filler in the oral mucosa is unclear. Therefore, the toxicity towards cells related to the oral mucosa should be clarified in the future. In this study, we did not perform elemental analysis of the S-PRG filler eluate. However, using data from a previous study17, the concentration of ions released can be estimated. In addition, it is necessary to determine the effect of each type of ion released on LIP expression in the future to clarify the mechanism of the effect of the S-PRG filler. However, the interactions among ions released from the S-PRG filler should be considered. Further in vitro, in situ, and animal studies are required before testing in humans. Additional studies should evaluate each material to confirm its efficacy and biosafety.

CONCLUSIONS

Diluted S-PRG filler eluates (1:32) showed a tendency to suppress lipase activity in C. albicans by downregulating LIP5 and LIP8 expression, which could reduce the pathogenicity of C. albicans.

ACKNOWLEDGMENTS

This work was supported by the Shofu Corporation. The Shofu Corporation also provided the materials for this project. The sponsor had no involvement in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

This work was supported by the JSPS Grant-in-Aid of Young Scientists (JP22K17129).

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

REFERENCES

1) Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence 2013; 4: 119-128.
2) Moutsopoulos NM, Konkel JE. Tissue-specific immunity at the oral mucosal barrier. Trends Immunol 2018; 39: 276-287.
3) Farah CS, Lynch N, McCullough MJ. Oral fungal infections: An update for the general practitioner. Aust Dent J 2010; 55: Supplement 1 48-54.
4) Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011; 20: 251-260.
5) Hube B, Stehr F, Bossenz M, Mazur A, Kretschmar M, Schäfer W. Secreted lipases of Candida albicans: Cloning, characterisation and expression analysis of a new gene family with at least ten members. Arch Microbiol 2000; 174: 362-374.
6) Stehr F, Felk A, Gäcser A, Kretschmar M, Mähnss B, Neuber K, et al. Expression analysis of the Candida albicans lipase gene family during experimental infections and in patient samples. FEMS Yeast Res 2004; 4: 401-408.
7) Gäcser A, Stehr F, Kröger C, Kredics L, Schäfer W, Nosanchuk JD. Lipase 8 affects the pathogenesis of Candida albicans.
Infect Immun 2007; 75: 4710-4718.

8) Fujimoto Y, Iwasa M, Murayama R, Miyazaki M, Nagafuji A, Nakatsuka T. Detection of ions released from S-PRG fillers and their modulation effect. Dent Mater J 2010; 29: 392-397.

9) Uo M, Wada T, Asakura K. Structural analysis of strontium in human teeth treated with surface pre-reacted glass-ionomer filler eluate by using extended X-ray absorption fine structure analysis. Dent Mater J 2017; 36: 214-221.

10) Shiiya 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: 70-75.

11) Nedeljkovic I, De Munck J, Slomka V, Van Meerbeek B, Teughels W, Van Landuyt KL. Lack of buffering by composites promotes shift to more cariogenic bacteria. J Dent Res 2016; 95: 875-881.

12) 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: 5056.

13) Tonprasong W, Inokoshi M, Tamura M, Uo M, Wada T, Takahashi R, et al. Tissue conditioner incorporating a nano-sized surface pre-reacted glass-ionomer (S-PRG) filler. Materials (Basel) 2021; 14: 6648.

14) Takakusaki K, Fueki K, Tsutsunami C, Tsutsunami Y, Iwasaki N, Hanawa T, et al. Effect of incorporation of surface pre-reacted glass ionomer filler in tissue conditioner on the inhibition of Candida albicans adhesion. Dent Mater J 2018; 37: 453-459.

15) Tsutsunami C, Takakuda K, Wakabayashi N. Reduction of Candida biofilm adhesion by incorporation of prereacted glass ionomer filler in denture base resin. J Dent 2016; 44: 37-43.

16) Hatano K, Inokoshi M, Tamura M, Uo M, Shimizu M, Tonprasong W, et al. Novel antimicrobial denture adhesive containing S-PRG filler. Dent Mater J 2021; 40: 1365-1372.

17) Tamura M, Cueto ME, Abe K, Kamio N, Ochiai K, Imai K. Ions released from a S-PRG filler induces oxidative stress in Candida albicans inhibiting its growth and pathogenicity. Cell Stress Chaperones 2018; 23: 1337-1345.

18) Koide T, Tamura M. Effect of diglycerol dicaprylate on Candida albicans growth and pathogenicity. Biosci Biotechnol Biochem 2021; 85: 2334-2342.

19) Li S, Brearker RR. Fluoride enhances the activity of fungicides that destabilize cell membranes. Bioorg Med Chem Lett 2012; 22: 3317-3322.

20) Kaloviti D, Tillmann A, Cook E, Jacobson M, You T, Lenardon M, et al. Combinatorial stresses kill pathogenic Candida species. Med Mycol 2012; 50: 699-709.

21) Pointer BR, Boyer MP, Schmidt M. Boric acid destabilizes the hyphal cytoskeleton and inhibits invasive growth of Candida albicans. Yeast (Chichester Engl.) 2015; 32: 389-398.

22) 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: 261-267.

23) 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 J 2016; 32: 1095-1102.

24) Bao X, Liu X, Zhang Y, Cui Y, Yao J, Hu M. Strontium promotes cementoblasts differentiation through inhibiting sclerostin expression in vitro. BioMed Res Int 2014; 2014: 487535.

25) Hakki SS, Bozkurt BS, Hakki EE. Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). J Trace Elem Med Biol 2010; 24: 243-250.

26) Varanasi VG, Leong KK, Dominia LM, Jue SM, Loomer PM, Marshall GW. Si and Ca individually and combinatorially target enhanced MC3T3-E1 subclone 4 early osteogenic marker expression. J Oral Implantol 2012; 38: 325-336.

27) Nakade O, Koyama H, Arai J, Ariji H, Takada J, Kaku T. Stimulation by low concentrations of fluoride of the proliferation and alkaline phosphatase activity of human dental pulp cells in vitro. Arch Oral Biol 1999; 44: 89-92.

28) Veron MH, Couble ML, Magboire H. Selective inhibition of collagen synthesis by fluoride in human pulp fibroblasts in vitro. Calcif Tissue Int 1993; 53: 38-44.

29) Movahedi Najafabadi BA, Abnosi MH. Boron induces early matrix mineralization via calcium deposition and elevation of alkaline phosphatase activity in differentiated rat bone marrow mesenchymal stem cells. Cell J 2016; 18: 62-73.

30) Kashiwagi K, Inoue H, Komasa R, Hosoyama Y, Yamashita K, Morisaki A, et al. Optimal dilutions of S-PRG filler eluate for experiments on human gingival fibroblasts in vitro. Dent Mater J 2021; 40: 136-142.

31) Rossoni RD, de Barros PP, Lopes LADC, Ribeiro PC, Nakatsuia T, Kasaba H, et al. Effects of surface pre-reacted glass-ionomer (S-PRG) eluate on Candida spp.: Antifungal activity, anti-biofilm properties, and protective effects on Galleria mellonella against C. albicans infection. Biofouling 2019; 35: 997-1006.