INTRODUCTION

Two major dental diseases are caries and periodontal disease, of which periodontal disease are inflammatory tissue-destructive diseases caused by bacteria. Periodontal disease begins with the loss of root and periodontal tissue attachments, and severe periodontitis is one of the major causes of tooth loss.

Periodontal disease progresses as a result of interactions between cytokines, growth factors, reactive oxygen species, and pro-inflammatory mediators, such as matrix metalloproteinases (MMPs), resulting in the destruction of periodontal tissue and alveolar bone. The levels of MMPs induced by inflammatory cytokines are elevated in the inflammatory gums of periodontal disease patients and contribute to periodontal tissue destruction. MMP-1 and MMP-2 activities were shown to be stronger in inflamed gingiva than in healthy gingiva, and MMP-1 and MMP-2 levels in gingival crevicular fluid positively correlated with the severity of periodontal disease. MMP-3 degrades proteoglycans, fibronectin, and collagen (e.g. III, IV, and VII) and has been implicated in the destruction of various tissues. Furthermore, MMP-3 activates many other MMPs (MMP-1, 7, 8, 9, and 13) by mutual activity and promotes tissue destruction. MMP-1 and MMP-3 protein levels and activities in gingival crevicular fluid and gingival tissue were previously reported to be higher in inflamed gingival tissues than in healthy sites. These findings suggest that MMP-1 and MMP-3 act synergistically and play an important role in the degradation and remodeling of the extracellular matrix (ECM) of periodontal tissue.

Tumor necrosis factor-α (TNF-α) is one of the pro-inflammatory cytokines that play a major role in tissue injury. It is produced by fibroblasts, epithelial cells, and macrophages, and exerts a number of effects, such as the activation of inflammatory leukocytes, modifications to vascular permeability, and the induction of bone resorption. Patients with gingivitis or periodontitis produce higher levels of inflammatory mediators, including TNF-α and interleukin (IL)-6, than healthy individuals. Excessive ECM degradation is characteristically observed in rheumatoid arthritis, tumor invasion, and periodontitis because TNF-α promotes the release of MMPs that destroy the gingival ECM as well as rapid disease progression. A stimulation with TNF-α was previously shown to increase the secretion of MMP-1 by human dermal fibroblasts (HDF) and that of MMP-3 by human dental pulp as well as the expression of MMP-1 and MMP-3 by dental pulp fibroblasts.

Surface pre-reacted glass ionomer (S-PRG) filler forms a stable glass ionomer phase on the surface of a glass core. It has a stable glass ionomer phase, and six types of ions (F, Na, Al, B, Sr and Si) are sustainably released from the filler surface layer. Previous studies reported that F exhibits ion-releasing and recharging abilities. The ions released from the surface of S-PRG filler exert a number of effects, such as caries prevention, the inhibition of bacterial adhesion, the suppression of decalcified lesions, antibacterial activity, anti-plaque formation activity, and acid-buffering capacity. S-PRG fillers are used in various dental materials, such as composite resins, fisher sealants, and tooth surface-coating materials, and are useful for improving the condition of the oral environment.

S-PRG filler eluate is prepared by mixing S-PRG filler with a solvent, such as distilled water or alpha minimum essential medium (α-MEM), for 24 h and collecting the supernatant. Na, F, Al, B, Sr and Si are released into S-PRG filler eluate, which has an acid-buffering ability. S-PRG filler eluate has been shown to induce the phosphorylation of ERK and promote the secretion of MMPs. These findings suggest that S-PRG filler eluate may be useful for the treatment of periodontal disease.
to promote the osteoblast differentiation of human mesenchymal stem cells\(^3\) and exerts inhibitory effects against enamel demineralization\(^4\). However, few studies have examined S-PRG filler eluate in detail. S-PRG filler eluate, which is a liquid, may be easily spread in solution throughout the oral cavity, with both hard tissue, such as enamel, and soft tissue as its treatment targets.

The aim of this study was to examine the effects of S-PRG filler eluate on MMP-1 and MMP-3 secretion by human gingival fibroblasts (HGF). Moreover, we investigated the effects of S-PRG filler eluate on the secretion of MMP-1 and MMP-3 by HGF stimulated with the inflammatory cytokine TNF-\(\alpha\).

**MATERIALS AND METHODS**

**Cell culture**

We used unlinkable and anonymized HGF approved by the Ethical Review Board of Osaka Dental University (approval number 070716). HGF were grown from explants obtained from the healthy marginal gingiva of healthy donors. Primary cultures were performed using a previously described method\(^3\) and was cryopreserved for this study. Experiments with HGF were performed between passage 3 and 10. HGF were maintained in \(\alpha\)-MEM (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 2 mM L-glutamine (Wako Pure Chemical Industries), 100 \(\mu\)g/mL penicillin (Wako Pure Chemical Industries), 100 \(\mu\)g/mL streptomycin (Wako Pure Chemical Industries), 100 \(\mu\)g/mL streptomycin (Wako Pure Chemical Industries), and 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA). HGF were incubated at 37°C under 5% CO\(_2\). The present study was approved by the Ethical Review Board of Osaka Dental University (Approval No.110879).

**S-PRG filler eluate preparation**

S-PRG fillers were prepared as previously reported\(^3\). Fluoroboroaluminosilicate glass was prepared by fusing silica (SiO\(_2\)), mullite (3Al\(_2\)O\(_3\)·2SiO\(_2\)), boric oxide (B\(_2\)O\(_3\)), cryolite (Na\(_3\)AlF\(_6\)), strontium fluoride (SrF\(_2\)) and strontium carbonate (SrCO\(_3\)). After grounding of glass frit, treatment of polysiloxane and polyacrylic acid was subsequently subjected to finally obtain S-PRG filler. S-PRG filler eluate was prepared using a previously described method\(^3\). Briefly, \(\alpha\)-MEM was mixed with S-PRG filler (1 \(\mu\)m average filler diameter) at a weight ratio of 1:1 (1 L:1,000 g), followed by gentle mixing with a tumbler mixer at room temperature for 24 h. After mixing, S-PRG filler was precipitated with a centrifuge and the supernatant was recovered. The supernatant collected was filtered through a chromatodisc (0.45 \(\mu\)m) to obtain S-PRG filler \(\alpha\)-MEM eluate (S-PRG filler eluate). An elemental analysis of five ions (Na, Al, B, Sr and Si) released from S-PRG filler was performed using inductively coupled plasma atomic emission spectroscopy (ICP-AES; ICPS-8000, Shimadzu, Kyoto, Japan). Analysis was conducted after preparing calibration curves corresponding to each element (standard solution concentration; Na: 0, 0.5, 20, and 50 ppm; Al: 0, 0.5, 5, and 10 ppm; B: 0, 10, 50, and 100 ppm; Sr: 0, 5, 20, and 50 ppm; Si: 0, 0.5, 1, and 5 ppm).

An elemental analysis was performed on \(\alpha\)-MEM released from S-PRG filler using an ion electrode method with a fluoride electrode (9609BNWP, Thermo Fisher Scientific). Similarly, concentration of F was determined after preparing its calibration curves (standard solution concentration: 0.1, 1, 5, and 10 ppm). Ion concentrations in S-PRG filler eluate were as follows: Al 12.0 ppm, B 1456.0 ppm, Na 3350.6 ppm, Si 7.3 ppm, Sr 792.8 ppm, and F 54.3 ppm (Table 1). All experiments used \(\alpha\)-MEM medium eluting S-PRG filler components.

### Western blotting

HGF were seeded onto a 12-well plate at a density of 1.0×10\(^5\) cells/well and cultured for 4 days to 90% confluency in \(\alpha\)-MEM containing 10% FBS. Then, HGF were incubated in serum-free \(\alpha\)-MEM for 1 h. After incubation, HGF cells were stimulated with 0.1% S-PRG filler eluate for 24 h. Supernatants were concentrated up to 10-fold with Amicon Ultra (Millipore, Billerica, MA, USA) and denatured with sample buffer (containing 0.25 M Tris HCl pH6.8, 8% SDS, 40% glycerol, 0.02% bromophenol blue, 4% 2-Mercaptoethanol). Equal amounts of each sample were then separated to 10% SDS/PAGE. Following separation, the proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore) and blocked with 20% Blocking One (Nacalai, Kyoto, Japan) in 50 mM Tris–HCl at pH 7.5 and 150 mM NaCl overnight. Membranes were treated with antibodies [anti-MMP-1; 1:1000 (Santa

| Table 1 | Comparison of various ion concentrations between S-PRG filler eluate and \(\alpha\)-MEM. |
|---------|---------------------------------------------------------------|
|         | Elements/Ion concentration (ppm)                              |
|         | Al    | B      | Na     | Si    | Sr    | F     | Ca    | P     | K     |
| \(\alpha\)-MEM (Lot. ECN7022) | 0     | 0      | 3,597.1| 0     | 0     | 0     | 77.2  | 33.5  | 246.5 |
| S-PRG filler eluate solution (Lot. 091503)| 12.0  | 1,456.0| 3,350.6| 7.3   | 792.8 | 54.3  | 1.9   | 0.2   | 42.4  |

S-PRG filler eluate was prepared using the following method. \(\alpha\)-MEM was mixed with S-PRG filler (1 \(\mu\)m average filler diameter) at a weight ratio of 1:1 (1 L:1,000 g), and the supernatant was collected. The collected supernatant was filtered to obtain S-PRG filler eluate. S-PRG, Surface pre-reacted glass ionomer; \(\alpha\)-MEM, Alpha Minimum Essential Medium
The amount of secreted MMP-1 increased in a concentration-dependent manner until the concentration of the S-PRG filler eluate was 0.5%. However, at a concentration of 1%, the amount of secreted MMP-1 was slightly less than that at a concentration of 0.5%. These results confirmed that the secretion of MMP-1 peaked in S-PRG filler eluate at a concentration of 0.5% (Fig. 1 upper). The secretion of MMP-3 increased in a concentration-dependent manner until the concentration of S-PRG filler eluate was 0.1%. At concentrations of 0.5% and 1%, the amount of MMP-3 secreted was less than that at concentrations lower than 0.1%. These results clearly demonstrated that the secretion of MMP-3 peaked in 0.1% S-PRG filler eluate (Fig. 1 middle). No significant changes were observed in the amount of actin in any samples (Fig. 1 lower).

Effects of S-PRG filler eluate on MAPK phosphorylation

Previous studies reported that the phosphorylation of MAPK (p38, ERK, and JNK) is involved in the secretion of MMP-1 and MMP-3. The role of MAPK (p38, JNK, and ERK) in enhancing the secretion of MMP-1 and MMP-3 by S-PRG filler eluate was examined using western blotting. The concentration of S-PRG filler eluate used was 0.1%, the concentration at which the amounts of MMP-1 and MMP-3 secreted were significantly enhanced. Time-dependent changes in MAPK phosphorylation up to 30 min after the stimulation were then examined. The phosphorylation of p38 and ERK was slightly enhanced after 1 min, and markedly increased from 5 to 30 min (Fig. 2 upper and middle). JNK was constitutively phosphorylated even without the stimulation. The change observed in the phosphorylation of JNK by S-PRG filler eluate was...
not observed from 0 to 30 min (Fig. 2 lower). In this experiment, peaks were noted in the phosphorylation of p38 and ERK after 30 min.

Effects of various MAPK inhibitors on MMP-1 and MMP-3 secretion by S-PRG filler eluate
Since the phosphorylation of p38 and ERK was enhanced by S-PRG filler eluate, the effects of p38 and ERK inhibitors on MMP-1 and MMP-3 secretion were examined. No changes were induced in the phosphorylation of JNK by S-PRG filler eluate; however, it was constitutively phosphorylated even without the stimulation. Therefore, the effects of JNK inhibitors on MMP-1 and MMP-3 secretion were also investigated. HGF was added to 0.1% S-PRG filler eluate and various MAPK inhibitors (p38 inhibitor: 10 μM SB203580, JNK inhibitor: 25 μM SP600125, ERK inhibitors: 10 μM U0126 and 25 μM PD98059), and the effects of these inhibitors were confirmed by western blotting. The enhanced secretion of MMP-1 by the stimulation with 0.1% S-PRG filler eluate was partially suppressed by the p38 inhibitor SB203580 (10 μM) (Fig. 3 upper). The JNK inhibitor SP600125 (25 μM) and ERK inhibitors U0126 (10 μM) and PD98059 (25 μM) almost completely inhibited the secretion of MMP-1 induced by the 0.1% S-PRG filler eluate stimulation (Fig. 3 upper). The JNK inhibitor SP600125 (25 μM) and ERK inhibitors U0126 (10 μM) and PD98059 (25 μM) almost completely suppressed the secretion of MMP-1 induced by the 0.1% S-PRG filler eluate stimulation. Similar to the secretion of MMP-1, the enhanced secretion of MMP-3 by the stimulation with 0.1% S-PRG eluate was partially suppressed by the p38 inhibitor SB203580 (10 μM) (Fig. 3 lower). Similarly, the secretion of MMP-3 induced by the stimulation with 0.1% S-PRG eluate was almost completely suppressed by the JNK inhibitor SP600125 (25 μM) and the ERK inhibitors U0126 (10 μM) and PD98059 (25 μM) (Fig. 3 lower). No changes were observed in the amount of actin in any samples (Fig. 3 lower).
**Fig. 4** Role of S-PRG filler eluate in the secretion of MMP-1 and MMP-3 from TNF-α-stimulated HGF. HGF cells were pretreated for 30 min with various concentrations (0, 0.01, 0.05, 0.1, 0.5, and 1%) of S-PRG filler eluate or were not treated. They were subsequently stimulated by TNF-α (10 ng/mL) for 24 h or received no stimulation, and the supernatant was concentrated. Western blotting was used to assess the secretion of MMP-1 (upper) and MMP-3 (middle) by HGF. To show that supernatants were obtained from equal amounts of cells, the amount of actin (lower) was evaluated by western blot analysis. Data presented are representative of four independent experiments.

S-PRG, Surface pre-reacted glass ionomer; MMP, matrix metalloproteinase; TNF-α, Tumor necrosis factor-α; HGF, human gingival fibroblasts

**Fig. 5** Involvement of S-PRG filler eluate in the ERK activation of TNF-α-stimulated HGF. HGF cells were pretreated for 30 min with S-PRG filler eluate (0.1 %) or were not treated, subsequently stimulated by TNF-α (5 ng/mL) for 5 to 60 min, and cell lysate samples were prepared. The phosphorylation level of ERK was examined by a western blot analysis (upper). To ensure that equal amounts of ERK were obtained from the lysates, the membranes were stripped and reprobed with anti-ERK antibodies (lower). Four samples were analyzed and a typical image is shown for each. S-PRG, Surface pre-reacted glass ionomer; TNF-α, Tumor necrosis factor-α; HGF, human gingival fibroblasts

**Effects of S-PRG filler eluate on MMP-1 and MMP-3 secretion by HGF stimulated by TNF-α**

We then examined the effects of S-PRG filler eluate on inflammation. TNF-α is an inflammatory cytokine produced by macrophages and HGF during gingivitis. The effects of S-PRG filler eluate on the secretion of MMP-1 and MMP-3 by TNF-α (10 ng/mL)-stimulated HGF were investigated using western blotting. The secretion of MMP-1 and MMP-3, which was significantly increased by the TNF-α stimulation, was suppressed by S-PRG filler eluate (Fig. 3 upper and middle). The inhibition of MMP-1 and MMP-3 secretion by S-PRG filler eluate was the strongest at a concentration of 0.05% for MMP-1 and 0.01% for MMP-3. No changes were observed in the amount of actin in any samples (Fig. 4 lower).

**Effects of S-PRG filler eluate on ERK phosphorylation in TNF-α-stimulated HGF**

We investigated the mechanisms by which S-PRG filler eluate suppresses enhanced MMP-1 and MMP-3 secretion by TNF-α-stimulated HGF. The phosphorylation of ERK by the TNF-α alone stimulation and co-stimulation of TNF-α and S-PRG filler eluate was observed at 15 and 30 min. In addition, the respective phosphorylation peaks were the same at 15 min (Fig. 5 upper). Equal amounts of ERK were obtained from the lysates (Fig. 5 lower). The phosphorylation of ERK by the co-stimulation with TNF-α and S-PRG filler eluate was slightly stronger than that by TNF-α alone. These results demonstrated that the S-PRG filler eluate stimulation exerted suppressive effects on TNF-α-stimulated enhancements in MMP-1 and MMP-3 secretion and these effects involved increases in ERK phosphorylation.

**DISCUSSION**

In this study, we observed that the S-PRG filler eluate containing 6 types of ions (F, Na, Al, B, Sr and Si) slightly enhanced the secretion of HGF into the culture supernatant of MMP-1 and MMP-3. The MAPK family consists of three major groups: p38, ERK and JNK, which have been implicated as key regulators of various cellular responses, including cell proliferation, apoptosis, differentiation, and migration. It is known that three MAPK activations, ERK, p38 MAPK and JNK, are involved in the secretion of MMP-1 and MMP-3. Furthermore, enhancements in the secretion of MMP-1 and MMP-3 by the stimulation with S-PRG filler eluate were partially suppressed by the p38 inhibitor (SB 203580) and almost completely suppressed by the ERK inhibitors (PD 098059 and U0126) and the JNK inhibitor (SP 600125). These results indicate that activation of ERK rather than p38 may be involved in the secretion of MMP-1 and MMP-3 induced by S-PRG filler eluate. Regarding JNK, JNK was constitutively
phosphorylated without a stimulation under these experimental conditions, and no changes were observed in its phosphorylation due to S-PRG filler eluate. However, in experiments using the JNK inhibitor (SP 600125), the secretion of MMP-1 and MMP-3 in the presence of S-PRG filler eluate was almost completely suppressed. This may have been because JNK, which was constitutively phosphorylated without a stimulation, was suppressed by SP600125. These results are consistent with previous findings showing that JNK phosphorylation in the secretion of MMP-1 and MMP-3.

It has been reported that 0.001% S-PRG filler eluate can stimulate the migration of fibroblast line HGF-1 via the ERK signaling pathway. Consistent with a previous report, our findings show that the S-PRG filler eluate enhanced ERK phosphorylation. When an experimental endodontic sealer containing S-PRG filler induces osteoblast differentiation, phosphorylation of ERK and p38 was induced by Sr released from S-PRG filler. In addition, NT-Sr promoted RANKL-induced ERK phosphorylation. Therefore, it is considered that Sr among the ions in the S-PRG filler eluate may be involved in promoting the activation of ERK.

TNF-α, an inflammatory cytokine, promotes the production of MMP-1 and MMP-3 by HDF and destruction of the gingival ECM. In the present study, we examined the effects of S-PRG filler eluate on inflammation by co-stimulating HGF with TNF-α and S-PRG filler eluate. The results obtained showed that the secretion of MMP-1 and MMP-3, which was significantly increased by the TNF-α stimulation, was suppressed by the co-stimulation with S-PRG filler eluate. A previous study demonstrated that activation of MAPK (p38, ERK and JNK) induced by TNF-α plays an important role in the secretion of MMP-1 and MMP-3. Interestingly, we found that the S-PRG filler eluate enhances TNF-α-induced phosphorylation of ERK. Akt pathway positively regulates MMP-1 and MMP-3 expression in HDF. In addition, NT-Sr promotes RANKL-induced phosphorylation of ERK, but inhibits osteoclast differentiation by repressing Akt pathways.

Therefore, it is possible that the enhancement of ERK phosphorylation by co-stimulation of S-PRG filler eluate and TNF-α may be the mechanism of Sr, and it is necessary to investigate the effect of the Akt pathway in future studies. In addition, it is necessary to investigate the influence of MAPK (p38 and JNK) other than ERK in the future studies. Western blotting is primarily a binary comparison method for demonstrating a change in a protein of interest, so MMP expression appeared to be different between Fig. 1 and Fig. 4. Therefore, quantitative analysis of MMP-1 and MMP-3 using ELISA should be assessed in future studies.

Since the S-PRG filler eluate contains F, Al, B, Na, Si, and Sr ions, it is thus suggested that the combination of these multiple ions suppresses MMP-1 and MMP-3 secretion which was induced by TNF-α. Extracellular ions are extracellular environment factors that have great influence on the physiological activity of cells. The efficacy of individual ions on cellular bioactivity results in different affects depending on various conditions such as concentration and combination with other ions. B, F, Al, and Sr are known to be a toxic agent when used in high concentrations. Similarly, we previously reported that the S-PRG filler eluate has cytotoxicity when used in culture media at high concentrations. In this experiment, enhanced phosphorylation of p38 and ERK was observed in HGF cells stimulated with 1000-fold diluted S-PRG filler eluate. However, ERK phosphorylation was observed in HGF-1 cells stimulated with 10000-fold diluted S-PRG filler eluate, but no p38 phosphorylation was observed. In this way, it is possible that different effects may be exhibited depending on the concentration of the S-PRG filler eluate. Furthermore, the current study did not focus on which ion affected MMP-1 and MMP-3 secretion from HGF. Mechanistic study to address whether the eluted multiple ions had a direct or indirect effect on MMP-1 and MMP-3 secretion is also lacking. In a future study, we will evaluate the function of individual ion and different combination of ions. In this study, S-PRG eluate containing multiple ions suppressed the secretion of MMP-1 and MMP-3 from HGF stimulated by TNF-α. This result suggests that the S-PRG filler eluate may suppress TNF-α-induced inflammatory in HGF.

CONCLUSION

The results of the present study demonstrated that the multiple ion-containing solution eluted from S-PRG filler suppressed the secretion of MMP-1 and MMP-3 induced by TNF-α, potentially suppressing gingival inflammation. These findings suggested that even without the S-PRG filler, it is possible to bring about biological activity if there is an S-PRG eluate containing multiple ions eluted from the S-PRG filler. In addition, the present study provides useful information for the development of novel therapeutic agents for gingival inflammation using solutions composed of multiple ions.

ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science KAKENHI Grant Number JP17K12027 (Grant-in-Aid for Scientific Research (C)).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1) Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 1990 1994; 5: 78-111.
2) Mariotti A. Dental plaque-induced gingival diseases. Ann Periodontol 1999; 4: 1-17.
3) Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809-1820.
4) Sapna G, Gokul S, Bagri-Manjrekar K. Matrix
metalloproteinases and periodontal diseases. Oral Dis 2014; 20: 538-550.
5. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. J Periodontol 1993; 64: 474-484.
6. Ding Y, Uitto VJ, Firth J, Salo T, Haapasalo M, Konttinen Y, et al. Modulation of host matrix metalloproteinases by bacterial virulence factors relevant in human periodontal diseases. Oral Dis 1995; 1: 279-286.
7. McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol 1994; 21: 497-506.
8. Rhim EM, Ahn SJ, Kim JY, Kim KH, Lee HW, Kim EC, et al. Stimulation of metalloproteinases by tumor necrosis factor-alpha in human pulp cell cultures. J Endod 2013; 39: 795-800.
9. Palosaari H, Pennington CJ, Larmas M, Edwards DR, Tjaderhane L, Salo T. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. Eur J Oral Sci 2003; 111: 117-127.
10. Lee A, Choi SJ, Park K, Park JW, Kim K, Choi K, et al. Detection of active matrix metalloproteinase-3 in serum and fibroblast-like synovocytes of collagen-induced arthritis mice. Bioconjug Chem 2013; 24: 1068-1074.
11. Ozeki N, Yamaguchi H, Kawai R, Hiyama T, Nakata K, Mogi M, et al. Cytokines induce MMP-3-regulated proliferation of embryonic stem cell-derived odontoblast-like cells. Oral Dis 2014; 20: 505-513.
12. Wisithphrom K, Murray PE, Windsor LJ. Interleukin-1 alpha alters the expression of matrix metalloproteinases and collagen degradation by pulp fibroblasts. J Endod 2006; 32: 186-192.
13. Ahn SJ, Rhim EM, Kim JY, Kim KH, Lee HW, Kim EC, et al. Tumor necrosis factor-alpha induces matrix metalloproteinase-3, -10 and -13 in human periodontal ligament cells. J Periodontol 2014; 85: 490-497.
14. Soell M, Elkaïm R, Tenenbaum H, Cathepsin C, matrix metalloproteinases, and their tissue inhibitors in gingiva and gingival crevicular fluid from periodontitis-affected patients. J Dent Res 2002; 81: 174-178.
15. Séguyer S, Gogly B, Bodineau A, Godeau G, Brousse N. Isolation of active matrix metalloproteinase-3 in serum and fibroblast-like synovocytes of collagen-induced arthritis mice. Bioconjug Chem 2013; 24: 1068-1074.
16. Itota T, Carrick TE, Yoshiyama M, McCabe JF. Fluoride release and recharge characteristics of denture base resins containing surface pre-reacted glass ionomer fillers. Dent Mater J 2009; 28: 295-302.
17. Kim HH, Shin CM, Park CH, Kim KH, Cho KH, Eun HC, et al. Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dental fibroblasts. J Lipid Res 2005; 46: 1712-1720.
18. Parikh AA, Moon MR, Kane CD, Salzman AL, Fischer JE, Hasselgren PO. Interleukin-6 production in human intestinal epithelial cells increases in association with the heat shock response. J Surg Res 1998; 77: 40-46.
19. Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: Anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. Br J Cancer 2014; 110: 469-478.
20. Assuma R, Outes T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. J Immunol 1998; 160: 403-409.
21. Zhu J, Guo B, Mu M, Guo W, Yuan Y, Yuan H, et al. Interleukin-6-174GC polymorphism contributes to periodontitis susceptibility: an updated meta-analysis of 21 case-control studies. Dis Markers 2016; 2016: 9612421.
22. Brenner DA, O’Hara M, Angel P, Chojkier M, Karin M. Prolonged activation of jun and collagenase genes by tumour necrosis-a. Nature 1989; 337: 661-663.
23. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. Front Biosci 2006; 11: 529-543.
24. Przybylowska K, Kluczna A, Zadrozny M, Krawczyk A, Kulig A, Rykala J, et al. Polymorphisms of the promoter regions of matrix metalloproteinases genes MMP-1 and MMP-9 in breast cancer. Breast Cancer Res Treat 2006; 95: 65-72.
25. Goda S, Kato Y, Domae E, Hayashi H, Tani-Ishii N, Iida J, et al. Effects of JNK1/2 on the inflammation cytokine TNF-alpha-enhanced production of MMP-3 in human dental pulp fibroblast-like cells. Int Endod J 2015; 48: 1122-1128.
26. Wisithphrom K, Windsor L. The effects of tumor necrosis factor-a, interleukin-6, and transforming growth factor-b1 on pulp fibroblast mediated collagen degradation. J Endod 2006; 32: 583-581.
27. Ikemura K, Tay FR, Endo T, Pashley DH. A review of chemical-approach and ultramorphological studies on the development of fluoride-releasing dental adhesives comprising new pre-reacted glass ionomer (PRG) fillers. Dent Mater J 2008; 27: 315-339.
28. Kawashima S, Shinkai K, Suzuki M. Effects of an experimental adhesive resin containing multi-ion releasing fillers on direct pulp capping. Dent Mater J 2016; 35: 479-489.
29. Wang Y, Kaga M, Kajiwara D, Minamikawa H, Kakuda S, Hashimoto M, et al. Ion release and buffering capacity of S-PRG filler-containing pit and fissure sealant in lactic acid. Nano Biomed 2011; 3: 275-281.
30. Han L, Cv E, Li M, Niwano K, Ab N, Okamoto A, et al. Effect of fluoride mouth rinse on fluoride releasing and recharging from aesthetic dental materials. Dent Mater J 2002; 21: 285-295.
31. Iota T, Carrick TE, Yoshiyama M, McCabe JF. Fluoride release and recharge in glass, comomer and resin composite. Dent Mater J 2004; 20: 789-795.
32. Kamijo R, Mukai Y, Tominaga T, Iwaya I, Fujino F, Hirata Y, et al. Fluoride release and recharge characteristics of denture base resins containing surface pre-reacted glass ionomer filler. Dent Mater J 2009; 28: 227-233.
33. Ito S, Iijima M, Hashimoto M, Tsukamoto N, Mizoguchi I, Saito T. Effects of surface pre-reacted glass-ionomer fillers on mineral induction by phosphoprotein. J Dent 2011; 39: 72-79.
34. Iwaya I, Mukai H, Fukukawa H, Teranaka T. Evaluation of enamel acid resistance acquired under a temporary esthetic coating material. J Dent Health 2009; 59: 125-131.
35. Shimazu K, Ogata K, Karibe H. Evaluation of the ion-releasing and releasing abilities of a resin-based fissure sealant containing S-PRG filler. Dent Mater J 2011; 30: 923-927.
36. Han L, Okamoto A, Fukushima M, Okiji T. Effect of calcium fluoride-releasing as one step adhesive. Dent Mater J 2006; 25: 509-515.
37. Saito T, Tomiyama K, Iizuka J, Hasegawa H, Kuramochi E, Saito T. Effects of surface pre-reacted glass-ionomer fillers on mineral induction by phosphoprotein. J Dent 2011; 39: 72-79.
38. Fujimoto Y, Iwasa M, Miyazaki M, Nagafuli A, Nakatsu T. Detection of ions released from S-PRG fillers and their modulation effect. Dent Mater J 2010; 29: 392-397.
39. Hosoya Y, Ando S, Otani H, Yukinari T, Miyazaki M, Garcia-
41) Kawasaki K, Kambara M. Effects of ion-releasing tooth-coating material on demineralization of bovine tooth enamel. Int J Dent 2014; 2014: 463149.

42) Iota T, Carrick TE, Rusby S, Al-Naimi OT, Yoshiyama M, McCabe JF. Determination of fluoride ions released from resin-based dental materials using ion-selective electrode and ion chromatograph. J Dent 2004; 32: 117-122.

43) 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: 750-754.

44) Mukai Y, Tomiyama K, Shiiya T, Kamijo K, Fujino F, Teranaka T. Formation of inhibition layers with a newly developed fluoride-releasing all-in-one adhesive. Dent Mater J 2005; 24: 172-177.

45) Nemoto A, Chosa N, Kyakumoto S, Yokota S, Noda M, et al. Water-soluble factors eluted from surface pre-reacted glass-ionomer filler promote osteoblastic differentiation of human mesenchymal stem cells. Mol Med Rep 2018; 17: 3448-3454.

46) Iino M, Murayama R, Shimamura Y, Kurokawa H, Furuichi T, Suzuki T, et al. Optical coherence tomography examination of the effect of S-PRG filler extraction solution on the demineralization of bovine enamel. Dent Mater J 2014; 33: 48-53.

47) Kagawa M, Goda S, Matsumoto N. The effect of phenytoin on the matrix metalloprotease-3 production in HGFs. J Oral Tissue Engin 2015; 13: 57-66.

48) Lu J, Guo JH, Tu X, Zhang C, Zhao M, Zhang QW, et al. Tiron inhibits UVB-induced AP-1 binding sites transcriptional activation on MMP-1 and MMP-3 promoters by MAPK signaling pathway in human dermal fibroblasts. PLoS One 2016; 11: e0159998.

49) Krems SF, Spanik HP, Snaar-Jagalska BE. Functions of the MAPK family in vertebrate-development. FEBS Lett 2006; 580: 4984-4990.

50) Schieven GL. The biology of p38 kinase: A central role in inflammation. Curr Top Med Chem 2005; 5: 921-928.

51) Lee J, Jung E, Lee J, Huh S, Hwang CH, Lee HY, et al. Emodin inhibits TNF alpha-induced MMP-1 expression through suppression of activator protein-1 (AP-1). Life Sci 2006; 79: 2480-2485.

52) Kida Y, Kobayashi M, Suzuki T, Takeshita A, Okamatsu Y, Hanazawa S, et al. Interleukin-1 stimulates cytokines, prostaglandin E2 and matrix metalloproteinase-1 production via activation of MAPK/AP-1 and NF-kappaB in human gingival fibroblasts. Cytokine 2005; 29: 159-68.

53) Migita K, Miyashita T, Ishibashi H, Maeda Y, Nakamura M, Yatsuhashi H, et al. Suppressive effect of leflunomide metabolite (A77 1726) on metalloproteinase production in IL-1beta stimulated rheumatoid synovial fibroblasts. Clin Exp Immunol 2004; 137: 612-616.

54) Bao S, Jung Y, Choi YM, Li S. Effects of er-miao-san extracts on TNF-alpha-induced MMP-1 expression in human dermal fibroblasts. Biol Res 2015; 48: 8.

55) Yamaguchi-Ueda K, Akazawa Y, Kawarabayashi K, Sugimoto A, Nakagawa H, Miyazaki A, et al. Combination of ions promotes cell migration via extracellular signal-regulated kinase 1/2 signaling pathway in human gingival fibroblasts. Mol Med Rep 2019; 19: 5039-5045.

56) 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.