ORIGINAL ARTICLE

Surface Pre-reacted Glass-ionomer (S-PRG) Filler Eluate Suppresses MMP-1 Secretion by TNF-α-stimulated Human Gingival Fibroblasts

Lan LAN, Hiroshi INOUE, and Seiji GODA

Department of Physiology, Osaka Dental University, Osaka, Japan

Synopsis

Tumor necrosis factor (TNF)-α is a major inflammatory factor that regulates tissue destruction in periodontitis and promotes the release of Matrix metalloproteinase (MMP) -1, which breaks down the gingival extracellular matrix. MMP-1 is one of the proteolytic enzymes that are capable of cleaving collagen and may play an important role in the destruction of connective tissue. The present study investigated the effects of Surface pre-reacted glass-ionomer (S-PRG) filler eluate on the secretion of MMP-1 by human gingival fibroblast (HGF) stimulated with the inflammatory cytokine TNF-α. S-PRG filler eluate did not alter Akt or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 phosphorylation levels in TNF-α-stimulated HGF. In the mitogen-activated protein kinase (MAPK) pathway, S-PRG filler eluate did not alter the phosphorylation level of p38 in TNF-α-stimulated HGF, but decreased that of c-Jun N-terminal kinase (JNK). These results will facilitate the development of new anti-inflammatory agents for periodontal disease using materials composed of S-PRG filler eluate.

Key words: *S-PRG filler eluate, Human gingival fibroblasts, Matrix metalloproteinase-1*

Introduction

Periodontal disease is an inflammatory disease caused by bacteria that results in tissue destruction and is one of the two main oral diseases [1, 2]. It is prevalent worldwide, affecting up to 90% of the world's population [3]. Periodontitis is a chronic inflammatory disease that is characterized by the deterioration and destruction of the tooth-supporting apparatus, the periodontium [4], and severe periodontitis is one of the main causes of tooth loss [3]. At sites infected with bacteria, macrophages produce the inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF). Matrix metalloproteinases (MMPs) induced by inflammatory cytokines play an important role in periodontal tissue destruction [5].

MMPs are a family of metal-dependent proteolytic enzymes that degrade the extracellular matrix (ECM) and basement membrane[6, 7]. MMP-1 is a major proteolytic enzyme that cleaves collagen and may be an important enzyme in the destruction of connective tissue[8]. The levels of MMPs induced by inflammatory cytokines were previously shown to be elevated in the inflammatory gums of periodontal disease patients and contributed to the destruction of periodontal tissue [5]. MMP-1 and MMP-2 activities were found 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 [9, 10].

TNF-α is one of the pro-inflammatory cytokines that play a major role in tissue injury[11, 12]. It is produced by fibroblasts and macrophages and exerts a number of effects, such as the induction of bone resorption [12-14].

Patients with gingivitis or periodontitis produce higher levels of inflammatory mediators, including TNF-α and IL-6, than healthy individuals [15]. The excessive degradation of the ECM is a characteristic of rheumatoid arthritis, tumor invasion, and periodontitis because TNF-α promotes the release of MMPs, which destroy the gingival ECM and induce rapid disease progression [16-19]. A stimulation with TNF-α was previously shown to increase the secretion of MMP-1 by human dermal fibroblasts (HDF) [11].

Surface pre-reacted glass-ionomer (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 hours and collecting the supernatant. Na, F, Al, B, Sr, and Si are released into S-PRG filler eluate, which has an acid-buffering ability [20]. Recent studies demonstrated that S-PRG filler eluate suppressed the pathogenicity of oral bacterial flora [21] and stimulated the migration of the human gingival fibroblast cell line, HGF-1 [22]. However, limited information is currently available on the effects of multiple ions in S-PRG filler eluate on human gingival fibroblast (HGF).

Therefore, the present study investigated the effects of S-PRG filler eluate on the secretion of MMP-1 by HGF stimulated with the inflammatory cytokine TNF-α. The effects of multiple ions in S-PRG filler eluate on signal transduction pathways were also examined.

Materials and Methods

1.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 as described previously [23]. Experiments were performed using HGF between passages 3 and 10. HGF were maintained in α-MEM (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 2 mM L-glutamine (Wako Pure Chemical Industries), 100 μg/mL penicillin (Wako Pure Chemical Industries), 100 μg/mL streptomycin (Wako Pure Chemical Industries), and 10% fetal bovine serum (Biowest, Rue de la Caille, France). 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.111111).

2. Preparation of S-PRG filler eluate

The multi-ion solution eluted from S-PRG fillers was provided by SHOFU Inc. (Kyoto, Japan). S-PRG filler prepared according to the method of Fujimoto et al. [20] was used for the preparation of the multi-ion solution. The filler material was removed by filtration, and the ion solution was centrifuged to remove any residual insoluble material. The supernatant collected was filtered through a chromatodisc (0.45 μm) to obtain S-PRG filler α-MEM eluate (S-PRG filler eluate). The clear supernatant was filtered to remove any residual insoluble material and used as the S-PRG filler elute. 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 (ICPS-8000, Shimadzu Co., Kyoto, Japan). An elemental analysis of F released from S-PRG filler was conducted using an ion electrode method with a fluoride electrode (9609BNWP, Thermo Fisher Scientific). 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 α-MEM medium eluting S-PRG filler components.

3. Western blotting

HGF were seeded on a 12-well plate at a density of 1.0×10^5 cells/well and cultured for 4 days to 90% confluency in α-MEM containing 10% FBS. HGF were then incubated in serum-free α-MEM for 1 hour. After the incubation, HGF were stimulated for 24 hours with various concentrations of S-PRG filler eluate (0.1, 0.5. and 1%) and 10 ng/mL of recombinant human TNF-α (Miltenyi Biotec, Auburn, CA, USA). After the stimulation, 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, and 4% 2-mercaptoethanol). Equal

Table 1 Comparison of various ion concentrations between S-PRG filler eluate and α-MEM.
S-PRG filler eluate was prepared using the following method. α-MEM was mixed with S-PRG filler (average filler diameter of 1 μm) at a weight ratio of 1:1 (1 L:1,000 g), and the supernatant was collected. The supernatant was filtered to obtain S-PRG filler eluate. S-PRG, Surface pre-reacted glass ionomer.; α-MEM, alpha minimum essential medium.

	Al	B	Na	Si	Sr	F	Ca	P	K
α-MEM (Lot. ECN7022)	0	0	3597.1	0	0	0	77.2	33.5	246.5
S-PRG filler eluate solution (Lot. 091503)	12.0	1456.0	3350.6	7.3	792.8	54.3	1.9	0.2	42.4

(ppm)

amounts of each sample were then separated by 10% SDS/PAGE. 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 an anti-MMP-1 antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature (RT) for 1 hour. Membranes were incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody, mouse anti-goat IgG-HRP (Santa Cruz Biotechnology) at a 1:2000 dilution at RT for 1 hour. After the incubation, immunoreactivity was visualized using the chemiluminescent reagent, Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore), and blot images were acquired using VersaDoc 5000 (Bio-Rad, Hercules, CA, USA). HGF were also seeded on a 12-well plate and cultured for 4 days. Following serum deprivation for 1 hour, HGF were pretreated for 30 minutes with S-PRG filler eluate (0.1%) or were not treated. HGF were then stimulated with 0.1% S-PRG filler eluate and 10 ng/mL of recombinant human TNF-α, and time-dependent changes in the phosphorylation of various signal transduction molecules for up to 60 minutes after the stimulation were examined. After the stimulation, total protein was isolated in sample buffer supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Equal amounts of each sample were then separated by 10% SDS/PAGE, transferred to PVDF membranes, and blocked with 20% Blocking One in 50 mM Tris–HCl at pH 7.5 and 150 mM NaCl overnight. Membranes were incubated with 1:1000-diluted primary antibodies against anti-phospho Akt (Cell Signaling Technology, Danvers, MA, USA), anti-phospho nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 (Cell Signaling Technology), anti-phospho p38 (Santa Cruz Biotechnology), and anti-phospho c-Jun N-terminal kinase (JNK) (Thermo Fisher Scientific, Waltham, MA, USA) at RT for 1 hour. Membranes were then incubated with the matching secondary antibody (HRP-labeled anti-mouse IgG or anti-rabbit IgG; 1:2000) (Jackson ImmunoResearch, West Grove, PA) at RT for 1 hour. Blot images were visualized using VersaDoc 5000. The same membrane was stripped and reprobed with the following antibodies: anti-Akt (1:1,000, Cell Signaling Technology), anti-NF-κB p65 (1:1,000, Cell Signaling Technology), anti-p38 (1:1,000, Santa Cruz Biotechnology), and anti-JNK (1:1,000, Santa Cruz Biotechnology)).

Results

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

We initially examined the effects of S-PRG filler eluate on inflammation. The effects of S-PRG filler eluate (0.1, 0.5, and 1%) on the secretion of MMP-1 by TMP-α (10 ng/mL)-stimulated HGF was investigated using Western blotting. MMP-1 secretion, which was increased by the TNF-α stimulation, was suppressed by S-PRG

filler eluate (Fig. 1, upper). The inhibition of MMP-1 secretion by S-PRG filler eluate was the strongest at a concentration of 0.1%. No significant changes were observed in the amount of actin in any sample (Fig. 1, lower).

2. Effects of S-PRG filler eluate on Akt activation in TNF-α-stimulated HGF

Next, we used Western blotting to investigate whether S-PRG filler eluate was involved in the activation of Akt in TNF-α-stimulated HGF. The concentration of S-PRG filler eluate used was 0.1%, which was a concentration at which the amount of MMP-1 secreted was significantly reduced. Time-dependent changes in Akt phosphorylation up to 60 minutes after the stimulation were investigated. Akt was constitutively phosphorylated without the stimulation, and no significant changes were observed in Akt phosphorylation levels, even following the stimulation with TNF-α (Fig. 2, upper). In addition, no significant changes were observed in the phosphorylation levels of Akt following a co-stimulation with TNF and S-PRG filler eluate (Fig. 2, upper). These results indicate that S-PRG filler eluate did not affect TNF-α-

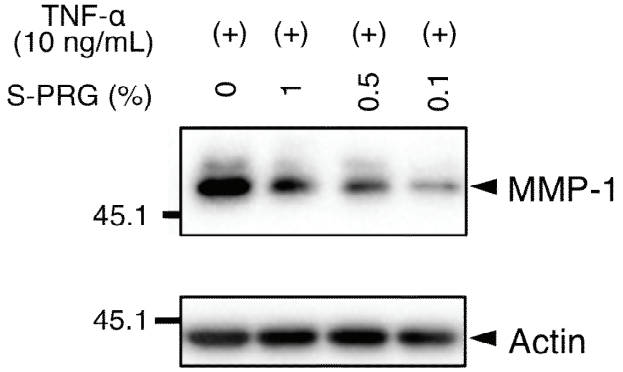

Fig. 1 Effects of various concentrations of S-PRG filler eluate on MMP-1 secretion by HGF
HGF were stimulated by various concentrations (0, 0.1, 0.5, and 1%) of S-PRG filler eluate for 24 hours, and the supernatant was collected. Western blotting was used to assess the secretion of MMP-1 (upper) by HGF. To show that supernatants were obtained from equal amounts of cells, the amount of actin (lower) was evaluated by Western blotting. Data presented are representative of four independent experiments. HGF, human gingival fibroblasts; MMP, matrix metalloproteinase; S-PRG, surface pre-reacted glass ionomer

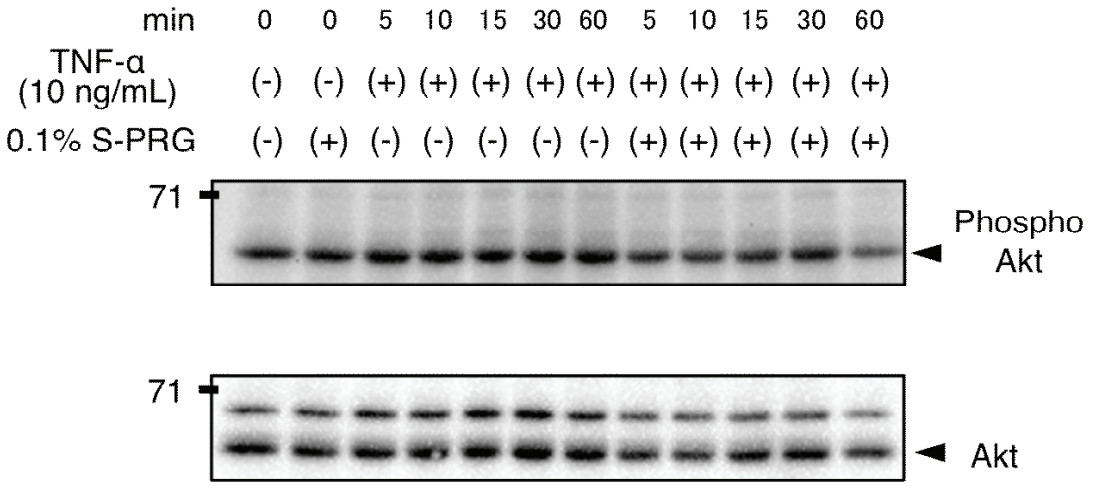

Fig. 2 Involvement of S-PRG filler eluate in Akt activation in TNF-α-stimulated HGF
HGF were pretreated for 30 minutes with S-PRG filler eluate (0.1%) or were not treated, and were then stimulated by TNF-α (10 ng/mL) for 0 to 60 minutes. Cell lysate samples were prepared. The phosphorylation level of Akt was examined by Western blotting (upper). To ensure that equal amounts of Akt were obtained from lysates, the membranes were stripped and reprobed with anti-Akt 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

stimulated Akt phosphorylation levels in HGF. The total amount of the Akt protein was not affected by any of the experimental conditions used (Fig. 2, lower).

3. Effects of S-PRG filler eluate on NF-κB activation in TNF-α-stimulated HGF

NF-κB p65 phosphorylation levels were investigated to determine if the S-PRG filler eluate modulates NF-κB activation in TNF-α-stimulated HGF. Using the same stimulus as that in Fig. 2, time-dependent changes in NF-κB p65 phosphorylation levels for up to 60 minutes after the stimulation were investigated. In the present study, the phosphorylation of NF-κB p65 was enhanced 5 minutes after the TNF-α stimulation and detected for up to 60 minutes (Fig. 3, upper). Phosphorylation levels peaked after 5 to 10 minutes, and then gradually decreased over time (Fig. 3, upper). However, the co-stimulation with TNF and S-PRG filler eluate did not induce any significant changes in NF-κB p65 phosphorylation levels from those with the TNF-α stimulation (Fig. 3, upper). These results indicate that S-PRG filler eluate did not affect the TNF-α-stimulated NF-κB p65 phosphorylation levels of HGF. The total amount of the NF-κB p65 protein was not affected by any of the experimental conditions used (Fig. 3, lower).

4. Effects of S-PRG filler eluate on mitogen-activated protein kinase (MAPK) activation in TNF-α-stimulated HGF

As shown in Fig. 4 (upper), the phosphorylation of p38 was enhanced 5 minutes after the TNF-α stimulation and was detected for up to 60 minutes, similar to the phosphorylation of NF-κB p65. p38 phosphorylation levels peaked 10 minutes after the TNF-α stimulation, and then gradually decreased over time. p38 phosphorylation levels decreased to the same level as that in unstimulated HGF (as a control) 30 minutes after the TNF-α stimulation. The co-stimulation with TNF-α and S-PRG filler eluate did not significantly change p38 phosphorylation levels from those with the TNF-α stimulation (Fig. 4, upper). These results indicate that S-PRG filler eluate did not affect TNF-α-stimulated p38 phosphorylation levels in HGF. The total amount of the p38 protein was not affected by any of the experimental conditions used (Fig. 4, lower).

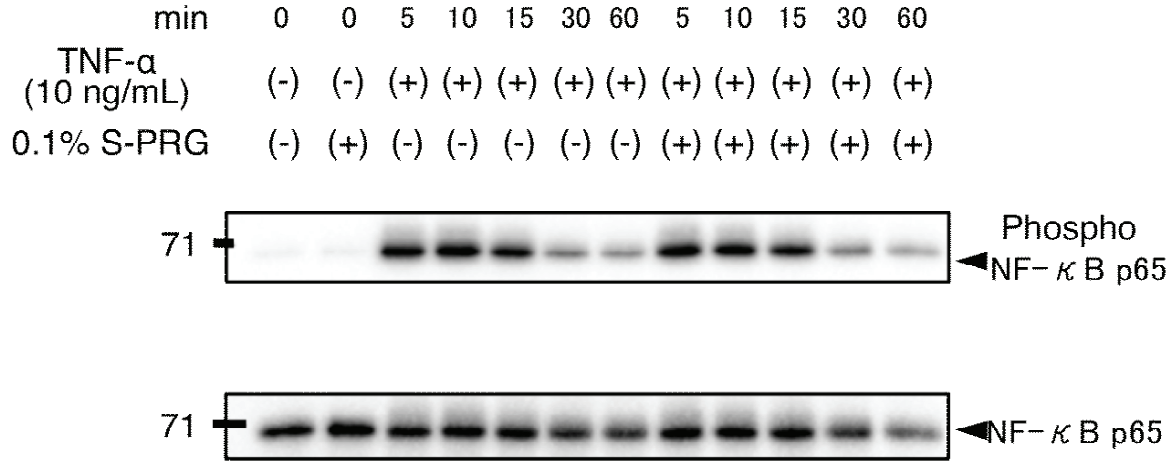

Fig. 3 Involvement of S-PRG filler eluate in NF-κB p65 activation in TNF-α-stimulated HGF
HGF were pretreated for 30 minutes with S-PRG filler eluate (0.1%) or were not treated, and were then stimulated with TNF-α (10 ng/mL) for 0 to 60 minutes. Cell lysate samples were prepared. The phosphorylation level of NF-κB p65 was examined by Western blotting (upper). To ensure that equal amounts of NF-κB p65 were obtained from the lysates, the membranes were stripped and reprobed with anti-NF-κB p65 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

We then examined the JNK pathway using the same approach. Time-dependent changes in the phosphorylation of JNK confirmed the involvement of S-PRG filler eluate in its activation in TNF-α-stimulated HGF. The phosphorylation of JNK was slightly enhanced 5 minutes after the TNF-α stimulation and was detected for up to 60 minutes (Fig. 5, upper). JNK phosphorylation levels peaked 15 minutes after the TNF-α stimulation, and then gradually decreased over time (Fig. 5, upper). JNK phosphorylation levels were maintained at a higher level than in unstimulated HGF (as a control) until 60 minutes after the TNF-α stimulation (Fig. 5, upper). The co-stimulation with TNF-α and S-PRG filler eluate decreased JNK phosphorylation levels

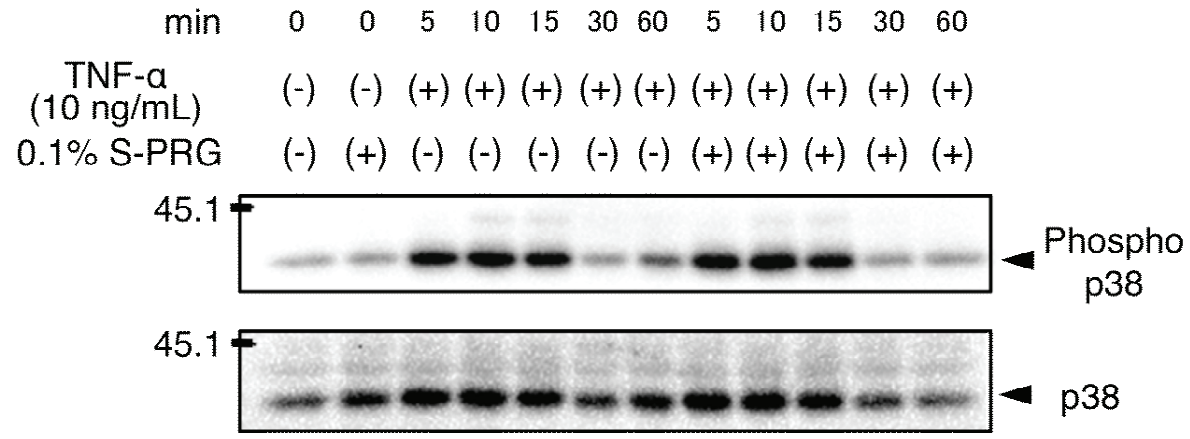

Fig. 4 Involvement of S-PRG filler eluate in p38 activation in TNF-α-stimulated HGF
HGF were pretreated for 30 minutes with S-PRG filler eluate (0.1%) or were not treated, and were then stimulated with TNF-α (10 ng/mL) for 0 to 60 minutes. Cell lysate samples were prepared. The phosphorylation level of p38 was examined by Western blotting (upper). To ensure that equal amounts of p38 were obtained from lysates, the membranes were stripped and reprobed with anti-p38 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

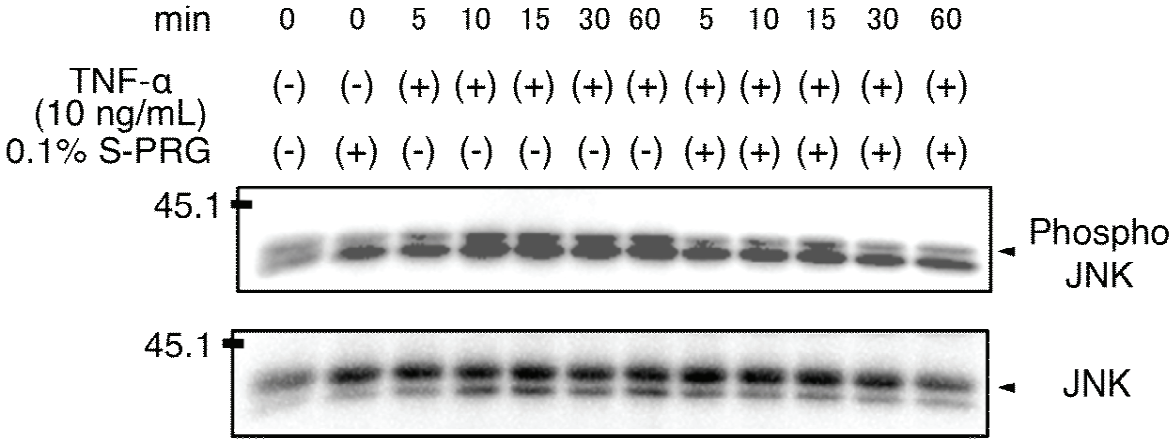

Fig. 5 Involvement of S-PRG filler eluate in JNK activation in TNF-α-stimulated HGF
HGF were pretreated for 30 minutes with S-PRG filler eluate (0.1%) or were not treated, and were then stimulated with TNF-α (10 ng/mL) for 0 to 60 minutes. Cell lysate samples were prepared. The phosphorylation level of JNK was examined by Western blotting (upper). To ensure that equal amounts of JNK were obtained from lysates, membranes were stripped and reprobed with anti-JNK 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

more than the TNF-α stimulation. Figure 5 shows that the co-stimulation with TNF-α and S-PRG filler eluate reduced JNK phosphorylation levels. The total amount of the JNK protein was not significantly affected by any of the experimental conditions used (Fig. 5, lower).

Discussion

The present results revealed that S-PRG filler eluate containing six types of ions (F, Na, Al, B, Sr, and Si) inhibited the secretion of MMP-1 by TNF-α-stimulated HGF. MMP-1 degrades type 1 collagen and is involved in the destruction of periodontal soft tissue in periodontal lesions [24, 25]. The protein levels and activation of MMP-1 were previously shown to be enhanced in inflamed gingival tissue [9, 26]. Therefore, the inhibition of MMP-1 expression in periodontal lesions is important for preventing the onset and progression of periodontal disease. Iwamatsu-Kobayashi et al. reported that S-PRG filler eluate suppressed the progression of bone resorption in a mouse model of ligation-induced periodontal disease [27]. This finding suggests the potential of S-PRG filler eluate to inhibit alveolar bone resorption in periodontal lesions. Based on these findings and the present results, the topical application of S-PRG filler eluate to periodontal lesions may help to prevent the breakdown of both alveolar bone and periodontal soft tissue.

TNF-α is an inflammatory cytokine produced by macrophages and HGF during periodontitis [28, 29]. TNF-α promotes the production of MMP-1 by HDF and the destruction of gingival ECM [17, 30]. In the present study, we investigated 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, which was increased by the TNF-α stimulation, was suppressed by the co-stimulation with S-PRG filler eluate. The Akt pathway was previously reported to play a role in the regulation of MMP-1 secretion, which was increased by a TNF-α stimulation, in human dermal fibroblasts [31]. We herein demonstrated that S-PRG filler eluate did not modulate Akt activation in TNF-α-stimulated HGF. AKT was constitutively phosphorylated without the TNF-α stimulation, and AKT phosphorylation levels did not significantly change with or without the co-stimulation with TNF-α and S-PRG filler eluate. The expression of MMP-1 in response to inflammation is regulated by the transcription factor NF-κB. In HDF, the activation of NF-κB is involved in the regulation of inflammation via various intracellular signaling pathways, including the MAPK pathway [32]. Previous studies demonstrated that the NF-κB pathway was involved in the production of MMP-1 in human periodontal ligament cells [33, 34]. Based on these findings, we investigated the possibility that S-PRG filler eluate modulates NF-κB activation in TNF-α-stimulated HGF. We showed that S-PRG filler eluate did not affect NF-κB p65 phosphorylation levels in TNF-α-stimulated HGF. The discrepancies between the present results and these findings were attributed to differences in the cells examined. The present results suggested that the Akt-NF-κB pathway was not involved in the inhibitory effects of S-PRG filler eluate on MMP-1 secretion by TNF-α-stimulated HGF.

A previous study demonstrated that the activation of MAPK (p38, JNK, and ERK) by TNF-α plays an important role in the secretion of MMP-1 [30]. MAPKs constitute a group of serine/threonine protein kinases that are subdivided into three subfamilies: p38, JNK, and ERK. MAPKs are activated by various extracellular stimuli and induce the phosphorylation of important signaling molecules associated with cell proliferation, inflammation, and apoptosis [35]. In the present study, the TNF-α stimulation increased the phosphorylation levels of p38 and JNK; however, the co-stimulation with S-PRG filler eluate only induced changes in JNK. We found that the co-stimulation with TNF-α and S-PRG filler eluate reduced JNK phosphorylation levels more than the stimulation with TNF-α. Regarding p38, the S-PRG filler eluate did not significantly affect TNF-α-stimulated p38 phosphorylation levels under these experimental conditions. Consistent with previous findings, the present results demonstrated that the activation of MAPK was involved in the secretion of MMP-1. In the present study, the MAPK pathway (particularly JNK) may have played a role in the inhibitory effects of S-PRG filler eluate on MMP-1 secretion by TNF-α-stimulated HGF. However, the mechanism of decreased phosphorylation levels of MAP kinase has not been completely elucidated by this experiment. In addition, further studies are needed to investigate the effects of MAPK

(e.g., ERK) other than p38 and JNK and the signaling pathways downstream of MAPK, such as c-Fos and c-Jun.

S-PRG filler eluate contains F, Al, B, Na, Si, and Sr ions. The present results suggest that the combination of these ions suppressed MMP-1 secretion by TNF-α-stimulated HGF. S-PRG extracellular ions markedly affect the physiological activity of cells [36, 37]. The effectiveness of individual ions on the biological activity of cells differs depending on a number of conditions, such as concentrations and combinations with other ions. B [38], F [39], Al [40], and Sr [41] are toxic when used at high concentrations. We also previously reported that S-PRG filler eluate exhibited cytotoxicity when used in culture media at high concentrations [42]. Micromolar concentrations of Al ions have been shown to directly affect osteoblasts and stimulate osteoblast proliferation and differentiation [36]. Lower concentrations of Sr were also found to facilitate the osteogenic differentiation of human adipose-derived stem cells [43]. Therefore, changes in extracellular ion compositions may have a significant positive or negative impact on cells. The present study did not focus on which ions suppressed MMP-1 secretion in TNF-α-stimulated HGF. Therefore, the functions of individual ions as well as different combinations of ions warrant further study.

Conclusion

The present results demonstrated that S-PRG eluate containing multiple ions suppressed the secretion of MMP-1 by TNF-α-stimulated HGF. Therefore, S-PRG filler eluate may suppress TNF-α-induced inflammation in HGF. We propose the development of S-PRG filler eluate as a component of oral rinsing solution, as previously suggested by Iwamatsu-Kobayashi *et al*. [27]. To achieve these goals, future studies using S-PRG filler eluate are needed to confirm its anti-inflammatory properties and cytokine responses and also to elucidate the mechanisms by which periodontal tissue destruction is suppressed.

Acknowledgments

We are grateful to Dr. Mao from the Graduate School of Dentistry, Department of Physiology, Osaka Dental University. This work was supported by the Japan Society for the Promotion of Science KAKENHI Grant Number JP21K10269 (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 2000 1994; 5: 78-111.
2) Mariotti A. Dental plaque-induced gingival diseases. Ann Periodontol 1999; 4: 7-17.
3) Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809-1820.
4) Mullally BH, Coulter WA, Hutchinson JD, Clarke HA. Current oral contraceptive status and periodontitis in young adults J Periodontol 2007; 78: 1031-1036.
5) Sapna G, Gokul S, Bagri-Manjrekar K. Matrix metalloproteinases and periodontal diseases. Oral Dis 2014; 20: 538-550.
6) Ravanti L, Kähäri VM. Matrix metalloproteinases in wound repair (review). Int J Mol Med 2000; 6: 391-407.
7) Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491-21494
8) Nares S. The genetic relationship to periodontal disease. Periodontol 2000 2003; 32: 36-49.
9) Ding Y, Uitto VJ, Firth J, Salo T, Haapasalo M, Konttinen Y, Sorsa T. Modulation of host matrix metalloproteinases by bacterial virulence factors relevant in human periodontal diseases. Oral Dis 1995; 1: 279-286.
10) McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol 1994; 21: 497-506.
11) Kim HH, Shin CM, Park CH, Kim KH, Cho KH, Eun HC, Chung JH. Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dermal fibroblasts. J Lipid Res 2005; 46: 1712-1720.
12) 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: 40-44.
13) 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 tu-

mour-stroma interaction. Br J Cancer 2014; 110: 469-478.
14) Assuma R, Oates 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.
15) Zhu J, Guo B, Fu M, Guo W, Yuan Y, Yuan H, Zhang S, Yu, H. Interleukin-6-174G/C polymorphism contributes to periodontitis susceptibility: an updated meta-analysis of 21 case-control studies. Dis Markers 2016; 2016: 9612421.
16) Itagaki M, Kubota T, Tai H, Shimada Y, Morozumi T, Yamazaki K. Matrix metalloproteinase-1 and -3 gene promoter polymorphisms in Japanese patients with periodontitis. J Clin Periodontol 2004; 31: 764-769.
17) Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M. Prolonged activation of jun and collagenase genes by tumour necrosis-α. Nature 1989; 337: 661-663.
18) Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. Front Biosci 2006; 11: 529-543.
19) Przybylowska K, Kluczna A, Zadrozny M, Krawczyk T, Kulig A, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J, Blasiak J. 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.
20) 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.
21) Kono Y, Tamura M, Cueno ME, Tonogi M, Imai K. S-PRG filler eluate induces oxidative stress in oral microorganism: suppression of growth and pathogenicity, and possible clinical application. Antibiotics (Basel). 2021; 10: 816.
22) Yamaguchi-Ueda K, Akazawa Y, Kawarabayashi K, Sugimoto A, Nakagawa H, Miyazaki A, Kurogoushi R, Iwata K, Kitamura T, Yamada A, Hasegawa T, Fukumoto S, Iwamoto T. 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.
23) 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.
24) Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 1996; 1: 821-878.
25) Beklen A, Ainola M, Hukkanen M, Gürgan C, Sorsa T, Konttinen YT. MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. J Dent Res. 2007; 86: 347-351.
26) Okuda K, Miyazaki A, Momose M, Murata M, Nomura T, Kubota T, Wolff LF, Yoshie H. Levels of tissue inhibitor of metalloproteinases-1 and matrix metalloproteinases-1 and -8 in gingival crevicular fluid following treatment with enamel matrix derivative (EMDOGAIN). J Periodontal Res 2001; 36: 309-316.
27) Iwamatsu-Kobayashi Y, Abe S, Fujieda Y, Orimoto A, Kanehira M, Handa K, Venkataiah VS, Zou W, Ishikawa M, Saito M. Metal ions from S-PRG filler have the potential to prevent periodontal disease. Clin Exp Dent Res 2017; 3: 126-133.
28) Han L, Cv E, Li M, Niwano K, Ab N, Okamoto A, Honda N, Iwaku M. Effect of fluoride mouth rinse on fluoride releasing and recharging from aesthetic dental materials. Dent Mater J 2002; 21: 285-295.
29) Itota T, Carrick TE, Yoshiyama M, McCabe JF. Fluoride release and recharge in giomer, compomer and resin composite. Dent Mater 2004; 20: 789-795.
30) Goda S, Kato Y, Domae E, Hayashi H, Tani-Ishii N, Iida J, Ikeo T. Effects of JNK1/2 on the inflammation cytokine TNF-α-enhanced production of MMP-3 in human dental pulp fibroblast-like cells. Int Endod J 2015; 48: 1122-1128.
31) Oh JH, Kim A, Park JM, Kim SH, Chung AS. Ultraviolet B-induced matrix metalloproteinase-1 and -3 secretions are mediated via PTEN/Akt pathway in human dermal fibroblasts. J Cell Physiol 2006; 209: 775- 785.
32) Lu Y, Wahl LM. Oxidative stress augments the production of matrix metalloproteinase-1, cyclooxygenase-2, and prostaglandin E2 through enhancement of NF-kappa B activity in lipopolysaccharide-activated human primary monocytes. J Immunol 2005; 175: 5423-5429.
33) Roupakia E, Markopoulos GS, Kolettas E. IL-12-mediated transcriptional regulation of matrix metalloproteinases. Biosci Rep 2018; 38: BSR20171420.
34) Lisboa RA, Andrade MV, Cunha-Melo JR. Toll-like receptor activation and mechanical force stimulation promote the secretion of matrix metalloproteinases 1, 3, and 10 of human periodontal fibroblasts via p38, JNK and NF-kB. Arch Oral Biol 2013; 58: 731-739.
35) Kim EK, Choi EJ: Pathological roles of MAPK signaling pathways in human diseases. Biochem Biophys Acta 2010; 1802: 396-405.

36) Lau KH, Yoo A, Wang SP. Aluminum stimulates the proliferation and differentiation of osteoblasts in vitro by a mechanism that is different from fluoride. Mol Cell Biochem 1991; 105: 93-105.
37) Quignard S, Coradin T, Powell JJ, Jugdaohsingh R. Silica nanoparticles as sources of silicic acid favoring wound healing in vitro. Colloids Surf B Biointerfaces 2017; 155: 530-537.
38) 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.
39) Veron MH, Couble ML, Magloire H. Selective inhibition of collagen synthesis by fluoride in human pulp fibroblasts in vitro. Calcif tissue Int 1993; 53: 38-44.
40) Zhu Y, Xu F, Yan X, Miao L, Li H, Hu C, Wang Z, Lian S, Feng Z, Li Y. The suppressive effects of aluminium chloride on the osteoblasts function. Environ Toxicol Pharmacol 2016; 48: 125-129.
41) 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.
42) Kashiwagi K, Inoue H, Komasa R, Hosoyama Y, Yamashita K, Morisaki A, Goda S. Optimal dilutions of S-PRG filler eluate for experiments on human gingival fibroblasts in vitro. Dent Mater J 2021; 40: 136-142.
43) Aimaiti A, Maimaitiyiming A, Boyong X, Aji K, Li C, Cui L: Low-dose strontium stimulates osteogenesis but high-dose doses cause apoptosis in human adipose-derived stem cells via regulation of the ERK1/2 signaling pathway. Stem Cell Res Ther 2017; 8: 282.

(Received: February 2, 2022/
Accepted: March 31, 2022)

Corresponding author:
Hiroshi Inoue, D.D.S.,Ph.D.
8-1 Kuzuha Hanazono-cho, Hirakata,
Osaka 573-1121, Japan
Department of Physiology, Osaka Dental University
+81-72-864-3054
+81-72-864-3154
E-mail: inoue-hi@cc.osaka-dent.ac.jp