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Original Article

Ultrasonic irrigation of periodontal pocket with surface pre-reacted glass-ionomer (S-PRG) nanofiller dispersion improves periodontal parameters in beagle dogs

Hirofumi Miyaji [a,*], Kayoko Mayumi [a], Yukimi Kanemoto [a], Ichie Okamoto [a], Asako Hamamoto [a], Akihito Kato [a], Tsutomu Sugaya [a], Tsukasa Akasaka [b], Saori Tanaka [a,c]

[a] *Department of Periodontology and Endodontology, Faculty of Dental Medicine, Hokkaido University, N13W7, Kita-ku, Sapporo, Hokkaido, 060-8586, Japan*
[b] *Department of Biomedical Materials and Engineering, Faculty of Dental Medicine, Hokkaido University, N13W7, Kita-ku, Sapporo, Hokkaido, 060-8586, Japan*
[c] *Division of General Dentistry Center for Dental Clinics, Hokkaido University Hospital, N14W5, Kita-ku, Sapporo, Hokkaido, 060-8648, Japan*

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ABSTRACT

Objectives: Surface pre-reacted glass-ionomer (S-PRG) nanofiller, an antibacterial ion-releasing bioactive glass, has been shown to adhere to tooth surfaces and reported to improve inflammatory parameters in experimental periodontitis. In this study, cementum substrate was irrigated ultrasonically with dispersion to examine in-vitro nanofiller adhesion and antibacterial activity. Moreover, periodontal pockets in a beagle dog were ultrasonically irrigated with dispersion to assess periodontal healing.
Methods: The morphology of human cementum irrigated with S-PRG nanofiller dispersion was examined by scanning electron microscopy and energy dispersive X-ray spectrometry. The antibacterial activity of the treated cementum was tested using *Actinomyces naeslundii*. In addition, experimentally formed periodontal pockets in beagle dog were ultrasonically irrigated with S-PRG nanofiller dispersion. Periodontal parameters (gingival index, bleeding on probing, probing pocket depth, and clinical attachment level) were measured from baseline (0 weeks) through 12 weeks. Moreover, the effects of irrigation with S-PRG nanofiller on changes in periodontal microflora and bone healing were analyzed.
Results: After ultrasonic irrigation, S-PRG nanofiller adhered to the cementum and exhibited antibacterial activity. The periodontal parameters were shown to improve following ultrasonic irrigation with S-PRG nanofiller dispersion. Analysis by next-generation sequencing revealed that the ratio of red-complex species decreased in the pockets irrigated with S-PRG nanofiller dispersion. In addition, the S-PRG nanofiller showed the potential to promote bone healing.
Conclusions: Ultrasonic irrigation with S-PRG nanofiller dispersion using an ultrasonic scaler system permitted delivery of the S-PRG nanofiller to the root surface, providing improved parameters in experimental periodontitis and modifying the composition of subgingival periodontal microflora.

1. Introduction

Periodontitis is an infective disease caused by the oral bacterial biofilm that forms on the root surfaces exposed in the periodontal pockets. Since the re-growth of pathogens on treated root surfaces would result in the return of periodontal disease, it is desirable to inhibit bacterial re-growth after periodontal treatments such as scaling and root planing. Several studies have reported that the application of heavy metals, polymers, and ceramics maintain the antimicrobial properties of the tooth surface [1–3]. Surface pre-reacted glass-ionomer (S-PRG) filler is a bioactive glass that releases antibacterial ions, such as fluoride and borate [4]. S-PRG filler has been applied clinically to dental materials, such as composite resins, and the antibacterial properties of such filler have been shown widely [5–8]. Recently, Mayumi et al. reported the use of S-PRG nanoparticulated filler as a coating agent for tooth surfaces [9]. In that report, a S-PRG nanofiller dispersion was injected (by syringe) into the periodontal pocket in dogs. Tooth surfaces coated with S-PRG nanofiller inhibited the growth of oral bacteria and contributed to the improvement of clinical inflammatory parameters in experimental periodontitis in that canine model.

* Corresponding author.
E-mail address: miyaji@den.hokudai.ac.jp (H. Miyaji).

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Abbreviations

BL	baseline
BOP	bleeding on probing
CAL	clinical attachment level
CFU	colony-forming unit, CT, computed tomography
Ctrl	control
EDX	energy dispersive X-ray spectrometry
GI	gingival index
MT	Masson's trichrome
PPD	probing pocket depth
SEM	scanning electron microscopy
S-PRG	surface pre-reacted glass-ionomer

Ultrasonic scaler systems are widely used as a treatment tool for periodontal diseases, where such systems serve to remove biofilm and calculus from the root surface. Since these systems function using water irrigation, antimicrobial agents easily can be irrigated into the subgingival regions [10]. Thus, in the present work, we sought to deliver the S-PRG nanofiller dispersion to the root surface using an ultrasonic scaler system, providing an antibacterial coating on the root surface. After ultrasonic scaling by irrigation of the human cementum with S-PRG nanofiller dispersion, the adhesion of nanofillers and antibacterial properties were investigated *in vitro*. In addition, the effect of ultrasonic irrigation using S-PRG nanofiller dispersion on periodontitis parameters and subgingival periodontal microflora was assessed in experimental periodontitis in beagle dog.

2. Materials and methods

The S-PRG nanofiller (mean volume diameter of nanofiller, 0.48 μm) was fabricated according to the method of Mayumi et al. [9] and dispersed in distilled water (1 wt.%) (Fig. 1(A)). First, tooth cementum substrates obtained from human teeth were irrigated ultrasonically using an ultrasonic scaler system (PIEZON 250, Electro Medical Systems S.A., Nyon, Switzerland) with a Piezon tip PS at power 3 for 10 s (while tracing the cementum surface) delivering the S-PRG nanofiller dispersion. S-PRG nanofiller adhesion to the cementum substrate then was examined using scanning electron microscopy (SEM; JSM-6500F, JEOL, Ltd., Tokyo, Japan) equipped with an energy dispersive X-ray spectrometer (EDX).

To test antibacterial activity, *Actinomyces naeslundii* (final concentration: 1×10^7 colony-forming units (CFU)/mL; ATCC 27039), an early colony-forming bacterium of the oral cavity [11], was inoculated onto S-PRG nanofiller-irrigated or non-irrigated human cementum surfaces and incubated anaerobically using actinomyces broth (BBL Actinomyces Broth, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 24 h. After staining with LIVE/DEAD BacLight Bacterial Viability Kit (Thermo Fisher Scientific, Waltham, MA, USA), fluorescence intensity was measured using ImageJ software (ver. 1.41, National Institutes of Health, Bethesda, MD, USA). For quantitative evaluation, the percentage of green (live cell) and red (dead cell) fluorescence intensity was calculated. Bacterial cells were collected from the cementum substrate by ultrasonic washing, diluted 10-fold in fresh broth, and spread onto BHI agar plates (Eiken Chemical Co., Ltd.). After 24 h of anaerobic incubation, colony counts were performed.

To assess the in vivo effects of ultrasonic irrigation using S-PRG nanofiller dispersion, one beagle dog (female, aged 12–16 months, weighing approximately 10 kg) with experimental periodontitis was used. After general and local anesthesia [9], class-II furcation defects (each 5 mm in height, 3 mm in horizontal length) were created surgically in the furcations of premolar teeth (P2, P3, and P4); silicone impressions were placed in the furcations for 1 month to obtain experimental periodontitis (Fig. 1(B)) [9]. One week after removal of the impressions, clinical periodontal parameters were examined as described previously [9], including gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). The resulting values were considered the baseline (BL) measurements. After clinical examination, the root surfaces exposed in the periodontal pockets were irrigated ultrasonically at power 3 with a Piezon tip PS (with tracing of the tooth root) for 30 s (per tooth). Irrigation was performed with S-PRG nanofiller dispersion (Fig. 1(C)) (right-side molars) or with distilled water only (control; left-side molars). Clinical parameters were recorded continuously by one blinded examiner at 1, 4, 8, and 12 weeks after BL. No oral cleaning was performed during the observation period.

To investigate the periodontal pocket flora using next-generation sequencing, a sterile paper point was inserted into the periodontal pocket of P2 for 30 s at BL and at 12 weeks, and subgingival plaque was collected and stored at −80 °C until analysis. After DNA extraction using a Gene find v2 kit (Beckman Coulter, Brea, CA), the 16S rDNA V3-V4 region of the plaque bacteria was amplified using universal primers 341F-805R and sequenced at Genome-lead Co., Ltd. (Takamatsu, Japan) using an Illumina Miseq system (Illumina, San Diego, CA). The sequences were clustered into operational taxonomic units (97% identity threshold) and analyzed using the QIIME pipeline (v.1.9.1) [12]. Finally, homology searches were performed against two databases: Green genes (v.13.5) and the Human Oral Microbiome Database (v.14.5) [13].

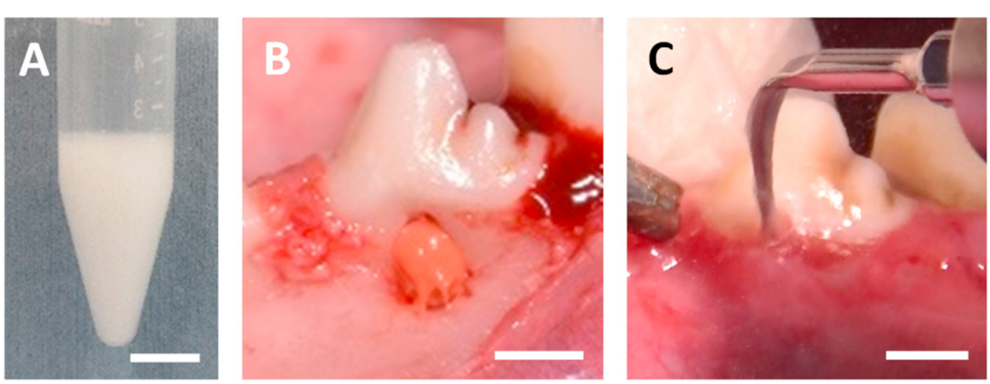

Fig. 1. (A) S-PRG nanofiller dispersion. Scale bar = 1 cm. (B) Creation of experimental periodontitis (injection of silicone impression). Scale bar = 5 mm. (C) Irrigation of periodontal pocket using S-PRG nanofiller dispersion and ultrasonic scaler system. Scale bar = 5 mm. S-PRG, surface pre-reacted glass-ionomer.

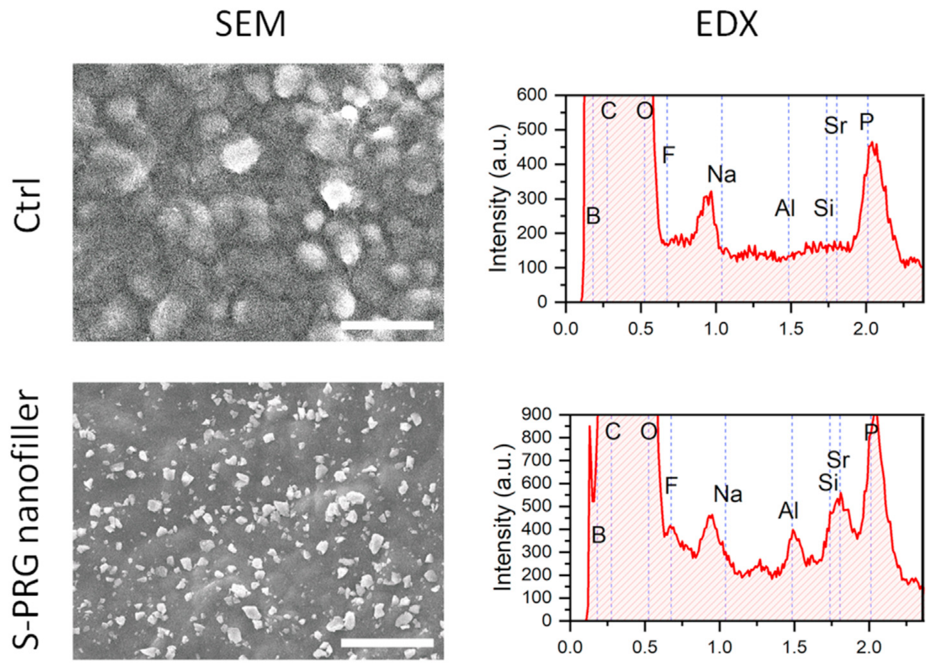

Fig. 2. Characterization of human cementum irrigated with S-PRG nanofiller dispersion SEM images and EDX intensity of control (unirrigated) and S-PRG nanofiller-irrigated cementum surface. Scale bar = 10 μm. EDX, energy dispersive X-ray spectrometry; SEM, scanning electron microscopy; S-PRG, surface pre-reacted glass-ionomer.

At 12 weeks, after clinical observation, scoring, and collection of plaque, the dog was subjected to general anesthesia, euthanasia, and perfusion-fixation with 10% buffered formalin. The teeth and surrounding tissues were collected, and bone defects were imaged using a microcomputed tomography (micro-CT; Latheta LCT-200; Hitachi, Ltd., Tokyo) to observe bone healing. Next, the tissues were demineralized with 10% ethylenediaminetetraacetic acid and paraffin-embedded by conventional procedures. Tissue sections were prepared by slicing thinly in the buccolingual direction, stained with Masson's trichrome (MT), and observed under a light microscope.

For each quantitative parameter, mean values and standard deviations were calculated, and two-tailed Mann-Whitney U test was used for statistical analysis using SPSS software package (SPSS

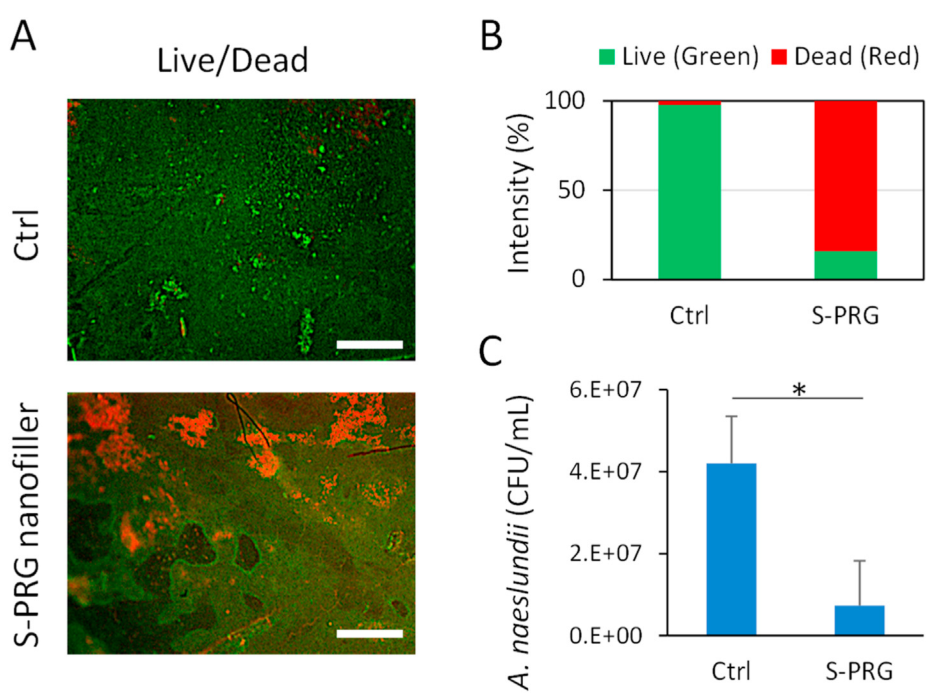

Fig. 3. Antibacterial properties of S-PRG nanofiller-irrigated cementum. (A) LIVE/DEAD BacLight staining of *A. naeslundii*. Scale bar = 500 μm. (B) Intensity of live (green) and dead (red) bacterial cells (n = 3, mean). (C) Bacterial counts of *A. naeslundii* (n = 3, mean + SD). *P < 0.05. Mann-Whitney U test. Ctrl, control; S-PRG, surface pre-reacted glass-ionomer; CFU, colony-forming unit.

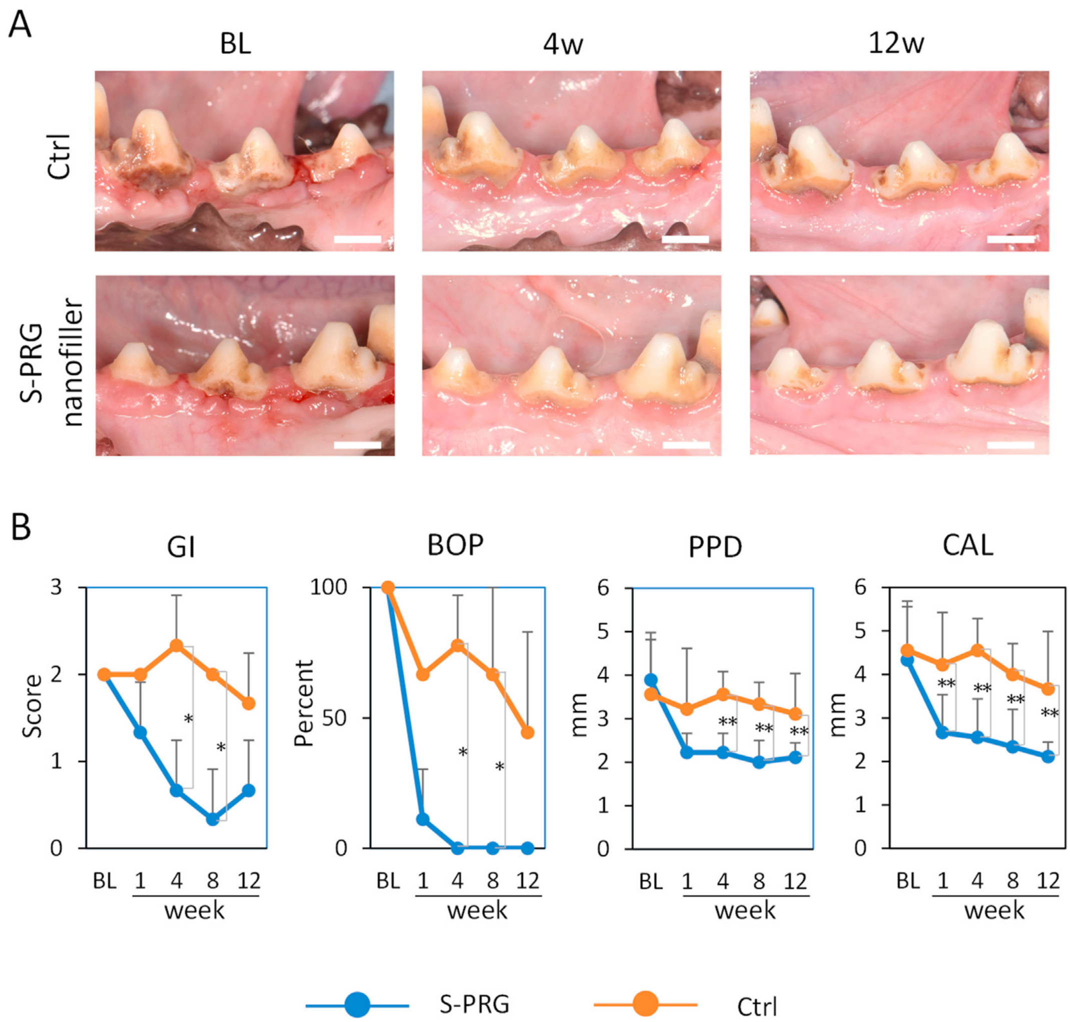

Fig. 4. ***In vivo* clinical examination.** (A) Digital photographs of gingival tissue at BL, 4-week (4w), and 12-week (12w) observation periods. Scale bar = 1 cm. (B) Periodontal parameters (mean value of three premolars + SD). *P < 0.05, **P < 0.01. Mann-Whitney U test. BOP, bleeding on probing; BL, baseline; CAL, clinical attachment level; Ctrl, control; GI, gingival index; PPD, probing pocket depth; S-PRG, surface pre-reacted glass-ionomer; w, week.

11.0; IBM Corporation, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

3. Results

Fig. 2 shows SEM images of human cementum surfaces irrigated ultrasonically with S-PRG nanofiller dispersion or distilled water (control). After irrigation with S-PRG nanofiller dispersion, fine particles frequently were observed on the cementum surface. In the EDX analysis, the main elements of S-PRG filler (including silica and strontium (which exhibit overlapping spectra), aluminum, and fluorine) were detected on the S-PRG nanofiller-irrigated cementum.

LIVE/DEAD BacLight staining of cementum surface showed that the irrigation using S-PRG nanofiller dispersion increased the number of dead (red-stained) bacteria (Fig. 3(A) and (B)). In addition, irrigation using S-PRG nanofiller dispersion significantly suppressed the number of bacterial colonies ($P < 0.05$, Fig. 3(C)), suggesting that the residual S-PRG nanofiller on the cementum exhibits bactericidal effects against *A. naeslundii*.

Clinical periodontal examination of the beagle showed that gingival redness persisted for 12 weeks on the control teeth (no application of S-PRG nanofiller dispersion), but had largely disappeared at 4 weeks on teeth irrigated ultrasonically with S-PRG nanofiller dispersion (Fig. 4A). Periodontal parameters showed that the application of S-PRG nanofiller dispersion at Week 0 resulted in significant decreases (compared to control) in GI, BOP%, PPD, and CAL (Fig. 4B). Bacterial species (phylum) ratios in the periodontal pockets are shown in Fig. 5. The phyla Bacteroidetes and Spirochaetes were observed at BL, and in the control (water-irrigated) teeth at 12 weeks. However, S-PRG nanofiller dispersion suppressed these periodontal pathogens to less than 0.1% at 12 weeks. Additionally, *Corynebacterium* and *Neisseria* were detected at higher percentages.

Evaluation by X-ray, micro-CT, and light microscopy of MT-stained images (Fig. 6) revealed bone and gingival tissue repair in the furcation defect region after ultrasonic irrigation using S-PRG nanofiller dispersion.

4. Discussion

In SEM images and EDX analysis of human cementum surfaces, fine particles were observed on the cementum surface, and the elements, such as silica, strontium, aluminum, and fluorine were detected. These elements reportedly are components of the S-PRG filler [4], suggesting that the fine particles observed on the

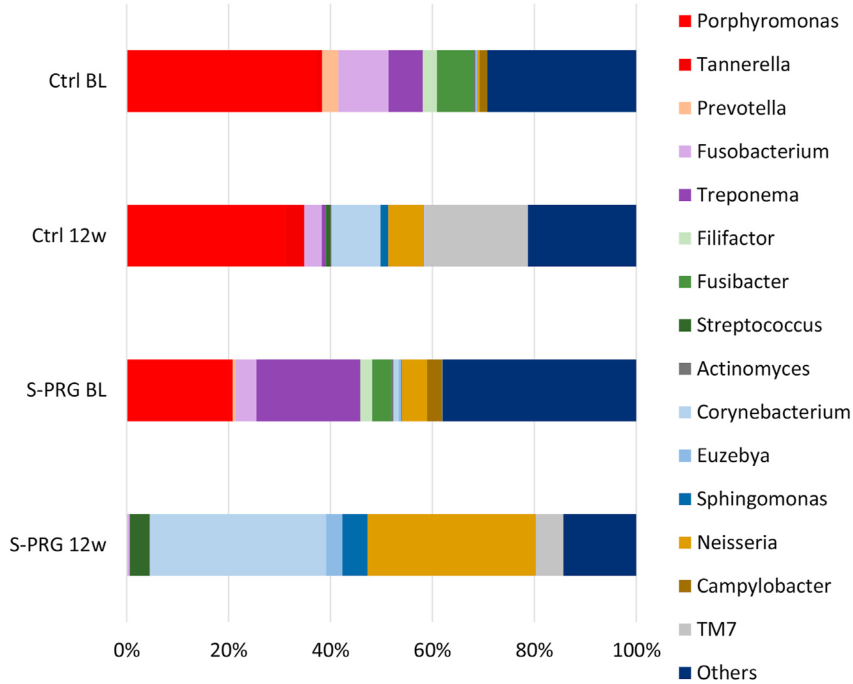

Fig. 5. Composition of microbiome of periodontal pocket at BL and 12 weeks. BL, baseline; Ctrl, control; S-PRG, surface pre-reacted glass-ionomer; 12w, 12 weeks.

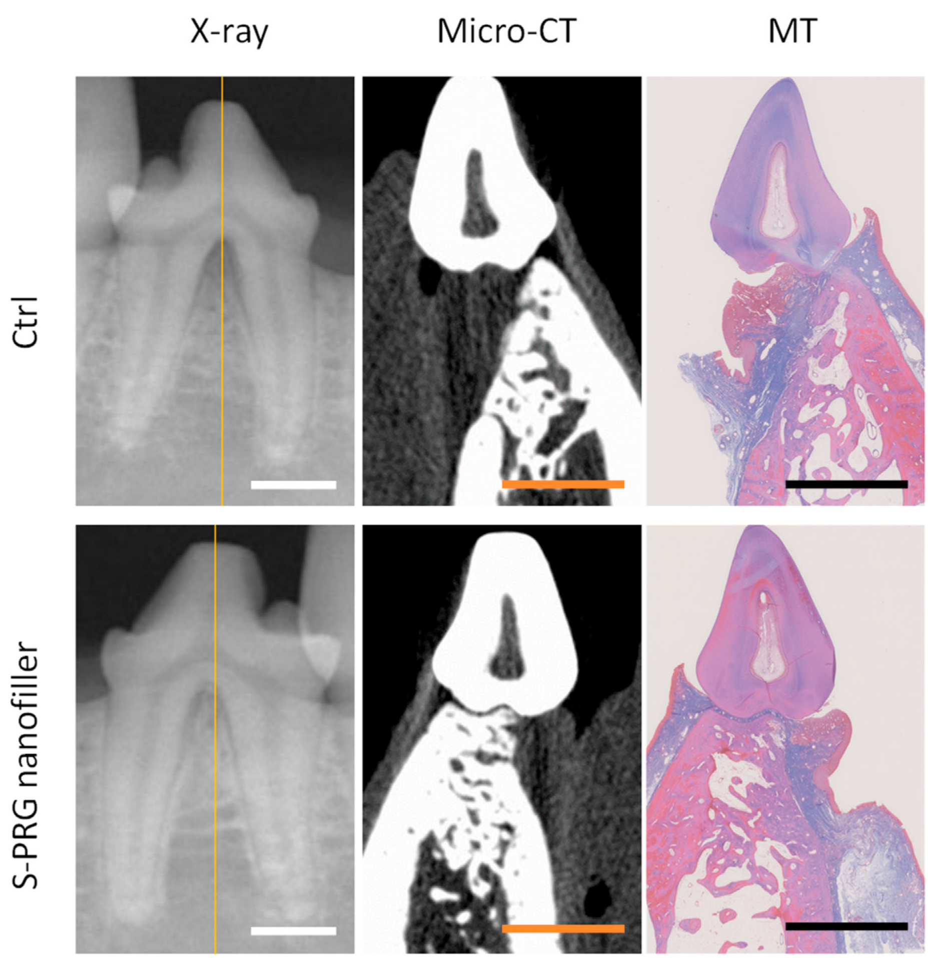

Fig. 6. X-ray, micro-CT, and histological analysis at 12 weeks. Micro-CT and MT images show the buccal-lingual cross section corresponding to yellow line in the X-ray images. Scale bar = 5 mm. Ctrl, control; CT, computed tomography; MT, Masson's trichrome; S-PRG, surface pre-reacted glass-ionomer.

cementum were S-PRG nanofiller. Mayumi et al. reported that S-PRG nanofiller is able bind to human dentin [9]; the present study further suggested that S-PRG nanofiller has the capacity to bind to human cementum, even when using an ultrasonic scaler device.

Irrigation using S-PRG nanofiller dispersion provided antibacterial effects (Fig. 3) and decreased the parameter values of experimental periodontitis (Fig. 4). In addition, S-PRG nanofiller dispersion suppressed the phyla Bacteroidetes (e.g., Porphyromonas) and Spirochaetes (e.g., Treponema), which contribute to periodontal disease [14], to less than 0.1% at 12 weeks (Fig. 5). In addition, *Corynebacterium* and *Neisseria*, known oral commensal bacteria [15,16], were detected at higher percentages, suggesting that the application of S-PRG nanofiller strongly affected the composition of the periodontal pocket microflora. Decreases in the presence of red complex periodontal pathogens, such as *Porphyromonas gingivalis* and *Treponema denticola*, play a key role in attenuating periodontal disease [14]. Thus, the present work represents the first report (to our knowledge) showing the potential of S-PRG nanofiller to modify the periodontal microflora. We speculate that the release of bacteriostatic or bactericidal borate and fluoride ions from S-PRG nanofiller may suppress the growth of periodontal pathogens [17,18]. Full sterilization of the oral cavity is difficult in practice, but the antibacterial effects of S-PRG nanofiller on the root surface may be effective in reconstructing the symbiotic biofilm in this environment [19].

Fig. 6 showed bone and gingival tissue repair in the furcation defect region by S-PRG nanofiller dispersion. Bacterial inflammatory parameters in periodontal tissue were largely eliminated by irrigation with S-PRG nanofiller, presumably facilitating the tissue healing process. Strontium ions released from S-PRG filler reportedly have anti-inflammatory effects [20]; S-PRG nanofiller therefore potentially provides complementary healing to furcation areas.

A limitation of the present study is that the sample size of the in vivo experiment (1 beagle) was small. Further experiments will be needed to elucidate the antibacterial relation between S-PRG nanofiller and periodontal microflora, in addition to the clinical efficacy and safety of this treatment. Nonetheless, we hope that S-PRG nanofiller irrigation using an ultrasonic scaler system may find wide use in clinical periodontal treatment.

5. Conclusions

Ultrasonic irrigation with S-PRG nanofiller dispersion using an ultrasonic scaler system could deliver S-PRG nanofiller to the cementum surface, thereby providing antibacterial effects. In support of this hypothesis, irrigation using S-PRG nanofiller dispersion in an in vivo model improved the parameters of experimental periodontitis and modified the composition of subgingival periodontal microflora.

Conflict of interest

The authors have no conflict of interest to declare.

Ethics approval

Human cementum was obtained from a patient attending Hokkaido University Hospital; this individual provided informed consent prior to sample collection. The protocol for the clinical study was reviewed and approved by the Hokkaido University Hospital Institutional Review Board for Clinical Research (Approval No. 17-222). In addition, the protocol for the animal study was reviewed and approved by the Animal Research Committee of Hokkaido University (Approval No. 17-93).

CRediT authorship contribution statement

Hirofumi Miyaji: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - review & editing. **Kayoko Mayumi:** Data curation, Investigation. **Yukimi Kanemoto:** Investigation. **Ichie Okamoto:** Investigation. **Asako Hamamoto:** Investigation. **Akihito Kato:** Investigation, Writing - review & editing. **Tsutomu Sugaya:** Writing - review & editing. **Tsukasa Akasaka:** Investigation, Writing - review & editing. **Saori Tanaka:** Investigation, Writing - review & editing.

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