Development of Redox Therapy for Periodontitis using Redox Injectable Gel

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ABSTRACT SUMMARY
The aim of this study was to investigate the possibility of Redox Injectable Gel (RIG) as a preventive and therapeutic drug for the periodontitis. We designed novel RIG as a specific ROS scavenger, used a rat model to determine the efficacy of RIG for protection against Porphyromonas gingivalis (Pg)-induced alveolar bone loss and gingival circulatory failure. Since the redox gel retained periodontal pocket for long term, the therapeutic efficiency improved significantly. One of the most important points of this therapy is to anticipate no or very less adverse effect to entire body because ROS-scavenging nitroxide radical groups were conjugated with matrix backbone, which thus does not interfere normal redox reaction inside cells.

INTRODUCTION
Reactive oxygen species (ROS) are considered to cause various disorders if they are produced in excess and become uncontrollable in the body. However, redox nanoparticle that effectively eliminates excess ROS has been developed and reported to be effective for the treatment of various systemic disorders. This particle is expected to be developed into drugs due to its high cost-performance, regional specificity, and few adverse effects. Therefore, to develop redox therapy for periodontitis, we designed novel RIG, which is formed after disintegration of the nano-assembled flower micelle at 37°C, allowing nitroxide radicals to locally act as a specific ROS scavenger, and evaluated inhibitory effects of RIG on Pg-induced bone loss in rat experimental periodontitis model, which has been reported to be related to ROS.

EXPERIMENTAL METHODS
【Redox Injectable Gel】
We designed novel RIG, which is formed after disintegration of the nano-assembled flower micelle at 37°C, allowing nitroxide radicals to locally act as a specific ROS scavenger (Redox +) (Fig. 1).

【Experimental Periodontitis】
4 weeks-old Sprague-Dawley rats were divided into 4 groups (n=6/group). Group A served as the Pg non-infected control, group B was infected orally with Pg, group C was administered RIG (-) extract in drinking water followed by Pg infection, and group D was administered RIG (+) extract in drinking water followed by Pg infection. The experimental procedures of this study were reviewed and approved by the committee of ethics on animal experiments of Kanagawa Dental University and were carried out under the guidelines for animal experimentation. Rats were infected orally with Pg ATCC 33277, which was suspended in 5% carboxymethylcellulose. Each rat, received 0.5ml (3.0 × 10^10 cells/ml) of the suspension by oral gavage at 8, 10, 12, 14, and 16 days.

【Gingival blood flow】
After the bacterial infection, six rats were extracted from each group and were examined a gingival blood flow. Gingival blood flow was measured the palatal and buccal gingiva by a laser Doppler flowmeter with a laser Doppler probe.
Alveolar bone loss was imaged by Microcomputerized tomography (Micro-CT). At horizontal bone loss around the maxillary molar, the distance from the cement-enamel junction (CEJ) to the alveolar bone crest (ABC) was measured at seven buccal sites per rat.

**Histological analysis**
The sections were stained with TRAP stain was performed in accordance with the manufactures instructions. Red-stained multinucleated (TRAP-positive) cells were defined as osteoclasts and examined under an optical microscope.

**RESULTS AND DISCUSSION**

**Measurement of alveolar bone resorption**
Alveolar bone loss was imaged by Micro-CT. These results suggest that RIG result in inhibition of Pg-induced bone loss (Fig. 2). The levels of bone loss were evaluated by the distance CEJ to ABC at the buccal area on the left side of maxilla. The level of bone loss was significantly lower in Control group and Pg + Redox (+) group than Pg group and Pg + Redox (-) group ($p < 0.05$).

**Gingival blood flow**
The gingival blood flow in Pg group and Pg + Redox (-) group decreased more than in Control group and Pg + redox (+) group, showed a remarkable reduction in gingival ($p < 0.05$).

**Histological findings**
We examined alveolar bone destruction caused by bacterial infection using TRAP staining, osteoclast forming resorption lacunae were observed in the interdental area of the upper second and third molar in Pg group and Pg + Redox(-) group. However, no notable microscopic changes in periodontal tissue were in control group and Pg + Redox (+) group ($p < 0.05$).

**CONCLUSION**
We evaluated inhibitory effects of RIG on Pg-induced bone loss in rat experimental periodontitis model. In this study, bone loss were evaluated by the distance CEJ to ABC at the buccal area and imaged by Micro-CT. The gingival blood flow was measured using laser Doppler flowmetry, and osteoclasts were evaluated with TRAP staining. These results suggest that RIG result in inhibition of Pg-induced bone loss, increase in gingival blood flow, and decrease in the number of osteoclasts. It can be anticipated as a novel therapeutic drug in the treatment on Pg-induced periodontitis.

**REFERENCES**