

Plasma Dental Treatment with the Reduced pH Method and the LF Plasma Jet ~ Disinfection of Dentin~

低 pH 法を用いたプラズマ歯科治療 ~感染歯質の殺菌~

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In the dental treatment, methods for sterilization of infected dentine have not been established yet. In the present study, we evaluated the bactericidal effect of low frequency atmospheric pressure plasma jets with low pH against *Enterococcus faecalis*, an oral pathogen causing incurable root-canal infection, using the dentine infection models. The results showed that the LF jet had bactericidal effects on *E. faecalis* in liquid with lower pH condition than 4.5 in one minute irradiation, and reached to the detection limit in 3-5 minutes. In the similar test using the infection model of hydroxyapatite pellets instead of dentine, the bactericidal effect was detected as 99.9%. With the infection model using the dentine slices of human molar teeth, the bactericidal effect of 99% was gained. These results indicate that LF jets might be applied to the clinical dentistry.

Introduction

The dental caries is a disease caused by demineralization with organic-acids metabolically produced by oral microorganisms, and the hard tissue of tooth is decayed. The therapeutic treatment is removing the infected enamel and dentine mechanically, and filling the cavity with dental materials or replacing the lost crown by prosthetic dentistry techniques. However, the methods for sterilization of infected dentine have not been established yet. If the removing of infected tooth is not enough or not adequate, it has been reported that some bacteria remain inside the dentinal tubules, and these remaining bacteria may subsequently lead to secondary caries. As the caries

progress, microorganisms gain entry into the dental pulp, leading to pulpal inflammation. Eventually, this requires a root canal treatment, but the success rate of root canal treatment is about 80%, apparently due to incomplete intra-canal sterilization and obturation. Hence, in order to obtain better results with caries and root canal treatment, it is necessary to invent new methods that can completely sterilize the infected dental tissues. We have already studied about the bactericidal effect on *Streptococcus mutans*, a major cariogenic bacteria [1]. In this study, we evaluated the bactericidal effect of low frequency atmospheric pressure plasma jets with low pH against *E. faecalis*, an oral pathogen causing incurable root-canal

infection, using the dentine infection models.

Materials and methods

Preparation of dentine slices

Dentine slices were prepared from human molar tooth by cutting into 2mm thick with micro-cutter. The surfaces of slices were polished with water-resist polishing paper #1000 and treated with ultrasonication. The hydroxyapatite pellet was high temperature calcinations to gain the same density as dentine. The slices and pellets were sterilized by autoclave and kept in water until to next step.

Sterilization test

E. faecalis (ATCC19433 strain) was cultured and adjusted to $10^5 \sim 10^6$ CFU/ml and 50 μ l of this microbial suspension was added to 450 μ l of citrate buffer (2.0mM) making a total volume of 500 μ l of the suspension liquid in 24 well plate. *E. faecalis* was also adjusted to 10^7 CFU/ 10μ l in BHI broth and inoculated onto the surfaces of slices or pellets for overnight at 37 °C. After slight washing with distilled water, the slices and pellets were immersed in citrate-Na buffer of pH3.5 and 4.5. The liquid of suspension or containing immersed slices and pellets were exposed to LF jet (Gas: Helium, Flow rate of Helium gas: 2.00l/min, High voltage: 7 kV, Low frequency: 16 kHz, consumption of electrical power: 22VA, inner and outer diameters of the glass tube, 4mm and 2mm).

After irradiation, 100 μ l of suspension was plated on the agar plates and the number of live cells was determined by the colony forming unit (CFU) assay.

After removing buffer from slices or pellets, 100 μ l of RPMI 1640 medium added by Alamar Blue (TREK Diagnostic Systems, CA, USA) as the redox indicator and aerobically incubated at 37°C for 6-8 hours, and the fluorescence was measured with a wavelength of Ex 530nm, Em : 590nm.

Results and Discussions

Effects of plasma irradiation on bacterial suspensions in the liquid phase

As shown in Fig.1, after irradiation from 2 to 3 minutes at pH 6.5 and in comparison with the control, there was a gradual reduction in viable cells which was not statistically significant. A 5-minute irradiation resulted in a significant reduction ($p < 0.001$). No viable cells could be detected at pH 4.5 or 3.5, for 2 and 3 minute irradiation times. When compared with the control, there was a significant difference for irradiation times from 1 to 5 minutes ($p < 0.001$). For *E. faecalis*, the D values at pH 3.5, 4.5 and 6.5 were calculated to be 0.30, 0.47 and 2.00, respectively.

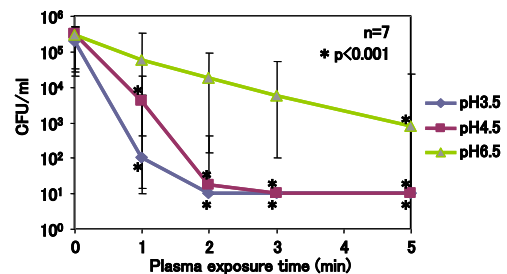


Fig. 1 CFU assay of *E. faecalis* in various pH solutions.

Effects of plasma irradiation on inoculated dentin models

As shown in Fig2, in the results of tests using the infection model of hydroxyapatite pellets instead of dentine, the bactericidal effect was detected as 99.9%. With the infection model using the dentine slices of human molar teeth, the bactericidal effect of 99% was gained.

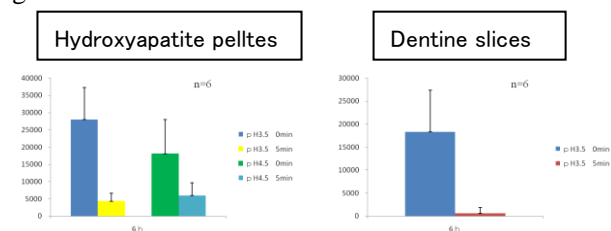


Fig2. REDOX indicator assay after plasma irradiation on dentine infection models

These results indicate that LF jets might be applied to the clinical dentistry to sterilize dentine tissues.

[1] H.Yamazaki, T.Ohshima, Y.Tsubota,

H.Yamaguchi, A.Jayawardena Y.Nishimura:
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