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Sterilization Effect of Plasma Using the Reduced pH Method for Treatment of Dental Diseases (Dental Caries and Root Canal Infection)
歯科疾患（う蝕および感染根管）の治療のための低pH法を用いたプラズマ殺菌効果

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The control of infectious microorganisms in dentistry is extremely important, but a sterilization method of dentin with its complicated fine and meshed structure is not yet established. Therefore we began examinations whether plasma is effective for sterilization of infected tooth tissue. We could confirm a sterilization effect of microbial suspensions *in vitro* with an atmospheric-pressure-low-temperature-plasma (LF jet) using the reduced pH method [1]. We tested and succeeded in the almost complete sterilization of a dental caries model with LF jet and an infected root canal model by using plasma treated water.

1. Introduction

In clinical dentistry, control of infectious microorganisms is extremely important, but a sterilization method of infected dentin with its complicated fine and meshed structure is not yet established.

Cariogenic bacteria metabolically produce acid after sugar uptake by food into the oral cavity. Dental caries occurs as a consequence of de-mineralization by this acid; subsequently the tooth structure with hard tissue of enamel and dentin collapses.

In dental treatment, infected dentin is removed as much as possible using drilling devices, then the cavity is filled with dental material. However, incomplete removal of the infected tooth tissue causes recurrence, on the other hand excessive cutting damages the pulp.

When caries reaches the pulp, the infection cannot be suppressed by immunological reactions but will induce inflammation and necrosis. A severe inflammation causes apical infection and abscess. In this case the infected pulp tissue must be completely removed and endodontic therapy is necessary.

A systematic review of 2007 and 2008 journals pointed out that, with the present technique of endodontic therapy, 20 to 30% of post-treatment patients had recurrent symptoms. Moreover, it was reported that powerful disinfectants lead to harmful incidents caused by residual toxicity.

There is a requirement for a novel sterilization technique besides chemicals, one which is highly effective and safe. Therefore, we started examination regarding the effectiveness of plasma sterilization on infected dentin and root canals. In general, plasma sterilization in liquid phase is difficult because direct irradiation of bacteria is not possible. However, we could confirm the effectiveness of plasma with the reduced pH method *in vitro* [1, 2]. In this study, we examined the sterilization effect on a dental caries model and on a root canal infection model.

2. Sterilization effect on the infected tooth tissue model

The infected tooth models were made by using baked hydroxyapatite, the main mineralized tooth structure, or human extracted tooth slices. *Streptococcus mutans* (cariogenic bacteria) and *Enterococcus feacalis* (a representative pathogen of refractory root canal infection) were inoculated at 10^5-10^7 CFU/10μl on hydroxyapatite pellets or dentin slices and cultured to make infected tooth models.

Those models were bathed in 500μl of citrate-Na buffer and the surface irradiated for 3-5 min with LF jet. Determination of living bacteria using fluorescent redox indicator was carried out. The result indicated that plasma jet had bactericidal activity in the order of 10^3-10^5CFU-reduction in both models (Fig. 1).

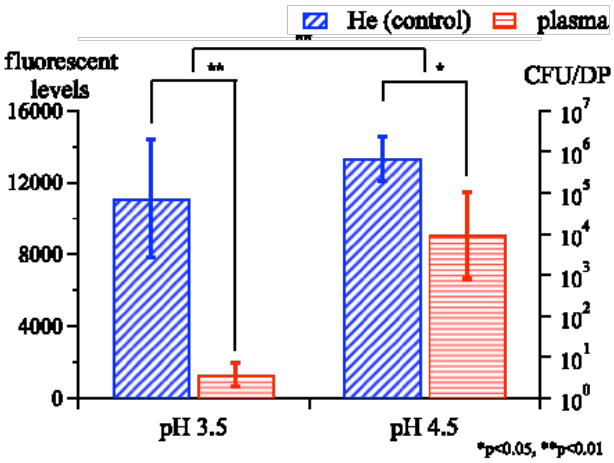

Fig. 1. The fluorescent level and CFU of *S. mutans* before and after plasma irradiation on infected dentin slices

3. Sterilization effect on the caries tooth model

An extracted human molar was drilled to make a cavity and inoculated with *S. mutans* every day for one week. Then the tooth cavity was filled with buffer of pH 3.5 or 6.5 and irradiated with LF jet (Fig. 2). The dentin was collected before and after irradiation, and cultured on an agar plate as a CFU assay. The result showed that CFU of *S. mutans*, recovered from the caries cavity, was reduced below the detection limit after irradiation for 3 min only at pH 3.5.

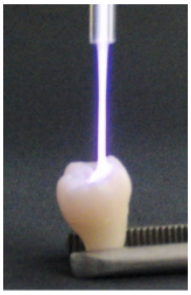

Fig .2. LF jet irradiation on the caries tooth model

4. Sterilization effect on the infected root-canal tooth model

Microbial suspensions of *E. faecalis* or *C. albicans* of 10^4-10^5 CFU/10μL were inoculated into the root canal of extracted human teeth and cultured for one day. The root canal was washed with distilled water once, then filled with buffer of pH 3.5 and irradiated with plasma. The remaining cells were collected with a paper point and cultured in broth for 48hrs as a turbidity test. Unfortunately, most of the samples were turbid, and an adequate sterilizing effect could not be confirmed.

We thought the reasons were insufficient diffusion or convective flow of gas and liquid due to the narrow area in a root canal.

Therefore, we used plasma-treated water (PTW), which is pure water irradiated with plasma. It is anticipated to contain plasma-generated radicals, which are delivered to the deep area of the root canal. PTW was prepared by irradiation of pure water with plasma jet for several minutes and cryopreserved. The disinfection property of PTW was confirmed by bacterial suspension culture of low pH.

The success rate (sterilizing effect) using undiluted plasma treated water was 100%. A success rate of 85.7% was achieved with a 60% concentration of PTW, and a 30% concentration led to a success rate of 60% (Fig. 3). This showed the sterilization effect of plasma treated water in a concentration -dependent manner.

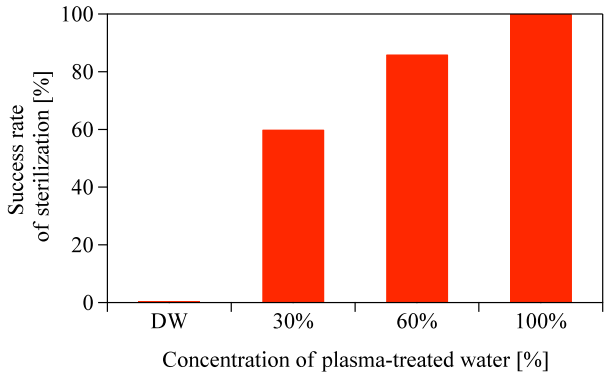

Fig. 3. Evaluation of the sterilization effect of PTW used for infected root canal models

5. Conclusion & Discussion

Use of LF jet and PTW by the reduced pH method was significantly effective to safely sterilize infected tooth and root canal models. Especially, PTW has the properties of immediate inactivation at body temperature and a high sterilizing effect. In other words, a clinical application in dentistry for a disinfectant without residual activity is considered possible and desirable. Therefore, plasma can be anticipated as a novel technique of sterilization in dental treatment. For verification of the effectiveness *in vivo*, further trials using animal models should be conducted.

References

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