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New Dental Drug Delivery System for Removing Mutans Streptococci from the Oral Cavity: Effect on Oral Microbial Flora

Hiroaki Takeuchi¹, Hidenobu Senpuku¹, Khairul Matin¹, Noboru Kaneko¹, Noriko Yusa¹, Eiji Yoshikawa², Hirohisa Ida², Susumu Imai¹, Tosiki Nisizawa¹, Yasuhito Abei¹, Yoshiharu Kono³, Takuji Ikemi³, Yoshihiro Toyoshima⁴, Kazuo Fukushima^{1,5} and Nobuhiro Hanada^{1*}

¹Department of Oral Science, National Institute of Infectious Diseases, Tokyo 162-8640,

²BML Co. Ltd., Tokyo 151-0051,

³Department of Operative Dentistry, Nihon University School of Dentistry at Matsudo, Chiba 271-8587,

⁴Dai-ichi Mutual Life Insurance Company Hibiya Medical Center, Tokyo 100-8411 and

⁵Department of Microbiology, Nihon University School of Dentistry at Matsudo, Chiba 271-8587

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Dental caries is caused by mixed infection of pathogenic indigenous microbiota and biofilm formation (1). *Streptococcus mutans* produces biofilm on the tooth surface, leading to the development of dental caries. Various approaches have been developed for removing *S. mutans* from the oral cavity in order to prevent dental caries (2-5). The present study examined effect of a dental drug delivery system (3DS) using 0.2% chlorhexidine (CHX) on *S. mutans* and other oral bacterial flora.

The 3DS consists of individual trays, called Drug Retainer, that fit onto and deliver small amount of anti-bacterial drugs (in high concentration) to the tooth surfaces. This system is easy to manipulate and keeps the drugs in direct contact with the tooth surface without being diluted by saliva so that the bacteria on the tooth surface, but not on the oral mucosa, are killed. The anti-microbial drug used was CHX which is widely accepted as an effective antiseptic in dentistry (5). Before application of 3DS, dental biofilms which prevent the penetration of anti-microbial agents (6) were removed by means of Professional Mechanical Tooth Cleaning (PMTc). The 3DS was applied for 5 min twice a day (morning and afternoon). In addition, the oral cavity was rinsed with 10 ml of 0.2% CHX gluconate mouthwash (Corsodyl; ICI, Maccksfield, England) for 1 min after lunch every day. The treatment was continued for 9 days.

Seven healthy human volunteers having *S. mutans* on the tooth surface participated in the study. Among them four

subjects (A, B, C, and F, see below) showed no identifiable recolonization by *S. mutans* on the tooth surface 60 days after the end of the treatment.

The effect of the treatment on the oral flora was examined as its perturbation may have adverse effects. Plaque and saliva samples were taken before the trial and at 1, 24, and 60 days after the treatment. Plaque samples were collected from the upper right second premolar and first molar teeth by rubbing with a cotton swab postero-anteriorly along the buccal surface. The samples were transferred into 1 ml of reduced transport medium (0.4% agar, 0.15% thioglycolate/phosphate buffered saline) in sterile tubes (SEEDSWAB No. 1, Eiken Chemical Co., LTD., Tokyo). At the same time, saliva secreted over a period of 3 min, while being stimulated by biting paraffin gum, was collected into ice-chilled sterile bottles. The sample was then transferred by using a cotton swab into the transport medium and 4 ml of Brain Heart Infusion (BHI) broth. The BHI broth containing the plaque or saliva samples was sonicated by ultrasonic dispersion (60 W power output) for 10 seconds, and poured onto blood agar plates or Mitis-Salivarius agar plates containing 0.02 M bacitracin (MSB), respectively, in a volume of 50 μ l by using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo). After anaerobic culture for 48 h, total bacterial colonies on the blood agar and total *Streptococci* colonies on the MSB plates were counted. *S. mutans* could be identified by their characteristic appearance.

The number of colonies of total *Streptococci* before the treatment in all the subjects ranged from $10^{6.5}$ to 10^8 cfu/ml (Fig. 1A) in the plaque samples. After the PMTC and 3DS

*Corresponding author: Fax: +81-3-5285-1172, E-mail: nhanada@nih.go.jp

treatment, the total *Streptococci* decreased to less than $10^{6.5}$ cfu/ml in 1 day, but recovered at least partially in 24 days and

completely in 60 days (Fig. 1A). Similar curves were obtained for the total bacteria (Fig. 1B). The effect on *Streptococci* (Fig. 1C) or on the total bacteria (Fig. 1D) in the saliva samples was minimal.

In conclusion, the 3DS in combination with PMTC which selectively killed *S. mutans* on the tooth surface is considered safe in that the oral flora is minimally perturbed.

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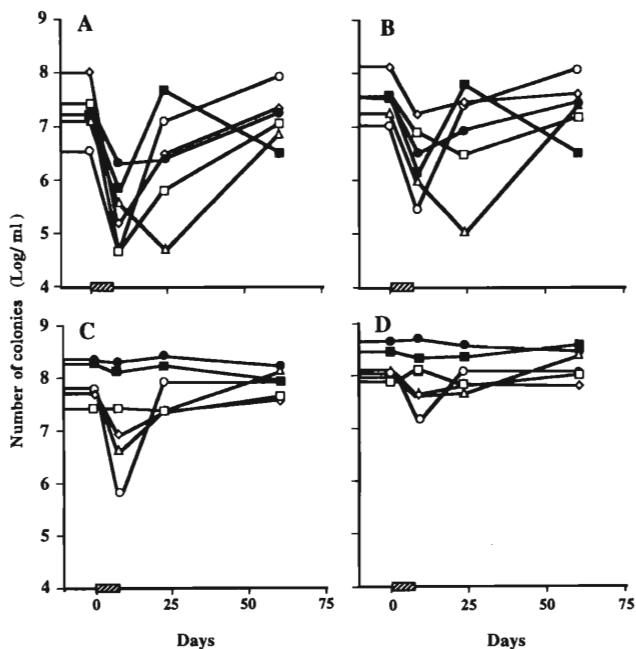


Fig. 1. Effect of 3DS combined with PMTC on bacterial flora in the oral cavity. A: total *Streptococci* on the tooth surface. B: total bacteria on the tooth surface. C: total *Streptococci* in saliva. D: total bacteria in saliva. □: subject A. ◇: subject B. ○: subject C. △: subject D. ●: subject E. ■: subject F. ▨: period of treatment. The data on subject G was omitted due to a technical failure.