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Effect of $ZnCl_2$ on plaque growth and biofilm vitality

Haijing Gu [a,b], Danni Fan [a], Jinlong Gao [c,e], Wei Zou [c,e], Zhixiang Peng [a], Ziming Zhao [d], Junqi Ling [a,*], Racquel Z. LeGeros [b,**]

[a] Guanghua School of Stomatology, Sun Yat-sen University, 56 Ling Yuan Xi Road, Guangzhou 510055, China
[b] Department of Biomaterials & Biomimetics, New York University College of Dentistry, NY, United States
[c] Faculty of Dentistry, University of Sydney, NSW, Australia
[d] Guangdong Provincial Institute of Traditional Chinese Medicine, 60 Heng Fu Road, Guangzhou 510095, China

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of $ZnCl_2$ on plaque-growth and vitality pattern of dental biofilm and to determine the optimum zinc concentration for the inhibition of plaque formation.

Design: Data were collected from nine volunteers for whom a special-designed acrylic appliance was prescribed after a careful dental check up. The volunteers rinsed twice daily for 2 min with $ZnCl_2$ of 2.5, 5, 10, 20 mM as treatment and double distilled water (DDW) as control in respective assigned test weeks. The plaque index (PI) was assessed after 48 h of appliance wearing. The glass discs with the adhered biofilm were removed from the splints and stained with two fluorescent dyes. The biofilm thickness (BT) and bacterial vitality of the whole biofilm as well as the mean bacterial vitality (BV) of the inner, middle and outer layers of biofilm were evaluated under confocal laser scanning microscope (CLSM).

Results: PI, BT and BV of biofilms treated by various concentrations of $ZnCl_2$ were reduced significantly when compared with the DDW group ($p < 0.05$). PI, BT and BV of the 2.5 mM $ZnCl_2$ group was significantly higher than groups of 5, 10, 20 mM $ZnCl_2$ ($p < 0.05$). The mean BV of the 3 layers (inner, middle and outer layers) showed that 2.5 mM $ZnCl_2$ was the lowest concentration to inhibit BV in the outer layer, 5 mM was the lowest concentration to extend this inhibition of BV to the middle layer, and none of the concentrations investigated in this study has shown any effect on bacteria inhibition in the inner layer.

Conclusion: Zinc ions exhibited possible inhibitory effects on plaque formation, and have a promising potential to be used as an antibacterial agent in future dentifrices and mouthrinses.

1. Introduction

Dental caries remains prevalent at plaque-retention sites despite various caries control treatments. For caries incidence to be decreased in high-caries-risk patients, additional antimicrobial treatments such as chlorhexidine rinses, high concentration fluoride gels, or varnishes are advised. As a antimicrobial component in some toothpastes, zinc has been shown to inhibit crystal growth by binding to the surfaces of solid calcium phosphates.[1] The concentration of zinc ions affects the types and amounts of formation of calcium phosphate crystals.[2] $ZnCl_2$ in mouthrinse was shown to function as an effective calculus control agent reducing the

* *Corresponding author*. Tel.: +86 2083880049; fax: +86 2083822807.
** *Corresponding author*. Tel.: +1 2129989580; fax: +1 2129954244.
E-mail addresses: Lingjq@mail.sysu.edu.cn (J. Ling), rzl1@nyu.edu (R.Z. LeGeros).
[e] These authors contributed equally to this study.
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formation of calculus significantly.[3–5] Previous clinical studies have examined the effects of dentifrices formulated with zinc ions on reducing the bacterial load of the dental plaque.[4,6] However, the microbiological efficacy of zinc ion on bacteria in different layers of the oral biofilm remains unknown.

The concentrations of zinc in the dentifrice vary in different studies. Santos et al.[5] reported that a 2.0% zinc citrate dentifrice significantly reduced supragingival calculus deposits, and a 0.5% zinc citrate had a similar effect in a three-month study.[7] However, Scruggs et al.[8] reported that a 0.5% zinc citrate dentifrice did not have the effect to reduce supragingival calculus. In this study, we explored the effects of zinc *in situ* biofilm at different layers with intra-oral devices which enable complete protection of the samples. This research can help us to understand how plaque is regulated and prevented by zinc as an inhibitor, and give directions on the concentration of zinc in anti-microbial agents.

There are several ways to study the effects of antimicrobial agents on plaque flora. Traditionally, scanning and transmission electron microscopes (EM) were employed to study biofilm composition and structure. Recently, confocal laser scanning microscopy (CLSM) with special staining techniques was widely employed in biofilm studies. Compared to EM, sample preparation of CLSM avoids dehydration and fixation during sample preparation which enables non-destructive visualizations of biofilms *in situ*.[9–11] To precisely mimic the biofilm formation *in vivo*, intra-oral devices were utilized to grow the biofilm on the supporting substrate.[12–15] Unlike *in vitro* studies, intra-oral devices for the biofilm study enable complete protection of the samples whilst worn by participants and during processing.

The aims of the present study were to investigate the effects of zinc ions on the dental biofilm formation *in vivo* using the CLSM combined with vital fluorescent technique and to optimize the concentration of zinc for inhibition of plaque formation by *in vivo* studies.

2. Materials and methods

2.1. Surveys

Nine volunteers (5 females and 4 males) aged between 25 and 42 years (average 29.7 years) were recruited from medical and dental faculty. Each participant reviewed and signed the informed consent form before taking part in this study. Selection criteria for volunteers include a full dentition, healthy gingiva and no clinically detectable caries. Exclusion criteria include: (1) any sign of destructive periodontitis or inflammatory symptoms; (2) history of antibacterial mouthwashes or antibiotics use in the last half year; (3) the usage of fixed or removable orthodontic appliances or partial/complete dentures; (4) known allergic reaction to the ingredients of mouthrinse; (5) systemic diseases, pregnancy, and lactation.

2.2. Test solution preparation

Test solutions containing various concentrations of $ZnCl_2$ (Fisher Scientific, USA) were freshly prepared in DDW. The pH value of the solutions was 6.1 ± 0.1. The DDW served as a negative control.

2.3. Biofilm sample collection

Prior to each test period, scaling and polishing were performed on all volunteers to remove dental plaque, calculus and stain. Individual clear acrylic appliance (Fig. 1) was then placed in the upper jaw of each volunteer over a 48-h period. Three sterilized glass discs (3 mm in diameter, 1.5 mm in thickness and free surface energy) were bonded with self-curing resin towards the interdental area between two adjacent molars or premolars to avoid disturbing the biofilm growth by the tongue or cheek. The positions of the glass discs were chosen to mimic approximal plaque formation. During the experimental process, all subjects maintained their regular diet and kept wearing the appliances (intra-orally). During meals and daily mechanical oral hygiene, the appliances were stored in sterile physiological saline solution. All subjects can only brush with DDW and floss with fluoride-free dental floss within the experimental 48-h period.

2.4. Zinc ion treatment and plaque measurement

The study design was a five-period cross-over double-blind randomized clinical trial. Five types of solution of DDW (as a control), 2.5, 5, 10 and 20 mM $ZnCl_2$ were coded randomly by a dentist. (Types of solution treatments would be decoded after statistical analysis.) At the first treatment, each subject randomly drew a code from one of the five solutions, and rinsed mouth with the corresponding solution as twice a day (morning and evening) for 2 days. When rinsing the mouth, each subject used 10 ml of the allocated mouthwash to rinse for 2 min each time whilst wearing the splint. The subjects were allowed for at least 10 days of routine mechanical oral hygiene with same toothpaste between each of 5 treatment periods to render a normal oral environment. Each individual was subject to all five different treatment solutions in a random sequence. Adverse responses were systematically recorded by a dentist after each treatment.

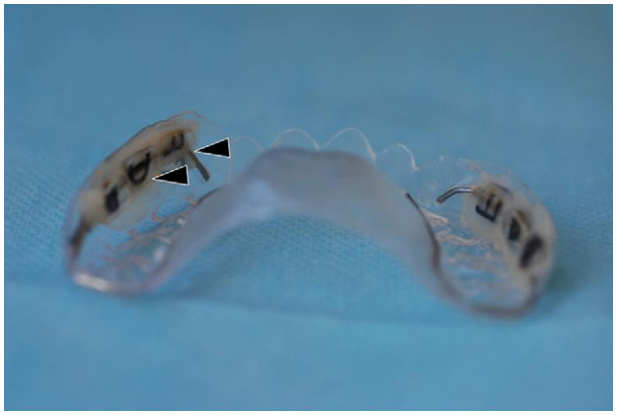

Fig. 1 – Two arrowheads show the special-designed elastic splint appliance placed in the upper jaw. Six circle glass discs (3 mm in diameter, 1.5 mm in thickness) were bonded at the interdental area between posterior teeth.