ARCHIVES OF ORAL BIOLOGY 60 (2015) 1059–1065

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

# Antimicrobial effects of a bioactive glass combined with fluoride or triclosan on *Streptococcus mutans* biofilm

Yu-Ting Xu [a,b], Qiong Wu [a,b], Ya-Ming Chen [a,b,*], Roger J. Smales [c,**], Shu-Ya Shi [a,b], Meng-Ting Wang [a,b]

[a] Jiangsu Key Laboratory of Oral Diseases, Nanjing Medical University, Nanjing, China
[b] Department of Polyclinics, Affiliated Hospital of Stomatology, Nanjing Medical University, Nanjing, China
[c] School of Dentistry, Faculty of Health Sciences, The University of Adelaide, Adelaide 5005, SA, Australia

ARTICLE INFO

*Article history:*
Accepted 16 March 2015

*Keywords:*
Bioactive glass
Bioglass
Antimicrobial effect
Biofilm

ABSTRACT

*Objective:* The objective of this study was to investigate the antibacterial effects on a cariogenic biofilm of a bioactive glass (BAG) combined with either sodium fluoride (NaF) or triclosan (TCS).

*Design:* According to minimal bactericidal concentrations, 37.5 mg/ml of BAG, 4.69 mg/ml of NaF, and 15.53 μg/ml of TCS solutions were prepared. When used alone, the three antimicrobial solutions were increased to double-dosage strength (2MBC). The study contained the following experimental groups: group 1, BAG (2MBC); group 2, NaF (2MBC); group 3, TCS (2MBC); group 4, BAG + NaF; group 5, BAG + TCS; group 6, control (saline). *Streptococcus mutans* biofilm was cultured with 0.1% sucrose anaerobically on 66 sterilized coverslips ($1 \times 1$ cm$^2$) for 24 h uninterrupted. After 10 min of exposure to the experimental groups, the microbial kinetics, morphology, and viability of the *S. mutans* biofilms were assessed by evaluation of colony-forming units (CFUs), scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM).

*Results:* BAG (2MBC) used alone showed significantly stronger antibacterial effects than the other two antimicrobials used alone. The combination groups also displayed the same or greater biofilm inactivation effects as BAG (2MBC) in the plate count test. SEM showed smaller stacks (towers) and fewer surrounding bacteria in groups BAG (2MBC), BAG + NaF, and BAG + TCS. Confocal microscopy also determined higher live/dead ratios in groups NaF (2MBC), TCS (2MBC), and control than in groups BAG (2MBC), BAG + NaF, and BAG + TCS.

*Conclusions:* The combinations of BAG with either NaF or TCS enhanced the inactivation effects of BAG (2MBC) on *S. mutans* biofilm, and these findings should be further investigated clinically for the control of dental biofilms.



* Corresponding author at: Department of Polyclinics, Affiliated Hospital of Stomatology, Nanjing Medical University, 136 Hanzhong Road, Nanjing 210029, China. Tel.: +86 25 85031832; fax: +86 25 86516414.
** Corresponding author.
E-mail addresses: yaming_chen@qq.com (Y.-M. Chen), roger.smales@adelaide.edu.cn (R.J. Smales).
http://dx.doi.org/10.1016/j.archoralbio.2015.03.007
0003–9969/

## 1. Introduction

Virulent biofilms firmly attached to tooth surfaces are the prime biological factor associated with dental caries,''[1] which is the most prevalent and expensive-to-treat oral infectious disease worldwide. Traditional biocides such as chlorhexidine, metals, sodium fluoride (NaF), and triclosan (TCS) have been shown to inhibit oral planktonic bacteria.[2–4] More recently, researchers have also investigated the remineralization and antiseptic actions of a bioactive glass (BAG).[5–8] The combination of two or three agents could improve their antibacterial effectiveness and, when added to BAG, $Ag_2O$ has been shown to boost the antimicrobial action of BAG without compromising its bioactivity.[9] Thus, the combinations of traditional biocides and BAG could be a new research approach. However, in most previous studies, bacteria were observed in the planktonic mode, with little known about the effects of BAG on cariogenic biofilms.

Therefore, we aimed to compare the antibacterial effects of BAG 45S5 and two traditional agents (NaF and TCS) used either alone or in combination (BAG with either NaF or TCS) on a cariogenic biofilm model.

## 2. Materials and methods

### 2.1. Minimal inhibitory concentration and minimal bactericidal concentration determinations for BAG, NaF, and TCS against Streptococcus mutans

A more recently available BAG powder (45S5; Datsing Bio-Tech Co. Ltd, Beijing, China) was investigated. According to the manufacturer, the maximum grain size is <90 μm, and the major ingredients are $SiO_2$, $Na_2O$, CaO, and $P_2O_5$. Laboratory standard BAG (purity: 100%), NaF (purity: 98%; Sinopharm Chemical Reagent Co. Ltd, Shanghai, China), and TCS (purity: 97%; Aladdin, Los Angeles, CA, USA) were diluted in distilled water for the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determinations. Lyophilized stocks of *Streptococcus mutans* (ATCC 25175) were provided by the China General Microbiological Culture Collection Center (Beijing, China) and reconstituted on brain–heart infusion (BHI) agar plates in an anaerobic chamber (Bugbox; Ruskinn, Bridgend, UK) filled with 80% $N_2$, 10% $H_2$, and 10% $CO_2$ at 37 °C. The new generation of bacteria was created by inoculation of fresh BHI broth in test tubes with some of the bacterial colonies.

A macrodilution method[10] was used for the determination of MIC. With twofold dilution, the concentrations of BAG, NaF, and TCS were 150 (~1.17 mg/ml), 75 (~0.59 mg/ml), and 250 (~1.95 μg/ml), respectively (eight concentrations each group). When using the macrodilution method, the minimum concentration of limpid liquid observed by the naked eye determined the MIC value. The MBC was assayed by growth tests of the bacterial colonies in the suspensions (100 μl each) of MIC and higher concentrations. The colonies were counted after anaerobic incubation for 48 h. A 99.9% decrease determined the MBC of BAG (45S5) against *S. mutans*. The experiments were performed in triplicate.

### 2.2. Antimicrobial effects on immature biofilm bacteria

The microorganisms were first diluted to the 0.5 McFarland turbidity standard (approximately $1.0 \times 10^8$ colony-forming units (CFU)/ml), and then the solution was further diluted with BHI broth to the ratio of 1:100 giving a concentration of $1.0 \times 10^6$ CFU/ml. Finally, 0.1 ml of solution ($1.0 \times 10^6$ CFU/ml) was incubated with 0.1 ml of sucrose and 1.5 ml of broth (approximately $1.0 \times 10^5$ CFU/ml). Biofilms were formed on 66 sterile coverslip pieces ($1 \times 1\ cm^2$) placed in three sterile 24-well polystyrene cell culture plates (Costar 3524; Corning, Tewksbury, MA, USA) with broth, sucrose, and *S. mutans* in the volumes described above. The cell culture plates were incubated without interruption in the anaerobic chamber for 6, 12, and 24 h at 37 °C.[11]

To assay the biofilm attached on the coverslips, liquid from three cell culture plates was removed separately after 6, 12, and 24 h. Then the coverslips were gently washed twice with phosphate-buffered saline (PBS) (NaCl, KCl, $Na_2HPO_4$, $KH_2PO_4$, pH = 7.0) at 1 drop/5 s. The concentrations used for each antimicrobial agent tested were based on the MBC data. The concentrations of each agent were doubled (2MBC) when used alone. The concentrations of the antimicrobials were 75 mg/ml of BAG (group 1), 9.38 mg/ml of NaF (group 2) and 31.25 μg/ml of TCS (group 3). Further, when used in combination, the concentrations were halved at 37.5 mg/ml of BAG combined with either 4.69 mg/ml of NaF (group 4) or 15.53 μg/ml of TCS (group 5).

Thirty-six coverslips were divided into six groups and three time points. The six coverslips treated with saline solution (group 6) were considered a negative control group. To insure homogeneity, the BAG 45S5 powders were well dispersed by continuous stirring before being poured into wells containing the biofilm plaques. The biofilms were treated only once when they reached either 6, 12, or 24 h of growth. After 10 min of exposure, each coverslip was washed with PBS for five times and transferred into a centrifuge tube filled with 1 ml of saline. The tubes were vortexed at high speed for 3 min and sonicated with an Aquasonic sonicator (KQ 500B; Kunshan Ultrasonic Instrument Co. Ltd, Jiangsu, China) for 10 min, and then the biofilms on the coverslips were detached. To count the number of biofilm-viable microorganisms, the cell broths in the tubes were first diluted using a 10-fold serial dilution technique, and the bacteria incubated in agar plates for 48 h at 37 °C. Using a stereomicroscope (SMZ1000; Nikon, Tokyo, Japan), the resultant colonies were counted, with identification based on the colony shape and Gram staining. Each experiment was performed in triplicate.

### 2.3. Scanning electron microscopy observations

Two coverslips from each group were fixed in 2.5% glutaraldehyde for 24 h at 4 °C. The samples were washed in distilled water and dehydrated in ascending concentrations (50%, 70%, 95%, and 100%) of ethanol solution, each for 30 min. The samples were then dried in a desiccator and sputter-coated with gold. The surface morphology of the biofilms was examined using a scanning electron microscope (SEM) (Leo 1530; LEO, Oberkochen, Germany) at 12 kV in high-vacuum mode.