Bactericidal Effect of Strong Acid Electrolyzed Water against Flow *Enterococcus faecalis* Biofilms

Xiaogang Cheng, DDS,[*†] *Yu Tian, PhD, DDS,*[*] *Chunmiao Zhao, MDS,*[*‡] *Tiejun Qu, PhD, DDS,*[*] *Chi Ma, PhD,*[†] *Xiaohua Liu, PhD,*[†] *and Qing Yu, PhD, DDS*[*]

Abstract

Introduction: This study evaluated the bactericidal effect of strong acid electrolyzed water (SAEW) against flow *Enterococcus faecalis* biofilm and its potential application as a root canal irrigant. **Methods:** Flow *E. faecalis* biofilms were generated under a constant shear flow in a microfluidic system. For comparison, static *E. faecalis* biofilms were generated under a static condition on coverslip surfaces. Both the flow and static *E. faecalis* biofilms were treated with SAEW. Sodium hypochlorite (NaOCl, 5.25%) and normal saline (0.9%) were included as the controls. Bacterial reductions were evaluated using confocal laser scanning microscopy and the cell count method. Morphological changes of bacterial cells were observed using scanning electron microscopy. **Results:** The confocal laser scanning microscopic and cell count results showed that SAEW had a bactericidal effect similar to that of 5.25% NaOCl against both the flow and static *E. faecalis* biofilms. The scanning electron microscopic results showed that smooth, consecutive, and bright bacteria surfaces became rough, shrunken, and even lysed after treated with SAEW, similar to those in the NaOCl group. **Conclusions:** SAEW had an effective bactericidal effect against both the flow and static *E. faecalis* biofilms, and it might be qualified as a root canal irrigant for effective root canal disinfection. *(J Endod 2016;42:1120–1125)*

Key Words

Bactericidal effect, *Enterococcus faecalis*, flow biofilm, microfluidic system, static biofilm, strong acid electrolyzed water

Significance

Under the conditions of this study, SAEW showed an effective bactericidal effect against both the flow and static *E. faecalis* biofilms. The SAEW might be a promising root canal irrigant for effective root canal disinfection.

An ideal root canal irrigant should possess proper properties such as lubrication, debridement, an antimicrobial effect, and no or low toxicity (1, 2). The root canal irrigants used currently include sodium hypochlorite (NaOCl), hydrogen peroxide, and EDTA. Among them, NaOCl is the most commonly used and effective irrigant. However, there is a concern about the toxicity of NaOCl to periapical tissue (2–4). Strong acid electrolyzed water (SAEW) is generated by electrolysis of a weak sodium chloride solution on the anode side, with a pH value ranging from 2.3 to 2.7 (5, 6). Because of its antimicrobial activity against bacteria and viruses, SAEW has been widely used in food safety and surgical site disinfection (5, 7–9). In recent years, SAEW has been investigated as a potential root canal irrigant (10, 11).

Enterococcus faecalis is usually chosen as the test strain of a potential root canal irrigant, which is 1 of the pathogens associated with persistent endodontic infections (12–14). It was reported that a bacterial biofilm possessed a drug resistance of 100 to 1000 times higher than its planktonic form (15). Laboratory studies of bacterial biofilms mainly focus on "static biofilms," which are grown in a stagnant culture condition without a continuous supply of fresh medium (16, 17). Because of its culture condition, the static biofilm may not be capable of truly mimicking the biofilm formed in the body. A continuous fluid flow is more suitable for developing a mature and robust biofilm (called "flow biofilm"). The fluid flow provides a nutrient exchange and shear flow, influences the density and strength, and affects the removal of cells from the biofilm (18, 19). Flow bacterial biofilms may exist inside an infected root canal. When pulpal necrosis or periradicular periodontitis takes place, exudate forms and causes a fluid exchange in and out of the root canal. This fluid exchange provides a sustainable nutrient source and shear flow for bacterial biofilm formation (16). In addition, the disintegration of the sealer or undetected voids in filling mass leads to leakage channels. The channels allow periradicular tissue fluids to reach the root canal system and provide nutrient and shear flow to flow bacterial biofilm

From the *State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi Key Laboratory of Oral Diseases, Department of Operative Dentistry and Endodontics, School of Stomatology, The Fourth Military Medical University, Shaanxi, People's Republic of China; †Biomedical Sciences Department, Texas A&M University Baylor College of Dentistry, Dallas, Texas; and ‡Department of Operative Dentistry and Endodontics, Baoji Stomatology, Baoji, People's Republic of China.

Address requests for reprints to Xiaohua Liu, Biomedical Sciences Department, Texas A&M University Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, or Qing Yu, State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi Key Laboratory of Oral Diseases, Department of Operative Dentistry and Endodontics, School of Stomatology, The Fourth Military Medical University, 145 West Chang-le Road, Xi'an 710032, PR China. E-mail address: xliu@bcd.tamhsc.edu, yuqing_fmmu@hotmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.04.009

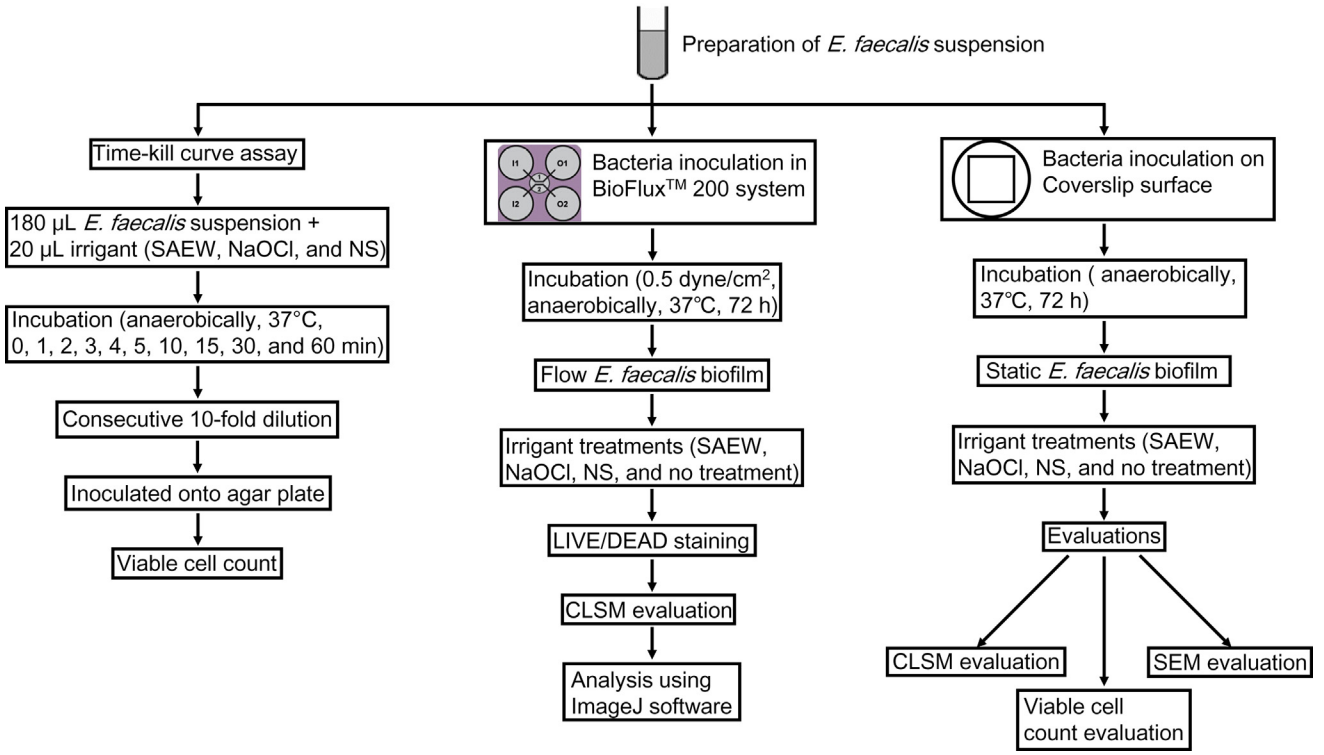

Figure 1. A flowchart of the experiment design. SAEW, NaOCl, and NS represent strong acid electrolyzed water, 5.25% NaOCl, and 0.9% normal saline, respectively.

growth (20). However, to date, all studies about the bactericidal effect of SAEW on *E. faecalis* have been limited to static biofilms (10, 21), and no results have been reported on flow biofilms.

In this study, we prepared flow *E. faecalis* biofilms using a microfluidic system and evaluated the bactericidal effect of SAEW against the flow *E. faecalis* biofilms (Fig. 1). For comparison, we also included static *E. faecalis* biofilms in the experiments.

Materials and Methods

Bacteria Preparation

E. faecalis (ATCC 29212) thawed from frozen stock was streaked onto a brain-heart infusion (BHI; Difco, Detroit, MI) agar plate and incubated anaerobically at 37°C for 24 hours. A single colony was inoculated into 5 mL BHI broth and cultured at 37°C for 24 hours.

Irrigants

SAEW was prepared by the electrolysis of tap water containing 0.05% sodium chloride with an exclusive electrolyzing apparatus (Alfa Light; Amano Co, Yokohama, Japan). Its physicochemical characteristics include a pH of 2.3 ± 0.15, an oxidation-reduction potential (ORP) of 1163 ± 3.7 mV, and a chlorine concentration of 49 ± 1.3 ppm. The pH value and ORP were determined using a pH meter (HM-14P; TOA Electronics Ltd, Tokyo, Japan). The available chlorine concentration was tested using a chlorine comparator (Portable Type II; Toyo Engineering Works Ltd, Chiba, Japan) based on the O-tolidine method. NaOCl (5.25%, pH = 12.3–12.6) was diluted from a 10% NaOCl solution (Sigma-Aldrich Co, St Louis, MO) in deionized water. SAEW and 5.25% NaOCl were used immediately after prepared; 0.9% normal saline (NS) (pH = 4.7–5.6, Kelun Pharmaceutical Co, Sichuan, China) was used after purchase without further treatment.

Time-kill Curve Assay

E. faecalis suspension was diluted to 10^5 colony-forming units (CFU)/mL in BHI. One hundred eighty microliters of the dilution and 20 µL of each irrigant (SAEW, NaOCl, and NS) were added into a well of a 96-well tissue culture plate (Costar; Corning Inc, Corning, NY). The plates were incubated anaerobically at 37°C. Bacterial survivals were obtained using the viable cell count method at 0, 1, 2, 3, 4, 5, 10, 15, 30, and 60 minutes (n = 6). Following the same procedure, 200 µL of the dilution without any irrigant was used as a blank control (n = 6). All of the procedures were repeated in triplicate.

Irrigant Treatment against Flow *E. faecalis* Biofilms

A microfluidic system (BioFlux 200 System; Fluxion Biosciences, South San Francisco, CA) that provides constant and controlled shear flows was used in this study. Forty-eight–well BioFlux plates with glass bottoms were used for flow *E. faecalis* biofilm formation. Initially, *E. faecalis* dilution (10^8 CFU/mL) was added to the outlet well and pumped to the inlet well for 6 seconds at 1 dyne/cm^2. Next, bacterial cells were left steady to colonize for 30 minutes. After that, BHI broth was constantly pumped from the inlet wells to the outlet wells at 0.5 dyne/cm^2. The plates were incubated anaerobically at 37°C for 72 hours, which was determined by the preliminary experiment to yield an adequate density of bacteria for the subsequent evaluation. The formed biofilms were treated with SAEW, NaOCl, and NS at 0.5 dyne/cm^2 for 10 minutes (n = 5). Sodium thiosulfate (50 µL) was applied for 5 minutes to terminate the residual antimicrobial activity of SAEW and NaOCl (22). In addition, 5 biofilms were left untreated. All the biofilms were then rinsed with distilled water and stained with a LIVE/DEAD BacLight Bacterial Viability Kit (L-13152; Molecular Probes, Invitrogen Inc, Carlsbad, CA) at 0.5 dyne/cm^2 for 15 minutes. The kit included SYTO 9 green fluorescent nucleic acid stain and propidium iodide (PI) red fluorescent stain. Bacteria with intact cell membranes were