Evaluation of the Effect of *Enterococcus faecalis* Biofilm on the 2% Chlorhexidine Substantivity: An *In Vitro* Study

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Abstract

Introduction: The aim of this study was to correlate the bacterial viability and the presence of 2% chlorhexidine (CHX) solution on dentin by means of confocal laser scanning microscopy and high-performance liquid chromatography for 48 hours, 7 days, and 30 days. **Methods:** One hundred twenty-three extracted human teeth were used. Samples were divided into 4 groups according to the solution (CHX or saline) and the presence of *Enterococcus faecalis* biofilm. Samples were kept in contact with 5 mL of the solution for 5 minutes. Each group was divided into 3 subgroups according to the evaluation period (n = 10). Statistical analysis was performed by using the Kruskal-Wallis test, the Mann-Whitney U test ($P < .05$), and the Spearman rank correlation coefficient ($P < .01$). **Results:** There was a negative correlation between the percentage of live cells and the amount of remaining CHX ($P = .000$). CHX significantly reduced the percentage of viable cells compared with saline after 48 hours ($P = .007$). Differences were maintained in the 7-day evaluation period ($P = .001$). After 30 days, the CHX group presented an increase of viable cells, thereby becoming similar to saline ($P = .623$). Simultaneously, the remaining CHX was significantly reduced in the 30-day specimens ($P = .000$). **Conclusions:** The results of this study indicate that 2% CHX solution was detected for 48 hours and 7 days with a low percentage of viable cells. The presence of microorganisms on human dentin did not affect 2% CHX maintenance. *(J Endod 2015;41:1364–1370)*

Key Words

Chlorhexidine, *Enterococcus faecalis*, substantivity

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Endodontic infection is characterized by the presence of microorganisms in the root canal system and is the major cause of apical periodontitis (1). Species interaction and nutrient supply are essential for the persistence of endodontic infection. Therefore, the root canal represents a special environment, which allows the survival of a restricted microbiota (2).

During endodontic treatment, anaerobiosis is disrupted when the pulp chamber is assessed. Furthermore, chemomechanical preparation eliminates microorganisms by interfering with microbial interaction and limiting the nutrient sources. However, even after root canal filling, penetration of periapical and dentinal fluids provides a favorable environment for recontamination when sealing fails (3). *Enterococcus faecalis*, a gram-positive and facultative microorganism, is commonly associated with endodontic treatment failure (4–7). It may also become resistant to endodontic disinfection protocols (7–10).

Different antimicrobial agents can be used for root canal system disinfection. Although intracanal medicaments will remain between appointments, the time of action of irrigants is restricted to root canal preparation procedures. If irrigants and/or intracanal medicaments have residual antimicrobial activity, prevention of recontamination may be achieved (11).

Studies have shown that chlorhexidine (CHX) is able to adsorb onto the dentin walls (substantivity), providing long-lasting antimicrobial effects (12–20). In this regard, different evaluation periods are considered. Moreover, the assessment of the CHX residual effect is mostly based on microbiological techniques. Although some studies quantify CHX, no study has precisely correlated the substantivity to the bacterial viability. A summary of the various study designs, methods, and evaluation periods of these previous studies are found in Table 1.

The aim of the present study was to determine the correlation between the bacterial viability and the presence of 2% CHX on dentin for 48 hours and 7 and 30 days after the disinfection protocol.

Materials and Methods

Specimen Preparation

This study was approved by the Ethics Committee in Research (Federal University of Rio Grande do Sul–UFRGS, protocol #770038). One hundred twenty-three extracted human teeth with a single root canal were selected. Roots with fracture lines or anatomic irregularities, previous endodontic treatment, calcifications, or curvatures were excluded from the study. The roots were irrigated with 2.5% sodium hypochlorite (Biodinâmica Química e Farmacêutica Ltda, Ibiporã, Brazil), and pulp remnants were removed using a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland).

The sample preparation was adapted from Ma et al (21). The root canals (N = 123) were enlarged up to #6 Gates-Glidden drills (Dentsply Tulsa, Tulsa, OK) with a low-speed handpiece. On the external surface, 2 grooves were produced following the root long axis with a round small-diameter drill (KG Sorensen, Cotia, Brazil). Each root was sectioned into 2 halves with the use of a chisel (2 specimens per root, N = 246). Standardized dentin blocks (4-mm length) were obtained from the cervical portion of the root. Sections were made by using a double-face diamond disc (Extec, Enfield, CT) with 0.3-mm thickness and a 120-mm diameter at a speed

TABLE 1. Summary of Studies Evaluating the Residual Antimicrobial Effect of Chlorhexidine (CHX)

Authors	Sample	Experimental groups	CHX contact time	Evaluation periods	Evaluation methodology	Conclusions
White et al, 1997 (12)	Extracted human teeth (n = 44)	2% CHX solution; 0.12% CHX solution; sterile deionized water	During preparation	6, 12, 24, 48, and 72 h after preparation	Inhibition zone	CHX Substantivity is maintained for 72 h
Leonardo et al, 1999 (13)	Human teeth with pulp necrosis and periapical lesion (n = 22)	2% CHX solution	During preparation	Before and 48 h after preparation	CFU counting and inhibition zone	CHX antimicrobial residual effect for 48 h
Komorowski et al, 2000 (18)	Bovine dentin (n = 60)	Saline; 0.2% CHX; 5.25% NaOCl	5 min; 7 d	21 d	Optical density	Authors recommend the use of 0.2% CHX as an intracanal medication for 7 d
Ercan et al, 2004 (19)	Necrotic human teeth (n = 30)	2% CHX solution; 5.25% NaOCl	During preparation	Before, immediately after and 48 h after preparation	CFU counting	CHX is an alternative for the use of NaOCl
Rosenthal et al, 2004 (16)	Bovine teeth (n = 60)	2% CHX solution; saline	10 min	1 d, 3, 6, and 12 wk	Spectrophotometry and CFU counting	The use of CHX presents advantages
Dametto et al, 2005 (14)	Extracted human teeth (n = 80)	2% CHX solution; 2% CHX gel; 5.25% NaOCl; distilled water; natrosol gel	During preparation	Before, immediately after, and 7 d after preparation	CFU counting	CHX solution and gel can be recommended as auxiliary substances during root canal preparation
Khademi et al, 2006 (15)	Infected bovine dentin (n = 80)	2% CHX solution; 2.6% NaOCl; doxycycline	5 min	Immediately after, 7, 14, 21, and 28 d after disinfection	CFU counting	CHX presented higher substantivity than doxycycline and remained for 28 d
Souza et al, 2012 (17)	Extracted human teeth (n = 45)	2% CHX solution; 2% CHX gel; distilled water	During preparation	24 h, 30 and 90 d	HPLC	CHX remained in root canal for 90 d
Ferrer-Luque et al, 2014 (20)	Extracted and infected human teeth (n = 86)	2% CHX solution; 0.2% CHX solution; 0.2% cetrimide	1 min	Immediately after and daily for 50 d after disinfection	Turbidity evaluation	2% CHX solution presented the highest residual effect in teeth infected with *E. faecalis*

CFU, colony forming units; CHX, chlorhexidine; HPLC, high-performance liquid chromatography; NaOCl, sodium hypochlorite.

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