Abstract

In endodontic practice, the continue endodontic failure can be produce by the presence of Enterococcus faecalis in the root complex. The aim of this study was to kill E. faecalis in biofilm state using a proteolytic agent. A biofilm model was conducted for E. faecalis growing and then was employed endodontic irrigants as clorhexidine 2%, sodium hypoclorite 5% and C. pubescens 2% for disrupt the E. faecalis biofilm at 2, 5 and 10 minutes. Turbidity method and colony formed unit where employed to measure the disruption. C. pubescens 2% showed the most disruption of E. faecalis biofilm at 2, 5 and 10 minutes. Sodium hypoclorite 5% showed bacterial disruption at 10 minutes. Clorhexidine 2% not showed biofilm disruption at all times. Conclusion: enzymatic treatment for total disruption of E. faecalis may be the ideal therapy for infected root canals.

Keywords: Biofilm disruption, Enterococcus faecalis, Carica pubescens

Introduction

Biofilm is a microbial community coated by a polysaccharide matrix (1). This kind of complex structure provides some means to microorganisms, such as nutrients, communication, heterogenicity, development, defence, and spread, in order that they can survive in hostile environments (2). In the mouth this biofilm can be found in dental surfaces (3), on the tongue (4), and in dental materials (5); and it causes many of oral diseases: caries (6), gingivitis (7), periodontal disease (8), endodontic infections (6), among others. When this biofilm becomes mature it can only be removed from the surfaces by using mechanical forces (9) like tooth brushing, endodontic instrumentation, removal of caries, and periodontal instrumentation; however, in these procedures a little part of the biofilm can still remain which elicits their development causing later on a failure therapy (10).

In endodontology many techniques have been proposed to remove this biofilm from the canal (11, 12). In fact, an endodontic failure therapy can be explained by the presence of Enterococcus faecalis biofilm into the root complex (13). Research has been performed with many endodontic irrigants aimed to kill the E. faecalis (14). However, most of these studies were performed with a non-sessile microorganism. In fact these tests do not obey the natural state of microorganisms: a biofilm state.

Papain, a proteolytic enzyme of Carica pubescens, is used for dental purposes to soften and remove just dental tissues infected of tooth decay. This therapy can proposed firstly by Dr. Rainey who promotes a minimally preparation of tooth for reconstruction (15). This philosophy is known as Minimally Invasive Dentistry.

The aim of this study was to kill E. faecalis in biofilm state using a proteolytic agent.

Material and method

This study was approved by Ethical Committee of Universidad Católica de Santa María, Arequipa, Perú, number UCSM-CE-2013-0006.

Dental preparation

Dentine tubules were prepared according to Vaghela (16) with some modifications. One hundred single root premolars were selected from a dental bank of the Facultad de Odontología – Universidad Católica de Santa María, Arequipa - Perú. These teeth were submerged in sodium hypochlorite 10% and EDTA to remove any rest of organic tissue. Then, teeth were decoronated 5 mm below the cement-enamel junction, followed by sterilization in autoclave 121°C for 15 minutes. At this time, endodontic instrumentation was performed using Gates Glidden drill nº 3, followed by apiceptomy, and obturation with MTA-Angelus (Angelus, Brazil). All surfaces of the teeth were prepared with phosphoric acid and then were sealed with bonding. All the teeth were stored in a bottle filled with sterile BHI (brain heart infusion broth).

Microbial procedures and biofilm growth

E. faecalis (ATCC 29212) was collected from the Microbiology lab of Facultad de Odontología – UCSM and were grown in BHI at 37ºC, 5% CO₂ for 24 hours. An inoculum was done at 1 x 10⁶ UFC/mL concentration and 20 uL of this inoculum were introduced into the root canal of the tooth and finally the coronal portion was closed with
dental wax. The teeth were cultivated for 21 days at 37°C with CO₂ 5% to produce a biofilm in the root (17).

**Disorganization of biofilms**

*E. faecalis* biofilm obtained were disorganized using a 40 Hedstrom file, then thirty teeth were treated with chlorhexidine 2%, another thirty teeth were treated with sodium hypochlorite 5% and the other thirty teeth with *C. pubescens* 2% in gel. Negative controls were not treated. Chlorhexidine, sodium hypochlorite and *C. pubescens* were studied at 2, 5, and 10 minutes. After that, the tooth specimen were cleaned with NaCl 0,9% and then cultivated in 10 mL of BHI at 37°C CO₂ 5% for 24 hours over 7 days. Negative control can be defined as not treated tooth. The presence of turbidity indicates bacterial growth and was measured by optical density 0,5 at 550 nm (this value is equivalent to 1,4 x 10⁷ CFU/mL). Colony formed units were performed to evaluated microbial growing.

**Statistical analysis**

All statistical tests were performed by one-way ANOVA and Tukey’s correction at 5% significance level using GraphPad Prism version 5.00 for Mac, GraphPad Software, San Diego California USA, www.graphpad.com.

**Results**

*C. pubescens* was the most effective substance to eliminate fully *E. faecalis* biofilm at 2, 5, and 10 minutes (Figures 1a-c). Sodium hypochlorite at 5% eliminated *E. faecalis* at 10 minutes (Figure 1c). Chlorhexidine at 2% does not eliminate *E. faecalis* biofilms at any length of time (Figures 1a-c). Colony formed units (table nº1) showed full disruption of *E. faecalis* at 5 and 10 minutes when used *C. pubescens*. Sodium hypochlorite at 5% eliminated *E. faecalis* at 10 minutes of treatment. Not fully elimination of *E. faecalis* were showed when used Chlorhexidine at 2% and controls groups.
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Figure 1: Optical density measured of grows of E. faecalis after C. pubescens, clorhexidine and sodium hypochlorite. **a** C. pubescens showed a great disruption of E. faecalis biofilm at 2 minutes. **b** C. pubescens showed a great disruption of E. faecalis biofilm at 5 minutes. **c** C. pubescens and sodium hypochlorite showed a great disruption of E. faecalis biofilm at 10 minutes. *** Indicate an significant differences.

Table 1: CFU/mL denotes that C. pubescens fully disrupted of E. faecalis biofilm at 5 and 10 minutes, in compare with sodium hypochlorite 5% and clorhexidine at 2%. Sodium hypochlorite at 5% disrupt well at 10 minutes. Clorhexidine at 2% and control group did not disrupt the E. faecalis biofilm.

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<tr>
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<th>2 minutes</th>
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<tr>
<td>Control</td>
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<td>Sodium hipoclorite 5%</td>
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<td>Clorhexidine 2%</td>
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<td>Papain</td>
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CFU/mL
Unc = uncountable

Discussion

E. faecalis is the principal microorganism isolated among the most persistent endodontical and post-treatment infections. Clorhexidine and sodium hypochlorite are fully used to remove the smear layer and disinfected root canal. Much research has been proposed to evaluate the antimicrobial capacities of both irrigants.

In this study clorhexidine 2% showed a partial elimination of E. faecalis at 10 minutes of treatment. This data does not accord with Bhardwaj, A et al (2012) that showed a 100% of microbial inhibition. However, the data does accord with Wang who showed a little E. faecalis inhibition in comparison with NaOCl 6%. Ariaz-Moliz showed no inhibition capacities of clorhexidine against E. faecalis biofilm (18).

Many data in literature corroborate the efficacy of NaOCl to kill completely the E. faecalis at 10 minutes of treatment. This data does not accord with Bhardwaj, A et al (2012) that showed a 100% of microbial inhibition. However, the data does accord with Wang who showed a little E. faecalis inhibition in comparison with NaOCl 6%. Ariaz-Moliz showed no inhibition capacities of clorhexidine against E. faecalis biofilm (18).

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Proteolytic treatment in killing microbial biofilms has been reported in industries (22), medical (23) and dental therapies (17, 24). Papain is a cysteine protease found in many concentrations as endodontic irrigants. The present study showed that NaOCl 5% was effective in killing E. faecalis biofilm at 10 minutes. However, some studies report some complication about the use of NaOCl as an irrigant. Witton showed tissue and neurological complication when using this agent in endodontic therapy (19). Pelka and Petschelt related a case report that presented a permanent mimic musculature and neurological damage cause by NaOCl (20). Studies report that NaOCl is an extremely tissue-cytotoxic chemical, killing fibroblast (in vivo) at 0,01%, causing hemolysis, ulceration, inhibition of neutrophil migration, endothelial damages in contact with vital tissues (21). In this way, authors believe that using NaOCl for up to 10 minutes vital tissues can suffer several damages.

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Further studies must be done with polimicrobial model biofilm using papain as a disrupter.

Based on this data, *C. pubescens* has a promise as a use as an enzimatic disrupter of *E. faecalis* biofilm in root canals.

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**References**


