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Rapid enzimatic disruption of *Enterococcus faecalis* biofilm using *Carica pubescens*: a pilot study

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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 endondontic 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% CO_2 for 24 hours. An inoculum was done at 1 x 10^6 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

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dental wax. The teeth were cultivated for 21 days at 37ºC with CO_2 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 clorhexidine 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. Clorhexidine, 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 37ºC CO_2 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 10^7 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). Clorhexidine 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 Clorhexidine at 2% and controls groups.

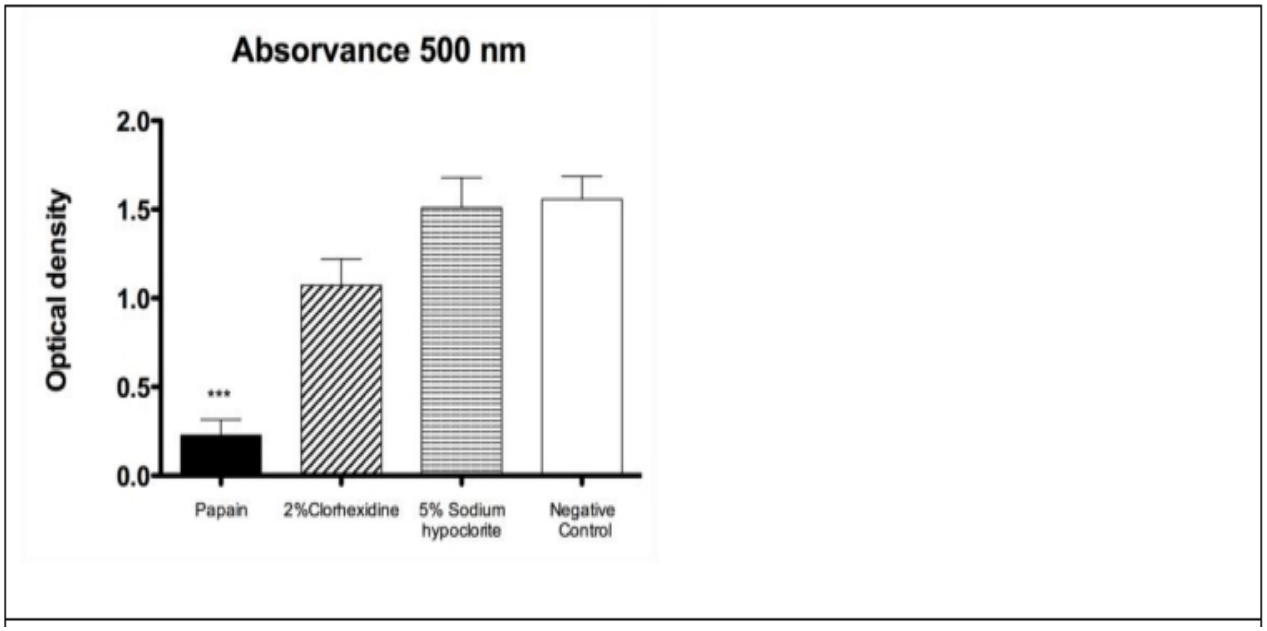

Figure 1a

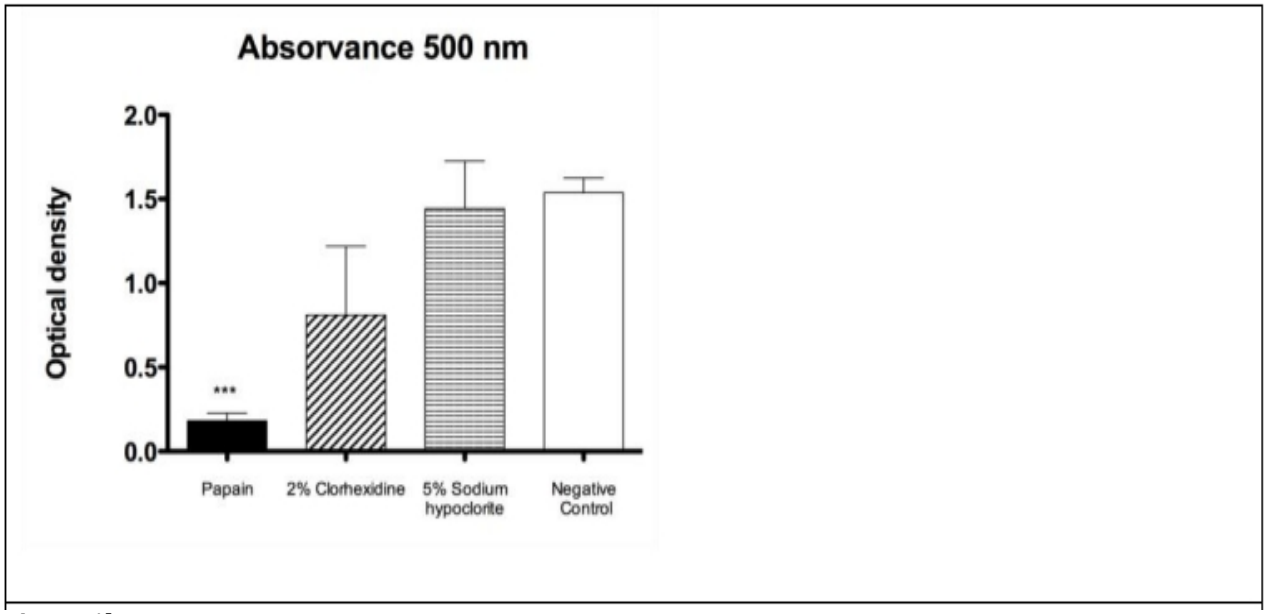

Figure 1b

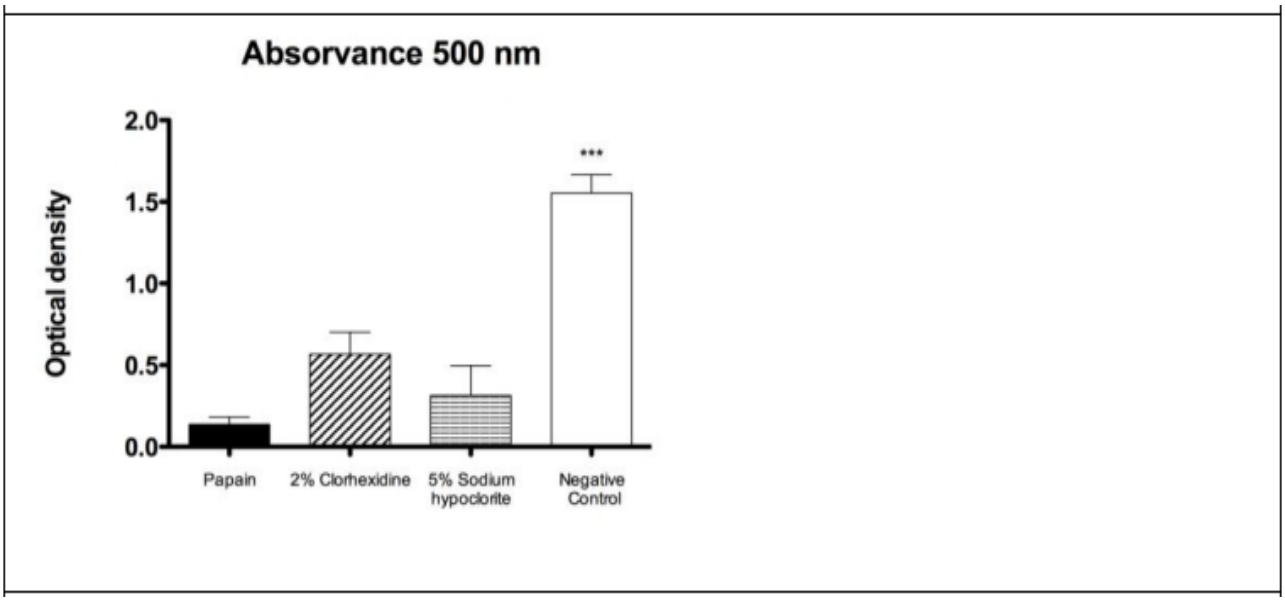

Figure 1c

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.

	2 minutes	5 minutes	10 minutes
Control	Unc	Unc	Unc
Sodium hipoclorite 5%	Unc	736 ±12,23	---
Clorhexidine 2%	Unc	Unc	Unc
Papain	5 ± 1,34	---	---

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* biofilms. Indeed, many authors recommend the use of NaOCl 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, endotelial 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.

Proteolytic treatment in killing microbial biofilms has been reported in industries (22), medical (23) and dental therapies (17, 24). Papain is a cysteine protease founded in microbes, plants, insects and animals (25). This protease in plants has been found as *Carica papaya*. This fruit contains papain and is used in dental sciences as a dental caries remover (26). Indeed, in South America exists a *Vasconcellea pubescens*, known as papaya arequipeña that showed more levels of papain than the *Carica papaya*. In the present study the *Vasconcellea pubescens* was used at 2% to remove the *E. faecalis* biofilm. The protease showed a great killing of *E. faecalis* biofilm at 2, 5, and 10 minutes, and because killing fully *E. faecalis* biofilm early, in comparison to clorhexidine and NaOCl, this protease can be used in minimum time in the root canal avoiding tissue damage. Some studies report antibacterial activity of papain. Anuj et al. report a medium antibacterial activity, however, he did not refer in his paper to the origin of the protease; these data in consequence are not in accord with data of the present study.

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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