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Evaluation of antimicrobial effectiveness and dentine mechanical properties after use of chemical and natural auxiliary irrigants

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ABSTRACT

Objective: To evaluate the effect of GSE, NaOCl, CHX and QMix as an antimicrobial agents against *Enterococcus faecalis* and their influence on flexural and ultimate tensile strength of root canal dentine.

Methods: Root canals were divided into five groups ($n = 10$) according to the substances used: 2.5% NaOCl, 2% CHX, 6.5% GSE, Qmix and control group (distilled water) (DW). Final irrigation was done with 17% EDTA in all groups, except when DW was used. The number of colony-forming units was used to evaluate the antimicrobial activity. Dentine beams were used to assess the flexural strength after treatment with substances as described before ($n = 10$). The UTS was evaluated after the treatment of root dentine hourglass shape sections with the same substances ($n = 30$).

Results: The lowest bacteria contamination was observed for CHX and GSE, while NaOCl and QMix showed an intermediate antimicrobial activity ($p > 0.05$). NaOCl and QMix significantly reduced the mechanical properties of dentine (flexural strength and UTS) ($p < 0.05$) and no statistical difference was found among CHX, GSE and DW ($p > 0.05$).

Conclusion: CHX and GSE were more effective than NaOCl and QMix against *E. faecalis*. Furthermore, they did not harm dentine mechanical properties as observed for NaOCl and QMix.

Clinical significance: The use of GSE can be recommended for endodontic procedures since it has good antimicrobial activity and does not interfere in the mechanical properties of dentine; similarly to CHX.

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1. Introduction

Although all the stages of endodontic treatment are important, the mechanical preparation of the root canal is one of the operative procedures that requires a great deal of professional skills to ensure that it is adequately shaped and cleaned.[1] For this, endodontic instruments are used in association with auxiliary chemical solutions.[2] When rotary endodontic instruments are employed, reciprocating and traditional stainless steel hand instruments fail in completely prepare all the walls of the root canal,[1,3] so the selection of an appropriate endodontic irrigant is required to assist in the removal of organic tissues and bacteria such as *Enterococcus faecalis*, which are commonly located deep in the dentinal tubules as well as in isthmus and ramification areas.[4] *E. faecalis* is an anaerobic facultative microorganism that is highly resistant to conventional chemomechanical preparation and is usually found in cases of failure of root canal treatment.[5] This microorganism has several virulence factors and is able to withstand prolonged periods of nutrient limitation, persisting as a pathogen in the root canal and is linked to failure of the root canal treatment.[6,7]

Sodium hypochlorite (NaOCl) in a concentration from 0.5% to 5.25% has been traditionally used for irrigation during root canal treatment because of its antimicrobial activity and ability to dissolve organic matter.[2] At low concentrations, it is ineffective against some specific microorganisms,[8,9] while severe irritation has been reported when high concentrated solutions were forced into the periapical tissues.[10] Furthermore, NaOCl reduces the mechanical properties of dentine,[11,12] as well as promotes structural changes in organic dentine components.[12–14] Because of the adverse effects of NaOCl, researchers have investigated alternative solutions.

Chlorhexidine digluconate (CHX) has been suggested as an auxiliary substance in endodontic treatment because of its antimicrobial activity[15,16] and substantivity.[17,18] Studies comparing the antimicrobial effectiveness of NaOCl and CHX are inconsistent.[18–22] The main advantage of using CHX as auxiliary chemical substance is that it does not interfere with collagen in the dentine organic matrix.[13] In this way, it maintains the properties of the dentine substrate for restoration of the tooth with resin-based materials.

QMix (Dentsply Tulsa Dental, Tulsa, OK) is also a solution with antimicrobial activity used for the smear layer removal in final irrigation. Its chemical composition includes EDTA, CHX, and a specific detergent.[23] Stojicic et al.[23] showed that the QMix and NaOCl were superior to CHX and MTAD under laboratory conditions in killing *E. faecalis* and plaque bacteria in planktonic and biofilm culture. However, QMix has not been studied as an endodontic irrigating solution during the treatment.

Proanthocyanidin (PAC)-rich plant extracts represent a rich source of antimicrobial natural compounds,[24] which have great applicability in the dental field for enhancement of dentine biomechanical properties and biostability.[25,26] PACs are known to have therapeutic activities, acting as immunomodulators, antioxidants, antimutagens and antibacterials.[24,27,28] Furiga et al.[29] showed that the properties of an original combination of a PACs-rich extract with amine fluoride can prevent oral diseases and associated oxidative damage. Furthermore, the toxicity to human cells are relatively low.[30,31] However, their application in endodontics has not been reported.

Another common clinical problem affecting root endodontically treated teeth is the possibility of root fracture.[14] Factors that may predispose to fracture have been identified as changes in the mechanical properties and composition of dentine because of the action of irrigants, medicaments or root canal–filling materials[11–14,32–34] as well as loss of structural integrity because of caries or access cavity preparation,[30] especially in teeth with thin root structure.[14] The flexural strength and ultimate tensile strength tests of dentine are commonly aplyoed to assess the effects of chemical agents on the in the remaining dentine.[12]

Therefore, the aim of this study was to evaluate the effect of GSE, NaOCl, CHX and QMix as an antimicrobial agents against *E. faecalis* and their influence on the flexural and the ultimate tensile strengths of dentine. The hypotheses tested were the following: 1. GSE shows similar antimicrobial activity to NaOCl, CHX and QMix. 2. GSE does not harm the mechanical properties of dentine when used as irrigant.

2. Material and methods

2.1. Samples selection

This study was approved by the Institutional Review Board of University of Passo Fundo (#441.313 and #696.604). The teeth were kept frozen for no longer than 3 months, thawed and cleaned of adhering soft tissues. Fifty-five and fifteen single-rooted human extracted teeth were selected for antimicrobial test and ultimate tensile strength, respectively. Inclusion criteria were sound teeth, no previous root canal treatment, root filling or restoration single canal. Visual and radiographic evaluations were employed to further select teeth with single canal, normal pulp chambers, patent root canals, and fully formed apices without any sign of resorption were used. Mid-coronal dentine from 25 non-carious of human molars was used for the evaluation of mechanical properties.

2.2. Antimicrobial activity

Dental crowns were sectioned to obtain 15 mm of root length, which was further reduced 1 mm for the working length. The cervical third were prepared using Largo drills #2 and #3 (Dentsply, Maillefer, Ballaigues, Switzerland). In order to standardize the root canal diameter, roots were prepared by serial instrumentation up to the instrument #45 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) using irrigation with distilled water (DW). Then, a final rinse with 17% EDTA (Sigma–Aldrich, St. Louis, MO, USA) for 3 min under agitation through passive ultrasonic irrigation (PUI) was performed for smear layer removal. The PUI was performed using the ultrasonic device (NacPlus ultrasonics - Adiel, Ribeirão Preto, SP, Brazil). The stainless-steel endodontic tip to a size ET40 (SatelecActeon, Mount Laurel, NJ, USA) was inserted 2 mm short of the working length and activated for 30 s. The scale power 2 for endodontics was used for ultrasonic activation of