Influence of gel irrigant agents in oral biofilm

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ABSTRACT

Introduction: The aim of this study was to evaluate the antimicrobial activity of 1% sodium hypochlorite, 2% chlorhexidine and 24% EDTA, all in the gel form on oral biofilm. **Methods:** Sterile bovine dentin blocks were allocated on an intraoral device that was used by a volunteer for 3 days. After biofilm formation, the blocks were immersed in 100 µl of evaluated substances, for 5 minutes. After treatment, the samples were stained with 50 µl of a solution of SYTO 9 / propidium iodide and evaluated in a confocal microscope, yielding 50 images per group immediately after removing the antimicrobial agent. Data were analyzed by Kruskal-Wallis and Dunn's tests ($\alpha = 0.05$). **Results:** Statistical differences between experimental groups and the control were verified. The 1% sodium hypochlorite was more effective than other evaluated substances ($p < 0.05$). The 2% chlorhexidine significantly reduced the percentage of live cells, in comparison to EDTA gel ($p < 0.05$). **Conclusions:** The irrigant agents used in this study showed no ability to dissolve induced *in situ* biofilm. However the sodium hypochlorite gel showed better results than chlorhexidine and EDTA.

Keywords: Biofilms. Dentin. Microscopy, confocal.

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Introduction

The clinical and radiographic success of endodontic treatment in teeth with pulp necrosis is closely related to the significantly reduction of bacterial infection to provide a favorable environment for perirradicular tissue healing.[1] During biomechanical preparation, the mechanical action of instruments cannot touch all the walls of the root canal system due to its complex anatomy.[2] Meanwhile, untouched areas can remain with necrotic tissue residues and microbial biofilm. The complex degree of organization of biofilms hinders the action and effectiveness of the antimicrobial agents[3,4,5] which has led to a relentless pursuit of substances and protocols that favor the elimination of the infecting microbiota.

Sodium hypochlorite is the most used antimicrobial irrigant to perform endodontic treatment due to its antimicrobial property[6] and dissolution of tissues.[7] However, a limitation of this material is its high toxicity to periapical tissue, which is closely related to its concentration. The accidental injection promote the NaOCl extrusion to periodontal tissue and it can cause serious consequences to patient —as severe pain, edema and even necrosis—, and extreme allergic reactions.[8,9,10] The potential of damage effects depend on the concentration[11,12] and volume of the extruded NaOCl beyond the limits of the root canal.

In pediatric patients, commonly endodontic procedure involves permanent teeth with incomplete apex or large foramina, and deciduous teeth affected by dental caries or dental trauma. In these situations, irrigate solution extrusion is favoured due to increased foraminal opening, or resorption in cases of primary teeth, which would lead to the aggression of the permanent tooth germ. The irrigant solutions commonly used in the pulpectomy of primary teeth show some level of toxicity.[13]

Irrigants in gel form present an easier handling and lowest flowability, in relation to liquid irrigants, thus providing to the clinician a greater control over the irrigating substance, preventing accidents and complications with their use.[14] Furthermore, irrigant gels provide better lubrication of the canal when mechanical instrumentation is used[15,16] and could be an effective and safe option for irrigation if their properties are maintained, mainly in pediatric patients.

There are no studies that evaluated the antimicrobial properties of NaOCl and chlorhexidine both in gel form with the use of *in situ* biofilm model. The aim of this study was to evaluate the antimicrobial activity of 1% sodium hypochlorite, 2% chlorhexidine and 24% EDTA, all in the gel form during 5 minutes on oral biofilm.

Materials and Methods

Biofilm formation

Forty sterile bovine dentine blocks (3 x 3 x 2 mm) were used. The blocks were pretreated with 17% EDTA (Biodinâmica, Ibiporã, PR, Brasil) for 4 minutes to remove the smear layer generated during the sample preparation. For the biofilm formation, an *in situ* model was used where a volunteer used a Hawley device with the samples fixed in sites created on the plate for 72 hours of continuous use,[17] except during meal times and sanitation, upon approval of the study by the Ethics Committee on Human Research (CEP 190/2011). The diet of the volunteer was maintained. After the intraoral contamination, the samples were incubated in 3 ml of BHI in an oven at 37° C for 48 hours. After this period, the samples were removed and washed in 1 ml distilled water.

Treatment of specimens

The samples were divided into four groups (n=10) according to irrigant used, as follow: G1 – 1% sodium hypochlorite gel (Farmácia Véritas de Manipulação, Bauru, Brazil), G2 – 2% chlorhexidine gel (Biodinâmica, Ibiporã, Brazil), G3 – 24% EDTA gel (Biodinâmica, Ibiporã, PR, Brazil) and G4 – distilled water. To perform the irrigation of the specimens, the contaminated blocks were arranged individually in the wells of the culture plates, were given 100 µl of the experimental material for 5 minutes and after this period, were washed with 1 ml of distilled water. Additionally, samples of the group treated with sodium hypochlorite, after contact with the substance, were immersed in 5% sodium thiosulfate for 5 min to neutralize the residual effect of the sodium hypochlorite.

Microscopic examination of specimens

The analysis of viability of the biofilm was carried out using a solution of SYTO 9 / propidium iodide (Live/Dead BacLigth kit, Invitrogen, USA) for 10 minutes.

The SYTO 9 is a green fluorescent selective nucleic acid dye, indicated to stain live and dead cells. The propidium iodide seeks to identify the microbial population of the affected cell membrane or dead cells, and presents red fluorescence. Upon entering the cells, the red fluorescence decreases the fluorescence of the SYTO 9, leaving the dead cells with a red fluorescence. The samples after staining were evaluated in a Confocal Laser Scanning Microscope (Leica TCS-SPE; Leica Biosystems CMS, Mannheim, Germany). The wavelengths of absorption and emission were 494/518 nm for the SYTO 9 and 536/617 nm for the propidium iodide. Five stacks were obtained from each sample using a 40x lens and oil immersion with a size of 1 mm and 512 x 512 pixels. Fifty images per group were obtained immediately after removal of the antimicrobial agent. To quantify the biofilm, the bioImage L software (www.bioImageL.com) was used to calculate the percentage of the volume of living cells found after the anti-microbial therapy.

Statistical analysis

The Prism 5.0 software (GraphPad Software Inc, La Jolla, CA, USA) was used for statistical evaluations. To evaluate the obtained data, the Kruskal-Wallis test was applied followed by the Dunn's test for multiple comparisons. The significance level was set at 5%.

Results

The median, minimum and maximum values of the percentages of living micro-organisms of each study group are described in Table 1. The distilled water presented 97.80% of live cells and the other groups differed statistically from the distilled water. In a statistical comparison between groups, a significant difference ($p < 0.05$) between the 1% sodium hypochlorite and all the other experimental groups was observed. Significant differences ($p < 0.05$) were also observed in the comparison between the 2% chlorhexidine gel and the 24% EDTA gel.

Figure 1 shows representative images of each group of samples after treatment with the respective irrigant for 5 min. Quantification of biomass noted that the chlorhexidine and EDTA were not able to dilute the oral plaque and had a high amount of viable bacteria. For the 1% NaOCl, it was observed that even though the biofilm remained intact, its antimicrobial effectiveness was evident.

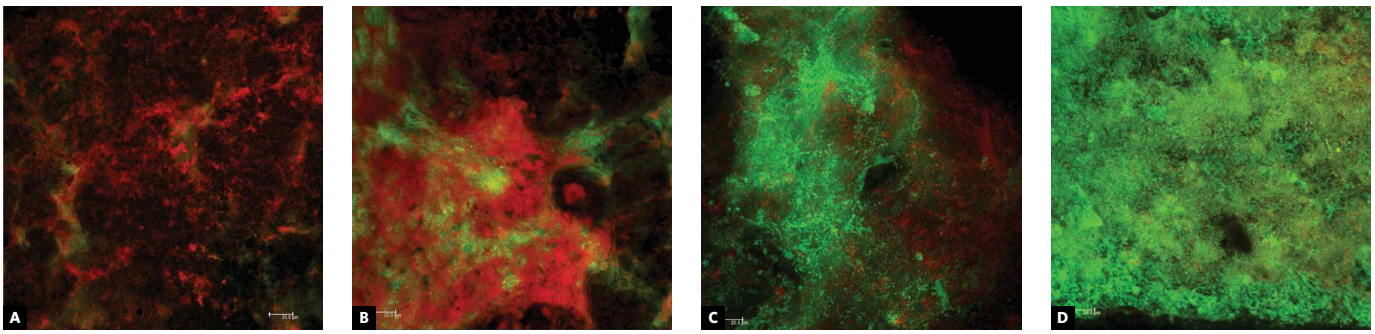

Figure 1. Confocal laser scanning microscopy images of the samples treated for 5 min with 1% sodium hypochlorite gel (**A**), 2% chlorhexidine gel (**B**), 24% EDTA gel (**C**) and distilled water (**D**). Live cells are seen in green, dead cells in red. Each image represents an area of 275 x 275 milimeters.

Table 1. Median and standard deviations values of percentages of live cells (Green), evaluated immediately after contact with irrigant agents. Different letters in each column indicates statistically significant differences ($p < 0.05$).

	Live cells (%)
1% Sodium Hypochlorite gel	20.98 (21.22)[a]
2% Chlorhexidine gel	51.58 (22.36)[b]
24% EDTA gel	78.52 (19.89)[c]
Distilled water	97.81 (10.55)[d]

Discussion

The biofilm is an organized formation of microorganisms in an organic matrix, covered by a polysaccharide layer,[18] and this formation is constituted in a form of microbial defense against the organism defense cells,[1] as well as the antimicrobial substances.[3,4,5,18] These structures can be found in the main canal, fins or isthmuses and dentinal tubules.[18,19]

When present in the main canal, the mechanical action of instruments can disorganize and remove it.[2] However, when the biofilm is present in the lateral areas as fins or isthmuses, the instruments fail to act, therefore, the action in the biofilm in these situations is the responsibility of the chemical action of the irrigant substance, but in this study, all the substances used in gel form were effective for dissolving the biofilm.

The proposed use of irrigants in gel form occurs by good lubricating action, particularly when employing rotary instrumentation[15,16] and by having a lower outflow for liquids. This low flow will favor the clinical control of the irrigator agent, especially in cases where this risk is eminent, such as teeth with broad or incomplete foramen and deciduous teeth with resorption. The chlorhexidine and EDTA tends to promote less cytotoxic potential to primary teeth cells.[13]

The use of sodium hypochlorite gel should be considered since this dedicated antimicrobial in its irrigant form could provide unpleasant situations when extruded to the periapical tissues.[15,20] Accidents and complications resulting from extrusion of NaOCl generate inflammation, ulceration and necrosis.[8,9,10,14] The cell destruction will cause an increase in vascular permeability, promoting the release of inflammatory mediators, which will generate an immediate swelling. However damage effects are closely dependent of NaOCl concentration[11,12] and volume.

The results of the present study verified that the 2% chlorhexidine presented low action on the biofilm in both the antimicrobial action and biofilm removal, confirming previous studies in which 2% chlorhexidine in liquid form was employed.[17,21] When the action was analyzed *in vitro*, this substance presented high antimicrobial action.[15,16] This demonstrates that in *in situ* situations of biofilm formation, where organic matter is present, this impairs the antimicrobial action of the chlorhexidine — situation that best reflects the clinical conditions in the root canal.

The 1% hypochlorite showed superior antimicrobial activity to 24 % EDTA and 2% chlorhexidine, however, NaOCl in gel form did not demonstrate the ability to dissolve the biofilm layers, different than the liquid form, as observed in previous studies.[6,17,21] This constitutes a problem because there is maintenance of the organic substrate with some living cells which can colonize and form new biofilm, in addition to the organic substrate occupy the voids which compromises the sealing of root canal obturation, thus creating empty spaces which can encourage bacterial infiltration.

Regarding the EDTA, its action was low with similar results to a previous study[21] that employed EDTA in liquid form, demonstrating that this substance presents weak antimicrobial activity and an absence of dissolved organic matter.

Conclusions

None of the substances analyzed in gel form presented dissolving capacity of *in situ* induced biofilm; however, the 1% sodium hypochlorite showed better antimicrobial effect than 2% chlorhexidine and 24% EDTA.

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