

Comparative Study of the Antimicrobial Efficacy of Chlorhexidine Gel, Chlorhexidine Solution and Sodium Hypochlorite as Endodontic Irrigants

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The purpose of this study was to assess the *in vitro* the antimicrobial efficacy of chlorhexidine gluconate gel as an endodontic auxiliary chemical substance compared to sodium hypochlorite (NaOCl) and chlorhexidine gluconate solution. The antimicrobial efficacy of the tested substances was evaluated using the agar diffusion test. The growth inhibition zones produced by 0.2%, 1% and 2% chlorhexidine gel were evaluated against 5 facultative anaerobic bacteria and 4 pigmented Gram-negative anaerobes, and compared to the results obtained by NaOCl and chlorhexidine solution. The largest growth inhibition zones were produced when the test bacteria were in contact with 2% chlorhexidine gluconate gel (11.79 mm), being significantly different ($p < 0.05$) from the growth inhibition zones produced by all NaOCl concentrations, including 5.25% (9.54 mm). However, there was no statistically significant difference ($p > 0.05$) between the growth inhibition zones obtained with equal concentrations of chlorhexidine solution and gel. The results of this study indicate that, as far as its antimicrobial properties are concerned, chlorhexidine gel has a great potential to be used as an endodontic auxiliary chemical substance.

Key Words: chlorhexidine gel, endodontics, antimicrobial activity, irrigants.

INTRODUCTION

Microbial control by biomechanical methods is very important to the effectiveness of the endodontic therapy (1). Anaerobic bacteria, mainly black-pigmented Gram-negative, have been related to the signs and symptoms of endodontic disease, but facultative bacteria, such as *Enterococcus faecalis*, have also been isolated from pathologically involved root canals, and may be involved in failure of endodontic therapy (2).

Root canals with complex anatomy limit the mechanical action of endodontic instruments and thus it is highly recommendable the use of chemical solutions with antimicrobial activity, ability to dissolve organic tissues, lubricant properties and low cytotoxicity as an adjunct to the mechanical preparation (3).

Sodium hypochlorite (NaOCl) has been widely used as the endodontic irrigant of choice, although alternative solutions have already been studied. The use of chlorhexidine gluconate as an irrigant during root canal therapy has been suggested based on its antibacterial effect, substantivity and less malodor and cytotoxicity than NaOCl (4).

Much attention has been directed towards the chemical properties of endodontic irrigants, but there has not been much research on improving their mechanical abilities. The substances used to irrigate the root canal system are most frequently solutions. The use of viscous irrigants, such as glycerin-based chlorhexidine gluconate or urea peroxide has been suggested demonstrating an exceptional lubricant action and improved antimicrobial properties (5). Nevertheless,

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the viscous bases used in these irrigants have little solubility in water, leaving residues on the dentinal walls that interfere with the adequate filling of the root canal system (6). Natrosol gel, however, is a highly efficient, inert, hydrosoluble, non-ionic thickening agent based on anionic substances, such as the chlorhexidine gluconate, which is largely used to thicken shampoos, gels and soaps. Chlorhexidine in natrosol base has already proved its excellent mechanical ability as an intracanal irrigant for smear layer removal (7).

The use of chlorhexidine gel as an endodontic irrigant has been recently proposed (7), which justifies the small number of studies comparing its antimicrobial activity to that of commonly used endodontic irrigants. Therefore, the purpose of this study was to assess *in vitro* the antimicrobial efficacy of chlorhexidine gluconate based on natrosol gel compared to NaOCl and chlorhexidine solution.

MATERIAL AND METHODS

The microbial species used in this study were 5 facultative anaerobic bacteria and 4 pigmented Gram-negative anaerobes commonly isolated from infected root canals, as follows: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Streptococcus sanguis* (ATCC 10556), *S. sobrinus* 6715, *Actinomyces naeslundii* M104, *Porphyromonas gingivalis*, *P. endodontalis*, *Prevotella intermedia* and *Prevotella denticola*. The last four microorganisms were clinical isolates. The facultative anaerobes were kindly donated by the Center of Oral Biology of the University of Rochester, NY, USA.

Each microbial strain was evaluated against the following irrigants: chlorhexidine gluconate gel (0.2%, 1% and 2%); chlorhexidine gluconate solution (0.2%, 1% and 2%); and NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%). All substances were prepared by the same manufacturer (Endogel; Essencial Frama Ltd., Itapetininga, SP, Brazil). Saline was used as a control.

All microorganisms were previously subcultured onto appropriate culture media and under gaseous conditions. The facultative strains were individually inoculated into tubes containing 5 mL of sterile 0.85% saline suspension, adjusted to a concentration of 0.5 of the McFarland BaSO₄ standard (1.5×10^8 colony-forming units). An amount of 500 μ L of each test microbial suspension were inoculated into glass flasks

containing 50 mL of Brain Heart Infusion (BHI) agar at 46°C, vortexed and poured onto 130 mm-plates containing solidified layer of Mueller Hinton (MH) agar. For the anaerobes, sterile swabs were dipped into the microbial suspension (adjusted to 1.0 McFarland BaSO₄ standard = 3.0×10^8 colony-forming units) and inoculated onto pre-reduced 70 mm-plates containing 5% sheep-blood-Fastidious Anaerobe Agar. The inoculum procedures were appropriate to provide a semi-confluent growth of the tested microorganisms.

Stainless steel tubes (8.0 x 1.0 x 10 mm; inner diameter = 6 mm) were placed onto the media surface and fulfilled with 40 μ L of each test substance, followed by incubation at 37°C in appropriate gaseous and time conditions (i.e., facultatives: 48 h at a CO₂ incubator, and anaerobes: 7 days in an anaerobic work station). After the incubation period, the growth inhibition zone was considered as the shortest distance (mm) from the outside margin of the cylinder to the initial point of the microbial growth. Kruskal-Wallis statistical test was used to evaluate the differences between the susceptibility of individual microbial species to the endodontic irrigants. Significance level was set at 5%.

RESULTS

The mean zones of microbial growth inhibition produced by the different concentrations of chlorhexidine gel/solution and NaOCl are presented in Tables 1 and 2, respectively. Figure 1 shows graphically the mean zones of microbial growth inhibition created by the

Table 1. Mean zones of microbial growth inhibition (diameter, in mm) produced by the different concentrations of chlorhexidine solution and gel.

Microbial strains	CLX-S	CLX-S	CLX-S	CLX-G	CLX-G	CLX-G
	0.2%	1%	2%	0.2%	1%	2%
<i>S.aureus</i>	4.00	8.67	8.67	6.67	9.33	9.67
<i>E.faecalis</i>	4.00	4.67	5.00	3.17	3.50	4.33
<i>S. sanguis</i>	6.00	8.67	9.33	6.33	8.00	8.33
<i>S. sobrinus</i>	9.00	9.00	10.33	6.33	10.00	10.33
<i>A. naeslundii</i>	5.83	8.67	8.67	5.17	6.00	6.67
<i>P. gingivalis</i>	11.17	16.50	17.00	14.00	17.83	20.33
<i>P. endodontalis</i>	10.00	12.67	15.67	11.00	13.67	17.83
<i>P. intermedia</i>	12.33	13.33	17.33	16.17	20.00	21.33
<i>P. denticola</i>	5.50	6.50	7.00	4.17	6.00	7.30

tested irrigants.

All tested microorganisms were affected by the different concentrations of chlorhexidine gluconate, either in the solution or gel form. Similar results were not observed for NaOCl, especially in low concentrations.

The largest growth inhibition zones were produced when the tested bacteria were in contact with 2% chlorhexidine gluconate gel (11.79 mm), being significantly different ($p < 0.05$) from the growth inhibition zones produced by all NaOCl concentrations, including 5.25% (9.54 mm).

There was no statistically significant difference ($p > 0.05$) between the growth inhibition zones obtained with equal concentrations of chlorhexidine solution and gel, though the gel form produced zones with larger diameters than the solution.

DISCUSSION

NaOCl is widely used in endodontics as a root canal irrigant at different concentrations. Laboratorial and clinical investigations have shown that NaOCl produces an effective chemomechanical debridement of the root canal system, due to its properties, such as lubricating action for instrumentation, antimicrobial activity and dissolution of pulp tissue (8).

Exactly how NaOCl destroys microorganisms has never been demonstrated experimentally. The dissociation of hypochlorous acid is dependent on the pH and maintenance of the equilibrium between HOCl and OCl⁻, even though HOCl is constantly consumed by its germicidal function (9). However, some microor-

ganisms are resistant to NaOCl, especially at low concentration (10), which was also demonstrated in this study. On the other hand, the usage of NaOCl in high concentrations is undesirable because it is irritating to the periapical tissues (9). Therefore, several attempts have been made in order to find other efficient irrigants that provide a high antimicrobial action with low toxicity.

Chlorhexidine gluconate is a cationic bisguanide that seems to act by adsorption onto the cell wall of the microorganism and causing leakage of intracellular components. At low concentrations, chlorhexidine has a bacteriostatic effect, causing the leaching of small molecular weight substances from microorganisms. At higher concentrations, chlorhexidine has a bactericidal effect due to cytoplasmic precipitation and/or coagulation, probably caused by protein cross-linking (11). The present work not only confirmed the efficiency of chlorhexidine as an antimicrobial agent against oral microorganisms, as reported elsewhere (12,13), but also demonstrated bacterial susceptibility (even resistant organisms) to chlorhexidine gel at both tested concentrations (0.2% and 2%).

In the present study, a modified agar diffusion test was used, which has been widely employed to assess the antimicrobial activity of several endodontic materials *in vitro* (14). The *in vitro* susceptibility of 9 bacterial species was tested against 3 concentrations of NaOCl (0.5%; 1%; 2.5%; 4%; 5.25%) and 3 concentrations of chlorhexidine gluconate gel or solution (0.2%, 1% and 2%). Differences in the bacterial vulner-

Table 2. Mean zones of microbial growth inhibition (diameter, in mm) produced by the different concentrations of NaOCl.

Microbial strains	NaOCl 0.5%	NaOCl 1%	NaOCl 2.5%	NaOCl 4%	NaOCl 5.25%
<i>S. aureus</i>	4.33	4.67	4.83	4.83	5.83
<i>E. faecalis</i>	0.00	0.00	1.50	2.17	3.17
<i>S. sanguis</i>	0.33	0.67	2.67	2.00	3.00
<i>S. sobrinus</i>	0.00	0.00	1.83	2.83	3.50
<i>A. naeslundii</i>	0.00	0.00	1.83	6.67	8.67
<i>P. gingivalis</i>	7.67	11.67	17.83	19.67	22.83
<i>P. endodontalis</i>	0.00	2.83	4.17	6.00	11.50
<i>P. intermedia</i>	0.00	10.33	11.33	12.83	24.50
<i>P. denticola</i>	0.00	1.33	2.00	2.33	2.83

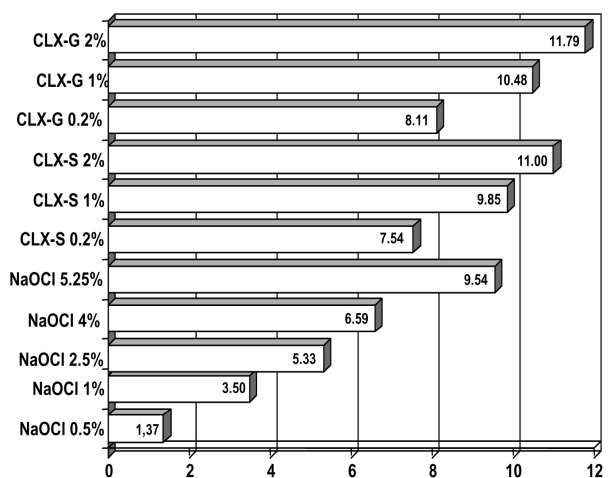


Figure 1. Graphic presentation of the mean zones of microbial growth inhibition (in mm) created by the tested irrigants. CLX-G = Chlorhexidine gluconate gel; CLX-S = Chlorhexidine gluconate solution; NaOCl = Sodium hypochlorite.

ability to the biomechanical procedures during the endodontic treatment have been identified (15). The present results also indicated great variations in the chemical susceptibilities of the tested microorganisms.

NaOCl has an organic tissue dissolving property that is desirable during its action into contaminated root canals, removing pulp remnants that frequently remain after the mechanical preparation. Nevertheless, this dissolving ability is not selective, which means that if NaOCl reaches the periapical region there may be tissue damage.

The toxic effects of NaOCl have been evaluated (16). The authors concluded that 0.025% is a safe concentration of NaOCl to clinical use, maintaining the antimicrobial action without harmful effects to the periapical tissues. Nevertheless, the results of the present study and others (8,10) indicate that NaOCl, mainly at low concentrations, has little or no effect against some microorganisms, such as *E. faecalis*, *S. sanguis*, *S. sobrinus*, and even fastidious bacteria, such as *P. denticola*. The present results showed that 5.25% was the most efficient concentration of NaOCl, which is consistent with the findings of a previous work (17).

Despite the large use of several concentrations of NaOCl as endodontic irrigants, other substances with less cytotoxicity and larger antimicrobial spectrum should be studied. Chlorhexidine gluconate has been used in Endodontics as an irrigating solution, but most frequently in a solution presentation. Ferraz et al. (7) assessed the mechanical action of chlorhexidine gel as an endodontic irrigant, demonstrating its ability to remove smear layer and capacity to eliminate *E. faecalis* from infected root canals *in vitro*. Chlorhexidine gel has also been evaluated as an intracanal medication, with a good performance (14). Natrosol gel, used as a base to the chlorhexidine gluconate in the present study, is a greatly efficient inert, water-soluble thickening agent, which is largely used to thicken shampoos, gels and soaps. The antimicrobial activity observed in the agar diffusion test revealed that the chlorhexidine gel produced larger zones of microbial growth inhibition than the solution with equal concentrations, but no statistically significant difference was confirmed. On the other hand, the growth inhibition zones promoted by both presentation forms of 2% chlorhexidine were significantly larger than those created by all NaOCl concentrations, including 5.25%. All microbial species tested here were sensitive to chlorhexidine gluconate, either in gel or in solution, in

all tested concentrations. Chlorhexidine demonstrated antimicrobial action with a broad spectrum, in agreement with the results of a previous study (12).

The lowest concentration of chlorhexidine gluconate evaluated in the present study (0.2%) showed an *in vitro* antimicrobial activity equivalent to that of 5.25% NaOCl, in agreement with which has been reported elsewhere (18). Delany et al. (12) studied the 0.2% chlorhexidine gluconate solution in extracted teeth, suggesting that this concentration could be used efficiently as either an irrigant solution or an intracanal medication.

The results of the present study confirmed those of Ohara et al. (19), though those authors tested only chlorhexidine solution. In both experiments, chlorhexidine gluconate was superior to NaOCl against various microorganisms in distinct *in vitro* experimental models. One of the parameters evaluated by Ohara et al. (19) was the contact time required by each solution to eliminate pathogens that are frequently isolated from infected root canals. Chlorhexidine gluconate was the most efficient substance, with better results than 5.25% NaOCl.

On the other hand, Gomes et al. (20) verified that 2% chlorhexidine solution and 5.25% NaOCl had similar antimicrobial performance, taking less than 30 s to eliminate *E. faecalis*. The authors also reported that 2% chlorhexidine gel took 1 min to kill the same bacterial strain. However, all irrigants proved to have potential to fulfill contact time necessary under clinical conditions because, during endodontic treatment, bacteria are in contact with irrigants for around 30 to 60 min (17).

Based on the antimicrobial efficacy observed in the present study allied to the excellent performance demonstrated in previous investigations (7,20), it may be concluded that chlorhexidine gel has a great potential to be used as an intracanal auxiliary chemical substance.

RESUMO

O objetivo deste estudo foi avaliar *in vitro* a atividade antimicrobiana do gluconato de clorexidina gel, como irrigante endodôntico, comparando-o ao hipoclorito de sódio (NaOCl) e ao gluconato de clorexidina líquido. A atividade antimicrobiana das substâncias testadas foi avaliada pelo teste de difusão em ágar. As zonas de inibição de crescimento bacteriano produzidas pela clorexidina gel a 0,2%; 1% e 2% foram observadas frente a 5 espécies de bactérias anaeróbias facultativas e 4 espécies de anaeróbios estritos, Gram-negativos e produtores de pigmento negro; e comparados com os resultados obtidos pelo NaOCl e

pela clorexidina líquida. As maiores zonas de inibição foram produzidas quando as bactérias testadas ficaram em contato com a clorexidina a 2% em gel (11,79 mm), apresentando diferença estatisticamente significativa ($p < 0,05$) quando comparados às zonas de inibição de crescimento bacteriano produzidas por todas as concentrações avaliadas de NaOCl, incluindo 5,25% (9,54 mm). No entanto, não houve diferença estatisticamente significativa ($p > 0,05$) comparando as zonas produzidas por concentrações equivalentes de clorexidina líquida ou gel. Os resultados indicaram que a clorexidina em gel tem grande potencial para ser usada como substância química auxiliar quanto às suas propriedades antimicrobianas.

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