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In Vitro Assessment of the Antimicrobial Action and the Mechanical Ability of Chlorhexidine Gel as an Endodontic Irrigant

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The objective of this study was to assess the chlorhexidine gluconate gel as an endodontic irrigant. First the ability of chlorhexidine gel to disinfect root canals contaminated in vitro with *Enterococcus faecalis* was investigated. A scanning electron microscope was also used to evaluate its cleansing ability compared with endodontic irrigants commonly used, such as sodium hypochlorite and chlorhexidine gluconate liquid. The results indicated that the chlorhexidine gel produced a cleaner root canal surface and had an antimicrobial ability comparable with that obtained with the other solutions tested. It was concluded that chlorhexidine gluconate in gel form has potential for use as an endodontic irrigant.

The role of bacteria and their byproducts in the initiation and perpetuation of pulpal and periapical disease is well-established. Thus microbial control by biomechanical procedures is very important for the effectiveness of root canal treatment.

Anaerobic bacteria, mainly black-pigmented Gram-negatives, have been linked 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 related to failure of endodontic therapy (1).

The majority of bacteria found in the root canal microflora may be removed simply by the mechanical action of endodontic instruments. Nevertheless due to the anatomical complexities of many root canals, even after meticulous mechanical procedures, organic residues and bacteria located deep in the dentinal tubules cannot be reached.

Therefore various substances have been used during and immediately after root canal preparation to remove debris and necrotic pulp tissue and to help eliminate microorganisms that cannot be reached by mechanical instrumentation (2). It is highly desirable that the chemical agents selected as endodontic irrigants possess four major properties: antimicrobial activity, dissolution of organic tissues, aid in debridement of the canal system, and nontoxicity to periapical tissues (3).

The most popular endodontic irrigant is sodium hypochlorite (NaOCl), which has been used for well over 4 decades. Although it is an effective antimicrobial agent and an excellent organic solvent (4) it is known to be highly irritant to the periapical tissues (5), mainly at high concentrations. For this reason the search for another irrigant with a lower potential to induce adverse effects is desirable.

Chlorhexidine gluconate has been recommended as a root canal irrigant (6, 7) and many studies have demonstrated its broad-spectrum antimicrobial action, substantivity, and low grade of toxicity (8, 9). However the inability of chlorhexidine to dissolve pulp has been a problem. Some attempts were made to solve this deficiency by the combined use of NaOCl and chlorhexidine (10).

The substances that have been used during chemomechanical preparation are usually in liquid form. Some authors have suggested the use of a viscous irrigant, such as urea peroxide or chlorhexidine gluconate based on anhydrous glycerin that might have better lubricant action and enhancement of the antimicrobial property (11). Nevertheless the viscous bases used in these irrigants are little soluble in water, leaving residues on the dentinal walls that damage the final obturation of the root canal system (12). Therefore the gel base used in the present study was the natrosol gel (hydroxyethyl cellulose) that is a nonionic, highly efficient, inert, water-soluble agent (13) widely used to thicken shampoos, gels, and soaps based on cationic substances such as chlorhexidine gluconate.

Chlorhexidine gluconate in gel has been extensively used in dentistry, showing good results in caries control by reducing *Streptococcus mutans* and *Lactobacillus* species, and as an aid in periodontal therapy by controlling Gram-positive and Gram-negative bacterial growth (14). In endodontics the application of chlorhexidine in gel form has already been suggested, but only as an intracanal medication (15), with no reports on its use to irrigate the root canal.

The purpose of this study was to assess in vitro the chemical (antimicrobial) and mechanical (cleansing) abilities of chlorhexidine gluconate based on natrosol gel as an endodontic irrigant.

MATERIALS AND METHODS

In Vitro Root Canal Disinfection

Seventy freshly extracted, straight, single-root teeth with complete apex formation were used. Conventional access was obtained through the crowns, and the teeth were instrumented to the apex using files size 40.

All teeth were submitted to an ultrasonic bath for 10 min in 17% EDTA followed by 10 min in a 5.25% NaOCl bath, according to Perez et al. (16) to eliminate the smear layer produced during the initial preparation. Their apical foramens were then sealed with epoxy resin to prevent bacterial leakage. The teeth were individually sterilized in bottles containing brain heart infusion broth (BHI) for 20 min at 121°C and the root canal systems were infected with *E. faecalis* according to the method of Siqueira et al. (17).

Microbial samples taken with sterile paper points were collected from all contaminated root canals before instrumentation to confirm the presence and purity of viable *E. faecalis* strains.

The teeth were divided into 3 groups of 20 teeth each and two control groups of five teeth each according to the irrigant used during root canal preparation as follows:

- Group 1: 20 teeth irrigated with 2% chlorhexidine gluconate gel
- Group 2: 20 teeth irrigated with 2% chlorhexidine gluconate liquid
- Group 3: 20 teeth irrigated with 5.25% NaOCl
- Negative control 1: 5 teeth irrigated with distilled water
- Negative control 2: 5 teeth irrigated with natrosol gel.

The same manufacturer (Drogal, Laboratory of Manipulation, Piracicaba, Brazil) prepared all the irrigants.

Each tooth was instrumented twice with a circular filing motion using #35 Hedstrom files for 30 s each time. Before, between, and immediately after the use of the Hedstrom files, 3 ml of the irrigants were injected into the root canal with a 26-gauge needle placed inside the canals as deep as possible without blockage.

At the end of the biomechanical preparation all root canals were flushed with 3 ml of the appropriate irrigant neutralizer followed by a final flush performed with 5 ml of sterile saline delivered in the same way. Neutralizer for NaOCl was 0.6% sodium thiosulfate, whereas 0.5% Tween 80 + 0.07% lecithin was used for chlorhexidine.

Root canals were dried with sterile paper points that were placed in flasks containing 5 ml of sterile BHI. These flasks were vortexed and incubated for 2 days at 37°C. The occurrence of broth turbidity was indicative of bacteria remaining in the root canal. The purity of the bacterial growth (*E. faecalis*) was also assessed as described previously.

Data were analyzed statistically using the SPSS for Windows (SPSS, Inc., Chicago, IL). The χ^2 test was applied, with the level of significance established at 5% ($p < 0.05$).

Cleansing Evaluation

This assessment was conducted on 25 freshly extracted straight single-root teeth with complete apex formation. The coronal access instrumentation procedures up to file #40 and irrigant delivery into the root canals were performed in the same way as described.

The teeth were divided into five groups of five teeth each.

TABLE 1. Turbidity of BHI medium containing postinstrumentation samples

Irrigant	Positive	Negative
2% Chlorhexidine gluconate liquid	9 (45%)	11 (65%)
2% Chlorhexidine gluconate gel	4 (20%)	16 (80%)
5.25% NaOCl	9 (45%)	11 (65%)
Distilled water	5 (100%)	0 (0%)

Control group (distilled water), $n = 5$.

χ^2 test showed no significant difference between the three irrigating agent solutions ($p > 0.1$).

The first group was irrigated with 2% chlorhexidine gel, whereas the second and the third groups were prepared using 5.25% NaOCl and 2% chlorhexidine gluconate liquid, respectively.

Ten teeth were irrigated only with distilled water during root canal instrumentation. Five of these teeth were used as a negative control. The other five teeth were submitted to an ultrasonic bath for 5 min in 5.25% NaOCl, followed by 1 min in 17% EDTA and were used as positive controls.

In all groups, 1 ml of irrigant was delivered between each file change, and a final flush was performed with 5 ml of distilled water delivered in the same way.

Each tooth was fixed in 2.5% glutaraldehyde for at least 30 min, grooved with a bur, and split buccolingually to expose the prepared canals. After dehydration to the critical point the teeth were coated with a thin film of gold and examined with a scanning electron microscope (Zeiss DSM 940A). Photographs of representative areas in the middle third of the root canals were taken at ×2000 magnification. The quantity of residual tissue debris and dentinal filings was assessed.

RESULTS

In Vitro Root Canal Disinfection

All BHI tubes containing the paper points of preinstrumentation samples presented positive turbidity after 72 h of incubation.

The χ^2 test failed to show any significant differences ($p > 0.1$) between the tested irrigants in suppressing bacterial growth (Table 1).

Cleansing Evaluation

The negative control group consisted of specimens irrigated only with distilled water. Figure 1 shows the smear layer, formed over the inner surface of dentinal walls, wherever the dentin was cut.

In the teeth of the positive control group the entire dentin surface was free of a smear layer after the ultrasonic bath with 5.25% NaOCl and 17% EDTA (Fig. 2).

The cleanest tubules were seen in the teeth of group 1 that were treated with 2% chlorhexidine gluconate gel with almost all tubules opened (Fig. 3).

The second group consisted of specimens irrigated with 5.25% NaOCl. These specimens showed a heavy smear layer that covered the apertures of the dentinal tubules; occasionally the location of some tubules was apparent (Fig. 4).

The specimens treated with 2% chlorhexidine gluconate liquid (group 3) presented a thin smear layer-covered surface with the tubular apertures being indicated by cracks (Fig. 5).

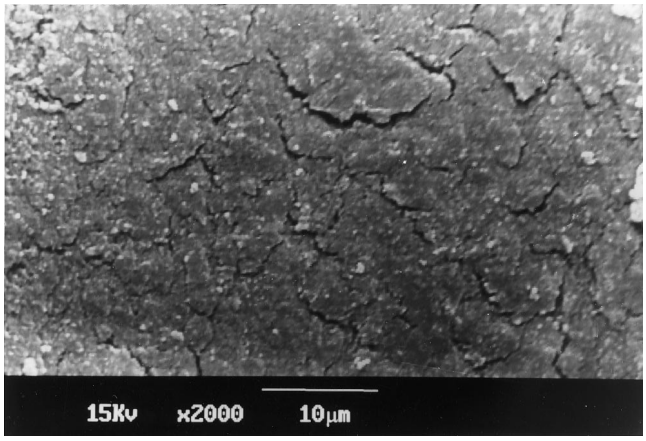

FIG 1. Smear layer covering the inner surface of tooth irrigated with distilled water (negative control group).

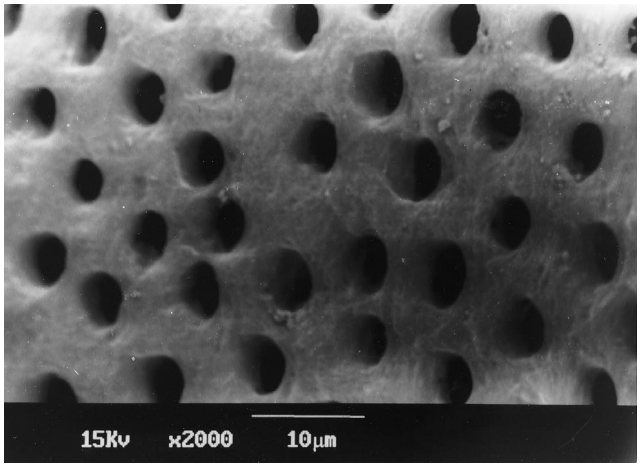

FIG 2. Dentinal tubules of tooth submitted to an ultrasonic bath in 5.25% NaOCl and 17% EDTA (positive control group).

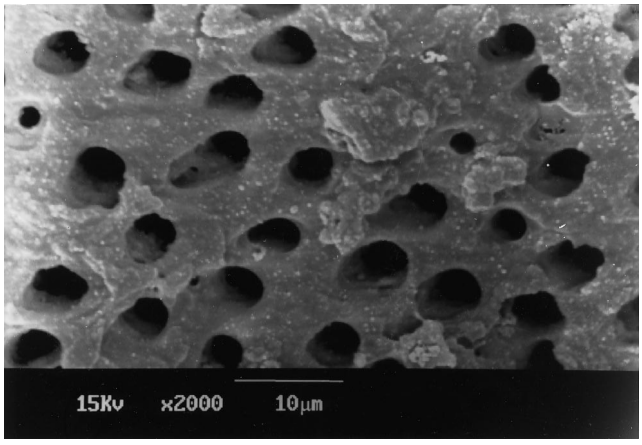

FIG 3. Open dentinal tubules of tooth irrigated with 2% chlorhexidine gluconate gel (group 1).

DISCUSSION

Many attempts have been made to find other efficient irrigants with a high antimicrobial action and low toxicity.

Chlorhexidine gluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At low concentrations of chlorhexidine, small molecular weight substances will leak out, resulting in a bacteriostatic effect. At higher concentrations chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking (7).

Chlorhexidine gluconate has been used in endodontics as an irrigant solution, but always in a liquid form. The chlorhexidine gel was only evaluated as an intracanal medication, demonstrating good performance (15).

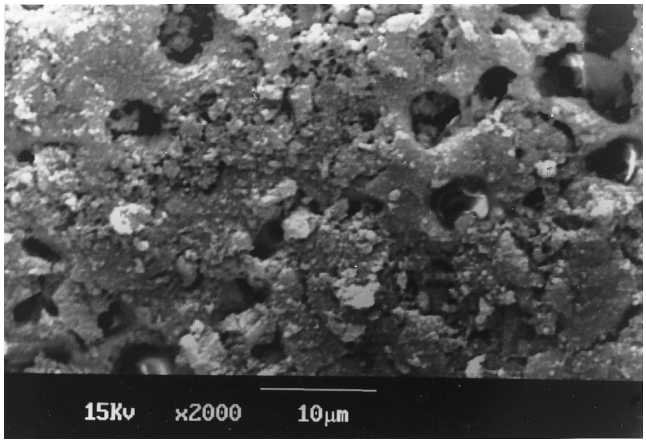

FIG 4. Inner canal surface of tooth irrigated with 5.25% NaOCl (group 2).

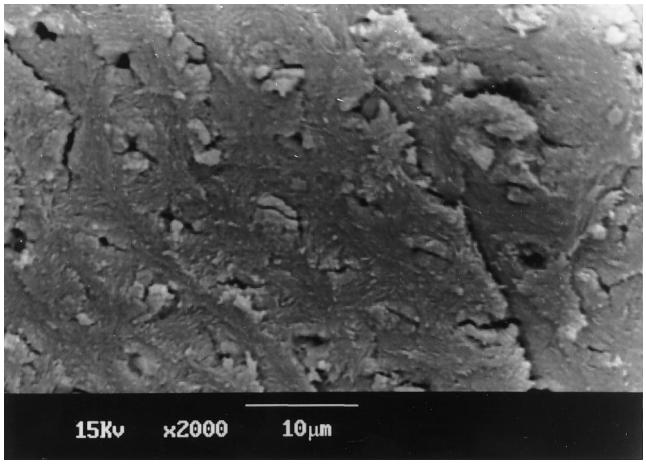

FIG 5. Thin smear layer covering dentinal tubules of tooth irrigated with 2% chlorhexidine gluconate liquid (group 3).

In Vitro Root Canal Disinfection

Various models of in vitro dentinal infection have been proposed, many of them using *E. faecalis*, a Gram-positive coccus, as the chosen bacterium (17).

The infection methodology adopted for the present investigation was adequate, because after 7-day incubation of the contaminated teeth, it was possible to recover pure cultures of viable *E. faecalis*.

Specific neutralizers were applied after the end of root canal instrumentation to make sure that any irrigant vestige have been transferred to the culture medium, altering bacterial growth. Therefore the irrigant substances acted only during the instrumentation procedures.

Cleansing Evaluation

The smear layer associated with root canal treatment consists not only of dentin as in the coronal smear layer, but also of remnants of the odontoblastic process, pulp tissue, and bacteria (18). Therefore an infected smear layer should be removed to eliminate bacteria, facilitate the antibacterial effect of intracanal disinfectants, and to improve the ultimate seal of the root canals (18).

Many irrigating solutions have been used during and after root canal preparation not only as antimicrobial agents, but also to increase the cutting efficiency of root canal instruments and flush away debris.

The SEM allows a detailed examination of the surface and is probably the best tool to identify organic and/or inorganic debris on the inner root canal wall after endodontic preparation.

NaOCl is a widely used irrigant; however it does not efficiently remove the smear layer (19). This fact was confirmed in the present investigation, even though NaOCl was able to reduce the smear layer when compared with the negative control group.

In this study the 2% chlorhexidine gluconate gel produced the cleanest dentin wall surface among the tested irrigants except for the positive control group. The mechanical properties of the gel seem to be the main factor for this difference, because the same chemical agent when used in liquid presentation displayed a lower cleanliness efficiency. Due to its viscosity the gel seems to compensate for chlorhexidine's inability to dissolve pulp tissue by promoting a better mechanical cleansing of the root canal and removing dentin debris and remaining tissues. In addition it has antimicrobial properties and a lubricant action during instrumentation.

Natrosol, a biocompatible carbon polymer (13), was used as a gel base for chlorhexidine gluconate. It is a water-soluble substance and therefore can be completely removed from the root canal with a final flush of distilled water. Viscous irrigants previously studied, even with chlorhexidine gluconate (11), were added to less soluble substances that left residues on the root canal surfaces, impairing the final obturation.

The present study confirmed some published work on the antimicrobial activity of chlorhexidine and sodium hypochlorite and demonstrated that the gel form may overcome the inability of chlorhexidine to dissolve organic tissues by its mechanical action.

Data of the present study indicate that chlorhexidine gel has potential as a routine endodontic irrigant, because it has proved to be of low toxicity and possess a wide antimicrobial spectrum (8, 9). However further studies on chlorhexidine gel use as an endodontic irrigant should be undertaken.

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