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Antimicrobial effects of root canal medicaments against *Enterococcus faecalis* and *Streptococcus mutans*

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Abstract

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Aim To compare the antimicrobial activities of Activ Point (Roeko, Langenau, Germany), Calcium Hydroxide Plus Point (Roeko, Langenau, Germany), calcium hydroxide, 1% chlorhexidine gel and bioactive glass (S53P4) against *Enterococcus faecalis* and *Streptococcus mutans*.

Methodology One hundred and twenty extracted single-rooted human teeth were used. After removing the crowns, root canals were prepared by using the Protaper rotary system. Following autoclave sterilization, root canals were incubated at 37 °C with *E. faecalis* ATCC 29212 and *S. mutans* RSHM 676 for 1 week. The specimens, which were divided into five treatment groups for each microorganism according to the intracanal medicament used, were tested in 10 experimental runs. In each experimental run, 10 roots were included as treatment, one root as positive control and one root as sterility control. Sterile paper points were utilized to take samples from root canals after the incubation of teeth in thioglycollate medium at 37 °C for 1 week. Samples taken from teeth by sterile paper points were inoculated onto sheep blood agar, and following an overnight incubation, the colonies grown on sheep blood agar were counted and interpreted as colony-forming units. Results were tested statistically by using Kruskal–Wallis and Conover's nonparametric multiple comparison tests.

Results CHX gel ($P < 0.001$ and $P < 0.001$), Activ Point ($P = 0.003$ and $P = 0.002$) and $Ca(OH)_2$ ($P = 0.010$ and $P = 0.005$) were significantly more effective against *E. faecalis* than that of $Ca(OH)_2$ Plus Point and bioactive glass, respectively. On the other hand, compared with $Ca(OH)_2$, CHX gel ($P < 0.001$), and Activ Point ($P < 0.001$), bioactive glass ($P = 0.014$) produced significantly lower colony counts of *S. mutans*. When compared with the positive control, treatment with $Ca(OH)_2$ Plus Point ($P = 0.085$ and $P = 0.066$) did not produce significantly lower colony counts of *E. faecalis and S. mutans*, respectively.

Conclusions Compared with the medicaments having an antimicrobial effect because of their alkaline pH, the medicaments containing chlorhexidine were effective against both *E. faecalis* and *S. mutans*.

Keywords: antimicrobial activity, infected dentine, intracanal medicament.

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Introduction

Bacteria in the root canal system are the primary aetiological factor in the development of periradicular inflammatory lesions (Byström *et al.* 1985). Root canal infections have a polymicrobial nature (Gomes *et al.* 2004); hence, anaerobic and facultative

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anaerobic microorganisms are usually found together in endodontic flare-ups and cases with post-treatment disease (Siqueira & Rôças 2009). *Enterococcus faecalis* is a Gram-positive facultative anaerobic bacterium, frequently isolated from persistent root canal infections (Siqueira & Rôças 2009). The fact that *E. faecalis* can withstand the ecologically amenable conditions within the root canal makes it challenging to eliminate (Williams *et al.* 2006). Another microorganism found in infected root canals associated with apical periodontitis is *Streptococcus mutans* (Gomes *et al.* 2004). Although relatively uncommon, *S. mutans* has been shown to be one of the most convenient microorganisms for use in the infected dentine model, mainly because of its ability to adapt to the laboratory setting, unlike strict anaerobic species (Kreth *et al.* 2008).

An important and fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent reinfection. Although cleaning and shaping of the root canal greatly reduce the number of bacteria, these procedures do not completely eliminate them because of the complex anatomy of the root canal system. Thus, the use of intracanal medicaments between appointments can enhance bacterial elimination before canal filling (Byström *et al.* 1985).

Calcium hydroxide ($Ca(OH)_2$) is widely used as an intracanal medicament in endodontic therapy. The high pH of $Ca(OH)_2$ has a destructive effect on bacterial cell membranes and protein structures (Siqueira & Lopes 1999). However, $Ca(OH)_2$ is not equally effective against all bacterial species found in root canals (Gomes *et al.* 2002). Chlorhexidine gluconate gel is an alternative for root canal medication because of its broad-spectrum antimicrobial effect (Ferraz *et al.* 2007). Chlorhexidine gluconate acts by adsorbing onto the cell wall of microorganism causing intracellular component leakage. Although chlorhexidine has several advantages, the additional use of 0.2% chlorhexidine solution for irrigation has been associated with a reduced success of treatment (Ng *et al.* 2011). The negative impact of using alternate irrigation with sodium hypochlorite and chlorhexidine solutions on root canal treatment outcome may be attributed to their interaction product (Ng *et al.* 2011). Gutta-percha points with $Ca(OH)_2$ and chlorhexidine diacetate have been developed to facilitate the application of root canal medicaments. Another alternative as an intracanal medicament is a bioactive glass of the SiO_2–Na_2O–CaO–P_2O_5 system (Zehnder *et al.* 2004). Although the antimicrobial effect of bioactive glasses is not fully understood, it may be based on the high pH environment it creates in aqueous suspensions (Stoor *et al.* 1998) or serving as a source of Ca and P ions to precipitate on bacterial cell wall surfaces, thereby destroying their cellular integrity (Zehnder *et al.* 2004).

The purpose of this study is to compare the disinfection capacities of $Ca(OH)_2$, 1% chlorhexidine gluconate gel, bioactive glass (S53P4), $Ca(OH)_2$ Plus Point (medicated gutta-percha with calcium hydroxide) (Roeko, Langenau, Germany) and Activ Point (medicated gutta-percha with chlorhexidine diacetate) (Roeko, Langenau, Germany) against *E. faecalis* and *S. mutans* in dentinal tubules.

Materials and methods

Teeth preparation

The study was approved by the Research Ethics Committee of Hacettepe University, Faculty of Dentistry, Turkey (Protocol number: 09/82-14). One hundred and twenty extracted, single-rooted human teeth were collected and stored in physiological saline. Bone, calculus and soft tissues on the root surface were removed. The crowns were removed with a diamond disc, and the root lengths were standardized to 16 mm.

Canals were evaluated for apical patency with a file of size 10. The root canals were instrumented to 15 mm using the ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) in conjunction with 2.5% sodium hypochlorite (NaOCl) irrigation. The ProTaper instruments were used in a crown-down manner according to the manufacturer's instructions. After root canal preparation was completed to a size F3 ProTaper instrument, the teeth were placed in an ultrasonic bath with 17% ethylenediamine tetraacetic acid (EDTA) for 4 min and 5.25% NaOCl for 4 min to remove the smear layer (Haapasalo & Ørstavik 1987) and then in physiological saline to remove remaining EDTA and NaOCl.

Each root specimen was placed in a microcentrifuge tube containing 1 mL of thioglycollate medium and then autoclaved for 15 min at 121 °C.

Inoculation

All microbiological experiments were conducted under aseptic conditions in a class II biosafety cabinet (Teknomar, Ankara, Turkey) to prevent airborne

bacterial contamination. *E. faecalis* (ATCC 29212) and *S. mutans* (RSHM 676) grown on sheep blood agar overnight were adjusted to 0.5 McFarland (1.5×10^8 cfu/mL) in thioglycollate medium.

Sterile thioglycollate medium was removed by using sterile micropipettes from microcentrifuge tubes and then replaced with 1 mL of bacterial suspension. The tubes were closed and incubated at 37 °C for 7 days. Contaminated thioglycollate was replaced with fresh sterile media every 3 days. At the end of each experimental run, bacterial viability and purity were checked by inoculating 10 μL of thioglycollate medium from each tube onto sheep blood agar.

Medication

At the end of the inoculation period, root specimens were removed from the microcentrifuge tubes and mounted in a sterile environment. The groups, each having 10 teeth samples for each microorganism, were medicated with five different medicaments and incubated for 1 week at 37 °C in microcentrifuge tubes with sterile thioglycollate medium. The specimens were tested in 10 experimental runs. Each experimental run consisted of 10 roots as treatment, one root as a positive control and one root as a sterility control. Positive controls were prepared via flushing 3 mL of sterile physiological saline into the root canals inoculated with bacterial suspensions. For testing sterility (negative control), 3 mL of sterile physiological saline was syringed into the root canals inoculated with sterile thioglycollate medium. Both controls were examined in each run for the elimination of any unexpected conditions such as contamination with other bacteria or misinoculation of the test microorganisms.

For the $Ca(OH)_2$-treated group, $Ca(OH)_2$ (Merck, Darmstadt, Germany) powder was mixed thoroughly with physiological saline (1 : 1), and the thick mixture was placed into the root canal with a lentulo spiral and a plugger. For the chlorhexidine gel group, 1% chlorhexidine gluconate gel (Drogsan, Ankara, Turkey) was placed into the root canal with a lentulo spiral.

For the $Ca(OH)_2$ Plus Point (Roeko, Langenau, Germany) and Activ Point (Roeko, Langenau, Germany) groups, medicated size 30 gutta-percha point trimmed to 15 mm was inserted into the root canals in the presence of physiological saline.

For the bioactive glass (S53P4) group, bioactive glass powder (Abmin Technologies Ltd, Turku, Finland) prepared in physiological saline (1 : 1) was placed into the root canal with a lentulo spiral and a plugger.

Bacterial sampling

The specimens were transferred from the microcentrifuge tubes to the sterile environment as before. Intracanal medicaments were removed according to the following protocol prior to bacterial sampling. For the removal of $Ca(OH)_2$ and chlorhexidine gel, a ProTaper F3 rotary instrument was used for 20 s. The neutralizer for $Ca(OH)_2$, was 3 mL of 0.5% citric acid and 3% Tween 80, and 0.3% L-α-lecithin mixture was used for chlorhexidine gel and then irrigated with 3 mL of physiological saline for both medicament groups. Medicated gutta-percha points were removed using tweezers. The canals were then irrigated with 3 mL of 0.5% citric acid for $Ca(OH)_2$ Plus Point, and 3 mL of 3% Tween 80 and 0.3% L-α-lecithin mixture for Activ Point followed by 3 mL of physiological saline for each one. Bioactive glass was removed using ProTaper F3 rotary files for 20 s. The canals were then irrigated with 6 mL of physiological saline. The root canals were irrigated with 6 mL of physiological saline only in the positive and negative control groups.

Bacterial sampling was performed using a sterile size 30 paper point. The paper point was left in the root canal for one minute and then transferred to a microcentrifuge tube containing 0.5 mL of physiological saline and then vortexed for 30 s. Samples of 0.1 mL of physiological saline from each tube were inoculated onto sheep blood agar, and after overnight incubation at 37 °C, the colonies grown on sheep blood agar were counted and interpreted as colony-forming units per millilitre (cfu/mL).

Statistical analysis

The median differences amongst groups were compared by the Kruskal–Wallis test and the Conover's multiple comparison test. A P value <0.05 was considered statistically significant.

Results

The colony counts (CFU) of *E. faecalis* and *S. mutans* after treatment with five different medicaments are presented in Fig. 1. Chlorhexidine gluconate gel ($P < 0.001$ and $P < 0.001$), Activ Point ($P = 0.003$

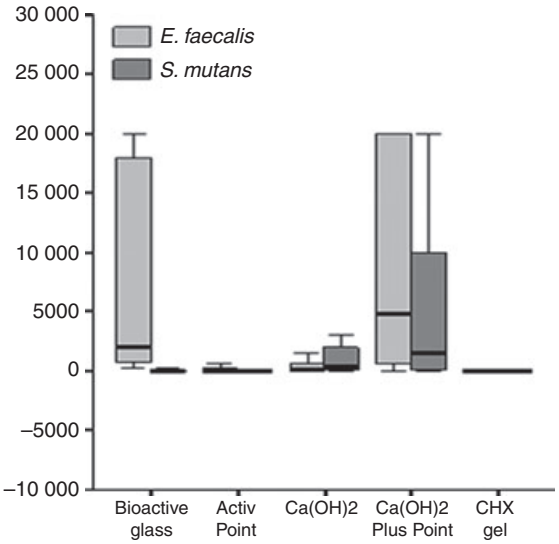

Figure 1 Box plot representing the number of CFU of *Enterococcus faecalis* and *Streptococcus mutans* in different treatment groups. The horizontal lines in the middle of each box indicate the median, whilst the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum number of CFU of *E. faecalis* and *S. mutans*.

and $P = 0.002$) and $Ca(OH)_2$ ($P = 0.010$ and $P = 0.005$) *were* significantly effective against *E. faecalis* when compared with $Ca(OH)_2$ Plus Point and bioactive glass, respectively. Chlorhexidine gluconate gel ($P < 0.001$), Activ Point ($P < 0.001$) and bioactive glass ($P = 0.014$) produced significantly lower colony counts of *S. mutans* than $Ca(OH)_2$. When compared with the positive control (2×10^4 cfu/mL), $Ca(OH)_2$ Plus Point allowed as much microbial growth of *E. faecalis* ($P = 0.085$) and *S. mutans* ($P = 0.066$). For each individual run, if contamination or misinoculation of experimental samples was suspected in control groups, results were excluded, and the experiment was repeated. Activ Point and chlorhexidine gluconate gel were found to be the most effective medicaments against both microorganisms.

Discussion

The prognosis of root canal treatment depends on root canal disinfection (Sjögren *et al.* 1997) with the additive effect of coronal and apical seal (Ray & Trope 1995). Many studies have shown the importance of intracanal medication during root canal disinfection (Trope *et al.* 1999, Figini *et al.* 2008). This study investigated the disinfection potential of five different medicaments. The *ex vivo* infected dentine model used was based on the model developed by Haapasalo & Ørstavik (1987) with modifications. This model is used for evaluation of antimicrobial effects of root canal disinfectants, medicaments and root canal sealers (Özcan *et al.* 2011). The study mimicked clinical conditions by using human teeth with the presence of cementum instead of bovine teeth (Lui *et al.* 2004). In addition, microorganisms were grown in liquid medium containing a tooth, which is similar to those exposed to the oral environment.

E. faecalis and *S. mutans* were chosen as test microorganisms because they are both common dental pathogens. *E. faecalis* is associated with persistent apical periodontitis and resists elimination from root canals (Dahlén *et al.* 2000). *Streptococcus* species were reported to be one of the most relevant taxa in symptomatic apical periodontitis (Rôças *et al.* 2011). *S. mutans* has been detected in root canal infections (Gomes *et al.* 2004) and was also reported to be a strong biofilm producer (Jiang *et al.* 2011), helping the bacteria to adapt and persist in root canals.

In this study, 0.3% of L-α-lecithin and 3% Tween 80 for chlorhexidine-containing medicaments (Zamany & Spangberg 2002) and 0.5% citric acid for calcium hydroxide-containing medicaments (Möller 1966) were used to eradicate any possible trace of residual antimicrobial agents. The neutralization effect of aforementioned solutions is important to assess the effect of antimicrobial disinfection capacities. They act by stopping the disinfection process at the time of use, thus enabling the evaluation of their net effect.

The results of this study clearly indicated that $Ca(OH)_2$ was not sufficient for the complete elimination of *S. mutans* from root canals (Fig. 1). The antimicrobial effect of $Ca(OH)_2$ against *E. faecalis* was lower than chlorhexidine-impregnated medicaments but not significantly different. This relative inefficacy of $Ca(OH)_2$ against *E. faecalis* was consistent with previous studies (Ercan *et al.* 2006, Delgado *et al.* 2010, Kandaswamy *et al.* 2010). Lui *et al.* (2004) reported that $Ca(OH)_2$ was effective in the superficial dentine compared with the deeper layers. The reason for this result was reported to be the superficial exposition of microorganisms to lethal levels of hydroxyl ions only at the tubule orifice (Siqueira & Lopes 1999). Sampling methods in this study, which included taking samples with paper points from superficial dentine, might be the reason of the comparable antimicrobial effect of $Ca(OH)_2$ with chlorhexidine gel and Activ

Point. In this study, $Ca(OH)_2$-containing gutta-percha points had the least favourable results. The likely explanation for this result could be the inadequate amount of $Ca(OH)_2$ released, which was insufficient to overcome the strong buffering capacity of dentine (Wang & Hume 1988, Fulzele *et al.* 2011).

The antimicrobial effect of chlorhexidine in the root canal has been reported to occur after medication of root dentine with chlorhexidine for 7 days (Komorowski *et al.* 2000). Lewinstein *et al.* (2012) found that the addition of chlorhexidine diacetate to provisional cements provided antimicrobial effects against *S. mutans.* Chlorhexidine delivery systems such as gels or gutta-percha have the advantage that they maintain the active agent in contact with the root canal walls for several days. Findings of this study indicated that 1% chlorhexidine gluconate gel was effective against both microorganisms tested. Gomes *et al.* (2001) found that chlorhexidine in the liquid and gel forms at different concentrations (0.2%, 1% and 2%) were effective against *E. faecalis* at different times. On the contrary, clinical use of 0.2% chlorhexidine for irrigation has been reported to reduce the success of treatment (Ng *et al.* 2011). This finding was attributed to the negative impact of using alternate irrigation with sodium hypochlorite and chlorhexidine or a lower concentration. In this study, Activ Point, which contains chlorhexidine diacetate, has also been shown to be effective against *E. faecalis,* in agreement with several previous studies (Lui *et al.* 2004, Ebert *et al.* 2008). Barthel *et al.* (2002) concluded that Activ Points were less effective than chlorhexidine gel in disinfecting root canals when infected by human oral flora. On the contrary, in this study, the antimicrobial effect of Activ Point was similar to chlorhexidine gluconate gel. This finding could be due to the monospecies microorganisms on the dentine model tested in this study. The antimicrobial effect of Activ Point was also dependent on the presence of moisture in and around the root canal to release chlorhexidine diacetate from the gutta-percha in this test method.

Bioactive glass was effective against *S. mutans* as reported previously (Stoor *et al.* 1998, Zehnder *et al.* 2004). The antimicrobial effect of bioactive glass might be due to its high pH, osmotic effects or the Ca^{+2} concentration in the dentine environment (Stoor *et al.* 1998). In addition, Zehnder *et al.* (2004) found that the antimicrobial effect of bioactive glass increased in the presence of dentine. The antimicrobial activity of bioactive glass against *E. faecalis* was moderate when compared with 2% chlorhexidine gel, 2% metronidazole gel and calcium hydroxide (Krithikadatta *et al.* 2007). On the contrary, there was no increase in antimicrobial effect of bioactive glass in the infected dentine model against *E. faecalis* compared with calcium hydroxide, chlorhexidine gel and Activ Point.

Conclusions

$Ca(OH)_2$ was effective against *E. faecalis* and moderately effective against *S. mutans,* whilst $Ca(OH)_2$-medicated gutta-percha showed inadequate antimicrobial activity for the elimination of both microorganisms. Bioactive glass showed antimicrobial effects against *S. mutans* but not against *E. faecalis*. Activ Point, and 1% chlorhexidine gel was microbiologically potent against both *E. faecalis* and *S. mutans.* It could be concluded that chlorhexidine-impregnated medicaments were more efficient than alkaline-pH-acting medicaments.

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