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Octenidine in root canal and dentine disinfection *ex vivo*

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Abstract

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Aim The aim of the present study was to investigate the antimicrobial activity of octenidine on *Enterococcus faecalis* ATCC 29212 in a dentine block model.

Methodology Fifty-six root segments of extracted human teeth were infected with *E. faecalis* for 4 weeks. Octenidine-phenoxyethanol gel (1 : 1) was applied for different timing: 1 min, 10 min, 7 days and in a different formula (1 : 3) for 10 min. Three samples were chosen for the group with placebo gel and for the group without infection (negative control). Dentine samples were collected, and the total count of bacteria and colony-forming units were determined. In addition, for controls and the 10 min group with 1 : 1 gel, the proportion of viable bacteria (PVB) was assessed.

Results Octenidine was particularly effective after incubation periods of 10 min and 7 days. The mean PVB decreased significantly from 57.2% to 5.7% after 10 min application. After 7 days, only one of 10 samples showed positive culture.

Conclusion The present study showed the effectiveness of octenidine against *E. faecalis* in dentine disinfection. Further laboratory and clinical studies are required.

Keywords: dentine disinfection, *Enterococcus faecalis*, octenidine, root canal dressing.

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Introduction

Mechanical instrumentation and chemical irrigation are essential in the reduction of bacteria in the root canal system. In order to eliminate remaining microorganisms, an interappointment dressing is commonly used. The effects of calcium hydroxide (CH) in root canal and dentine disinfection are well documented. The antimicrobial effects of CH may be directly related to its high alkalinity, and a great majority of the microbial species isolated from root canals shows susceptibility both clinically and *ex vivo* (Byström & Sundqvist 1981, 1983, Byström *et al.* 1985, Waltimo *et al.* 2005). However, several studies demonstrated that CH fails to eradicate *Enterococcus faecalis* residing in infected root canal systems (Byström *et al.* 1985, Haapasalo & Ørstavik 1987, Safavi *et al.* 1990, Ørstavik & Haapasalo 1990, Evans *et al.* 2002, Peters *et al.* 2002, Weiger *et al.* 2002, Saleh *et al.* 2004). This is of clinical importance since *E. faecalis* is the most frequently isolated species from teeth associated with failed root canal treatment (Molander *et al.* 1998, Sundqvist *et al.* 1998, Pinheiro *et al.* 2003, Lui *et al.* 2004).

An explanation for the resistance against CH might be the ability of *E. faecalis* to invade dentinal tubules, isthmuses and other ramifications of a root canal system (Love 2001). Furthermore, it has been documented to be able to survive for prolonged periods in high alkalinity (Byström *et al.* 1985, Haapasalo & Ørstavik 1987) and harsh nutrient conditions (Hartke *et al.* 1998, Figdor *et al.* 2003, Portenier *et al.* 2005).

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Evans *et al.* (2002) reported the survival of *E. faecalis* at high pH was due to a functioning proton pump with the capacity to acidify cytoplasm. On the other hand, the buffering capacity of dentine can inhibit the pH increase and the antimicrobial activity of CH in the root canal (Haapasalo *et al.* 2000).

Many alternative antimicrobial agents have been tested for their ability to eliminate *E. faecalis* from the root canal system. *In vitro* studies have indicated that chlorhexidine and camphorated paramonochlorophenol (CMCP) may be potent in the elimination of *E. faecelis* from the root canal system (Byström *et al.* 1985, Haapasalo & Ørstavik 1987, Ørstavik & Haapasalo 1990, Basrani *et al.* 2003, Gomes *et al.* 2003). However, other species, such as *Pseudomonas aeruginosa* may be show resistance to CMCP (Ørstavik & Haapasalo 1990). Furthermore, in contrast to CH long-term effects of CMCP and CHX in interappointment dressings are not clear (Ørstavik & Haapasalo 1990, Gomes *et al.* 2003). Several studies investigated combinations of two medicaments to get additive or synergistic effects. Combinations of CH with iodine potassium iodide or CHX seem to provide increased antibacterial activity of a broad spectrum (Waltimo *et al.* 1999, Basrani *et al.* 2003, Gomes *et al.* 2003, Sirén *et al.* 2004).

Octenisept is an antiseptic for skin burns, wound disinfection and mouth rinses consisting of octenidine hydrochloride and phenoxyethanol. Octenidine hydrochloride [*N*,*N*′-(1,10 decanediyldi-1[4*H*]-pyridinyl-4-ylidene)bis(1-octanamine)dihydrochloride] belongs to the bipyridines carrying two cationic active centres per molecule and demonstrates broad spectrum antimicrobial effects covering both Gram-positive and Gram-negative bacteria, fungi and several viral species (Sedlock & Bailey 1985). The mode of action is bactericidal/fungicidal by interfering with cell walls and membranes. Phenoxyethanol, an ethanol derivate, serves as a preservative component in Octenisept which is also supposed to improve the antibacterial activity of octenidine synergistically. Previous studies showed the efficacy of octenidine against dental plaque-associated bacteria, such as *Streptococcus mutans* and *Actinomyces viscosus* comparable to chlorhexidine digluconate (Slee & O'Connor 1983, Decker *et al.* 2003). According to the manufacturer (Schülke & Mayr, Norderstedt, Germany), the toxicity parameters of Octenisept are $EC_{50} > 3200$ mg L^{-1} assessed by OECD 209-standards and LD_{50} for rats >45 000 mg kg^{-1}. No carcinogenic or mutagenic effects have been registered.

The aim of the present study was to assess the antimicrobial effect of octenidine as an intracanal medicament against *E. faecalis*, using a dentine block model by culturing and by vital-dead staining.

Material and methods

Root dentine specimens

Fifty-six straight-rooted teeth extracted because of orthodontic or periodontal reasons were collected. The teeth had no carious lesions and were previously not root filled. After extraction, they were stored in hydrogen peroxide (3%). The teeth were incubated in an excess of physiological saline for 14 days before their preparation. Calculus and tissue remnants were removed with curettes. The crowns were removed with a rotating diamond saw (EXAKT mikro 40, Norderstedt, Germany) under water cooling, and the roots shortened apically, resulting in 7 mm long segments. These specimens were kept in tap water during all procedures to avoid dehydration.

The root canal was enlarged with Hedstroem files (Dentsply Maillefer, Ballaigues, Switzerland) to a size 60 under constant irrigation with a total of 10 mL NaOCl (1%). Subsequently, the smear layer was removed by rinsing with 10 mL citric acid (10%) for 5 min and, finally, with 20 mL physiological saline (Baumgartner *et al.* 1984, Machado-Silveiro *et al.* 2004). The root surface was coated with nail varnish, which was allowed to dry at room temperature for 1 h.

Six test specimens were randomly assigned to one tube containing 20 mL Schaedler broth (Oxoid Ltd, Basingstoke, UK), and, in order to improve broth penetration into dentinal tubules, treated ultrasonically (130 W, Sonics vibra cell, Sonics & Materials Inc., Newtown, CT, USA) for 30 s. Subsequently, the test specimens were autoclaved at 121 °C for 15 min. For sterility check, which was uniformly negative, the tubes were incubated at 37 °C for 72 h. After sterilization, the tubes were handled aseptically, and all subsequent procedures took place in a laminar airflow chamber to avoid contamination.

Infection of root specimens

Enterococcus faecalis ATCC 29212 grown on tryptic soy agar at 37 °C for 48 h was suspended in Schaedler broth, corresponding to an optical density of 0.5 on the McFarland scale. Each of the tubes containing six test specimens in 20 mL of Schaedler broth were inoculated

Table 1 Test groups and controls

Group	G-NO	G(1:1)-1min	G(1:1)-10min	G(1:3)-10min	G(1:1)-7d	Co-p	Co-n
N	10	10	10	10	10	3	3
Infection (days)	28	28	28	28	28	28	–
Dressing	–	O 1 : 1	O 1 : 1	O 1 : 3	O 1 : 1	PG	–
Incubation	–	1 min	10 min	10 min	7 days	7 days	–

G, group; G-NO, without Octenisept (=baseline infection control); Co-p, control positive; Co-n, control negative; O, Octenisept, PG, placebo gel.

with 200 μL of this suspension, resulting in an initial density of approximately 2.4×10^5 CFU mL^{-1}. The specimens were then incubated at 37 °C for 4 weeks, refreshing the nutrient broth twice a week. The purity of the cultures was controlled once a week, based on the colony morphology and cellular characteristics on Schaedler agar-cultivated samples. After 4 weeks of infection, the test specimens were irrigated with 10 mL physiological saline and dried with sterile paper points.

Study design

The specimens were randomly assigned to test groups and control groups, as listed in Table 1. The medicaments used and their exact constituents are listed in Table 2. Each canal was completely filled with gel, using a syringe. Extruded gel was carefully removed, and the apical and cervical surfaces were sealed with sterile aluminium foil. The test specimens were then incubated under humid conditions at 37 °C for 1 min, 10 min or 7 days.

Prior to bacterial sampling, the gel was removed by irrigating with 10 mL physiological saline. Subsequently, the canal was dried with sterile paper points. Sampling was carried out by preparing the root canals circumferentially from size 60 to size 90, with a sterile size 25 Hedstroem file. The fine dentine chips obtained were collected into a tube containing 1 mL phosphate-buffered saline (Oxoid Ltd) and three small glass beads. The suspension was homogenized by a vigorous vortexing for 3 min, followed by an ultrasonic treatment (26 W) for 10 s. The dentine chips were allowed to sediment for 5 min, and the supernatant was used for microbiological analyses. In pilot experiments, this procedure yielded uniform and high bacterial counts, indicating an effective homogenization of the sample without disturbing the integrity of bacterial cells.

Table 2 Composition of the gels used as dressings

	Octenisept 1 : 1 (g)	Octenisept 1 : 3 (g)	Placebo (g)
Octenidine	0.5	0.5	–
Phenoxyethanol	0.5	1.5	–
Glycerol	44.5	44.5	9.0
Siliciumdioxid	5.0	5.0	1.0

Microbiological analysis

Total count

Undiluted samples (3.5 μL) were analysed in a Neubauer chamber under a phase contrast microscope (Provis AX 70, Olympus AG, Volketswil, Switzerland). A total of 10×16 squares was counted, and the average value multiplied with 1.25×10^6 to calculate the total count (TC/mL).

Colony-forming unit

A dilution series of the original sample was prepared in sterile saline and, consequently, aliquots of 10 μL were inoculated onto Schaedler agar and incubated at 37 °C for 48 h. Visible colonies from appropriated dilutions were counted, and colony-forming unit (CFU mL^{-1}) was calculated.

Proportions of viable bacteria

Proportions of viable bacteria (PVB) was determined for groups G-NO and G(1:1)-10min. Undiluted samples were centrifuged at 8000 *g* for 5 min, washed once with sterile saline, harvested by centrifugation and resuspended in 100 μL of staining solution (Syto 9 and propidium iodide, Live/Dead BacLight Bacterial Viability Kit, MoB/Tec, Göttingen, Germany). After incubation in a dark chamber for 15 min, the samples were centrifuged, supernatant was discarded and the pellet was resuspended in 10 μL distilled water and immediately analysed under an epifluorescence microscope (Provis AX 70, Olympus AG). Syto 9-labelled viable bacteria by green fluorescence (excitation FITC 450–490 nm) in combination with propidium iodide which marked dead bacteria by red fluorescence (excitation Rhodamin 540 nm). Ten visual fields at a magnification of 800× were recorded. PVB was calculated as the number of bacterial cells associated with green

fluorescence divided by the total number of emitting either green or red fluorescence.

Data analysis

TC $mL^{-1}_{(mean)}$ ± SD and CFU $mL^{-1}_{(mean)}$ ± SD, which were calculated for all groups, were log_{10}-transformed. PVB values were only recorded for the groups G-NO and G(1:1)-10min. $PVB_{(mean)}$ ± SD and $CFU_{(mean)}$ ± SD, and their corresponding 95% confidence intervals were calculated.

Results

The log_{10} TC $mL^{-1}_{(mean)}$ values were homogenous in all test groups, and ranged between 6.8 and 7.1 (Table 3). The CFU $mL^{-1}_{(mean)}$ determinations revealed counts lower than the corresponding TC mL^{-1}. In all test groups, the log_{10} CFU $mL^{-1}_{(mean)}$ was significantly lower in comparison to that of G-NO (Fig. 1).

Table 4 illustrates the number of samples with and without bacterial growth. Incubation for 7 days revealed the highest number of negative growth samples. The negative control group remained uniformly free of growth, whereas the positive control group showed log_{10} CFU $mL^{-1}_{(mean)}$ of 5.9. PVB was evaluated for G-NO and G(1:1)-10min. After 10 min of incubation PVB decreased significantly from 57.2% to 5.7% (Fig. 2).

Table 4 Growth after sampling

Group	*N*	CFU-negative	CFU-positive	Log_{10} CFU/$mL^{a}_{(mean)}$
G-NO	10	0	10	6.5
G(1:1)-1min	10	0	10	4.7
G(1:1)-10min	10	5	5	3.1
G(1:3)-10min	10	4	6	3.5
G(1:1)-7d	10	9	1	3.0
Co-p	3	0	3	5.9
Co-n	3	3	0	0

[a]CFU-negative samples excluded.

Discussion

The dentine block model used in this experiment was modified from the one developed by Haapasalo & Ørstavik (1987). In the present study, extracted human teeth were used instead of bovine incisors (Safavi *et al.* 1990, Weiger *et al.* 2002, Lui *et al.* 2004, Saleh *et al.* 2004). Furthermore, instead of round burs, fine Hedstroem files were used for sampling. These achieved smaller particle sizes in the samples that enabled further homogenization of the samples and more reliable and repeatable quantification of bacterial yields. *Enterococcus faecalis*, which is commonly used as a reference organism in dentine disinfection studies, was chosen for the present study (Haapasalo & Ørstavik 1987, Ørstavik & Haapasalo 1990, Siqueira & de Uzeda 1997, Gomes *et al.* 2003,

Table 3 Total counts of bacteria in the samples

Group	G-NO	G(1:1)-1min	G(1:1)-10min	G(1:3)-10min	G(1:1)-7d	Co-p	Co-n
TC $mL^{-1}_{(mean)}$ (±SD)	7.0 (0.39)	7.1 (0.35)	6.9 (0.18)	7.0 (0.24)	6.9 (0.19)	7.0 (0.16)	6.4 (0.11)

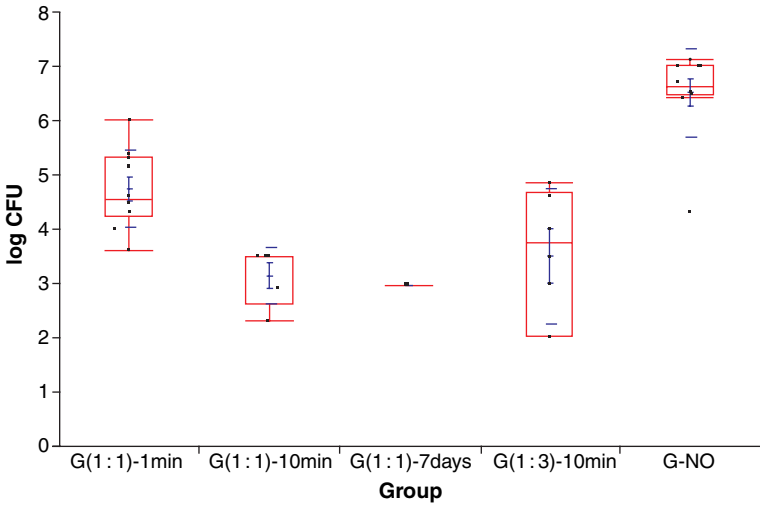

Figure 1 Distribution of log10 CFU values. Samples without bacterial growth are not included (see Table 4).

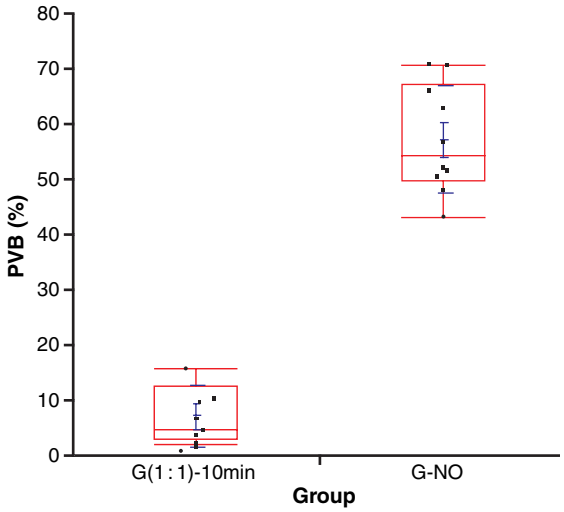

Figure 2 Comparison of proportion of viable bacteria between the groups G(1:1)-10min and G-NO.

Vivacqua-Gomes *et al.* 2005). It is commonly associated with persistent cases of apical periodontitis and considered to be difficult to eradicate from infected root canals and dentine (Molander *et al.* 1998, Sundqvist *et al.* 1998). *Enterococcus faecalis* is a tolerant bacterium easy to culture with a documented capability to invade into dentinal tubules *ex vivo* (Akpata & Blechman 1982, Haapasalo & Ørstavik 1987). In a preliminary experiment, the minimal inhibitory concentration (MIC) of Octenisept was determined for five *E. faecalis* strains: two root canal isolates, two nonoral clinical isolates and a laboratory strain (ATCC 29212). No difference was detected as the MIC varied only slightly and ranged between 0.0625 and 0.1875 μg mL^{-1}. Therefore, *E. faecalis* ATCC 29212 can be considered as a representative strain in this regard.

An exposure time of 7 days was chosen to allow the direct comparisons of the results with those of other investigations applying CH. There are differing reports concerning the efficacy of CH to disinfect dentine: some report only a minor antibacterial activity against *E. faecalis* without effective killing in infected dentinal tubules (Gomes *et al.* 2003, Saleh *et al.* 2004, Sirén *et al.* 2004). However, in a recent paper by Zehnder *et al.* (2006) a strong antibacterial effect of CH dressing against human root canals and dentinal tubules residing *E. faecalis* cells was detected, *ex vivo*.

The present study aimed to quantify surviving bacteria by CFU of the homogenized dentine filings. In addition to the parameter CFU, fluorescence labelling of the bacteria give valuable information about their vitality status (Weiger *et al.* 2002). In the present study, both of these techniques were applied in order to exclude false-negative CFU-based determinations.

Possible inhibition of the growth by the carry-over effect of the Octenisept remainders was controlled by an agar diffusion test. The dentine samples in PBS from the groups G-1min, G(1:1)-10min, G(1:3)-10min, Co-p and Co-n showed no inhibitory effect. On the contrary, all G(1:1)-7d samples inhibited *E. faecalis* growth on Schaedler agar. Possibly, a dressing for 7 days may allow higher saturation of dentinal tubules by the gel compounds. Complete removal of the gel by rinsing with physiological saline solution may be impossible.

A dressing for 7 days was highly efficient against *E. faecalis* residing in the root canal and dentinal tubules, eliminating all the cultivable bacteria in nine of the 10 test specimens. A complicated root canal anatomy or incomplete dressing may have contributed to the survival of bacteria in one test specimen.

In the present study, Octenisept gel showed modest antimicrobial activity against *E. faecalis* in root canal and dentine after 1 min, and a more pronounced effective dentine disinfection after incubation for 10 min and 7 days. These results substantiated the previously reported potency of octenidine hydrochloride against various microorganisms (Bailey *et al.* 1984, Decker *et al.* 2003). Octenisept 1 : 3 gel [G(1:3)-10min] with a higher phenoxyethanol concentration did not appear to be more effective than the 1 : 1 gel (Tables 2–4). Therefore, it can be assumed that octenidine itself is the active agent and that the synergistic antimicrobial effect of phenoxyethanol is limited.

The antibacterial potential of octenidine has been well documented and compared to some other disinfectants used in endodontics. The susceptibility of *S. mutans* and *A. viscosus* to octenidine has been reported to be comparable to chlorhexidine digluconate (Slee & O'Connor 1983, Decker *et al.* 2003). Furthermore, it has been shown that octenidine resists an organic challenges, i.e. maintains its antimicrobial efficacy in the presence of organic material comparably to chlorhexidine and iodine (Pitten *et al.* 2003). This is of interest, as, in a root canal system both organic and inorganic inhibitory factors are present that may weaken the antimicrobial efficacy (Haapasalo *et al.* 2000). The efficacy observed in the present study indicates the performance of octenidine was sufficient in this biologically complex environment. This indicates the justification for further comparative studies, including common antimicrobial agents used in endodontics.

Conclusion

This study indicated the potential of octenidine to eradicate *E. faecalis* from the root canal and dentine *ex vivo*. Further studies are necessary in order to evaluate the toxicological aspects and the efficacy of octenidine to eradicate other bacteria from infected dentine and root canals in comparison to antimicrobial agents commonly used in endodontics.

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