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Effect of electrophoretically activated calcium hydroxide on bacterial viability in dentinal tubules – *in vitro*

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Abstract – To evaluate the ability of electrophoretically activated calcium hydroxide (CH) to eliminate bacteria in dentinal tubules. In an *in vitro* model of dentinal tubule infection, 18 cylindrical root specimens prepared from freshly extracted bovine teeth were used. After removal of the smear layer, intracanal dentinal tubules were infected with *Enterococcus faecalis* for 21 days. In 12 specimens, CH paste was placed in the root canals for 7 days. In six of these, an electrophoretic current (10 mA per 10 min), using two electrodes, was applied after placing the medicament in the canal. Powder dentin samples obtained from within the canal lumina using ISO 025, 027, 029, 031 and 033 burs were examined for the presence of vital bacteria by inoculating agar plates and counting colony forming units. ANOVA with repeated measures was used to analyze results. A significant difference was found between experimental groups and the positive control group. CH and electrophoretically activated CH significantly ($P < 0.001$) reduced bacterial viabilities in dentinal tubules to a depth of 200 μm. Treatment with electrophoresis was significantly ($P < 0.001$) more effective than pure CH in depths of 200–500 μm. Specimens treated with electrophoretically activated CH showed no viable bacteria in dentinal tubules to a depth of 500 μm from the root canal space within 7 days. The time required for treatment of pulpal infection root resorption may be decreased, thus minimizing the risk of coronal fractures in young patients with traumatized teeth.

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Key words: calcium hydroxide; dentinal tubules; intracanal medicament; root canal treatment; *Enterococcus faecalis*; electrophoretic current

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Pulpal infection resorption is a common complication following traumatic injuries to anterior teeth in young patients (1). Prevention and repair of this pathologic process is to remove the stimulating factor, i.e. bacteria in the root canal space and dentinal tubules (2, 3). Bacteria can be removed from the root canal space by mechanical instrumentation and irrigation. The use of intracanal medicaments is essential to kill bacteria in infected dentinal tubules and root canal ramifications, which cannot be eliminated mechanically (4).

A range of intracanal medicaments can be used to disinfect the root canal dentinal walls. *In vitro* tests have shown that bacteria are killed when in direct contact with low concentrations of certain medicaments (5). However, intracanal medicaments, such

as calcium hydroxide (CH) or Ledermix® have limited ability to penetrate and disinfect dentinal tubules (6–9).

One disadvantage of using pure CH to repair pulpal infection resorption is the long-term treatment (at least 6 months). During this period young teeth with thin dentinal walls may be fractured (10). In a previous study, electrophoretically activated CH mixed with copper eliminated bacteria in dentinal tubules to a depth of 0.5 mm within 7 days (11). However, the use of copper may discolor the teeth and its toxicity to the periapical tissues is questionable.

The aim of the present study was to evaluate the antibacterial effect of electrophoretically activated CH without additives to disinfect dentinal tubules within 7 days.

Materials and methods

Preparation of specimens

The present study used the *in vitro* model for dentinal tubule infection of root canals originally described by Haapasalo and Ørstavik (6) and modified by Assouline et al. (12). Root canals of intact bovine incisors, sliced 4 mm thick, were enlarged to 2.3 mm in diameter with a round bur ISO 0.23 (EAL, Munich, Germany) using a slow-speed dental hand-piece. Cementum was removed using a polish paper (Ecomet 3, variable speed grinder-polisher, Buehler, IL, USA) under water cooling, resulting in a center-holed root dentin piece, with a 6 mm outer diameter. Organic and inorganic debris, including the smear layer, were removed by placing the specimens in an ultrasonic bath with 17% EDTA (pH - 7.8) followed by 0.5% NaOCl, each for 5 min.

Specimens were autoclaved in the vials for 30 min at 121°C to ensure sterilization. Six specimens, serving as negative control, were incubated on brain heart infusion (BHI, Difco, Detroit, MI, USA) agar plates at 37°C for 48 h to confirm sterilization.

Inoculation

A total of 18 sterile specimens were transferred to separate test tubes containing 1.5 ml BHI inoculated with *Enterococcus faecalis*. The medium was changed every 2 days for 21 days. In the present study, a clinical isolate of *E. faecalis* T2 (Tel-Aviv University stock, Israel), which is resistant to 2 mg ml^{-1} streptomycin (13–15) was used. Streptomycin sulfate (Sigma, Holon, Israel), at a concentration of 0.5 mg ml^{-1}, was included in all growth media throughout this study to overcome possible contamination in the experimental setup (13).

Medication

The 18 infected specimens were divided into three groups: groups 1 and 2 ($n = 12$) contained CH in aqua paste following mixture of powder (4 mg) with distilled water to a creamy consistency; group 3 ($n = 6$) served as positive control with no medicament. In group 2, ions derived from CH paste were mobilized through a low current electric field (10 mA min^{-1}) – one electrode inserted into the root canal space, the other electrode located outside the specimen. For antibacterial properties of the intracanal medicaments to be expressed in clinically used conditions, all specimens were incubated for 7 days at 37°C under humid condition. At the end of the experimental period, after replacing CH with freshly mixed paste, the low current electric field (10 mA min^{-1}) was used again.

Sample recovery

A sterile round bur, ISO size 023, which has the same diameter of the root canal, was used to remove the intracanal medicaments at the end of the experiment. This ensured complete removal of the medicaments from the inner canal surface.

Dentinal samples were taken from each test specimen with sterile round burs mounted in a hand-piece at low speed. Sterile forceps held specimens in place during sampling, using burs in the following ISO size sequence: 025, 027, 029, 031, and 033. Each bur removed a dentinal layer from the inner canal surface in the shape of a hollow cylinder, 100-μm thick, with increasing radius as the bur size increased. The powder dentin samples obtained with each bur were immediately collected in separate test tubes containing 3 ml of BHI broth and streptomycin at a concentration of 0.5 mg ml^{-1}. Tubes were vigorously mixed by vortex for 20 s before and after incubation for 1 h at 37°C.

The content of each test tube was serially diluted (100 l) in an eppendorf tube with 100 l of saline (five times). Triplicate samples of 0.01 ml were spread on BHI agar plates from each dilution, which were incubated for 24 h at 37°C. Growing colonies were counted and recorded as colony forming units (cfu). For each dentinal layer at least triplicate samples from two agar plates were counted. Data were analyzed using ANOVA (on logarithmic transformation) with repeated measures to indicate differences between treatment groups. One-way ANOVA was carried out on logarithmic transformation (Tukey's method) to indicate differences within each layer.

Results

The number of cfu obtained from five consecutive dentinal layers is presented in Table 1 and Fig. 1. In the positive control specimens, heavy bacterial infection was observed at the layer close to the lumen. This decreased from layer to layer up to the deepest layer tested (400–500 μm) which contained several hundred cfu. CH without electrophoretical activation significantly ($P < 0.001$) reduced the amount of viable bacteria in the first to second layers compared with the positive control group, but did not eliminate them completely. Low electric current applied to CH completely eliminated bacterial survival in the dentinal tubules at least up to 500 μm from the root canal lumen. In the second to fifth layers, it was statistically ($P < 0.001$) more effective than the control group and the CH group without electric activation. The negative control group showed no bacterial growth, which indicated sterilization of the process and prevention of biased results.

Table 1. Logarithmic transformation of the number of colony forming units (cfu+1) at different dentin layer depths

Dentin layer depth (μm)	CH + E	CH	Control	Significance* (*P*)
0–100	0	0	7.9 ± 1.57	<0.001
100–200	0	0.21 ± 0.45	7.5 ± 1.49	<0.001
200–300	0	1.7 ± 1.11	6.5 ± 1.42	<0.001
300–400	0	2.95 ± 0.85	5.37 ± 0.81	<0.001
400–500	0	3.76 ± 0.42	3.85 ± 0.96	<0.001

*One-way ANOVA was performed on log transformation of cfu for each layer.
Horizontal lines above data connect values which do not show significant differences (Tukey's method).

Discussion

The *in vitro* model for dentinal tubule infection described by Haapasalo and Ørstavik (6) is effective in detecting viable bacteria in various depths of dentinal tubules (6, 8). The initial model yielded only information regarding the presence of bacteria. In the present study, a modification was used, i.e. inoculating the bacteria in agar plates and counting the cfu (12). This resulted in a quantitative measure of vital bacteria in various dentin depths.

The dentinal infection model represents an ideal condition in which the root canal is short (4 mm length) and the application of medicaments and electrophoretical activation is simple. In this model, cementum is removed to allow bacteria to colonize the patent dentinal tubules (6). A similar condition occurs during pulpal infection root resorption where the external protective cementum layer is injured or absent and bacteria from the dentinal tubules stimulate advanced resorption (2). Further *in vivo* studies on injured teeth with root resorption are required to elucidate the effect of electrophoretical activation on intracanal medicaments.

Although CH has a lasting antibacterial activity in the root canal space because of its high pH (16, 17), it has poor solubility and unsatisfactory bactericidal effect in dentinal tubules (6). Iodine potassium iodine (IKI) and electrophoretically activated copper improve efficacy in bovine dentinal tubules (11), but there are possible side-effects, e.g. allergic reaction to iodine or tooth discoloration from the copper. The present study demonstrated a strong antibacterial effect in dentinal tubules of electrophoretically activated CH

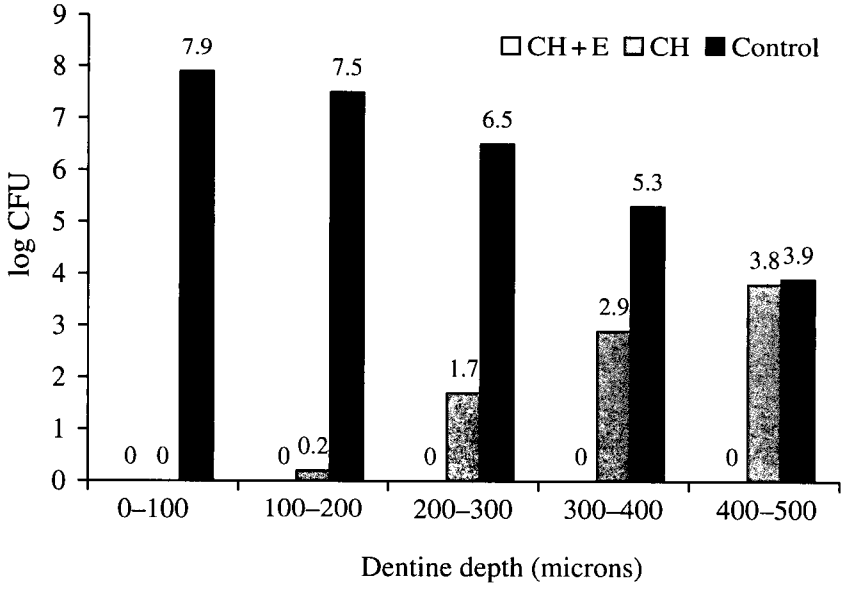

Fig. 1. Colony forming units (cfu) of bacteria in dentinal tubules facing the root canal space following treatment with calcium hydroxide with no additives (CH), and calcium hydroxide with electrophoretic-activated ions (CH+E). Each column represents the log transformation (cfu+1) obtained from six dentinal specimens.

with no additives. Bacteria were eliminated to a depth of 500 μm within 7 days after two applications of electrophoresis.

The protocol for treating inflammatory root resorption indicates root canal therapy with CH applied as an intracanal medicament for at least 6 months (18). The rationale of using intracanal medicament in these cases is to cease the stimulation by disinfecting the dentinal tubules. As CH has low solubility, an extended time is needed to penetrate and disinfect the dentinal tubules (6). Electrophoretically activated CH used on young traumatized teeth with pulpal infection root resorption may significantly shorten the treatment time. The final coronal restoration could soon follow, thus minimizing the risk of coronal root fractures during the treatment period.

Conclusions

Electrophoretically activated CH eliminates viable bacteria up to at least 500 μm in the dentinal tubules within 7 days. The time required for treatment of pulpal infection root resorption could be decreased, thus minimizing the risk of coronal fractures in young patients with traumatized teeth.

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