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Additional disinfection with a modified salt solution in a root canal model

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ABSTRACT

Objectives: The aim of this study is to investigate the disinfecting properties of a modified salt solution (MSS) and calcium hydroxide ($Ca(OH)_2$) in a non-direct-contact *ex-vivo* model.
Methods: Seventy-four single-canal roots infected with *Enterococcus faecalis* were treated with 1% sodium hypochlorite (NaOCl) irrigation or with NaOCl irrigation with subsequent dressing with MSS or $Ca(OH)_2$. After removal of the dressings, the roots were filled with bacterial growth medium and incubated for seven days to enable the surviving bacteria to repopulate the root canal lumen. Growth was determined by sampling the root canals with paper points before treatment (S1), after treatment (S2) and incubation after treatment (S3). The colony forming units were counted at S1 and S2. At S3, growth was determined as no/yes regrowth. The Kruskal–Wallis, McNemar and χ^2 test were used for statistical analyses.
Results: At S2, in the NaOCl group, growth was found in 5 of 19 root canals. After the removal of MSS or $Ca(OH)_2$ bacteria were retrieved from one root canal in both groups. At S3, repopulation of the root canals had occurred in 14 of 19 roots after sole NaOCl irrigation, 6 of 20 roots after MSS-dressing and in 14 of 20 roots after $Ca(OH)_2$-dressing. MSS was more effective in preventing regrowth than $Ca(OH)_2$ ($P = 0.009$).
Conclusions: The modified salt solution prevented regrowth in roots which indicates that it can eliminate persistent bacteria. Dressing the root canals with $Ca(OH)_2$ did not provide additional disinfection after NaOCl irrigation.

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1. Introduction

Apical periodontitis (AP) is the inflammatory response to microbial infection of the root canal system [1]. Accordingly, cleaning and disinfection is an important aim of root canal treatment. Currently, surface disinfectants such as sodium hypochlorite (NaOCl) are used to clean and disinfect the root canal system but they often appear insufficient to eliminate microorganisms due to the complex shape of the root canal system [2]. Calcium hydroxide ($Ca(OH)_2$) is a frequently used temporary dressing with the aim to further reduce the microbial load [3]. Unfortunately, at present in 20% of AP cases the AP does not resolve after root canal treatment [4] and therefore, there is a need for improved root canal disinfection.

Hypertonic salt solutions inactivate biofilm bacteria [5,6] which has prompted the development of a modified salt solution (MSS) [7,8] MSS contains sodium chloride and potassium sorbate and its mode of action is based on a multiple-hurdle strategy which combines a series of stress factors (hurdles) that the micro-organisms are unable to overcome (jump over). Examples of such hurdles include low or high temperature, osmotic pressure, pH, lack of oxygen and the use of preservatives [9]. While being confronted with these hurdles, the microorganisms try to adapt like they would do in the lag-phase of the bacterial growth cycle. Adaptation however, demands energy and once there are several hurdles to overcome the microorganisms can become exhausted. This strategy is applied in the food industry to inhibit microbial growth in freshly prepared foods [9]. The charm of a multiple-hurdle disinfection approach is that the right combination of safe components can yield a synergistic effect [8].

MSS inactivates multi-species biofilm bacteria when in direct contact with the biofilms [7]. Since, it is a mixture of highly concentrated salts, it is also expected to diffuse past the main root canal lumen into more distant areas. However, the hypothesis that MSS may eliminate microorganisms beyond the main root canal

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lumen is still to be investigated. Moreover, eventually the efficacy of MSS is to be tested in a clinical study, but for a consent of a medical ethical committee data must be generated in models with well-controlled parameters.

Previously, *ex-vivo* infected root canals were disinfected with for example NaOCl 10]. Immediately after disinfection, the root canals seemed free of bacteria with paper point sampling (PPS). In spite of that, after days repopulation of the canals by the previously irretrievable bacteria had occurred. Such a model may be suitable to explore the efficacy of MSS in areas beyond the main root canal. If after treatment, micro-organisms persist in the irregularities of the root canal system, then the efficacy of MSS in eliminating those micro-organisms can be put to the test.

Therefore, the aim of this paper is to investigate whether MSS and $Ca(OH)_2$ applied as an inter-appointment root canal dressing can eliminate 'distant' root canal infections.

2. Materials and methods

2.1. Group size and groups

The group size was determined as $N = 16$ for the experimental groups with G*Power 3.9.1.2 software (Franz Faul, Universitaet Kiel, Germany) with an α-value of .05, a power of 80%, an effect size of 0.8 and 2 degrees of freedom. Round and oval canals were equally distributed between the groups. Round was defined as height: width = 1:1; oval was defined as 'not round'.

The groups were: (1) irrigation 1% NaOCl; (2) irrigation 1% NaOCl plus MSS dressing; (3) irrigation 1% NaOCl plus $Ca(OH)_2$ dressing; (4) negative control and (5) sterile control. Group 1 served as a negative control to Groups 2 and 3 (Fig. 1).

2.2. Sample preparation

Eighty single-rooted teeth with one root canal were selected from the dental school collection of extracted teeth where they were stored in tap water. By decoronation, the root lengths were standardized to 12 mm. The working length (WL) was determined by subtracting 0.5 mm from the length where a size-10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was visible at the apical foramen. The canals were prepared with Mtwo rotaries till 40.04 (VDW GmbH, München, Germany). After each instrument change, the canals were irrigated with 2 mL 2.5% NaOCl. After iodometric titration [11], the NaOCl stock solution (Orphi Farma, Lage Zwaluwe, The Netherlands) was diluted to obtain the required concentrations. The irrigation needle (NaviTip 30 gauge; Ultradent Products Inc, South Jordan, UT, USA) was inserted 1 mm short of the WL. Final rinses were 3 mL 17% EDTA (Pulpdent Corporation, Watertown, WA, USA), 10 mL 2.5% NaOCl and 10 mL saline (Versylene®, Fresenius Kabi, Sevres, France).

On the outside, the apical foramen was sealed with composite (Bisco Ælite Flo™, Bisco Inc., Schaumburg, IL, USA) and the dentinal tubules were sealed with nail varnish (HEMA, Amsterdam, The Netherlands). Then, the roots were embedded in Futar D Slow

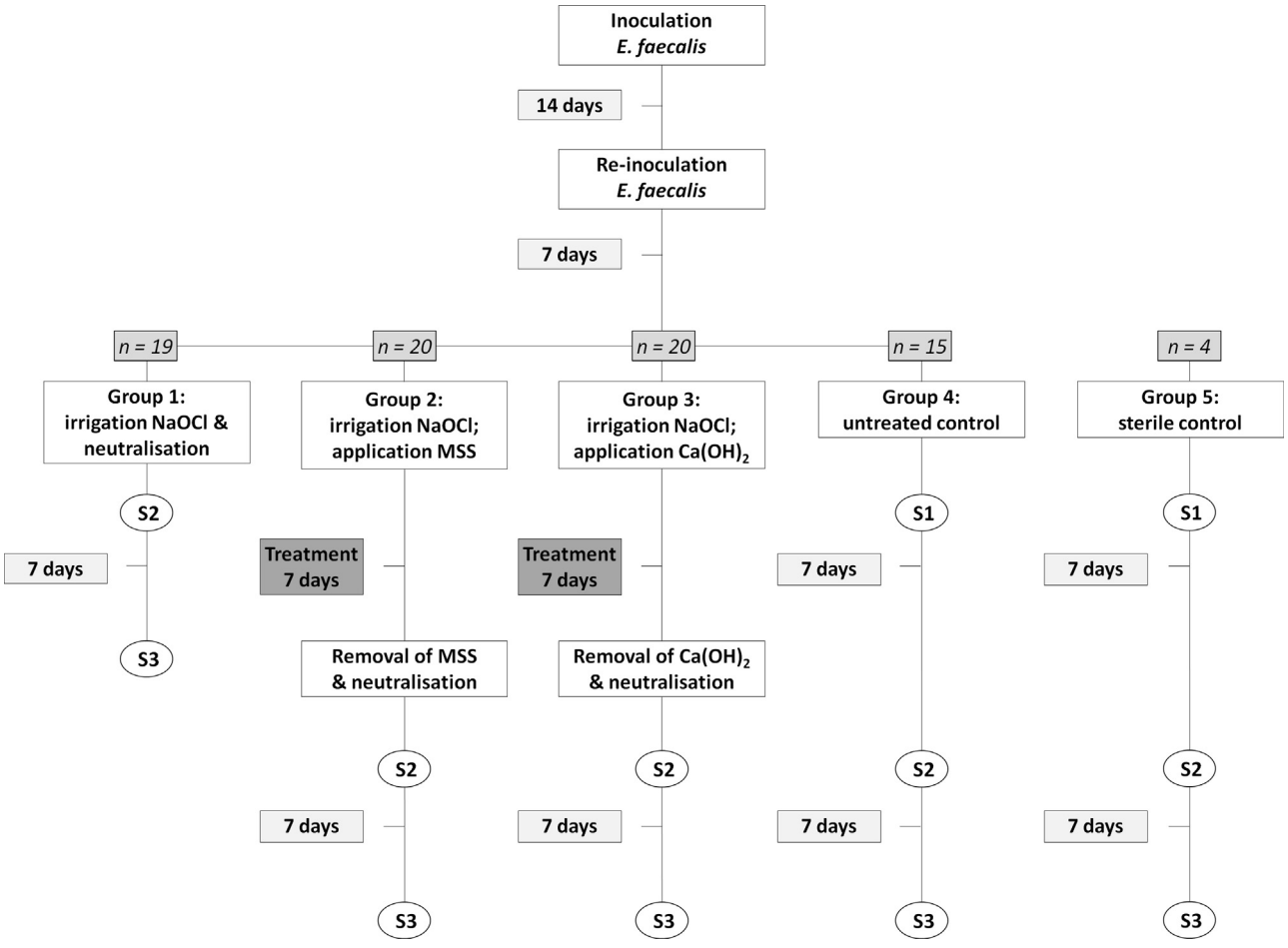

Fig. 1. Scheme of study design including sampling moments. From top to bottom: after an inoculation period of three weeks, the roots were treated with NaOCl alone, Group 1, or NaOCl with MSS or $Ca(OH)_2$, Groups 2 and 3. After each treatment, bacteria were allowed to repopulate the root canals during a 7-day incubation period. Group 4 represents the untreated control. At S1–S3 the root canal were sampled. Group 5 represents the sterile controls.

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