Journal of Dentistry 43 (2015) 1280–1284

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

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

Suzette V. van der Waal[a,b,*], Charlotte A.M. Oonk[b], Selma H. Nieman[b], Paul R. Wesselink[b], Johannes J. de Soet[a], Wim Crielaard[a]

[a] Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), Gustav Mahlerlaan 3004, 1081 LA Amsterdam, The Netherlands
[b] Department of Cariology, Endodontology and Pedodontology, Academic Centre for Dentistry Amsterdam (ACTA), Gustav Mahlerlaan 3004, 1081 LA Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 28 March 2015
Received in revised form 22 July 2015
Accepted 28 July 2015

Keywords:
Endodontic
Root canal
Disinfection
Modified salt solution
Calcium hydroxide
Enterococcus faecalis

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.

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

* Corresponding author.

http://dx.doi.org/10.1016/j.jdent.2015.07.015
0300-5712/

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

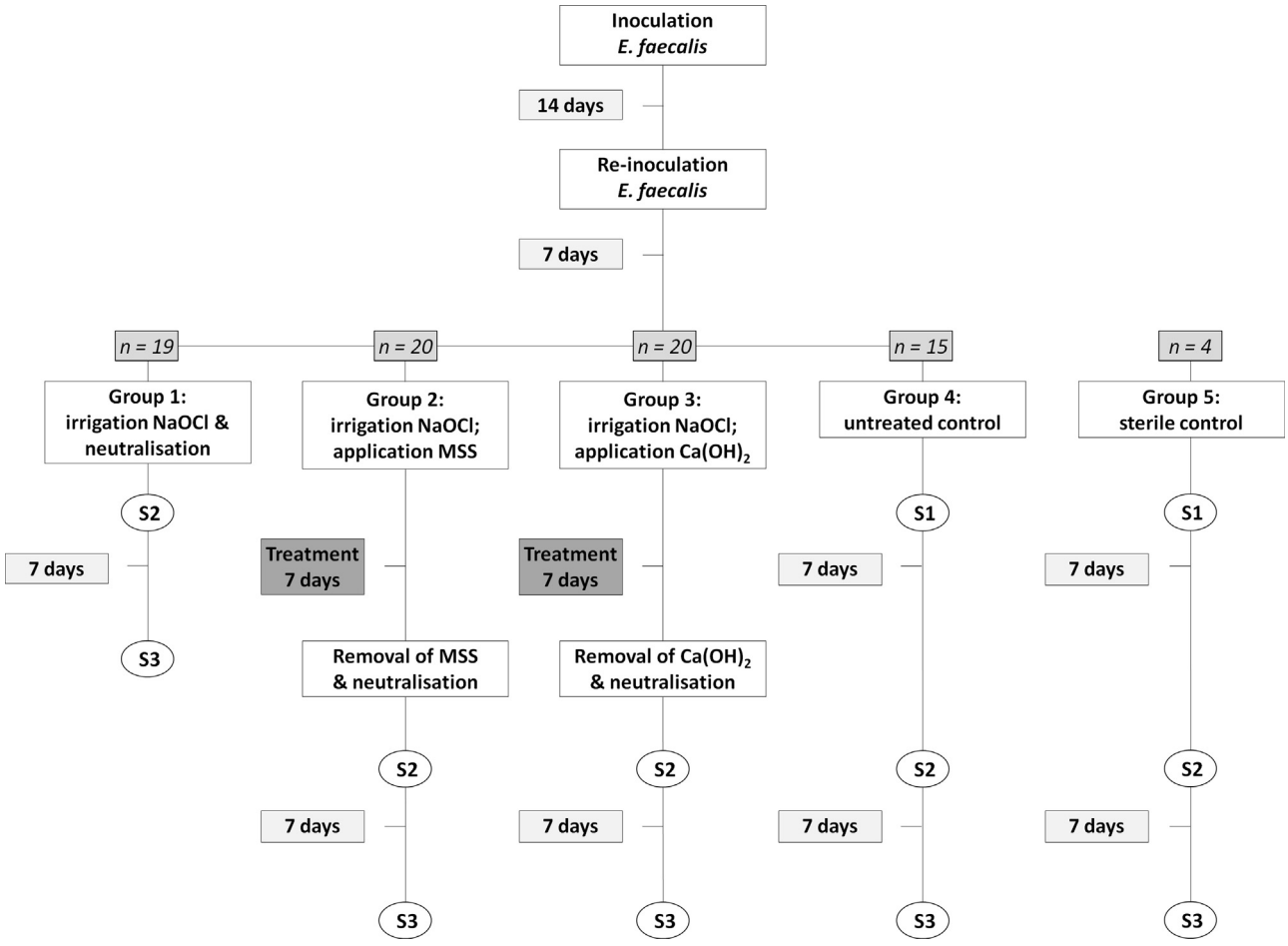

Fig. 1. Scheme of study design including sampling moments. From top to bottom: after an inoculation period of three weeks, the roots were treated with NaOCl alone, Group 1, or NaOCl with MSS or $Ca(OH)_2$, Groups 2 and 3. After each treatment, bacteria were allowed to repopulate the root canals during 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.

(Kettenbach GmbH & Co. KG, Eschenburg, Germany) in a 2-mL microtube with screw cap (Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany). The specimens were kept moist with sterile saline and autoclaved.

2.3. Inoculation

Seventy-four roots were inoculated with a fresh culture containing approximately 5.0×10^6 cells/mL *Enterococcus faecalis* ER5/1[12] in tryptic soy broth (TSB; Bacto™, Becton, Dickinson and Company, Le Pont de Claire, France) and incubated anaerobically (80% N_2, 10% H_2 and 10% CO_2 with a palladium catalyst) at 37 °C. After two weeks, the inoculation procedure was repeated. Fresh medium was added to the roots twice weekly for 3 weeks. In the anaerobic jar, open containers with demineralized water prevented dehydration of the specimens. Once a week, two random roots were checked for purity of the culture. The sterile controls were not inoculated.

2.4. Treatments

For each root, a sterile irrigation needle was used until 1 mm short of the WL.

Group 1: the canals were irrigated with 2 mL 1% NaOCl. To prevent carry-over, the canals were irrigated with 5 mL demineralized water, dried and sampled. After sampling, the canals were filled with TSB and the roots were incubated at 37 °C anaerobically for 7 days.

Groups 2 and 3: the canals were irrigated with NaOCl and thoroughly dried with paper points. In Group 2, the root canals were subsequently dressed with 10 μL MSS; in Group 3, with 10 μL $Ca(OH)_2$ (Ultracal® XS, Ultradent Products Inc.). For dressing the canals, the irrigation needle was introduced until 1 mm from WL. The roots were dressed for 7 days at 37 °C anaerobically. After the treatments, MSS or $Ca(OH)_2$ were removed by irrigation with 5 mL demineralized water. The canals were dried and sampled. Then, the canals were filled with TSB (see further Group 1). At S3, again samples were taken.

Groups 4 and 5 received fresh TSB instead of a treatment (Fig. 1).

2.5. Sampling and microbial processing

Bacterial samples were taken from the root canals after infection (S1), after treatment *i.e.* NaOCl irrigation or root canal dressing (S2) and after 7-days incubation for regrowth (S3) (Fig. 1). To halt the antimicrobial action of NaOCl a neutralizer containing 1% sodium thiosulphate was used [13,14]. The samples were obtained by filling the canal with the neutralizer (±10 μL) for 2 min. Then, three sterile paper points size 40 (Henry Schein, Melville, NY, USA) were inserted until WL. After 1 min, all canal contents had been absorbed and the paper points were collected in vials containing 2 mL phosphate buffered saline (PBS). The paper points in PBS were sonicated on ice (45 pulses in 90 s.). After ten-fold dilution, aliquots were seeded in duplicate on brain-heart-infusion-agar plates (Bacto™). At S3 of Groups 1–3, bacterial growth was noted as growth or no growth. Of the other samples, the colony forming units (CFU) were counted and the numbers of CFU were log transformed.

2.6. Data analysis

IBM SPSS software (version 21; International Business Machines Corp, Armonk, NY, USA) was used for statistical analyses. The log numbers of CFU at S1, S2 and S3 of the negative controls were compared with paired *T*-tests. Due to the not normal distribution of the data in Groups 1–3, the differences between the Groups at S2 were calculated with the Kruskal–Wallis test and differences between S2 and S3 and between Groups 1–3 at S3 with the McNemar and χ^2 tests. Post-hoc tests were performed as Fisher's exact tests on all three pairwise 2×2 comparisons. $\alpha < 0.05$ was considered significant.

3. Results

This study was undertaken to investigate whether MSS and Ca $(OH)_2$ applied as an inter-appointment root canal dressing can eliminate 'distant' root canal infections.

After autoclaving, two roots were discarded due to the occurrence of cracks; therefore the numbers per group as indicated in Fig. 1 are the numbers of included specimen after the last step in sample preparation, *i.e.* autoclaving. Then, the decision was made to make the NaOCl group and the negative control group smaller in order to keep the MSS and $Ca(OH)_2$ groups equal.

After inoculation during growth and treatment, samples were taken from the root canals after infection (S1), after treatment *i.e.* NaOCl irrigation or NaOCl irrigation plus root canal dressing (S2) and after 7-days incubation for regrowth (S3) (See Section 2 for details).

The negative controls contained on average $6.2 \times 10^6 \pm 1.4 \times 10^7$ CFUs *E. faecalis* per root canal. In Table 1 the mean log number of CFUs of the negative controls at S1, S2 and S3 are presented. The average log number of CFUs increased between S1 and S3; $P = 0.03$.

Table 2 shows the number of roots with growth at S2. The regrowth, S3, is presented in Table 3. MSS showed less regrowth compared to NaOCl irrigation ($P = 0.014$) or compared to NaOCl and NaOCl plus $Ca(OH)_2$ ($P = 0.009$). In the root canals where no bacteria were sampled at S2, bacteria were again recovered at S3 in 64% of canals with NaOCl (9 of 14), 26% with MSS (5 of 19) and 68% with $Ca(OH)_2$ (13 of 19). This shows that elimination of micro-organisms from areas that are more distant from the main root canal lumen is possible.

4. Discussion

The present study was aimed at and proofs that the studied Modified Salt Solution is able to penetrate into areas behind the lumina of main root canals and kill *E. faecalis*. Because of the highly concentrated formula of MSS, it is targeted to diffuse into these areas beyond the main root canal lumen. Indeed, migration of molecules from an area of high concentration to an area of a lower concentration is a law of physics. Diffusion resulting in hyper-osmotic stress in micro-organisms is one of the hurdles of the multiple-hurdle strategy of MSS application. Other hurdles are weak-acid stress and the growth inhibitory properties of sorbic acid.

The model used in this study is developed on the basis of an earlier *ex-vivo* study [10]. At present, there are no valorized endodontic models that can quantify the efficacy of medicaments behind or past the root canal lumen. For instance in a dentin-block model, smearing of bacteria from the inner layer to the outer layers can be an issue [15]. Microscopy models where biofilms are stained with viability stains may not accurately reflect the viability state of the cells [16,17]. Also, with microscopy the efficacy of medicaments

Table 1
In the negative controls (Group 4), the average log number of CFUs per root canal.

	Mean	SD
S1	5.22	1.28
S2	5.86	1.18
S3	6.15	1.00

Average log number of CFUs increased between S1 and S3; $P = 0.03$.

Table 2
Growth in number of roots after the treatment with NaOCl, NaOCl plus MSS or NaOCl plus $Ca(OH)_2$ (S2).

	1% NaOCl	1% NaOCl + MSS	1% NaOCl + $Ca(OH)_2$	Untreated control
No growth	14	19	19	0
Growth	5	1	1	15

Table 3
Number of roots with regrowth after 7-days incubation with growth medium (S3).

	1% NaOCl	1% NaOCl + MSS	1% NaOCl + $Ca(OH)_2$	Untreated control
No growth	5 [a]	14 [b]	6 [a]	0
Growth	14 [c]	6 [d]	14 [c]	15

In the same row, different letters represent a statistically significant difference between the groups.

cannot be quantified. The idea of a root-canal model might at first sight seem to be a paradox because only superficial bacteria can be retrieved by paper point sampling (PPS) [2,18–20]. Indeed, the root canal lumen may seem free of bacteria while biofilms in irregularities of the root canal system can still be present [10]. These residual biofilms however, can be suitable for testing the depth action of MSS or $Ca(OH)_2$ because living biofilms disseminate living cells which can then be isolated from the main root canal with PPS. So, although the employed model is not a realistic endodontic setting, a good feature is that where sampling of deep biofilms with PPS is not possible, by measuring regrowth the viability of the deeper biofilms can be proven. The current data show that at S2 almost all planktonic cells were removed, but that the attached biofilms behind the lumen were less affected because the presence of planktonic *E. feacalis* cells at S3 in the control Group 4 points to these live residual biofilms at S2.

E. faecalis was used in this study because this species attaches to a surface, produces biofilms, disseminates to planktonic cells and is found in endodontic infections (all own unpublished data and [12]). The current strain has been isolated from an endodontic infection and the stock was stored at −80 °C. To limit mutations during frequent subculturing of a strain, the cultures were taken directly from this stock and subcultured once. A single-species biofilm was employed to avoid the chance of overlooking the presence of bacteria in mixed-species populations that cannot be cultured. Another debatable variable in the model is the removal of the active agents with 5 mL of demineralized water. This volume was chosen to dissolve the highly concentrated MSS. Consequently, the other groups were handled identically. Although, $Ca(OH)_2$ can be difficult to remove after application, in this assay rinsing with 5 mL of demineralized water was enough to allow regrowth.

In the current study at S1, Groups 1–3 were not sampled because the results of the negative controls served as baseline measurements for the experimental groups. At S3, regrowth was regarded as a dichotomous variable because when the medicaments are neutralized and the conditions for bacterial growth are favourable, then regrowth occurs until nutrient depletion. Consequently, quantification of regrowth in the present study, but possibly also in other similar studies, provides no valid information about the number of residual bacteria at S2. This also entails that the current results cannot be compared to studies which did quantify regrowth [21,22]. In the current assay after culturing on agar plates, all regrowth was more than 10^5 cells per root canal.

Although our model was similar to another study [10], in our previous studies with 2%-NaOCl irrigation bacteria were recovered from only 36% of infected roots. To increase this number of infected roots after NaOCl treatment, a pilot study was performed on the maximal concentration necessary to obtain no growth at S2 but more than 80% *E. faecalis* positive root canals at S3. We found that 1% NaOCl was able to achieve this goal and therefore this concentration was used in the current study. Differences in regrowth between our and other studies can be explained by variations in root canal anatomy or different susceptibilities of the *E. faecalis* strains. The chemical instability of NaOCl can also have played a role [23,24] which is why, shortly before the experiments, the concentration of our NaOCl stock was measured.

In the present study, $Ca(OH)_2$ treatment after NaOCl irrigation resulted at S2 in 1 of 20 roots positive for bacterial regrowth, which is comparable to other reports [25–27]. Although other studies may have reported about the absence of unculturable species after $Ca(OH)_2$ application, the current high regrowth rate of 14 positive canals out of 20 at S3, however, is a new finding [25,28]. On the other hand, in a histological *in-vivo* study, residual bacteria have been found after $Ca(OH)_2$ dressing [20] and *in-vitro* after $Ca(OH)_2$ application bacteria remained viable in the dentinal tubules which can explain the current regrowth data [15,29,30].

This study was not aimed at testing the antimicrobial activity of MSS on multispecies biofilms since this has been done before [7]. However, we did find supportive evidence that MSS is able to reach more complex root canal structures behind the main canal lumen thus enhancing disinfection of the root canal system in situ. Eventually, MSS is to be tested in a true endodontic setting: a clinical study.

5. Conclusion

In this model, the modified salt solution prevented regrowth of residual bacteria in about 50% of root canals. $Ca(OH)_2$ gave no additional disinfection after irrigation with NaOCl. The efficacy of MSS is likely related to the ability to diffuse. New models should be developed to monitor and quantify the effect of diffusion of MSS or other medicaments into multispecies biofilms or other defined areas.

Clinical significance statement

With the currently used surface disinfectants such as sodium hypochlorite, complex-shaped infected root canal systems often cannot be completely disinfected. This paper shows that the modified salt solution has the potential to disinfect in areas beyond the main root canal.

Acknowledgements

Authors SVvdW and JJdS, are inventors of the intellectual property described in the pending patent: Composition, used *e.g.* as disinfectant or antimicrobial in dental treatment (*e.g.* root canal

treatments), comprises at least one tonicity agent (organic acids and/or their salts *e.g.* sodium acetate) rendering the composition hypertonic. WO2011102724-A2; NL2004260-C; WO2011102724-A3; US3012328708-A1; EP2536378-A2; CN102985051-A. The patent is owned by the University of Amsterdam, Amsterdam, The Netherlands.

References

[1] S. Kakehashi, H.R. Stanley, R.J. Fitzgerald, Effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats, Oral Surg. Oral Med. Oral Pathol. 20 (1965) 340–346.

[2] P.N. Nair, S. Henry, V. Cano, J. Vera, Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after one-visit endodontic treatment, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 99 (2005) 231–252.

[3] Z. Mohammadi, P.M. Dummer, Properties and applications of calcium hydroxide in endodontics and dental traumatology, Int. Endod. J. 44 (2011) 697–730.

[4] Y.L. Ng, V. Mann, K. Gulabivala, A prospective study of the factors affecting outcomes of non-surgical root canal treatment: part 2: tooth survival, Int. Endod. J. 44 (2011) 610–625.

[5] S.V. van der Waal, L.W. van der Sluis, A.R. Özok, R.A. Exterkate, J. van Marle, P.R. Wesselink, et al., The effects of hyperosmosis or high pH on a dual-species biofilm of *Enterococcus faecalis* and *Pseudomonas aeruginosa*: an in vitro study, Int. Endod. J. 44 (2011) 1110–1117.

[6] J.J. Wijnker, G. Koop, L.J.A. Lipman, Antimicrobial properties of salt (NaCl) used for the preservation of natural casings, Food Microbiol. 23 (2006) 657–662.

[7] S.V. van der Waal, N. Scheres, J.J. de Soet, P.R. Wesselink, W. Crielaard, Cytotoxicity, interaction with dentine and efficacy on multi-species biofilms of a modified salt solution intended for endodontic disinfection in a new in vitro biofilm model, Int. Endod. J. 48 (2015) 153–161.

[8] S.V. van der Waal, L.M. Jiang, J.J. de Soet, L.W. van der Sluis, P.R. Wesselink, W. Crielaard, Sodium chloride and potassium sorbate: a synergistic combination against Enterococcus faecalis biofilms: an in vitro study, Eur. J. Oral Sci. 120 (2012) 452–457.

[9] L. Leistner, Basic aspects of food preservation by hurdle technology, Int. J. Food Microbiol. 55 (2000) 181–186.

[10] A.R. Aranda-Garcia, J.M. Guerreiro-Tanomaru, N.B. Faria-Junior, G.M. Chavez-Andrade, R.T. Leonardo, M. Tanomaru-Filho, et al., Antibacterial effectiveness of several irrigating solutions and the Endox Plus system—an ex vivo study, Int. Endod. J. 45 (2012) 1091–1096.

[11] A. Vogel, A Textbook of Quantitative Inorganic analysis, 3rd ed., Longmans, London, UK, 1962.

[12] J.M. Duggan, C.M. Sedgley, Biofilm formation of oral and endodontic *Enterococcus faecalis*, J. Endod. 33 (2007) 815–818.

[13] A. Zamany, L.S. Spångberg, An effective method of inactivating chlorhexidine, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 93 (2002) 617–620.

[14] G.K. Kemp, K.R. Schneider, Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing, Poult. Sci. 79 (2000) 1857–1860.

[15] M. Haapasalo, D. Ørstavik, In vitro infection and disinfection of dentinal tubules, J. Dent. Res. 66 (1987) 1375–1379.

[16] Z. Wang, Y. Shen, M. Haapasalo, Effect of smear layer against disinfection protocols on *Enterococcus faecalis*-infected dentin, J. Endod. 39 (2013) 1395–1400.

[17] L. Netuschil, T.M. Auschill, A. Sculean, N.B. Arweiler, Confusion over live/dead stainings for the detection of vital microorganisms in oral biofilms—which stain is suitable? BMC Oral Health 14 (2014) 2.

[18] C. Reit, A. Molander, G. Dahlén, The diagnostic accuracy of microbiologic root canal sampling and the influence of antimicrobial dressings, Endod. Dent. Traumatol. 15 (1999) 278–283.

[19] C. Sathorn, P. Parashos, H.H. Messer, How useful is root canal culturing in predicting treatment outcome, J. Endod. 33 (2007) 220–225.

[20] J. Vera, J.F. Siqueira Jr., D. Ricucci, S. Loghin, N. Fernandez, B. Flores, et al., One- versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study, J. Endod. 38 (2012) 1040–1052.

[21] M.M. Menezes, M.C. Valera, A.O. Jorge, C.Y. Koga-Ito, C.H. Camargo, M.N. Mancini, In vitro evaluation of the effectiveness of irrigants and intracanal medicaments on microorganisms within root canals, Int. Endod. J. 37 (2004) 311–319.

[22] R.K. Lima, J.M. Guerreiro-Tanomaru, N.B. Faria-Junior, M. Tanomaru-Filho, Effectiveness of calcium hydroxide-based intracanal medicaments against *Enterococcus faecalis*, Int. Endod. J. 45 (2012) 311–316.

[23] S. van der Waal, T. Connert, A. Laheij, J. de Soet, P. Wesselink, Free available chlorine concentration in sodium hypochlorite solutions obtained from dental practices and intended for endodontic irrigation: are the expectations true? Quintessence Int. 45 (2014) 467–474.

[24] S.V. van der Waal, N.E. van Dusseldorp, J.J. de Soet, An evaluation of the accuracy of labeling of percent sodium hypochlorite on various commercial and professional sources: is sodium hypochlorite from these sources equally suitable for endodontic irrigation? J. Endod. 40 (2014) 2049–2052.

[25] C.R. Barthel, S. Zimmer, S. Zilliges, R. Schiller, U.B. Göbel, J.F. Roulet, In situ antimicrobial effectiveness of chlorhexidine and calcium hydroxide: gel and paste versus gutta-percha points, J. Endod. 28 (2002) 427–430.

[26] L.B. Peters, P.R. Wesselink, Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms, Int. Endod. J. 35 (2002) 660–667.

[27] U. Sjögren, D. Figdor, L. Spångberg, G. Sundqvist, The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing, Int. Endod. J. 24 (1991) 119–125.

[28] C. Reit, G. Dahlén, Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis, Int. Endod. J. 21 (1988) 291–299.

[29] J.M. de Lucena, E.M. Decker, C. Walter, L.S. Boeira, C. Löst, R. Weiger, Antimicrobial effectiveness of intracanal medicaments on *Enterococcus faecalis*: chlorhexidine versus octenidine, Int. Endod. J. 46 (2013) 53–61.

[30] J. Krithikadatta, R. Indira, A.L. Dorothykalyani, Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments, J. Endod. 33 (2007) 1473–1476.