Evaluation of the antimicrobial effect of super-oxidized water (Sterilox®) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model

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ABSTRACT

Ideally root canal irrigants should have, amongst other properties, antimicrobial action associated with a lack of toxicity against periapical tissues. Sodium hypochlorite (NaOCl) is a widely used root canal irrigant, however it has been shown to have a cytotoxic effect on vital tissue and therefore it is prudent to investigate alternative irrigants. Sterilox's Aquatine Alpha Electrolyte® belongs to the group of the super-oxidized waters; it consists of a mixture of oxidizing substances, and has been suggested to be used as root canal irrigant. Super-oxidized waters have been shown to provide efficient cleaning of root canal walls, and have been proposed to be used for the disinfection of medical equipment. Objective: To compare the antimicrobial action against *Enterococcus faecalis* of NaOCl, Optident Sterilox Electrolyte Solution® and Sterilox's Aquatine Alpha Electrolyte® when used as irrigating solutions in a bovine root canal model. Methodology: Root sections were prepared and inoculated with *E. faecalis* JH2-2. After 10 days of incubation the root canals were irrigated using one of three solutions (NaOCl, Optident Sterilox Electrolyte Solution® and Sterilox's Aquatine Alpha Electrolyte®) and subsequently sampled by grinding dentin using drills. The debris was placed in BHI broth and dilutions were plated onto fresh agar plates to quantify growth. Results: Sodium hypochlorite was the only irrigant to eliminate all bacteria. When the dilutions were made, although NaOCl was still statistically superior, Sterilox's Aquatine Alpha Electrolyte® solution was superior to Optident Sterilox Electrolyte Solution®. Conclusions: Under the conditions of this study Sterilox's Aquatine Alpha Electrolyte® appeared to have significantly more antimicrobial action compared to the Optident Sterilox Electrolyte Solution® alone, however NaOCl was the only solution able to consistently eradicate *E. faecalis* in the model.

Key words: Sodium hypochlorite. Super-oxidized water. Electrochemically activated solutions. Root canal irrigation. *Enterococcus faecalis*.

INTRODUCTION

Endodontic irrigants should have, amongst other properties, a broad antimicrobial spectrum of activity against anaerobic and facultative micro-organisms growing in biofilms and a relative absence of toxicity against periapical tissues and oral mucosa[25].

Sodium hypochlorite (NaOCl) is recommended as the main root canal irrigant because of its broad antimicrobial activity, the capacity to prevent formation of and dissolve the smear layer, in association with chelating agents, and its ability to dissolve tissue remnants[25]. However, NaOCl has

been shown to have a cytotoxic effect on vital tissue and can therefore elicit inflammatory reactions if it reaches the periapex[13]. Furthermore, NaOCl has been shown to cause a change in the force required to fracture dentin[24], and a reduction of the elastic modulus and flexural strength of dentin[5,21]. Furthermore NaOCl corrodes Protaper NiTi Rotary[1] (Dentsply/Maillefer, Baillagues, Switzerland) and carbon steel instruments[3], cause early fractures of ProTaper (Dentsply/Maillefer) instruments[1] and, when heated, reduce resistance to cyclic fatigue of nickel-titanium files[16].

Sterilox's Aquatine Alpha Electrolyte® (Optident Dental, Ilkley, West Yorkshire, UK) is a super-oxidized water that consists of a mixture of oxidizing substances including hypochlorous acid (HOCl) at a concentration of 144 mg/L, with a pH of 5.0-6.5 and a redox potential of >950 mV20. The manufacturer suggests that the production of HOCl in the Sterilox Dental System (Optident Dental) does not produce free radical Cl and that the available free chlorine in the solution is 200 PPM, that is larger than the concentration reported in literature.

Super-oxidized water has been suggested as an alternative to NaOCl, as it provides efficient cleaning of root canal walls[22], and has been recommended for the disinfection of endoscopes[19], dental unit water lines[10] and dental impression materials[11].

The aim of this study was to compare the antimicrobial action against Enterococcus faecalis of NaOCl, Optident Sterilox Electrolyte Solution® (Optident Dental) and Sterilox's Aquatine Alpha Electrolyte® when used as irrigating solutions in an E. faecalis infected bovine root canal model.

MATERIAL AND METHODS

Bovine incisors were used throughout this study. The study exerted no influence on the animal's fate at any stage as they were previously slaughtered in an Italian slaughterhouse for commercial purposes. The apical 5 mm and the crown of each incisor were dissected and the remaining root was cut into 1 cm slices with a diamond disc (Abrasive Technology Inc, Westerville, OH, USA). Subsequently the canal lumen was widened to a minimal diameter of 1.4 mm using the ParaPost® XP™ Endodontic post system drills (Coltene/Whaledent, Konstanz, Germany).

Finally the smear layer was removed via copious irrigation in an EDTA solution (Smear Clear, SybronEndo, Scafati, Italy) (4 min) and NaOCl (Teepol Bleach, Teepol, Orpington, UK) (4 min) in an ultrasonic bath.

Fifteen roots were placed individually in 10 mL of Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) and autoclaved. These were left to cool to room temperature and then incubated overnight at 37°C to verify the sterility of the samples. The BHI broths containing the roots were inoculated with 100 µL of an overnight culture of *E. faecalis* JH2-2[7] and incubated for 10 days at 37°C to allow for bacterial growth, infiltration of the dentin tubules and *E. faecalis* JH2-2 biofilm formation.

The roots were divided into 3 groups, according to the irrigant used: group 1 was irrigated with the Optident Sterilox Electrolyte Solution® (this is essentially saline and was used as our negative control), group 2 was irrigated with 4% NaOCl (Teepol Bleach) and group 3 was irrigated with freshly prepared Sterilox's Aquatine Alpha Electrolyte® solution. The concentration of the NaOCl solution was tested by iodometric titration. The obtained value amounted to 3.9.

After sealing the apical portion with autoclaved physiowax (RA Lamb Ltd, Eastbourne, UK), 5 cc of the selected irrigant was dispensed using a 27 gauge Monoject syringe (Kendall, Tyco, Mansfield, MA, USA) in an up-and-down motion, and left *in situ* for 3 min.

Following the removal of the apical seal to allow for the irrigation solution to drain, the coronal 5 mm portion of the specimen was sampled by grinding dentin and canal contents using ParaPost® XP™ Endodontic post system drill (Coltene/Whaledent) with a diameter of 1.5 mm.

Debris collected in the flutes of each drill was placed in a 1.5 mL microcentrifuge tube containing 1 ml BHI broth. After vortexing for 10 sec, a serial dilution of the debris containing BHI broth was made and 100 µL of neat, 10^{-2}, 10^{-4} and 10^{-6} dilutions were plated in duplicate onto fresh BHI agar plates and incubated overnight at 37°C. To confirm the morphology and Gram group of the bacterial cells, Gram staining was performed.

In order to determine if the carry-over of NaOCl could prevent the growth of cells in the broths an additional experiment was carried out on sterile bovine teeth. These had been treated the same as the teeth used above except that they had not been inoculated with *E. faecalis*. After irrigation with NaOCl, the debris from the drill flutes were put into 900 µL of BHI and 100 µL of stationary phase E. faecalis culture was added. This was serially diluted as above and dilutions plated out as before. In addition, one group of teeth was irrigated with sterile water to provide a negative control.

All data were compared stratified by dilutions. Colony-forming units (cfu) with too many to count (TMTC) (defined here as >800 cfu per agar plate) were attributed the highest rank in a non-parametric approach with ANOVA on ranks with a Duncan *post-hoc*. Differences were considered significant at $p<0.05$. To double check the results an additional non parametric approach was conducted. All data was compared stratified by dilutions. The Kruskal-Wallis test was used to compare the three groups involved followed by Mann-Whitney's test as a *post-hoc* procedure adjusted with Bonferroni correction for multiple comparisons. Significance

Table 1- Colony-forming units (cfu) in serial dilution plates. Each group contained 5 roots and serial dilutions and plating were carried out in duplicate. Whilst NaOCl (Group 2) was the only irrigant to eradicate the *E. faecalis* a significant difference was seen between the Optident Sterilox Electrolyte Solution® (Group 1) and the Sterilox's Aquatine Alpha Electrolyte® irrigation (Group 3)

Group 1 - Optident Sterilox Electrolyte Solution® irrigation (negative control)										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	TMTCa	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	Root Shattered sample lost	
10^{-2}	178	188	285	49	532	578	28	38		
10^{-4}	9	0	0	0	7	3	2	0		
10^{-6}	0	0	0	0	0	0	0	0		
Group 2 - Sodium hypochlorite irrigation										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	0	0	0	0	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0
Group 3 - Sterilox's Aquatine Alpha Electrolyte® irrigation										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	146	230
10^{-2}	4	4	6	14	42	50	17	16	1	2
10^{-4}	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0

[a] *TMTC = too many to count (>800 cfu per plate).*

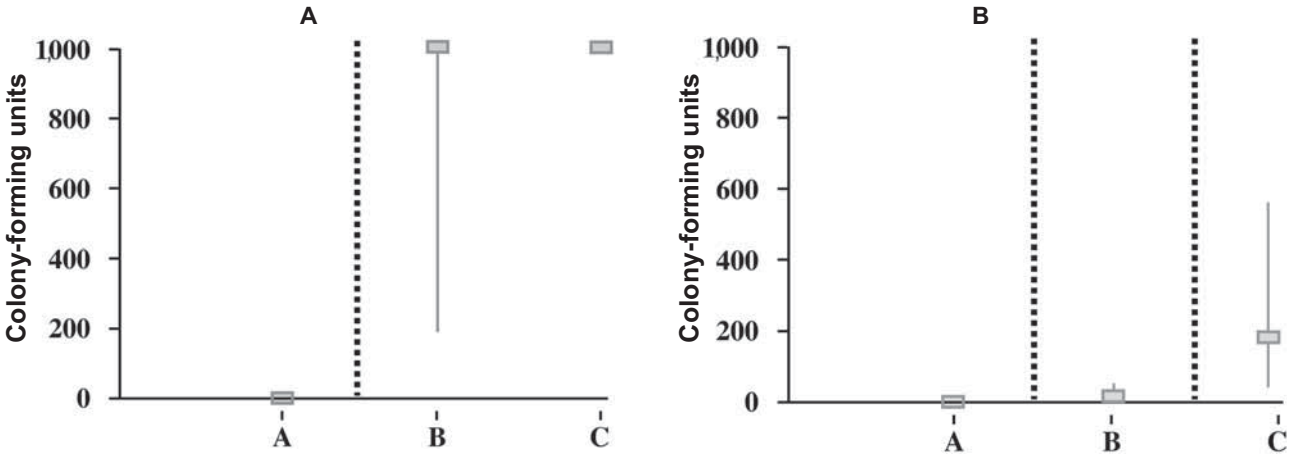

Figure 1- Mean number of colony-forming units recovered from debris after dentine grinding and root canal irrigation with NaOCl (A) - Sterilox's Aquatine Alpha Electrolyte® (B) - or Optident Sterilox Electrolyte Solution® (C) - 1A; Neat broth (no dilution). 1B; 10-2 dilution. The vertical dashed line shows significant difference between the groups on either side ($P<0.05$).

level was set at $\alpha=0.05$. Data were analyzed using SPSS software v. 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The overnight incubation of the sectioned bovine root canals resulted in no growth in any of the samples. This indicates that all the root sections were sterile at the start of the experiment.

Gram staining of a number of the resulting colonies showed the presence of Gram-positive cocci, consistent with the *E. faecalis* inoculum. The bacteria were morphologically identical to the *E. faecalis* used in the inoculum.

Results of the dilution series are presented in Table 1, while statistical analysis of the raw data for neat and 10^{-2} dilution is presented in Figure 1. Results for statistical analysis coincided for both approaches.

NaOCl was the only irrigant to eliminate all bacteria and was significantly better at killing *E. faecalis* than both the Optident Sterilox Electrolyte Solution® (saline) and Sterilox's Aquatine Alpha Electrolyte®. Additionally Sterilox's Aquatine Alpha Electrolyte® solution was superior to Optident

Sterilox Electrolyte Solution®.

The experiment to determine the effect of carry-over of NaOCl resulted in similar colony counts for the samples from teeth irrigated with NaOCl and water (results not shown) indicating that carry over of NaOCl had no noticeable effect in our experiment.

DISCUSSION

This study evaluated the antimicrobial action of Sterilox's Aquatine Alpha Electrolyte®, a commercially available super-oxidized water in the United Kingdom, in bovine root canals.

E. faecalis was selected as the test species, because it is commonly detected in asymptomatic, persistent root canal infections[12,15]. The bovine root model was chosen as it is clinically relevant, although the large root canal preparation size allows for more favorable dynamics of irrigation for the solution tested than is likely to occur *in vivo*. Additionally the number of bacteria present is likely to be artificially high compared to the *in vivo* situation. Despite these limitations, the *ex vivo* model has been successfully used previously to test the ability of *E. faecalis* to survive diverse root canal irrigations[17].

Our study suggested that the protocol followed was either able to prevent carry-over of the antimicrobial effect of NaOCl onto the BHI plates, possibly due to drainage of the solutions after irrigation or any carry over had no effect on the viability of the organisms, possibly due to the immediate dilution of the samples in the BHI broth. Furthermore, based on pilot studies, it was decided to collect samples at a single depth as no difference was found between different depths of sampling when NaOCl was used as irrigant; this is consistent with the results form other investigators on a similar bovine tooth model[8].

Sterilox's Aquatine Alpha Electrolyte® is obtained by passing a sodium chloride solution (Optident Sterilox Electrolyte Solution®) over coated titanium electrodes at 9 amps in a specifically made device (Optident Sterilox Dental Generator®; Optident Dental). Optident Sterilox Electrolyte Solution® (non-activated) was used as the negative control as we did not expect any antimicrobial action from this irrigant. NaOCl was tested, because it is largely recommended as the main root canal irrigant[25].

Endodontic literature suggests that infection of the root canal at the time of obturation has a negative influence on the prognosis of endodontic treatment[23]; NaOCl was the only irrigant tested which was consistently associated with negative cultures in our study. However, Sterilox's Aquatine Alpha Electrolyte® might be able to reduce the bacterial load to levels that could influence treatment outcome.

These results are not consistent with those of a previous study where different irrigants, including NaOCl, where tested against *E. faecalis* in a bovine tooth model, in fact Krause, et al.[8] (2007) suggest that 5.25% NaOCl was not able to render the dentinal shavings obtained sterile, it was however, significantly more effective than the other solutions tested[8]. The major difference between the models is the difference in volumes of irrigation used; 60 µL twice against 5 cc in our study, therefore suggesting a role for the amount of irrigant used on the ability to eliminate root canal infection in the bovine root model; in the same way a previous investigation indicates that the volume of irrigation has a significant influence in removing a bio-molecular film from root canal walls[6].

The disinfecting actions of super-oxidized water are heavily reduced in the presence of organic contamination[11,20]. The model used in our study allows for a greater bacterial growth than one might expect in an *in vivo* situation. It further excludes the mechanical aspect of root canal preparation, so that the bacterial biomass present in the root canal is likely to be greater than in normal clinical conditions. Consequently, its elimination will depend exclusively on the flushing and chemical effects of the irrigation solution tested. The importance of instrumentation in obtaining a significant reduction in bacterial content has been shown[14]. Therefore we hypothesize that, in the presence of a reduced bacterial load, as a result of a chemo-mechanical preparation super-oxidized water irrigation might have the ability to eradicate a more clinically relevant root canal infection.

The result of a previous study[2] showed that super-oxidized water had no ability to prevent the growth of *E. faecalis* using paper disks as the delivery method on Petri dishes, a protocol more favorable to the irrigant when compared to the bovine root model due to long time of contact with the micro-organisms, absence of interaction with dentin and cells in a metabolically active phase, therefore more susceptible to antimicrobials[9]. Nonetheless, a different irrigation source was tested (Dermacyn, Oculus Innovative Sciences, Petaluma, CA, USA). Different super-oxidized waters are produced by a similar electrolysis process but, due to a difference in the active concentration and the pH of the final solution, the product can have a different anti-microbial activity[18].

One of the suggested advantages of super-oxidized water, when compared to NaOCl, is its level of toxicity[20]. It is worth noting, that the mechanism of action of super-oxidized water involves oxidative damage which might cause ageing and irreversible dysfunctions that eventually produce cellular death[4]. A pH-neutral super-oxidized solution (Microcyn; Dermacyn, Oculus Innovative Sciences, Petaluma, CA, USA) has been tested. It was found to be significantly less cytotoxic than antiseptic hydrogen peroxide concentrations (used as a positive control for oxidative damage) because it does not induce genotoxicity or accelerated ageing

in vitro[4]. However, Microcyn has a different pH than Sterilox's Aquatine Alpha Electrolyte® and this needs to be taken into account when comparing the two irrigants.

CONCLUSIONS

Under the conditions of this study Sterilox's Aquatine Alpha Electrolyte® appeared to have significantly more antimicrobial action when used as an irrigant in the root canal system compared to the non-activated Optident Sterilox Electrolyte Solution®, but NaOCl was the only irrigant able to eliminate all bacteria in our experiments. Sterilox's Aquatine Alpha Electrolyte® caused a bacterial load decrease although being less effective than NaOCl.

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