Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution

J. T. Marais & W. P. Williams
Department of Restorative Dentistry, Faculty of Dentistry, University of Pretoria, Pretoria, South Africa

Abstract

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Aim The aim of this *in vitro* study was to evaluate the antimicrobial effectiveness of electro-chemically activated water (ECA) as an endodontic irrigation solution.

Methodology The root canals of 60 caries-free, single-rooted, adult, maxillary, anterior human teeth were instrumented and irrigated in a similar method to that used for *in vivo* root canal treatment. The external root surface of each tooth was sealed, maintaining the access cavities patent and the root canals were inoculated with a suspension containing four bacteria. The teeth were randomly divided into four groups ($n = 15$). Each group was irrigated ultrasonically with one of the following solutions: distilled water (control), NaOCl (3.5%), and ECA, the latter at pHs 7.0 and 9.0. Antimicrobial effectiveness was established directly after irrigation and again 7 days later, by counting colony-forming units on blood agar plates and by spectrophotometric analysis.

Results Large numbers of bacteria were present in the canals of teeth irrigated with distilled water. No bacteria were observed following irrigation with NaOCl. Neither of the ECA solutions were found to be effective against all the bacteria. Although some reduction in the number of bacteria was evident in the ECA groups, this was not statistically significant ($P > 0.05$) when compared to sodium hypochlorite.

Conclusion Within the confines of this study ECA did not demonstrate antimicrobial effectiveness.

Keywords: antimicrobial, colony-forming units, electro-chemically activated water, irrigation solution, root canals.

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Introduction

Sodium hypochlorite (NaOCl) at a concentration of between 2.5 and 5.25% is universally accepted as the irrigant of choice for root canal treatment (Cheung & Stock 1993, Gulabivala & Stock 1995, Walton & Rivera 1996). NaOCl is also associated with several complications and problems. First, NaOCl is extremely toxic to living tissue and extrusion of NaOCl through the apices of teeth causes severe pain, swelling and necrosis (Brown *et al.* 1995). Accidental extrusion of NaOCl into the maxillary sinus may cause serious complications (Cymbler & Ardakani 1994). Secondly, because of the corrosive nature of NaOCl, ultrasonic units used for canal irrigation are prone to mechanical breakdown. Thirdly, spillage and damage to operators', patients' and assistants' clothing is not uncommon. Fourthly, substrate adherence capacity of inflammatory macrophages has been shown to decrease significantly with the use of NaOCl (Jimenez-Rubio *et al.* 1997), a fact that may explain delayed healing of periapical tissues.

Antimicrobial properties of calcium hydroxide, chlorhexidine and camphorated paramonochlorophenol were examined by Barbosa *et al.* (1997). Nd-YAG laser irradiation (Fegan & Steiman 1995), citric acid (Georgopoulou *et al.* 1994), oxidative potential water (Hata *et al.* 1996), chlorhexidine gluconate (Jeansonne & White 1994) and gluteraldehyde (Jimenez-Rubio *et al.* 1997) have also been described as possible intracanal agents.

Over the course of the past 28 years Russian scientists

Correspondence: Dr W. P. Williams, PO Box 1977, Brooklyn Square 0075, South Africa (fax: +27 12 326 2754; e-mail: wwilliam@mweb.co.za).

have developed and refined the process of electro-chemically activating water. (Leonov 1997, Bakhir 1997).

Electro-chemically activated water (ECA) is the subject of more than 300 Russian patents and more than 20 000 units producing ECA are in operation in Russian hospitals today. ECA is produced from tap water and saline solution by a special unit that houses a unique flow-through electrolytic module (FEM). The FEM contains the anode, made from titanium and coated with ruthenium–oxide, iridium and platinum, and the cathode, made from titanium coated with pyro-carbon and glass-carbon. A diaphragm consisting of ultra-filtration, electro-catalytic ceramics on a bed of zirconium, yttrium, aluminium and niobium-oxides separates the anode and cathode.

The physical and chemical nature of ECA is not yet fully understood. The solution supposedly exists in a metastable or disequilibrious state after production and contains many free radicals and a variety of molecules and ions. In the metastable state the solution has a very high oxidation-reduction potential. Two types of ECA solution are produced. *Anolyte* has a high oxidation potential (plus 400 to plus 1200 millivolts). The manufacturers claim that it is possible to produce acidic, neutral or alkaline anolyte (pH 2–9), and that anolyte is antimicrobial. *Catholyte* is an alkaline solution (pH 7–12) with a high reduction potential (minus 80 to minus 900 millivolts). Catholyte is reputed to have a strong cleaning or detergent effect. Both these solutions remain in the metastable state for approximately 48 h before the solution returns to the stable state, becoming inactive once more.

Recently, strong evidence for the antimicrobial nature of anolyte was reported (Selkon *et al*. 1999, Shetty *et al*. 1999).

A 5 $\log_{10}$ reduction (99.999% effective kill rate) was reported for anolyte solution, produced by a device based upon the Russian invention, against *Mycobacterium tuberculosis*, *M avium-intracellulare*, *M chelonae*, *Escherichia coli* (including type 0157), *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* var *niger* spores, methicillin resistant *Staphylococus aureus*, *Candida albicans*, poliovirus type 2 and human immunodeficiency virus (HIV-1). This was accomplished in the relatively short period of 2 min. It was also found that high organic loading diminished the antimicrobial activity.

In another study (Marais 1998), one group of root canals were irrigated with ECA (catholyte followed by anolyte) and another with NaOCl (3.5%). Root canal surfaces were examined using a scanning electron microscope. ECA produced surfaces that appeared (visually) as clean as those for the NaOCl group. It was concluded that ECA removed the smear layer from some surfaces of the canals.

The research of Hata *et al*. (1996), reported on the effectiveness of so-called oxidative potential water (OPW) as a root canal irrigant. Hata and coworkers reported favourably on the cleaning ability of OPW (Super Mini-Water, Janix Inc., Atsugi, Japan) to clean debris from canal walls. OPW is manufactured with an electrolysis device. The electrode system is of a totally different configuration to that of the Russian system.

Interestingly, other investigators have also studied the antimicrobial effects of electrolysed water (Shimizu & Furusawa 1992) and there have also been attempts to study the effects of electrolysed neutral water against bacteria isolated from root canals (Horiba *et al*. 1999). So far these efforts have failed to effectively kill all microorganisms. Significantly Horiba *et al*. (1999) found electrolysed neutral water ineffective against *Bacillus subtilis* and *Candida albicans*, whereas in the study by Selkon *et al*. (1999), super-oxidized water produced by using the Russian technology was found to effectively kill these same bacteria.

The purpose of this *in vitro* study was to evaluate the antimicrobial effectiveness of ECA on a selected group of anaerobic bacteria in root canals of extracted human teeth.

Materials and methods

Sixty caries-free, single-rooted, adult maxillary anterior human teeth were collected from the extraction clinic of the University of Pretoria and rinsed under cold running water and stored in distilled water at 4 °C for 1 week. The teeth were examined radiographically and visually to eliminate any teeth with conditions that could influence the experimental procedure. Only teeth with straight, single canals were used.

Access cavities were prepared using diamond and round burs. Canals were explored using number 06 K-files, confirming apical patency and establishing working length visually, for each individual tooth.

The coronal two-thirds of the canals were prepared using a series of nickel–titanium files in a rotary handpiece (ProFile, Dentsply, Maillefer, Ballaigues, Switzerland) in a crown-down technique. After each size file, and intermittently during the preparation procedure, canals were irrigated with a 3.5% NaOCl solution (Jik, Reckitt Colman (PTY) LTD., Elandsfontein, South Africa) delivered by an ultrasonic unit (Piezon®Master, Siemens, Germany). The apical third of each canal was prepared

to a minimum ISO size 30, using a series of hand-files (Hyflex X-File, Hygenic Corporation, Akron, OH, USA) in a serial procedure. Once again, after each size file, the canals were thoroughly irrigated as described above. Following apical preparation, the canals were smoothly tapered using a step-back procedure and rotary nickel–titanium files, again with copious irrigation (NaOCl).

The apices of the teeth were sealed with a resin modified glass-ionomer cement (Advance, Caulk/Dentsply, Milford, DE, USA). The remaining root surface was coated with a double layer of nail varnish, to isolate the internal root canal environment (Behrend *et al.* 1996). Each canal was finally flushed with NaOCl, dried with absorbent paper points and filled with a creamy mixture of calcium hydroxide powder (Calcium hydroxide, Merck, D-6100, Darmstadt, Germany) (Turkun & Cengiz 1997), mixed with sterile water. A sterile cotton wool pellet was placed into the pulp chamber and the access cavity sealed with a reinforced zinc oxide eugenol material (IRM, Caulk/Dentsply, Milford, DE, USA). After a seven-day storage period in deionized water (37 °C), the canal systems were reaccessed.

Sterility was maintained for the duration of the experiment, conducted in a positive sterile airflow laboratory, by working in a laminar flow cabinet, and using sterilized instrumentation as well as gloves and masks.

The teeth were placed in sterile Ringer's solution (22 °C) for five 24-h cycles; Ringer's solution being replaced with a fresh solution after each cycle, thus allowing for the removal of NaOCl and calcium hydroxide from the canals and dentinal tubules. Twenty-five microlitres (μL) of sterile Ringer's solution was injected, using a sterile pipette, into each canal and aspirated (Fegan & Steiman 1995). This procedure was repeated three times, serving as a rinsing procedure. The third and final aspirated Ringer's solution was placed onto a blood agar plate and spread, using a sterile glass rod (Harrigan & McCance 1970). The plates were incubated anaerobically (Anerocult A®, Merck GaA, 64271 Darmstadt, Germany) at 37 °C for 7 days. All cultures were negative at 7 days, confirming absence of anaerobic bacteria within the root canals. Bacterial standardization was completed by using a MacFarland Standard-1 suspension and spectrophotometry (GBC -uv/vis 916. Spectrophotometer (Wirsam), GBC Scientific Equipment, Melbourne, Australia) at 540 nm.

Calibrated suspensions of two obligate (*Prevotella intermedia* ATCC No. 25611 and *Porphyromonas gingivalis* ATCC No. 33277) and two facultative (*Enterococcus faecalis* ATCC No. 49474 and *Actinobacillus actinomycetemcomitans* ATCC No. 33384) anaerobic bacteria were prepared and inoculated into 500 mL brain-heart-infusion (BHI) to form a broth (Kwang-Shik *et al.* 1997, Siqueira *et al.* 1997, Siqueira & de Uzeda 1996).

Table 1 Irrigation solutions and groups

Group	Solution
Group A (n = 15)	Distilled water (control group) (obtained from laboratory)
Group B (n = 15)	Sodium hypochlorite (3.5% concentration)
Group C (n = 15)	ECA solution 1 (pH 7.0)
Group D (n = 15)	ECA solution 2 (pH 9.0)

ECA, electro-chemically activated water.

The teeth were placed into a glass beaker containing 500 mL BHI broth (inoculated with bacteria) and incubated anaerobically at 37 °C, in a shake-incubator, for 7 days. Anaerobic incubation was accomplished by placing the glass beaker into a sealed anaerobic flask containing an Anerocult insert and the flask was placed into an incubator. After 7 days the teeth were removed from the broth in an upright position, retaining the suspension within the canals. The suspension was aspirated from each canal and 25 μL (dilution factor of 40 was determined to be the most efficient for this study) spread onto marked blood agar plates.

The plates were incubated anaerobically at 37 °C for 7 days and the presence of organisms inside the canal of each tooth was confirmed by formation of colony-forming units (exceeding 1000 per plate). Immediately following aspiration, each tooth was placed into a sterile, marked container that corresponded to the marked agar plate for that tooth. The teeth were randomly divided into four groups (n = 15). All teeth were irrigated for 5 min, each group with one of the four different irrigation solutions (Table 1) being delivered from an ultrasonic unit.

The ECA was produced by a STEDS unit (STEDS, Radical Waters (Pty) Ltd, Vorna Valley, South Africa). The STEDS unit was operated with a current of 3.5 A and 24 volt. A 5% saline solution and potable water from the local supply was used. Two solutions of anolyte were produced, solutions 1 and 2. In order to produce ECA solution 1, the outflow valve of the catholyte solution was adjusted to produce anolyte with a pH of 7.0 (increasing the flow of catholyte lowers the pH of anolyte and decreasing the flow of catholyte increases the pH of catholyte; the pH of catholyte cannot be manipulated).

In order to produce ECA solution 2 the catholyte outflow valve was closed totally, raising the pH of the anolyte solution maximally, to pH 9. This decision was made on an empirical basis, in order to make some assessment of the influence of manipulation of pH on

the effectiveness of this new technology. All solutions were produced at a flow of 2 L min^{-1}. ORP levels and conductivity of solutions were not recorded.

Directly following irrigation, 25 µL sterile BHI solution was injected into each canal and aspirated immediately. This procedure was repeated three times (rinsing the canal). The third 25 µL aspirated BHI solution was inoculated into 5.0 mL BHI broth and measured by spectrophotometry. In the same manner, a further 25 µL BHI solution was aspirated and placed onto a blood agar plate and incubated anaerobically at 37 °C for 7 days. The access cavities were sealed, the teeth placed in distilled water in individual, marked containers and incubated anaerobically at 37 °C for 7 days. Canals were accessed after 7 days and spectrophotometric analysis and microbial counts for colony-forming units repeated, as described above.

Results of the standard plate counts and spectrophotometry were analysed using analysis of variance (ANOVA) and Dunnett's one-tailed *t*-test to detect differences between the four groups and in particular to test for significant differences between the control group (group A) and the other three groups (groups B, C and D). Multiple comparison analysis, using Scheffe, Duncan and Tukey tests, was done to pinpoint differences between groups. A paired *t*-test was performed on spectrophotometric readings for each group, to determine whether growth of bacteria took place between the period directly following irrigation and the seventh day postirrigation.

Results

Results of the standard plate counts (colony-forming units) and spectrophotometric analysis are given in Table 2. All of the canals in group A showed more than 40 000 colony-forming units per millilitre of suspension, indicating the inability of distilled water to destroy bacteria. The canals in group B however, showed no colony formation and confirm the bactericidal action of NaOCl. Groups C and D showed fewer numbers of colony formation, as well as reduced spectrophotometric values compared to the control group (group A). This reduction however, did not approach the negative (zero) values as recorded for group B and in fact are closer to those values obtained for the control group (group A). All four groups showed an increase in colony-forming units and spectrophotometric values when compared to the results of the immediate postirrigation analysis. Values for groups C and D once again approximated the values for group A, and the values for group B were almost negligible in comparison.

Discussion

The selection of the four bacteria used in this study was based on the fact that they are all widely implicated pathogens in diseases of the pulp and periapical tissues. In addition, these bacteria have also been used, either as individual strains or in combination, in many *in vitro*

Table 2 Colony-forming units and spectrophotometric values measured immediately after irrigation of root canals with four different solutions (and measured 7 days after irrigation)

	No. of teeth (*n*)	Colony-forming units		Spectrophotometric values	
		Mean (40)/mL	SD	Mean	SD
Group A (control)	13	>1000[c] (>1000[c])	0 (0)	0.6585 (0.8458)	0.0959 (0.1138)
Group B (NaOCl)	14	0 (114)[e]	0 (291)	0.0442 (0.0654)	0.0134 (0.0138)
Group C ECA solution 1 (pH 7.0)	14	693 (814)	253 (183)	0.5785 (0.7524)	0.1914 (0.1670)
Group D ECA solution 2 (pH 9.0)	14	525 (721)	418[d] (286)	0.6251 (0.8082)	0.1377 (0.2467)

[a]ECA, electro-chemically activated water.
[b]SD, standard deviation.
[c]A standardized microbiological counting technique was applied (Harrigan & McCance 1970). Plates were divided into quadrants and a count exceeding 250 for a quadrant was recorded as exceeding 1000 for that plate.
[d]A high SD (standard deviation) value was recorded for this group, indicative of a high variation within the group (CV = 79.6)
[e]Two of the 14 plates had values of 800, resulting in a mean of 114 for the entire group. These colonies could be clearly identified as contaminants.

studies of this nature (Safavi *et al.* 1990, Yesilsoy *et al.* 1995, Siqueira & de Uzeda 1996, Siren *et al.* 1997, Siqueira *et al.* 1997, Kwang-Shik *et al.* 1997, Barbosa *et al.* 1997, Siqueira & de Uzeda 1997, Dougherty *et al.* 1998, Molander *et al.* 1998).

All of the cultures for the control group had bacterial counts exceeding 40 000 colony-forming units, representing active bacterial growth. The fact that these bacteria successfully withstood vigorous irrigation (ultrasonics) with distilled water and to a lesser extent, irrigation with ECA, proves that the four selected species of bacteria are a useful model for a study of this kind. Taxonomic identification was not carried out for growth on the culture medium and thus, any and all remaining bacteria were included in the colony-forming unit count.

The fact that colony-forming units consistently exceeded 40 000 (undefined) in the control group (group A), was reason to consider a larger dilution factor (i.e. >40). Should defined counts have been possible, this would have made statistical evaluation even more accurate. However, the probability of recording 'false-negative' (decreased sensitivity for the test) counts for groups C and D would have been more detrimental to the results and the dilution factor thus used was 40 (0.25 μL).

Under the conditions of this study, ECA failed to destroy all of the bacteria within the root canals. On the other hand, NaOCl reduced the bacterial counts to less than one colony-forming unit 25 μL^{-1} in all of the specimens. This was the case both directly following irrigation and, very importantly, also 7 days postirrigation.

The control group consistently (15 from 15 counts at each analysis) produced bacterial counts greater than 40 000 colony-forming units mL^{-1}, confirming the accuracy and usefulness of this model for similar studies. The results of the spectrophotometric analysis correlated well with those for the colony-forming units (as seen in Table 2), further confirming the relevance of this model.

The large variation (SD) recorded for colony-forming units for group D (Table 2) could not be accounted for. The very low (groups A and B) and low (group C) variation, of all three other groups, seems to indicate that the experimental model and techniques used were accurate. This, together with the fact that values for spectrophotometry indicate an acceptable level of variation (2× SD) in all four groups. Furthermore, the fact that this high variation was not seen in the measurements taken 7 days postirrigation, give further reason to accept this model as accurate.

Although fewer bacteria were measured in the groups irrigated with ECA (compared to group A, control) both directly following irrigation and 7 days postirrigation, ECA was not as effective in destroying bacteria as was NaOCl.

A probable explanation for this apparent failure of ECA to effectively destroy all the bacteria may be one of the following:

1 Only anolyte was used in this study, as opposed to the use of catholyte followed by anolyte in the study by Marais (2000). Catholyte is claimed by the inventors to have a cleaning or detergent effect. It is an accepted principle of disinfection that all matter should first be cleaned before disinfection is attempted. It seems logical (with the benefit of hindsight) that the canals should first have been cleaned with catholyte before anolyte was used. In the studies by Selkon *et al.* (1999) and Shetty *et al.* (1999) the antimicrobial activity of the superoxidized water was also shown to inhibit antimicrobial activity by high organic loading.

2 At the time of preparation of the ECA solutions used in this study, a technical failure of the unit may have resulted in an altered ECA solution.This would indicate the extreme technique sensitivity in the production of ECA for use as an endodontic solution and indicates the need for quality control of the technology.

3 The specific ECA parameters (pH, current, temperature, exposure time of canal to irrigant, flow rate) used in this study may need to be varied and will be studied in future research.

Of particular importance, again with the benefit of hindsight, are the flow rate amperage saline concentration, ORP level and total dissolved solid content. Recent reports have stressed the importance of a flow rate of less than 1 L min^{-1}, amperage of 4.5 A, ORP level above 750 mL and total dissolved solid content of less than 3 g L^{-1} (Panicheva 1999, Bakhir & Zadorozhny 1999).

As can be expected, in any system with so many variables, and given the metastable state of the solution, one can expect to have initial difficulties in identifying the exact parameters required for a successful antimicrobial solution for use in the root canal system.

Conclusions

1 In accordance with many other studies, NaOCl (3.5%) was shown to be bactericidal within the root canal system.

2 The use of ECA caused a reduction in the number of anaerobic bacteria within the root canal system, but

this was not statistically significant ($P > 0.05$) when compared to sodium hypochlorite.

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