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Validation of an extracted tooth model of endodontic irrigation

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Abstract

An extracted tooth model of endodontic irrigation, incorporating reproducible inoculation and irrigation procedures, was tested against *Enterococcus faecalis* using a variety of different irrigants in a Latin square methodology. ANOVA revealed no significant variations between the twelve teeth or experiments undertaken on different occasions; however, variation between irrigants was significant.

Keywords

Endodontic irrigants

Enterococcus faecalis

In vitro modelling

In dentistry, the treatment of the tooth pulp, root canals and surrounding tissue is termed endodontics. Bacteria established within the root canals of the teeth are beyond the reach of host defences or systemic antibiotics and so require effective disinfection using endodontic files and irrigation for successful treatment (Sjogren et al., 1997). *Enterococcus faecalis* (*Ef*) is frequently isolated from failed endodontic restorations (Zoletti et al., 2006) and is implicated in persistent root canal infections, often occurring as a monoseptic infection (Fabricius et al., 1982). A typical sequence for endodontic restoration would be to remove the infected pulp tissue and flush the root canal with an antimicrobial agent, such as sodium hypochlorite (NaOCl) (Walker, 1936). However, the dangers associated with exposing soft-tissues to NaOCl (Spangberg et al., 1979) makes the use of less cytotoxic irrigants such as chlorhexidine (CHX) (Siqueira et al., 2007) and electrochemically activated water, also known as super-oxidised water ($SOXH_2O$) (Gulabivala et al., 2004) attractive alternatives. A laboratory model capable of evaluating the antimicrobial efficacy of endodontic irrigants needs to be not only clinically relevant, but it should also be reproducible and demonstrate minimal variability between experiments.

Twelve extracted, single rooted teeth were prepared for endodontic treatment using modified double flare technique to a size 40 master apical file. This process represents the standard preparatory procedure at Liverpool University Dental Hospital during endodontic restoration which basically makes a hole in the occlusal (biting) surface of the tooth in order to gain access to the root canal. The roots and apices of all teeth were sealed using Prime and Bond NT (Dentsply, Weybridge, Surrey, UK) to allow them to retain liquid. The teeth were then mounted in cold cure silicone (Zhermack, Badia Polesine, Veneto, Italy) to hold them vertically and placed in glass jars before autoclaving. Liquid cultures of *Ef* (NCTC 775) were grown at 37ºC in 10 ml of brain heart infusion (BHI) broth (Sigma-Aldrich Company Ltd., Poole, Dorset, UK). After 16 hours, the cultures were centrifuged and the cell pellet washed in phosphate-buffered saline (PBS) (Oxoid, Basingstoke, Hampshire, UK) before being re-suspended in fresh BHI. The final optical density of the culture was

adjusted to 0.1 units (OD 650 nm) with additional BHI; this contained 5 x 10^7 colony forming units (cfu) ml^{-1}.

Each tooth was inoculated with 20 µl of the *Ef* culture and incubated aerobically for 48 hours before irrigation with either PBS (control), 1% NaOCl (Sigma), 2% CHX (Sigma) or $SOXH_2O$ (Sterilox®, Optident, Ilkley, West Yorkshire, UK). A Latin-square was formulated so that each of the four irrigants were used on three of the twelve teeth each week. The clinical irrigation procedure was mimicked in the laboratory by using an electronic dispensing pipette (Proline 50 – 1000 µl, Biohit, Torquay, Devon, UK) to supply irrigant at a reproducible flow rate. A standard 'blue' laboratory pipette tip was loaded with 1 ml of irrigant, which was in turn push fitted into an endodontic capillary tip (0.014" / 356 µm, Ultradent Products Inc, South Jordan, UT, USA) (Figure 1). With this pipette set at an output speed setting #3 the flow rate was 0.5 ml s^{-1} (manufacturer's data) which corresponded to a flow velocity at the capillary tip of ~1 m s^{-1}. The pipette was then primed to purge air from the tips. Irrigant was dispensed into the tooth as a series of five consecutive 200 µl aliquots; the final aliquot of irrigant was allowed to remain for one minute. Upon completion of irrigation, the root canal was flushed with casein peptone lecithin polysorbate broth (CPLP) (Fluka, Buchs, St. Gallen, Switzerland) containing 40 ml l^{-1} polyoxyethylene sorbitan monolaurate (Tween® 20, Sigma, Gillingham, Kent, UK) and 0.1 g l^{-1} sodium metabisulfite (Sigma), which was applied in a manner identical to that used during the irrigation procedure. The formulation of CPLP is similar in composition to Letheen broth, which is used to neutralise quaternary ammonium compounds (el-Falaha et al., 1987) and also served to flush residual NaOCl from the root canal in order to minimise its carry-over into the sampling and dilution phases. Bacteria were recovered from the teeth using a series of five, ISO 40 paper points (Dentsply) which were in turn rubbed against the walls of the root canal and allowed to draw up their full capacity of liquid before being transferred to PBS and vortex-mixed for 30 seconds. Viable counts of *Ef* were performed by serial dilution and growth on nutrient agar (Oxoid) after 48 hours aerobic incubation at 37ºC. On completion of the Latin-square, the entire experiment was repeated using the same teeth (n=96 in total; n=24 per irrigant). The counts

of viable bacteria recovered from the teeth following *in vitro* endodontic irrigation were used to investigate the three null hypotheses that had been postulated:

1. There are no significant differences between the different teeth within the cohort.
2. There are no significant differences between each leg of the experiment (i.e. week-to-week)
3. There are no significant differences between the four irrigants.

Three-way ANOVA of between subject effects (Table 1) showed that there were no significant differences between the twelve teeth, likewise there were no significant differences between each leg of the experiment over eight weeks. However, the differences between the four irrigants were significant which shows that the model is capable of elucidating the effectiveness of antimicrobial endodontic irrigants.

The number of viable bacteria recovered from the teeth following irrigation (Table 2) with the PBS control was 3.329 (log_{10} cfu), whilst the antimicrobial irrigants 1% NaOCl, 2% CHX (Sigma) and $SOXH_2O$ yielded 0.552, 1.441 and 1.577 (log_{10} cfu) respectively. However, only the difference between PBS and 1% NaOCl was statistically significant following using Tukey's post-hoc pairwise test.

In conclusion, the extracted tooth model is a useful method for evaluating the effectiveness of antimicrobial endodontic irrigants. In these preliminary experiments, the most effective irrigant was 1% NaOCl.

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Figures and Tables

Figure 1. *In vitro* modelling of endodontic irrigation using an electronic laboratory pipette fitted with an endodontic capillary tip to irrigate an extracted tooth previously inoculated with *E. faecalis*.

Table 1: Three-way ANOVA of between subject effects (n=96)

Variable	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Significance (p-value)
Irrigant	97.190	3	32.397	14.449	0.000*
Tooth	26.897	11	2.445	1.091	0.381
Week-to-week	28.956	7	4.137	1.845	0.091
Error	165.915	74	2.242		

Table 2: Post-hoc pairwise testing of irrigants, measured as mean log_{10} cfu of *Ef* recovered from root canals following endodontic irrigation, using Tukey's test (n=24 per irrigant)

Irrigant A	Mean Log cfu Recovered	Irrigant B	Mean Difference (A-B)	95% Confidence Interval		Significance (p-value)
				Lower	Upper	
PBS	3.329	$SOXH_2O$	1.887	0.751	3.023	0.000*
		CHX	1.751	0.6152	2.887	0.001*
		NaOCl	2.776	1.640	3.913	0.000*
$SOXH_2O$	1.441	PBS	-1.887	-3.023	-0.751	0.000*
		CHX	-0.136	-1.272	1.000	0.989
		NaOCl	0.889	-0.247	2.025	0.177
CHX	1.577	PBS	-1.751	-2.887	-0.615	0.001*
		$SOXH_2O$	0.136	-1.000	1.272	0.989
		NaOCl	1.025	-0.111	2.161	0.092
NaOCl	0.552	PBS	-2.776	-3.913	-1.640	0.000*
		$SOXH_2O$	-0.889	-2.025	0.247	0.177
		CHX	-1.025	-2.161	0.111	0.092

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