

**An *in-vitro* Evaluation of the Effectiveness
of Endodontic Irrigants, with and without
Sonic and Laser Activation, in the
Eradication of *Enterococcus faecalis*
Biofilm**

**A report submitted to the University of Adelaide in partial
fulfilment of the requirements of the Degree of Doctor of
Clinical Dentistry (Endodontics)**

**Dr Aaron Nicholas Seet
BSc (Hons) (S'pore), BDS (Adel)**



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Declaration

I, Aaron Seet, declare that this work to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text. It contains no material which has been accepted for the award of any other degree of diploma in any university or tertiary institution.

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Aaron Seet

Date: _____

Witnessed by: _____

Date: _____

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Abstract

Introduction

It is well established that the causative agent of endodontic disease is the presence and growth of bacteria (Kakehashi *et al.*, 1965; Möller *et al.*, 1981). Therefore, eradication of bacteria is essential to prevent or eliminate apical periodontitis. Studies have shown that elimination of bacteria prior to obturation has resulted in a more favourable outcome for endodontic therapy (Sjögren *et al.*, 1997). When endodontic treatment fails, bacteria is often isolated from the root canals of these teeth. One of the most commonly isolated bacteria is *Enterococcus faecalis* (Molander *et al.*, 1998; Sundqvist *et al.*, 1998). As such, endodontic therapy is founded upon three principles: mechanical instrumentation; irrigation with antimicrobial agents and placement of an intracanal medicament (Haapasalo *et al.*, 2005). However, the complex anatomy of the root canal system often prevents the penetration of irrigants and medicaments into recesses that cannot be accessed by mechanical instrumentation. The advent of sonic, ultrasonic and laser instruments has led to many investigations looking at their potential for the activation of irrigants (Lee *et al.*, 2004; de Gregorio *et al.*, 2009; De Moor *et al.*, 2009). However, most of these studies have concentrated on the removal of dentinal debris and smear layer (Lee *et al.*, 2004).

Aim

The aim of this study was to evaluate and compare the effectiveness of three modes of irrigation: syringe irrigation; sonic activation and laser activation of the irrigant in eradicating *E. faecalis* that had been cultivated within the root canals of extracted single rooted teeth.

Methodology

A flow cell was designed and constructed. The extracted teeth were decoronated, and prepared with rotary instruments to #40 to 1 mm beyond the apex of the tooth. This was to allow nutrient media to flow through the root canals. The flow cell was connected to a nutrient reservoir containing Todd Hewitt Broth, which was pumped into the flow cell via a peristaltic pump.

The flow cell was inoculated with *E. faecalis* (ATCC 700802) and cultivated for a period of four weeks. The flow cell was then dismantled and the teeth were assigned to 6 treatment groups:

1. syringe irrigation with saline
2. syringe irrigation with 4% sodium hypochlorite
3. sonic activation of saline (EndoActivator, Dentsply)
4. sonic activation of 4% sodium hypochlorite
5. laser activation of saline (Er,Cr:YSGG Waterlase, Biolase Technology)
6. laser activation of 4% sodium hypochlorite

Teeth were irrigated with 5 ml of either saline or 4% sodium hypochlorite for 1 minute. The 4% sodium hypochlorite solution was inactivated with 5% sodium thiosulphate. Teeth that received sonic activation were irrigated by hand for 5 seconds, followed by 10 seconds of sonic activation, and this was repeated four times over 1 minute. Laser activation of the irrigants was also performed. Irrigant was introduced into the canal for 10 seconds, followed by 5 seconds of laser activation, (0.25W, 20 Hz) this cycle was repeated four times.

Teeth were then crushed and serial dilutions were performed to determine the number of viable bacteria (CFU/ml) remaining in the root canals. Protein assays were conducted to quantitate the amount of biofilm obtained. Samples were also taken from each treatment group and the radicular dentinal surfaces of the root canals were viewed under scanning electron microscopy (SEM).

Results

The root canals that were syringe irrigated with saline were the positive controls. Activation of the irrigants with either the sonic or laser instruments resulted in reduced cellular viability of *E. faecalis*. The most dramatic reduction in viability of *E. faecalis* was seen when the Er,Cr:YSGG laser was used to activate 4% sodium hypochlorite, resulting in $99.93\% \pm 0.14\%$ percentage kill.

SEM analysis showed that sonic activation with saline only caused minimal disruption to the biofilm. Teeth irrigated with sodium hypochlorite showed fewer bacterial cells on the radicular dentine but was not effective in eliminating *E. faecalis* that had invaded the dentinal tubules. Laser activation of sodium hypochlorite resulted in clean dentine walls and minimal bacteria within the dentinal tubules.

Conclusion

Sonic or laser activation of an antimicrobial irrigant resulted in more effective bacterial elimination compared to hand irrigation. Compared to syringe irrigation and sonic activation of sodium hypochlorite, laser activation of sodium hypochlorite was able to effectively disinfect the root canal.