Investigation of the Effect of Rapid and Slow External pH Increases on *Enterococcus faecalis* Biofilm Grown on Dentine

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

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Table of Contents

Abstract

Objectives: Calcium hydroxide is a common endodontic medicament that produces a localized rise in hydroxyl ion concentration. *Enterococcus faecalis* has shown some resistance to calcium hydroxide. The aim of this study was to compare the survival of an *E. faecalis* biofilm that had been grown on dentine when exposed to rapid or slow increases in external pH.

Method: A flow cell apparatus was used to grow single species *E. faecalis* biofilm on dentine discs. Following four weeks growth in Todd Hewitt Broth (THB), flow cells were exposed to either a rapid or slow increase to pH 11.5 or 12.5 using pH buffered growth medium. After four days exposure to pH 11.5 or 12.5, the flow cells were dismantled and the dentine discs were sonicated in saline solution to dislodge the attached biofilm. Viability of *E. faecalis* was established by serial dilution and plating onto THB agar plates. Viability was then normalised to total protein as determined by protein assay. Scanning electron microscopy (SEM) and confocal laser microscopy (CLSM) was also carried out to qualitatively observe the effects of the different rates of pH increase.

Results: A significant difference in viability between a slow or rapid increase in pH has not been shown by this study. pH 12.5 solutions were more effective at killing bacteria than pH 11.5 but even at this high pH some *E. faecalis* still survived. Exposure to high pH drastically reduced the numbers of bacteria observed on the dentine discs by SEM and CLSM although some did persist.

Conclusion: Based on the results of this study, *E. faecalis* located at sites within the root canal where a slower rise in pH is likely following application of a high pH medicament such as calcium hydroxide, do not seem any more likely to undergo an adaptive response that will increase their resistance and survival than the same bacteria in locations where the pH rise will be rapid. The demonstrated survival of *E. faecalis* in a high pH environment similar to that experienced clinically may help explain the problems associated with current treatment protocols when retreating root filled teeth.

Declaration

I, Mark Stenhouse, 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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Mark Stenhouse

Date: _____ Date: _____

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