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

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Table of Contents

Abstract................................................................................................................................................. 4
Declaration .................................................................................................................................................. 5
Acknowledgements .................................................................................................................................. 6
1.1 Introduction ....................................................................................................................................... 7
1.2 Microbiological Basis of Endodontic Disease ................................................................................. 8
  1.2.1 Microbiological Identification- Culturing vs Molecular Techniques ...................... 11
  1.2.2 Microbiology of the Root Canal ......................................................................................... 13
    1.2.2.1 Primary Infections ........................................................................................................ 14
    1.2.2.2 Persistent Infections ...................................................................................................... 16
  1.2.3 Endodontic Therapy .................................................................................................................. 19
1.3 Enterococcus faecalis ......................................................................................................................... 22
  1.3.1 E. faecalis in the Oral Cavity ................................................................................................. 23
  1.3.2 E. faecalis in Primary Root Canal Infections ........................................................................ 25
  1.3.3 E. faecalis in Persistent Root Canal Infections ................................................................. 26
  1.3.4 Why is E. faecalis so prevalent in root filled teeth? ......................................................... 28
  1.3.5 E. faecalis Virulence Factors ................................................................................................. 30
    1.3.5.1 Aggregation Substance ................................................................................................ 30
    1.3.5.2 Surface Adhesins ........................................................................................................ 31
    1.3.5.3 Gelatinase .................................................................................................................... 31
    1.3.5.4 Cytolysin ...................................................................................................................... 32
    1.3.5.5 Lipoteichoic Acid ........................................................................................................ 32
1.4 Biofilm .............................................................................................................................................. 34
  1.4.1 Biofilm Mechanisms of Antimicrobial Resistance ............................................................. 36
    1.4.1.1 Diffusion Barrier .......................................................................................................... 36
    1.4.1.2 Antimicrobial Deactivation ........................................................................................ 37
    1.4.1.3 Slow rate of Bacterial Growth ..................................................................................... 37
    1.4.1.4 Expression of Resistant Phenotypes ...................................................................... 38
    1.4.1.5 Quorum Sensing ....................................................................................................... 38
    1.4.1.6 Genetic Transfer ........................................................................................................ 39
  1.4.2 Evidence of Biofilms in Endodontontology .............................................................................. 39
1.5 Calcium Hydroxide .......................................................................................................................... 42
  1.5.1 Efficacy of Calcium Hydroxide .............................................................................................. 44
  1.5.2 Challenges to Calcium Hydroxide ......................................................................................... 52
    1.5.2.1 Dentine Tubules ........................................................................................................... 52
    1.5.2.2 Diffusion ...................................................................................................................... 52
    1.5.2.3 Buffering .................................................................................................................... 54
    1.5.2.4 Time ........................................................................................................................... 55
1.6 Survival Mechanisms of E. faecalis Biofilm Against Calcium Hydroxide ................................. 57
  1.6.1 Innate Resistance to High pH .............................................................................................. 57
  1.6.2 Starvation Resistance .......................................................................................................... 59
  1.6.3 Tubule invasion ..................................................................................................................... 59
  1.6.4 Biofilm .................................................................................................................................. 59
  1.6.5 Viable But Non-Culturable (VBNC) .................................................................................. 60
1.7 Justification for this Study ............................................................................................................... 61
1.8 Aim .................................................................................................................................................. 62
2. Methods and Materials
2.1 Test Organism
2.2 Dentine Disc Preparation
2.3 Dentine Disc Serial Transfer Biofilm Growth Model
2.4 pH Adjusted Buffer Solutions Used for Alkaline Test Agents
2.5 Planktonic Growth Curves
2.6 Flow Cell Biofilm Growth Model
2.7 Alkaline pH Exposure
2.8 Biofilm Examination and Measurement
  2.8.1 Viability Plating
  2.8.4 Scanning Electron Microscopy (SEM)
  2.8.3 Confocal Laser Microscopy (CLSM)
2.9 Statistical Analysis
3. Results
3.1 Dentine Discs
3.2 Dentine Disc Serial Transfer Biofilm Growth Model
3.3 Planktonic Growth Curves
3.4 Flow Cell Biofilm Growth Model
  3.4.1 Control Flow Cell- Biofilm Grown in Unbuffered THB
  3.4.2 Flow Cell 1- Rapid Increase in pH to 11.5
  3.4.3 Flow Cell 2- Slow Increase to pH 11.5
  3.4.4 Flow Cell 3- Rapid Increase in pH to 12.5
  3.4.5 Flow Cell 4- Slow Increase in pH to 12.5
3.5 Comparison of Bacterial Viabilities Between Different Flow Cell Protocols
  3.5.1 Comparison of Rapid versus Slow Increases in pH
  3.5.2 Comparison of Rapid versus Slow Increase to pH 12.5 when 3 Discs Were Used per Sample
  3.5.3 pH 11.5 vs pH 12.5
4. Discussion
  4.1 Dentine Disc Serial Transfer Biofilm Growth Model
  4.2 Flow Cell Biofilm Growth Model
  4.3 Bacterial Reduction
  4.4 Bacterial Survival
  4.5 Rapid versus Slow Increase in pH
  4.6 pH 11.5 vs pH 12.5
  4.7 SEM Imaging
  4.8 Confocal Laser Microscopy
  4.9 Limitations of Study
  4.10 Directions for Future Research
  4.11 Conclusion
Appendix
  Flow Cell Viability Counts and Protein Assays
Bibliography
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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Declared by:_______________ Witnessed by: ____________

Mark Stenhouse

Date: _______           Date: _______
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