

EVALUATION OF SALIVARY FLORA ACIDOGENICITY UNDER ACIDIC CONDITIONS FOR PREDICTION OF CARIOGENIC POTENTIAL DURING FIXED ORTHODONTIC TREATMENT

Submitted in partial fulfilment to the degree of Doctor of Clinical Dentistry
(Orthodontics)

By

Sara A. Roberts
B.D.S.



School of Dentistry
Faculty of Health Sciences
The University of Adelaide
South Australia
5005

2010

Table of Contents

Figures and Tables	5
SECTION 1	5
SECTION 2	5
Abstract	7
Declaration	9
Acknowledgements	10
SECTION 1	11
Orthodontics and caries risk	11
Introduction	11
Incidence	11
Brackets and bands	13
Cements	14
Plaque and bacterial numbers	15
Distribution of teeth affected	16
Post orthodontic treatment analysis	17
Multifactorial aetiology	18
Oral flora associated with dental caries	18
Diversity of micro-organisms involved in dental caries	19
Historical and current nomenclature of mutans streptococci	19
Mutans streptococci	20
Lactobacilli	22
Other cariogenic bacteria	23
Variation within individual microbial species	24
Mutans streptococci	25
Lactobacilli	26
Caries Prediction	27
Prediction studies	28
Predictors	28
Accuracy of caries prediction	29
Sensitivity and specificity in a clinical setting	30

Mutans streptococci and lactobacilli as caries predictors.....	31
Variation in prediction	34
Saliva Testing.....	35
Tests based on culturing and bacterial numbers	36
Dip-Slide test	36
Limitations	37
Colour Indicators	38
Limitations	38
Historical tests	39
Tests based on acid production	39
Colour indicators.....	40
Historical tests	40
Limitations	41
Other parameters measured in saliva tests	42
Conclusion	44
References.....	45
Statement of Purpose	61
Hypothesis	61
Aims.....	61
SECTION 2	62
Article 1	62
Salivary pH change as a predictor of white spot lesion development in patients undergoing orthodontic treatment	63
Abstract.....	65
Introduction	66
Materials and Methods.....	68
Results.....	70
Discussion	74
Conclusion	77
References	78
Article 2	82
Salivary analysis of patients undergoing fixed orthodontic treatment; characterization of cariogenic bacterial strains.....	83

Abstract.....	85
Introduction	86
Method and Materials	88
Results.....	90
Comparison among populations	90
Association between, salivary mutans levels, biomass, terminal pH and organic acid production.....	91
Discussion.....	93
Limitations.....	95
Conclusion	96
References.....	97
Summary.....	101
APPENDIX 1	103
Materials	103
TSY20B agar plates.....	103
Solutions	104
0.4M potassium phosphate buffer solution 5.7 pH-control	104
0.4M potassium phosphate buffer solution 5.7 pH plus 10% sucrose-test..	104
Tryptone-Soya Broth.....	104
APPENDIX 2	105
End-product acid analysis.....	105
APPENDIX 3	107
Information sheet for participants.....	107
APPENDIX 4	108
Contact information for independent complaints procedure given to participants	108
APPENDIX 5	109
Consent forms for participants and guardians of a participant.....	109

Figures and Tables

SECTION 1

Figures

1. Cell-surface and secreted virulence factors of *Streptococcus mutans* (Mitchell, 2003). 22
2. Typical follow-up for evaluating the predictive power of a dichotomous risk marker for caries (Hausen, 1997) 29

Tables

1. Sensitivity, Specificity, positive and negative predictive values for different types of studies with lactobacilli and/or mutans streptococci as caries-risk predictors (van Houte, 1993) 33

SECTION 2

ARTICLE 1

Tables

1. Initial pH change, risk category, and white spot lesion development in patients undergoing fixed orthodontic treatment. 71
2. Change in salivary pH for each subject over time. 72

ARTICLE 2

Tables

1. Results for high risk group, showing mutans streptococci CFU/ml, optical density of culture, terminal pH of broth, production of lactic and acetic acid from each strain of mutans streptococci. 91

2. Results for low risk group, showing mutans streptococci CFU/ml, optical density of culture, terminal pH of broth, production of lactic and acetic acid from each strain of mutans streptococci. 91

Abstract

Orthodontic treatment is a common occurrence with up to 29.7% of the adolescent population (Bollen, Cunha-Cruz *et al.*, 2007) and 1% of the adult population (Whitesides, Pajewski *et al.*, 2008) receiving fixed braces. This type of treatment poses significant risks to the hard and soft tissues. One of the most common complications of fixed orthodontic appliance treatment is the demineralization and subsequent white spot lesion development in the enamel (Travess, Roberts-Harry *et al.*, 2004). White spot lesions are the early sign of dental caries and the incidence of white spot lesions in orthodontic patients has been reported as being as high as 50 per cent (Gorelick, Geiger *et al.*, 1982; Lundström and Krasse, 1987; Lovrov, Hertrich *et al.*, 2007) with white spot lesions sometimes occurring as early as 1 month after banding (Ogaard, Rølla *et al.*, 1988).

Currently available chair-side saliva tests measure bacterial counts or acid production of the entire oral microflora. These tests tend to be able to predict patients who are at a low risk of demineralization more accurately than those at an increased risk. There is no single test to suit all individuals that can reliably identify at risk patients (Hausen, 1997; Reich, Lussi *et al.*, 1999; Zimmer, Bizhang *et al.*, 2008).

The aim of this short-term study was to evaluate a technique to predict white spot lesion development in patients undergoing fixed appliance orthodontic treatment, and to analyse salivary bacteria to determine any differences in their metabolism.

Fifty-two patients due to start fixed appliance orthodontic treatment agreed to participate in the study. Saliva samples collected before braces were placed

and during treatment at six-eight week intervals, were mixed with a potassium phosphate buffer solution containing sucrose (10% w/v), at pH 5.7, and rate of pH change was measured over 30 minutes. Demineralisation development was determined from standardized intra-oral photographs.

Subjects whose samples showed the greatest pH change towards acid production were selected for further salivary analysis. Ten of the higher risk individuals were further analysed, along with ten low risk individuals. Samples were grown on TSY20B plates, and pure strains of mutans streptococci were isolated and re-grown. These were then suspended in tryptone-soya broth and after 48 hours optical density, terminal pH and acid analysis using HPLC were measured and analysed.

Of the fifty-two participants, three developed demineralisation. Two were identified as high risk from their initial saliva test, one as low risk, giving the test a sensitivity of 67% and specificity of 94%. There was no statistically significant change over time in the subjects, which indicated the risk status is unlikely to change.

There was no statistically significant difference between the high and low risk groups in salivary microflora metabolism. The major acid produced in each case was lactic acid, with acetic acid being produced at lower concentrations.

This test has the potential to be developed into a commercial chair-side saliva test. However, further testing is continuing and aims to follow the cohort of patients through the entirety of their treatment.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Sara Roberts and, 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.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Dr Sara Roberts

Dated

Acknowledgements

I would like to thank the following people for their support during the past three years.

Professor W. J. Sampson, P.R. Begg Chair in Orthodontics, The University of Adelaide, for his time, advice and expert opinion.

Dr N.J. Gully, Senior Lecturer in Microbiology, The University of Adelaide, for his time, advice, laboratory assistance, guidance and expert opinion.

Associate Professor C. Dreyer, Senior Lecturer in Orthodontics, The University of Adelaide, for his advice and expert opinion.

Thomas Sullivan, Statistician, Data Management & Analysis Centre, Discipline of Public Health, University of Adelaide for statistical advice and analysis.

The ASO foundation for Research and Education, for financial support.

To my family for all their support and understanding over the past three years.