

# **Expression of rheumatoid arthritis markers in normal and inflamed gingival tissues**

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

Geoffrey Harvey



# Table of Contents

<b>Declaration .....</b>	<b>i</b>
<b>Acknowledgements .....</b>	<b>ii</b>
<b>Chapter 1. Literature Review .....</b>	<b>1</b>
1.1 Introduction.....	1
1.1.1 Periodontitis .....	1
1.1.2 Rheumatoid arthritis .....	2
1.2 Rheumatoid Arthritis markers .....	3
1.2.1 Rheumatoid Factor.....	3
1.2.2 Antibodies against citrullinated proteins .....	3
1.2.3 Peptidyl arginine deiminase.....	5
1.2.3.1 Peptidyl arginine deiminase type 2.....	5
1.2.3.2 Peptidyl arginine deiminase type 4.....	6
1.3 Rheumatoid arthritis and periodontitis .....	7
1.3.1 Studies examining prevalence/severity of periodontitis in RA patients .....	7
1.3.2 Studies examining prevalence/severity of RA in periodontitis patients .....	10
1.3.3 Effect of periodontal treatment on RA .....	10
1.3.4 Other links between periodontitis and RA.....	12
1.4 Periodontal pathogens and RA .....	13
1.4.1 Porphyromonas gingivalis .....	13
1.4.2 Aggregatibacter actinomycetemcomitans.....	15
1.4.3 Other oral bacteria .....	16
1.5 Drug treatment of RA and effects on periodontitis.....	16
1.5.1 Non-steroidal anti-inflammatory drugs .....	16
1.5.2 Disease-modifying anti-rheumatic drugs.....	19
1.5.3 Antibiotics.....	20
1.5.4 Cytokine blocking agents.....	21
1.6 Rheumatoid Arthritis markers in periodontitis patients.....	22
1.6.1 Rheumatoid Factor in serum of periodontitis patients.....	22
1.6.2 Local production of RF in periodontal tissues.....	23
1.6.3 Inflammatory markers in periodontitis and RA patients .....	23
1.6.4 Antibodies to citrullinated proteins in serum of periodontitis patients.....	24
1.6.5 Local production of anti-CCP in the periodontium .....	25
1.7 Conclusion .....	25
1.8 Hypothesis .....	26
1.9 Aims of the study .....	26
1.10 References.....	27
<b>Chapter 2. Expression of rheumatoid arthritis markers in normal and inflamed gingival tissues.....</b>	<b>40</b>
2.1 Introduction.....	40
2.2 Materials and methods .....	41
2.2.1 Patient selection .....	41
2.2.2 Gingival tissue collection and processing.....	42
2.2.3 Routine histological staining .....	42
2.2.4 Immunohistochemistry for RF, CCP, PAD2 and PAD4 .....	43
2.2.4.1 Staining procedures.....	43
2.2.4.1.1 Anti-Citrulline.....	43
2.2.4.1.2 Peptidylarginine deiminase 2.....	44
2.2.4.1.3 Peptidylarginine deiminase 4.....	44
2.2.4.1.4 Rheumatoid Factor.....	44

2.2.4.1.5 Histologic analysis .....	45
2.2.5 GCF collection and processing .....	45
2.2.6 Detection of anti-CCP antibodies and Rheumatoid Factor by ELISA in GCF ..	45
2.2.7 Statistical analysis .....	46
2.3 Results.....	46
2.3.1 Analysis of GCF for presence of RF and anti-CCP.....	52
2.4 Discussion.....	55
2.5 Future directions .....	59
2.6 Conclusion .....	60
2.7 References.....	61
<b>Appendix 1. Figures and Tables .....</b>	<b>67</b>

# Declaration

I, Geoffrey Harvey, 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.

I give consent to this copy of my thesis to be made available to the University Library, for loan or photocopying, subject to the provisions of the Copyright Act 1968, as well 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.

Declared by: \_\_\_\_\_

Geoffrey Harvey

Date: \_\_\_\_\_

Witnessed by: \_\_\_\_\_

Date: \_\_\_\_\_

# Acknowledgements

I would like to take this opportunity to thank a number of people, whose support and input was crucial in the completion of my research:

First and foremost, I would like to express my utmost gratitude to Professor Mark Bartold for devising the original concept for this research, and for being a constant source of wisdom, guidance and expert advice throughout the duration of my studies.

I would like to extend special thanks to Dr Kencana Dharmapatni and Dr Tracy Fitzsimmons, whose expertise, unwavering patience, and support in the laboratory was of vital importance to the success of this project. Both Kencana and Tracy also provided me with constructive feedback which was highly valuable in the compilation of this report. I would also like to thank Ms Ceilidh Marchant for her technical assistance with the Periotron 8000, and advice on immunohistochemistry.

I am grateful to Dr Ghafar Sarvestani, who provided me exceptional instruction in the use of the NanoZoomer for digitising the immunohistochemistry slides, and also to Mr Yen-Liang Liu, who assisted with statistical analysis.

Many thanks go to my colleagues, Mrs Kerry Page, Ms Kylie Jones, Dr Kere Kobayashi, Dr Emma Megson, Dr Tina Choo, Dr Alexandre Hermann du Bois, Dr Siobhan Gannon, Dr Chris Bates and Dr Raymond Chan. The assistance with sample collection was much appreciated, but the friendship and memories will last for many years to come.

Thanks must also go to Ms Catherine Offler for her assistance in the final editing and preparation of this manuscript.

This project was funded by a grant from the Australian Dental Research Foundation, and I am sincerely thankful for this financial assistance. I would also like to acknowledge the Royal Australian Army Dental Corps, for providing excellent support throughout my studies and my career to date.

Finally, I'd like to convey my greatest appreciation and thanks to my family, and to my beautiful partner, Brittny Roberts. The support, kindness and understanding shown in the last three years has been fantastic, and has been an ongoing source of inspiration for which I am extremely grateful.