AN INVESTIGATION INTO THE ROLE OF RANKL AND SCLEROSTIN IN DENTOALVEOLAR ANKYLOSIS

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Clinical Dentistry (Orthodontics)

Dr Shelley Coburn, BDS (University of Otago)

Orthodontic Unit
School of Dentistry
Faculty of Health Science
The University of Adelaide
South Australia
AUSTRALIA

June 2015

1 CONTENTS

1.1 Table of Contents

1.2 List of Figures

Literature Review:

Article 1:

Article 2:

1.3 List of Tables

Literature Review:

Article 1:

Article 2:

1.4 List of abbreviations

Ab	Antibody
ABC	Avidin-biotin complex
AEC	3-Amino-9-ethylcarbazole
Ag	Antigen
ALP	Alkaline phosphatase
ATP	Adenosine-5'-triphosphate
BMP	Bone morphogenetic protein
BMU	Basic metabolic unit
CSF	Colony stimulating factor
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
ERM	Epithelial rests of Malassez
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IL	Interleukin
M	Molar (molarity)
M-CSF	Macrophage colony-stimulating factor
MMP	Matrix metalloproteinases
*m*RNA	Messenger ribonucleic acid
OPG	Osteoprotegerin
OY	Osteocyte
PBS	Phosphate buffered solution
PDL	Periodontal ligament

PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related protein
RANK	Receptor activator of nuclear factor kappa-β
RANKL	Receptor activator of nuclear factor kappa-β ligand
RNA	Ribonucleic acid
SCL	Sclerostin
SOST	The gene encoding for sclerostin
TGF	Transforming growth factor
TNF	Tumour necrosis factor
TRAP	Tartrate-resistant acid phosphatase

Abbreviations of length

mm	Millimetre
μm	Micrometre

Abbreviations of volume

ml	Millilitre
µl	Microlitre

Abbreviations of weight

g	Gram
kg	Kilogram
mg	Milligram
ng	Nanogram

2 ACKNOWLEDGEMENTS

I wish to express my appreciation and gratitude to the following people for their invaluable assistance in the completion of this thesis.

Professor W.J. Sampson, Emeritus Professor and Visiting Research Fellow, The University of Adelaide, for his advice and guidance. His patience and enthusiasm for teaching and research is invaluable.

Professor C.W. Dreyer, P.R. Begg Chair of Orthodontics, The University of Adelaide, for his comprehensive knowledge of the subject matter and pragmatic advice.

Dr Kencana Dharmapatni, School of Medical Sciences, The University of Adelaide, for her expertise and assistance especially with the laboratory work. Without her dedicated input this project would never have been completed.

Ms Suzanne Edwards, Discipline of Public Health, The University of Adelaide, for her expert statistical advice.

Mr Jim Manavis, Laboratory Manager, Hanson Institute Centre for Neurological Diseases, for his assistance in using the Nanozoomer Digital Microscope.

And finally, to my parents, Marion and Derek. Thank you for raising me to believe I can accomplish whatever I set my mind to.

3 THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

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 Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

_____

Shelley Coburn

30th June 2015

4 ABSTRACT

Background: Dentoalveolar ankylosis may occur spontaneously or as a sequela to dental trauma and results in an area of bone fusing to the tooth (Kurol 1981). Infra-occlusion following dentoalveolar ankylosis results in a number of significant orthodontic sequelae. These include over-eruption of the opposing tooth, tipping of adjacent teeth towards the ankylosed tooth, a loss of arch length, space loss and a shift of the dental midline (Messer and Cline 1980; Andlaw 1974; Ponduri et al. 2009). Vertical alveolar growth may also be hindered (Kjaer et al. 2008). The understanding of the biological processes behind the formation and repair of the ankylotic lesion is incomplete. Following dental trauma, the periodontal ligament (PDL) may be the source of the cells that repopulate a tooth root defect that determine whether ankylosis occurs (Erausquin and Devoto 1970; Lin et al. 2000; Melcher 1970; Line et al. 1974). When the PDL space is repopulated by cells from a source outside the true PDL tissues (such as the alveolar bone) healing may occur by way of dentoalveolar ankylosis.

Research in the field of bone biology has recently focused on the role of the osteocyte. This cell, with its unique location embedded in bone, may have an essential role in bone metabolism. Osteocytes produce sclerostin, a protein that inhibits bone formation. There is also evidence that the osteocyte may be a major source of receptor activator of NF-κβ (RANKL) which is essential for osteoclastogenesis. This project aims to investigate the expression of RANKL and sclerostin in a rat model of dentoalveolar ankylosis induced by a hypothermal insult.

The null hypothesis is that an applied cold insult and subsequent ankylosis does not affect the expression of RANKL and sclerostin within the dentoalveolar complex.

Methods: Dentoalveolar ankylosis was induced in fifteen, eight week old, male, Sprague-Dawley rats (5 groups of 3 rats each) by application of dry ice to the upper right first molar tooth. An additional 3 rats served as untreated controls and the experimental rats were sacrificed at days 0, 4, 7, 14 and 28. Immunohistochemical detection of RANKL and sclerostin was performed and the number of RANKL and sclerostin positive and negative cells as well as the number of empty lacunae representing dead osteocytes were calculated and compared between groups.

Results: The cold insult resulted in dentoalveolar ankylosis, with the periodontal ligament (PDL) almost completely replaced by bone in the furcation region of the root 14 days after injury with regeneration of the PDL evident after 28 days. Resorption of the ankylotic bone and cementum was evident in the furcation region. There was also a statistically significant increase in the number of empty lacunae due to osteocyte death that coincides with the incidence of maximal ankylosis.

RANKL was detected in bone marrow stromal cells, osteoblasts and bone lining cells, osteoclasts, endothelial-like cells lining vessels, epithelial cells, odontoblasts and periodontal fibroblasts. However, clear staining in osteocytes was not evident. Epithelial rests of Malassez showed strong expression of RANKL.

When ankylosis was present, there was a statistically significant difference in sclerostin expression between the areas of bone closest to, and farthest away, from the furcation area. There was a non-statistically significant trend towards reduced sclerostin expression at days 7 and 14 followed by a slight increase in expression at day 28. The slight increase in sclerostin expression at day 28 may indicate the establishment of a healing response.

In considering these results, it should be noted that this experiment uses a model of ankylosis in which the ankylotic lesion develops following a thermal insult. The factors that initiate ankylosis in a clinical situation are incompletely understood and may differ from this model.

Conclusions: Whilst RANKL was not detected in osteocytes in this model of ankylosis there was strong expression of RANKL by ERM in the PDL and a significant change in sclerostin expression near the area of ankylosis. This may contribute evidence that RANKL, sclerostin and the osteocyte might have a role in influencing the regeneration of the PDL following dentoalveolar ankylosis.

The null hypothesis that an applied cold insult and subsequent ankylosis does not affect the expression of RANKL and sclerostin within the dentoalveolar complex is rejected.