



**THE UNIVERSITY
OF ADELAIDE
AUSTRALIA**

CLAST CELL ACTIVITY IN A MODEL OF ASEPTIC ROOT RESORPTION

**CRAIG WILLIAM DREYER
B.D.S.(Adel), M.D.S.(Adel), F.R.A.C.D.S.**

**A research report submitted in partial fulfilment for the
Degree of Doctor of Philosophy in Dentistry**

**DENTAL SCHOOL
FACULTY OF HEALTH SCIENCES
THE UNIVERSITY OF ADELAIDE**

2002

TABLE OF CONTENTS

	Page
TABLE OF CONTENTS	2
ABSTRACT	7
SUMMARY	9
RESEARCH PRESENTATIONS	13
SIGNED STATEMENT	14
ACKNOWLEDGEMENTS	15
ABBREVIATIONS	17
 CHAPTER 1 INTRODUCTION	 19
1.1 The periodontium	19
1.1.1 Periodontal fibres	20
1.1.1.1 Collagen	20
1.1.1.2 Oxytalan, elaunin and elastin fibres	22
1.1.2 Cells of the periodontal ligament	23
1.1.2.1 Synthetic cells	23
• The fibroblast	23
• The osteoblast	24
• The cementoblast	25
• Epithelial Cells	27
1.1.2.2 Destructive Cells	27
1.1.3 Cementum	28
1.1.4 Blood vessels	29
 1.2 The Osteoclast	 30
1.2.1 The origin of osteoclasts	30
1.2.2 Osteoclast morphology	33
1.2.3 Mechanisms of resorption	37
1.2.3.1 Osteoclast recruitment	39
1.2.3.2 Osteoclast attachment	40
1.2.3.3 Osteoclast polarization	42
1.2.3.4 Dissolution of bone material	43
1.2.3.5 Dissolution of organic matrix	44
1.2.3.6 Removal of degradation products	47
1.2.3.7 The fate of osteoclasts	48
1.2.4 Osteoprotegerin and the regulation of osteoclasts	49
1.2.4.1 Osteoprotegerin	51
1.2.5 Identification of osteoclasts	60
1.2.5.1 Osteoclast enzyme histochemistry ...	60
• Tartrate-resistant acid phosphatase	60
• Succinic dehydrogenase	64
• Carbonic anhydrase II	66
• Miscellaneous enzymes	67
1.2.5.2 Immunolabelling of osteoclasts	67

	• KP1	73
	• Cysteine proteinases (cathepsins)	75
	• The vitronectin receptor	75
	• ED1	77
	• CD45 - Leucocyte common antigen	79
	• CD13	81
	• CD15	81
1.3	Root resorption	82
1.3.1	The odontoclast	83
1.4	Models of root resorption	90
CHAPTER 2	AIMS	97
2.1	Justification	97
2.2	Aims of the study	101
2.3	Null hypothesis	102
CHAPTER 3	MATERIALS AND METHODS	103
3.1	Materials.....	103
3.2	Methods	104
3.2.1	Anaesthesia	104
3.2.2	Thermal insult	105
3.2.3	Sacrifice	107
3.2.3.1	Carotid perfusion	107
3.2.3.2	Cardiac perfusion	110
3.2.4	Fixation	111
3.2.5	Decalcification	112
3.2.6	Tissue processing	112
3.2.6.1	Histology and Immunocytochemistry	112
3.2.6.2	Transmission electron microscopy..	113
3.2.7	Tissue sectioning and staining	114
3.2.7.1	Resin embedded material	114
3.2.7.2	TRAP enzyme histochemistry	115
3.2.7.3	Immunolabelling of sections for light microscopy	118
3.2.8	Photography and image capture	122
3.3	Experimental studies	123
3.3.1	Experiment 1	123
3.3.2	Experiment 2	123

3.3.3	Experiment 3	124
3.3.4	Experiment 4	124
3.3.5	Experiment 5	124
3.3.6	Experiment 6	125
3.3.7	Experiment 7	125
3.3.8	Experiment 8	126
CHAPTER 4	RESULTS	127
4.1	Animals	127
4.2	Results of method trials	127
4.2.1	Anaesthesia	127
4.2.2	Pilot study of TRAP enzyme histochemistry	129
4.2.3	Antigen retrieval techniques	129
4.3	Results of experimental procedures	130
4.3.1	Experiment 1	130
4.3.1.1	Light microscopy	130
4.3.2	Experiment 2	140
4.3.2.1	Light microscopy	140
4.3.3	Experiment 3	140
4.3.3.1	Light microscopy	140
4.3.4	Experiment 4	143
4.3.4.1	Light microscopy	143
4.3.4.2	TRAP enzyme histochemistry	144
4.3.4.3	Immunolabelling	152
	• KP1 antibody	152
	• ED1 antibody	157
	• Anti-CD45 antibody (Anti- leucocyte common antigen)	170
	• Anti-CD13 and anti-CD15 antibodies	172
	• Anti-cathepsin L, anti-CD61 and anti-carbonic anhydrase II antibodies	172
	• AE1/AE3 antibody	172
4.3.4.4	Transmission electron microscopy..	176
	• Bone surface	191
	• Bone marrow	197
	• Ankylosis	197
	• Pulp	197
4.3.5	Experiment 5	201
4.3.5.1	Light microscopy	201
4.3.5.2	TRAP enzyme histochemistry	201
4.3.5.3	Immunolabelling	202
	• ED1 antibody	202
4.3.5.4	Transmission electron microscopy..	202

4.3.6	Experiment 6	204
4.3.6.1	Light microscopy	204
4.3.6.2	TRAP enzyme histochemistry	204
4.3.6.3	Immunolabelling	205
	• ED1 antibody	205
4.3.7	Experiment 7	205
4.3.7.1	Light microscopy	206
4.3.8	Experiment 8	210
4.3.8.1	Light microscopy	210
4.3.8.2	TRAP enzyme histochemistry	217
4.3.8.3	Immunolabelling	223
	• ED1 antibody	223
4.3.8.4	Transmission electron microscopy..	230
	• Bone marrow	231
	• Pulp	231
	• Bone surface	245
	• Ankylosis	245
CHAPTER 5	DISCUSSION	251
5.1	Anaesthesia	251
5.2	The frozen tooth model	252
5.2.1	Usefulness of model	252
5.2.2	Cryotherapy	254
5.2.3	Thermal conductivity and radial effects	256
5.2.4	Effects of different experimental protocols on the resorption model	259
5.2.5	Simulation of orthodontic tooth movement and subsequent root resorption	261
5.3	The chronology of PDL change	264
5.4	Ankylosis	267
5.5	The identification of clast cells	270
5.5.1	Microscopy	270
5.5.1.1	Light microscopy	270
5.5.1.2	Transmission electron microscopy..	271
5.5.2	Enzyme histochemistry	273
5.5.2.1	Succinic dehydrogenase	273
5.5.2.2	TRAP labelling	275
5.5.3	Immunolabelling studies	280
5.5.3.1	Effects of tissue processing on antigenicity	281
	• Fixation	281
	• Decalcification	284
	• Tissue processing	285
5.5.3.2	The avidin-biotin-peroxidase complex	286

5.5.3.3	KP1 antibody	287
5.5.3.4	Anti-CD13 antibody	288
5.5.3.5	Anti-CD15 antibody	289
5.5.3.6	Anti-cathepsin L, anti-CD61, and anti-carbonic anhydrase II antibodies	290
5.5.3.7	ED1 antibody	291
5.5.4	Are osteoclasts and odontoclasts the same cells?	297
5.6	Osteoprotegerin	305
5.6.1	Tumour necrosis factor	309
5.6.2	Role of RANKL	312
5.6.3	Penetration of OPG	313
5.7	Future research directions	315
CHAPTER 6	CONCLUSIONS	317
CHAPTER 7	APPENDICES	321
CHAPTER 8	BIBLIOGRAPHY	355

ABSTRACT

Bone resorption by osteoclasts facilitates orthodontic tooth movement. However, an adverse sequela of tooth movement is the possibility of inflammatory root resorption. In order to examine aseptic hard tissue resorption an investigative rat model was developed using *in situ* freezing of the upper right molar by the application of solid carbon dioxide to the tooth crown. Variations in duration and frequency of application permitted the investigation of the cellular reactions responsible for the progression and repair of resorption and periodontal healing using tartrate-resistant acid phosphatase and succinic dehydrogenase enzyme histochemistry and a concert of antibodies directed at specific osteoclast antigens in control and osteoprotegerin-affected animals. In addition, an electron microscopic investigation was undertaken to assess variations in the morphological features of the resorptive cells in the experimental model systems.

The development of the model revealed a pattern of root resorption dissimilar to previously reported patterns caused by orthodontic means. Hard tissue resorption was generated by short-term freezing of molar crowns while ankylosis was a feature of an intense, prolonged insult as well as in all osteoprotegerin-affected rats. TRAP activity was found to be to be a useful clast cell marker but not in areas of ankylosis where ED1 (a macrophage lysosomal marker) proved to be more valuable. ED1 label indicated that macrophages or, at least, clast cell precursors are more involved in hard tissue resorption than previously considered, suggesting that odontoclasts are likely derived from the macrophage lineage of clast cell differentiation.

While osteoprotegerin inhibited osteoclastogenesis and resorptive activity in the skeletal bones and periodontium of unfrozen control molars in rats, it did not appear to inhibit ultrastructural signs of resorption in the frozen rat molars. In addition, TRAP activity was present but diminished while ED1 activity was still evident in the resorptive areas. This study concluded that osteoclasts and odontoclasts, while appearing to be similar cells, are possibly not the only cells responsible for hard tissue resorption. Furthermore, the observation that osteoprotegerin failed to prevent inflammatory resorption in the frozen tooth model suggests that there are possibly other extraclastic mechanisms or factors in this experimental model that influence resorption. Hence, the usefulness of OPG as a potential inhibitor of hard tissue resorption and, in particular, orthodontic root resorption remains to be established.