OSTEOCLAST-ASSOCIATED INTRACELLULAR ITAM SIGNALLING MOLECULES IN HUMAN PERI-IMPLANT OSTEOLYSIS AND RHEUMATOID ARTHRITIS

Ekram Alias

BSc. (Biomedical Sc.), BHSc. (Hons.)

DISCIPLINE OF ANATOMY AND PATHOLOGY

SCHOOL OF MEDICAL SCIENCES

Thesis submitted for the Doctor of Philosophy in Medicine

in The School of Medical Sciences at The University of Adelaide

November 2013

TABLE OF CONTENTS

TABLE OF CONTENTS ..... **i**

ABSTRACT ..... **vi**

STATEMENT OF ACCESS ..... **viii**

DECLARATION OF ORIGINALITY ..... **ix**

ACKNOWLEDGEMENT ..... **x**

PUBLICATIONS ..... **xii**

SCIENTIFIC COMMUNICATIONS ..... **xiii**

ABBREVIATIONS ..... **xvi**

LIST OF FIGURES ..... **xix**

LIST OF TABLES ..... **xxii**

1. LITERATURE REVIEW **1**

1.1. Bone Remodeling **2**

1.2. Inflammation-Associated Bone Diseases **3**

1.2.1. Peri-implant Osteolysis 4

1.2.2. Rheumatoid Arthritis 6

1.2.3. Osteoarthitis 11

1.3. Osteoclasts- The Bone Resorbing Cells **13**

1.3.1. Differentiation and Maturation of Osteoclasts 14

1.3.1.1. Recruitment and Targeting of Osteoclasts 15

1.3.1.2. Multinucleation 17

1.3.1.3. Osteoclast-associated Cell Markers 18

1.3.2. Mechanism of Bone Resorption 19

1.3.3. Osteoclasts in Pathology of Inflammation-Mediated Bone Loss 22

1.3.4. Regulation on Osteoclastogenesis and Bone Resorbing Activity by Cytokines 25

1.3.4.1. Tumor Necrosis Factor-Alpha (TNFα) 27

1.3.4.2. Interleukin-1 (IL-1) 29

1.3.4.3. Macrophage-Colony Stimulating Factor (M-CSF) 31

1.3.5. Molecular Regulation and Intracellular Signalling in Osteoclastogenesis 32

1.3.5.1. RANK/RANKL-dependent Pathway in Osteoblast/Osteoclasts Interaction 32
1.3.5.1.1. RANKL 33
1.3.5.1.2. Receptor Activator of NF-κB (RANK) 34
1.3.5.1.3. Osteoprotegerin (OPG) 35
1.3.5.1.4. OPG/RANKL/RANK System 36
1.3.5.1.5. Intracellular Signalling Post-RANK Activation 37
1.3.5.1.6. NFATc1- The Key Transcriptional Factor in Osteoclastogenesis 38
1.3.5.1.7. Pathological Relevance of OPG/RANKL/RANK System 41
1.3.5.2. ITAM-mediated Signalling Pathway 44
1.3.5.2.1. TREM2 and DAP12 49
1.3.5.2.2. OSCAR and FcRγ 54
1.4. Study Outline 64
1.4.1. Hypotheses 64
1.4.2. General Aims 65
1.4.2.1. Aim 1 65
1.4.2.2. Aim 2 65
1.4.2.3. Aim 3 65
1.4.2.4. Aim 4 65
1.4.3. Significance of the Study 66
2. EXPRESSION OF NFATc1, OSTEOCLAST-RELATED ITAM-RELATED MOLECULES AND MARKERS IN HUMAN IN TISSUES ADJACENT TO PERI-IMPLANT OSTEOLYSIS 69
2.1. Introduction 70
2.1.1. Hypothesis 73
2.1.2. Aims 73
2.2. Methods 74
2.2.1. Subjects 74
2.2.2. Immunohistochemistry and Reagents 75
2.2.2.1. Antibodies and Reagents 77
2.2.2.2. TRAP Staining and Serial Labeling of Osteoclast Cell Markers 78
2.2.2.3. Scoring of Immunostaining Results 79
2.2.3. Quantitative Real Time Reverse Transcription Polymerase Chain Reaction 79
2.2.3.1. RNA Extraction from Frozen Tissues and Spectrophotometry 79
2.2.3.2. Reverse Transcription 81
2.2.3.3. Real Time Polymerase Chain Reaction 82
2.2.4. Statistical Analysis 83
2.3. Results 84

2.3.1. Histological Features of Peri-Implant Osteolysis and Osteoarthritis Tissues 84
2.3.2. Expression of NFATc1 and osteoclast ITAM-associated molecules in peri-implant osteolysis tissues in comparison to osteoarthritis tissues 86
2.3.2.1. NFATc1 86
2.3.2.2. TREM2 and DAP12 89
2.3.2.3. OSCAR and FcRγ 92
2.3.3. Detection of osteoclast-cell lineage expressing TREM2 and OSCAR in peri-prosthetic tissues with TRAP and cathepsin K 95
2.3.4. Detection of polyethylene particles within immunostained multinucleated cells 98
2.3.5. mRNA expression of osteoclast ITAM-associated molecules in peri-implant osteolysis and osteoarthritic tissues 101
2.4. Discussions 103
2.5. Conclusions 115
3. EFFECT OF POLYETHYLENE ON THE EXPRESSION OF ITAM-RELATED MOLECULES IN OSTEOCLASTS *IN VITRO* 117
3.1. Introduction 118
3.1.1. Hypothesis 122
3.1.2. Aims 122
3.2. Methods 123
3.2.1. Preparation of PE Particles 123
3.2.2. Cell Culture 123
3.2.3. TRAP Staining 124
3.2.4. Dentine Pit Resorption Assay 125
3.2.5. qRT- PCR 125
3.2.5.1. RNA Extraction 126
3.2.5.2. Reverse-Transcription 126
3.2.5.3. Real Time PCR 126
3.3. Results 126
3.3.1. Effect of PE particles exposure on osteoclast formation 127
3.3.2. Effect of PE particles exposure on the osteoclast resorption activity 133
3.3.3. Effect PE particles on gene expression of osteoclast ITAM-related molecules in PBMC-derived osteoclasts 139
3.4. Discussions 146
3.5. Conclusions 159
4. DETECTION OF NFATc1 AND OSTEOCLAST ITAM-RELATED MOLECULES IN HUMAN RHEUMATOID ARTHRITIS 163

4.1. Introduction ... 164
4.1.1. Hypothesis ... 166
4.1.2. Aims ... 166
4.2. Methods ... 166
4.2.1. Subjects ... 166
4.2.2. Immunohistochemistry ... 169
4.2.2.1. Antibodies and Reagents ... 169
4.2.2.2. Scoring of tissue immunostaining ... 170
4.2.3. ELISA of OSCAR in Synovial Fluids from RA and OA Patients ... 171
4.2.4. Statistical Analysis ... 172
4.3. Results ... 172
4.3.1. Immunostaining of cathepsin K ... 173
4.3.2. Expression of NFATc1 by immunohistochemistry in RA and control tissues ... 177
4.3.3. Expression of ITAM-related molecules in RA synovial tissues ... 179
4.3.3.1. TREM2 and DAP12 ... 179
4.3.3.2. OSCAR and FcRγ ... 184
4.3.3.3. Immunostaining of OSCAR and TREM2 Associated with Vasculature ... 189
4.3.3.3.1. TREM2 ... 191
4.3.3.3.2. OSCAR ... 193
4.3.4. Level of soluble OSCAR levels in synovial fluids of RA and OA ... 195
4.4. Discusssion ... 197
4.5. Conclusion ... 207
5. REGULATION OF OSCAR EXPRESSION IN ENDOTHELIAL CELLS ... 211
5.1. Introduction ... 212
5.1.1. Hypothesis ... 214
5.1.2. Aims ... 215
5.2. Methods ... 215
5.2.1. Cell Culture ... 215
5.2.2. Real Time qRT-PCR ... 216
5.2.2.1. RNA Isolation and Spectrophotometry ... 217
5.2.2.2. Real Time Reverse Transcription ... 217
5.2.2.3. Polymerase Chain Reaction (PCR) ... 217
5.2.3. ELISA of OSCAR and OPG in Cell Culture Supernatant ... 217
5.2.4. Immunofluoresence on Monolayer Cell Culture ... 218
5.2.5. Statistical Analysis ... 219
5.3. Results ... 219

5.3.1. OSCAR and OPG mRNA Expression in HUVECs 219
5.3.2. Stimulation of OSCAR and OPG mRNA expression in BMEC by cytokines TNFα and IL-1ß 223
5.3.3. Expression of soluble OSCAR following TNFα and IL-1β treatment 231
5.3.4. Detection of OSCAR expression in BMEC *in situ* 233
5.4. Discussions 235
5.5. Conclusion 241
6. SUMMARY AND CONCLUDING REMARKS 242
REFERENCE LIST 247
APPENDICES 303

ABSTRACT

Peri-implant osteolysis (PO) and rheumatoid arthritis (RA) are examples of local inflammation-mediated bone loss, in which osteoclasts are believed to mediate the osteolysis. Besides the well-established OPG/RANK/RANKL system, ITAM-mediated signalling pathway has been found to be the co-stimulatory intracellular pathway mediating osteoclast differentiation and activity. TREM2, DAP12, FcRγ and OSCAR are components of the ITAM-mediated signalling pathway identified in osteoclasts. Another important molecule in the osteoclasts regulation is NFATc1, the key transcriptional factor mediating osteoclastogesis. Despite their known importance in the regulation of osteoclast and bone resorption, little is known if there any alteration in the expression of these molecules could be associated with the progression of bone loss in PO and RA.

In relation to study in context of PO, the expression of ITAM-related molecules, TREM2, DAP12, OSCAR and FcRγ, along with NFATc1 and osteoclast cell marker cathepsin K in PO tissues in comparison to OA tissues was examined at protein level through immunohistochemistry as well as at mRNA level using qRT-PCR. The effects of PE particles, a common PO-induced wear particles, on osteoclast formation and resorption activity as well as mRNA expression of NFATc1 and ITAM-associated molecules were studied *in vitro* using a novel collagen gel PBMC assay. As for studies on RA, the expression of all those molecules in RA (active and inactive) tissues was compared to OA and normal tissues. The levels of soluble OSCAR in synovial fluids from RA and OA patients was also measured through ELISA and compared. Following observation on immunostaining of RA tissues, the regulation on the expression of OSCAR in endothelial cells following TNFα and IL-1β stimulation was studied in BMEC culture *in vitro*. OSCAR protein expression was analysed through immunofluoresence and ELISA on the cell culture supernatants meanwhile mRNA level was measured using qRT-PCR.

Higher level of protein and mRNA for all those ITAM-associated molecules and cathepsin K was found in PO compared to OA tissues. Closer examination on tissue immunostaining found presence of PE particles inside and close to some cells positive for ITAM-related

molecules. Investigation on the effect of PE in culture of PBMC-derived osteoclast cells found that the particles promote more osteoclasts formed and higher resoprtion activity. The PE particles also appeared to stimulate the mRNA expression of cathepsin K and all ITAM-associated molecules studied. Examination on the immunostaining indicated that highest number of cells positive for NFATc1, TREM2, DAP12, OSCAR and FcRγ in active RA tissues compared to inactive RA, OA and normal tissues. High concentration of soluble OSCAR was found in synovial fluids of both RA and OA groups. Study on the expression OSCAR in BMEC demonstrated that TNFα and IL-1β could upregulate the expression of mRNA and protein in secreted form.

In general the expression of NFATc1, TREM2, DAP12, OSCAR and FcRγ was found high in PO and RA. Induction in expression of ITAM-associated molecules by PE particles and stimulation of OSCAR expression in endothelial cells by pro-inflammatory cytokines may suggest that these molecules may have role in the progression of PO and OA.

STATEMENT OF ACCESS

I, undersigned, the author of this thesis, understand that the University of Adelaide will make it available for use within the University library. All users consulting this thesis will have to sign the following.

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author, and make proper written acknowledgement for any assistance I have obtained for it."

Beyond this, I do not wish to place any restriction on access to this thesis.

Ekram Alias

DECLARATION OF ORIGINALITY

I certify that 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 in my name and, to the best of my knowledge and belief, contains no material 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 will, in the future, be used in a submission in my name, for any other degree or diploma in any university 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 catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Ekram Alias

ACKNOWLEDGEMENT

This study was supported by the Adelaide Fee Scholarship International (AFSI) from The University of Adelaide, Public Institutions of Higher Leaning Academic Training Scheme (SLAI) from Ministry of Higher Education Malaysia and The National University of Malaysia. Funding for the experimental work of this study was obtained through research grants from the National Health and Medical Research Council (NHMRC). I would also like to express gratitude to my current employer, The National University of Malaysia for granting me a leave for pursuing study at the PhD level.

My highest appreciation to my principal supervisor, Associate Professor Dr. David R. Haynes for being a wonderful and caring supervisor, as well as for all the continuing guidance and advices that I need throughout the PhD candidature.

I would like to express my special acknowledgement to my co-supervisors Dr. Tania Narelle Crotti and Dr. Kencana Dharmapatni, who have been very supportive supervisors. Their opinions, ideas and helps as well as expertise have been great assets towards my completion of project and PhD. I would like to acknowledge them for their contribution in efforts and precious time spent for consultations, from SQA scoring, statistics to guidance in the writing of the thesis right until the end. Their constant concern on my life has inspired and motivated me as well as made the long journey for PhD wonderful, sweet and memorable.

I would like to acknowledge Christopher A. Holding for his work and some of the data presented in Chapter 3 of this thesis (as indicated in some particular sections in the chapter). As part of this PhD project, the work has been extended to analysis on mRNA expression as well as on the images and earlier data provided by him. I would also like to extend my gratitude to him for all the helps, teachings and being a great technical “guru” even before the commencement of my PhD project.

I would like to thank Professor Malcolm D. Smith from Rheumatology unit in the Repatriation Hospital in Daw Park, SA for supplying his precious sample collection of tissues and synovial fluids from patients.

Many thanks go to Helen Weedon for her valuable assistance in preparing tissue sections, synovial fluids and RNA samples as well as for sharing information with us.

I owe a big credit to Professor Andrew C. W. Zannetino for his very supportive collaboration and brilliant ideas. His enthusiasm towards research has taught me a lot of lessons in my life.

My high gratitude to Jenny Drew from SA Pathology for giving a lot of helps and support, particularly for the *in vitro* work on endothelial cells presented in Chapter 5. Hope she enjoys her retirement days.

A credit to Dr. Susan Neale in the Discipline of Orthopaedic and Trauma, The University of Adelaide, for arranging the tissue collection used and presented in Chapter 2 together with the corresponding patient information. I would also like to acknowledge Ali Shah for his summer vacation scholarship 2006/07 work on testing several OSCAR antibody, which has given some insights towards the project.

I am very grateful to Dr. Ghafar Sarvestani and his colleagues from Detmold Imaging Facility in SA Pathology, for the assistance on Nanozoomer Digital Pathology facility used for capturing images for the immunohistochemistry work presented in thesis.

Also thank to Dale Caville from The Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, for his assistance with photography and time you spent for printing my diagrams. My acknowledgement also goes to Tavik Morgestern for helping me in compiling the thesis together.

Many thanks to my colleagues and peers, especially Melissa Cantley, Syahrul Fitri Zawawi and Arshad Sidek for their continuing morale support and help as well as for the friendship. I did enjoy the fun we had together. A big thank also for the other member of School Medical Sciences for the continuing support since the first day I came to the school.

Lastly, I would like to specially dedicate my success in completing the PhD project and thesis to my beloved wife, Haniza Hassan who has been beside me and encouraging me during those hard days and to my inspiring daughter, Iffah. Also to my beloved parents, siblings and in-law family for their continuing prayer, support and the long wait for my success during my 8-years stay in this wonderful city Adelaide, Australia.

PUBLICATIONS

Alias E*, Dharmapatni ASSK*, Holding AC, Atkins GJ, Findlay DM, Howie DW, Crotti TN, Haynes DR. Polyethylene particles stimulate expression of ITAM-related molecules in peri-implant tissues and when stimulating osteoclastogenesis in vitro. Acta Biomater 2012; 8:3104-12

Crotti TN, Dharmapatni ASSK*, **Alias E***, Zannettino ACW, Smith MD, Haynes DR. The immunoreceptor tyrosine-based activation motif (ITAM)-related factors are increased in synovial tissue and vasculature of rheumatoid arthritic joints. Arthritis Research and Therapy 2012; 14:R245

* These authors contributed equally to this work

SCIENTIFIC COMMUNICATIONS

Alias E[*], Dharmapatni K, Smith MD, Weedon H, Crotti TN, Haynes DR. **ELEVATED NUMBER OF CELLS EXPRESSING NFATc1 IN THE HUMAN ACTIVE RHEUMATOID ARTHRITIS TISSUES**. *Australia and New Zealand Orthopaedics Research Society (ANZORS), Adelaide, Australia 2009*
(Oral Presentation)

Alias E, Dharmapatni AASK, Smith MD, Weedon H, Crotti TN, Haynes DR[*]. **THE EXPRESSION OF NFATc1 AND OSCAR IN THE SYNOVIAL TISSUE OF PATIENTS WITH RHEUMATOID ARTHRITIS**. *Australian Rheumatology Association SA Branch Meeting, Adelaide, Australia 2009*
(Oral Presentation)

Alias E[*], Dharmapatni K, Smith MD, Weedon H, Crotti TN, Haynes DR. **THE EXPRESSION OF NFATc1 AND ITAM-RELATED MOLECULES IN THE SYNOVIAL TISSUE OF PATIENTS WITH RHEUMATOID ARTHRITIS**. *Australian Rheumatology Association (ARA), Melbourne, Australia 2010*
(Oral Presentation)

Alias E[*], Dharmapatni AASSK, Neale SD, Crotti TN and Haynes DR. **HIGHER EXPRESSION OF ITAM-RELATED OSTEOCLASTOGENESIS CO-STIMULATORY FACTORS IN PERI-IMPLANT OSTEOLYSIS TISSUES IN COMPARISON TO OSTEOARTHRITIS TISSUES**. *Australian and New Zealand Bone Mineral Society (ANZBMS), Adelaide, Australia 2010*
(Poster Presentation)

Crotti TN[*], **Alias E**, Dharmapatni AASK, Weedon H, Smith MD, Haynes DR. **THE INCREASED EXPRESSION OF ITAM FACTORS IN ACTIVE RA MAY BE INVOLVED IN MODULATING THE PATHOPHYSIOLOGICAL PROCESS OF**

RHEUMATOID ARTHRITIS. *ANZORS-Australian Health and Medical Research Congress (AHMRC), Melbourne, Australia 2010*
(Poster Presentation)

Haynes DR*, Alias E, Dharmapatni ASSK, Holding CA, Shah A, Howie DW, Findlay DM, Crotti TN. **ELEVATED EXPRESSION OF OSCAR, TREM-2 AND THEIR ADAPTOR MOLECULES MOLECULES IN HUMAN PERI-IMPLANT TISSUES ADJACENT TO FOCAL BONE RESORPTION**. 7th Combined Meeting of the Orthopaedics Research Societies, *Kyoto, Japan 2010*
(Oral Presentation)

Alias E*, Holding CA, Dharmapatni ASSK, Neale SD, Crotti TN and Haynes DR. **HIGHER EXPRESSION OF OSTEOCLAST ITAM-RELATED MOLECULES IS ASSOCIATED WITH HUMAN POLYETHYLENE (PE)-INDUCED PERI-PROSTHETIC OSTEOLYSIS**. *Australia and New Zealand Orthopaedics Research Society, Brisbane, Australia 2011*
(Poster Presentation)

Crotti TN*, Alias E, Dharmapatni AASK, Weedon HM, Zannettino A, Smith MD, Haynes DR. **EVIDENCE THAT ITAM RELATED FACTORS IN SYNOVIAL TISSUE AND VASCULATURE OF ACTIVE RHEUMATOID ARTHRITIS MAY BE INVOLVED IN MODULATING LOCAL BONE EROSION**. *American Society for Bone and Mineral Research, San Diego, US 2011*.
(Poster Presentation)

Dharmapatni K, Findlay DM*, **Alias E**, Holding CA, Atkins GJ, Howie DW Crotti TN, Haynes DR. **POLYETHYLENE PARTICLES STIMULATE OSTEOCLASTS IN VIVO AND IN VITRO IN ASSOCIATION WITH ENHANCED EXPRESSION OF ITAM-RELATED MOLECULES**. 39th Annual Congress of the European-Calcified-Tissue-Society (ECTS), *Stockholm, Sweden 2012*
(Poster Presentation)

Crotti TN*, **Alias E**, Dharmapatni AASK, Zannettino ACW, Howie DW, Haynes DR. **EXPRESSION OF OSCAR BY HUMAN ENDOTHELIAL CELLS FOLLOWING**

INDUCTION BY PRO-INFLAMMATORY CYTOKINES. 8th Clare Valley Bone Meeting, *South Autralia, Australia 2012*
(Poster Presentation)

Crotti TN*, Dharmapatni K, **Alias E**, Weedon H, Zannettino A, Smith MD. **EVIDENCE THAT VASCULAR EXPRESSION OF THE ITAM RECEPTOR, OSCAR, IN ACTIVE RHEUMATOID ARTHRITIS IS MODULATED BY INFLAMMATORY MEDIATORS**. *Australian Rheumatology Association, Brisbane, Australia 2012*
(Poster Presentation)

* All scientific communications were presented by the indicated (*) authors

ABBREVIATIONS

ACPA	anticitrullinated protein antibodies
ACR	American College of Rheumatology
AEC	3-amino-9-ethylcarbzole
AP1	activator protein-1
APTS	aminopropyltriethoxy-silane
Atpv0d2	ATPase V_0 domain
BMD	bone mass density
BMEC	bone marrow endothelial cell line
BMMs	bone marrow-derived monocyte/macrophage cells
BMUs	basic multicellular units
BSA	bovine serum albumin
Ca^{2+}	calcium
CCR	C-C chemokine receptor
cDNA	complementary deoxyribonucleic acid
ChIP	chromatin immunoprecipitation
CO_2	carbon dioxide gas
COCr	cobalt chromium
cpTi	commercially pure titanium
CRP	C-reactive protein
CsA	cyclosporin A
C_T	comparative threshold
CTR	calcitonin receptor
CVD	cardiovascular disease
DAP12	DNAx-protein 12kDa
DAPI	4', 6-Diamidino-2-phenylindole
DC-STAMP	dendritic-cell transmembrane protein
DEPC	diethylpyrocarbonate
DMARD	disease modifying antirheumatic drug
DNA	deoxyribonucleic acid
DPX	dibutyl phthalate xylene
DTT	dithiothreitol
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	endothelial cell growth factor
ELISA	enzyme-linked immunoabsorbant assay
ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FBGCs	foreign-body giant cells
FBLC	fibroblast-like cell
FBS	fetal bovine serum
FcRγ	Fc receptor common gamma-subunit chain
FCS	fetal calf serum
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase

GM-CSF	granulocyte-macrophage colony-stimulating factor
H&E	hemotoxylin eosin
hARP	human acidic ribosomal protein
HBSS	Hank's balanced salt solution
HMVEC	human microvascular endothelial cells
HRP	horse radish peroxidase
HUVECs	human umbilical vein endothelial cells
ICAM	intracellular adhesion molecules
IgG	immunoglobulin G
IL	interleukin
IL-1R	IL-1 receptor
IL-1ra	IL-1 receptor antagonist
IP_3	inositol triphosphate
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
LDL	low density lipoprotein
LPS	lipopolysaccharide
M-CSF	macrophage colony stimulating factor
mAb	monoclonal antibody
MCP-1	monocyte chemoattractant protein-1
MDL-1	myeloid DAP12-associated lectin-1
mg	milligram
MIP-1	macrophage inflammatory protein 1
MIP-1α	macrophage inhibitory factor-1α
MIP1γ	macrophage inflammatory protein 1-gamma
MITF	microphthalmia transcription factor
ml	milliliter
mm	milimeter
MMPs	matrix metalloproteinases
MNC	multinucleated cell
mRNA	messenger ribosomal nucleic acid
MTX	methotrexate
NFATc1	nuclear factor activated T-cell 1
NFκB	nuclear factor-kappa-B
ng	nanogram
NK	natural killer
NO	nitric oxide
NRS	normal rabbit serum
NSAIDs	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
OCT	Optimal Cutting Temperature medium
OPG	osteoprotogerin
OPG-Fc	OPG-fusion protein
ORO	Oil Red O
OSCAR	osteoclasts-associated receptor
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PE	polyethylene
PECAM-1	platelet-endothelial cell adhesion molecule 1

PGE_2	prostaglandin E_2
PIAS3	protein inhibitor of activated STAT 3
PIR-A	paired Ig receptor-A
PLCγ	phospholipase Cγ
PLOSL	polycystic lipomembranous osteodysplasia with sclerosis leukoencephalopathy
PMMA	polymethylmethacrylate
PO	peri-implant osteolysis
PP	peri-prosthetic
qRT-PCR	quantitive reverse-transcription polymerase chain reaction
RA	rheumatoid arthritis
RANK	receptor activator of NF kappa B
RANK-Fc	RANK fusion protein
RANKL	receptor activator of NF kappa B ligand
RANTES	regulated upon activation, normal T cell expressed and secreted
RF	rheumatoid factor
RGD	tripeptide arginine-glycine-aspartic acid
RNA	ribosomal nucleic acid
rpm	rotations per minute
RT	reverse-transcription
SDF-1	stromal-cell derived factor-1
SEM	standard error of mean
SH2	Src homology 2
SIRPβ	signal regulatory protein β
sOSCAR	soluble/ secreted form of OSCAR
SQA	semiquantitaive analysis
sRANKL	soluble RANKL
TACE	TNF-α converting enzyme
TMB	3, 3', 5, 5'-tetramethylbenzidine
TNF	tumor necrosis factor
TNFR	TNF receptor
TNFα	tumor necrosis factor-α
TRAF	TNF receptor activating factor
TRAP	tartrate-resistance acid phosphotase
TREM2	triggering receptor expressed by myeloid cells-2
USFs	upstream stimulating factors
V-ATPase	vacuolar (H^+) ATPase
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
ΔC_T	delta/difference in the comparative threshold

LIST OF FIGURES

Figure 1.1: Main stages in osteoclast differentiation.....15
Figure 1.2: Transportation of bone resorbing agents through a trans-Golgi trafficking network in osteoclasts and release across the ruffle border via exocytosis.....21
Figure 1.3: Overview on the interaction between pre-osteoclasts and osteoblasts, involving molecules like RANKL and OPG, which regulate the osteoclastogenesis process.....36
Figure 1.4: ITAM signalling plays role as costimulatory pathway involved in osteoclast differentiation.....47
Figure 1.5: Polypeptide structure and domains of human OSCAR structure and domains.....55
Figure 1.6: Positive feedback loop between NFATc1 and OSCAR expression and activity...59
Figure 1.7: Binding sites for transcription factors to bind on human OSCAR gene.....60
Figure 2.1: Representative histology of tissues from PO and OA groups following H&E staining.....85
Figure 2.2: NFATc1 expression in human peri-implant osteolysis and osteoarthritis tissues..88
Figure 2.3: TREM2 expression in human peri-implant osteolysis and osteoarthritis tissues...90
Figure 2.4: Representative pictures of DAP12 immunostaining in human peri-prosthetic and osteoarthritis tissues studied.....91
Figure 2.5: OSCAR expression in human peri-prosthetic and osteoarthritis tissues.....93
Figure 2.6: FcRγ expression in human peri-implant osteolysis and osteoarthritis tissues.....94
Figure 2.7: Serial tissue immunolabeling for TREM2 and OSCAR with osteoclast cell markers cathepsin K and TRAP.....97
Figure 2.8: Detection of polyethylene particles within multinucleated cells positively stained for osteoclasts-associated ITAM related molecules in human peri-prosthetic tissues.....99
Figure 2.9: Detection of PE particles in close proximity of multinucleated cells expressing cathepsin K (A) and TRAP (B).....100
Figure 2.10: Relative mRNA expression for NFATc1, TREM2, OSCAR, DAP12 and FcRγ in PO and OA tissues.....102

Figure 3.1: TRAP staining of PBMC-derived osteoclast cell culture at different time points following 3-days exposure with and without PE particles (between columns) in collagen gel. 128
Figure 3.2: TRAP staining of PBMC-derived osteoclast culture from each donor (between rows) at 14 days post 3-days collagen gel with and without PE particles (between columns) 129
Figure 3.3: Detection of PE particles within and in close proximity to TRAP-positive mutlinucleated PBMC-derived osteoclast-like cells at 14 days post 3-days exposure to PE in collagen gel 130
Figure 3.4: Counting of TRAP-positive multinucleated osteoclast-like cells following 14 days post 3-day culture in collagen gel 131
Figure 3.5: Representative pictures of TRAP staining in response to different doses of RANKL.....132
Figure 3.6: Dentine resorption pit assay of PBMC-derived osteoclast cell culture at different time points following 3-day exposure (beteen rows) with and without PE particles (between columns) in collagen gel 134
Figure 3.7: Dentine resorption pit assay of PBMC-derived osteoclast culture from each donor (between rows) at 14 days post collagen gel with and without PE particles (between columns) 135
Figure 3.8: Total resorption areas on dentine assay by PBMC-derived osteoclast-like cells following 14 days post 3-day culture in collagen gel in response to stimulation with and without PE 136
Figure 3.9: Representative picture of dentine resorption pits in response to different doses of RANKL.....137
Figure 3.10: Expression level of genes investigated in three donors at 10, 12 and 14 days post collagen gel 141
Figure 3.11: Expression level of genes investigated at 10, 12 and 14 days post collagen gel in three donors 143
Figure 3.12: Expression level of genes investigated in three donors at 14 days post collagen gel in response to different dose of RANKL 145
Figure 4.1: Cathepsin K expression in human RA, OA and normal tissues.....174
Figure 4.2: Panel of cathepsin K immunostaining in fibroblast-like synoviocytes in human active (A) and inactive RA (B), OA (C) and normal tissues (D). 176
Figure 4.3: NFATc1 expression in human RA, OA and normal tissues 178

Figure 4.4: TREM2 expression in human RA, OA and normal tissues.....181
Figure 4.5: DAP12 expression in human RA, OA and normal tissues.....183
Figure 4.6: OSCAR expression in human RA, OA and normal tissues 187
Figure 4.7: Panel of FcRγ immunostaining in human RA, OA and normal tissues 188
Figure 4.8: Immunostaining of TREM2 and OSCAR associated with blood vessel structures across different groups of human tissues.....190
Figure 4.9: TREM immunostaining associated with vasculature.....192
Figure 4.10: Immunostaining of OSCAR in human tissues of RA and OA.....194
Figure 4.11: Level of soluble OSCAR in synovial fluid of patients with RA and OA 196
Figure 5.1: Fold change of OPG and OSCAR mRNA level in 3 donors of primary HUVECs over 48 hours period post-treatment with cytokines TNFα and IL-1β.....220
Figure 5.2: OSCAR mRNA level in relative to GAPDH in in 3 donors primary HUVECs over 48 hours period post-treatment in 3 treatment groups.....222
Figure 5.3: OPG and OSCAR mRNA expression in BMEC following treatment with either 5ng/mL or 20ng/mL of TNFα or IL-1β 224
Figure 5.4: OPG and OSCAR mRNA expression in BMEC in response to 5ng/mL of TNFα or IL-1β up to 72 hours post-treatment.....226
Figure 5.5: OPG and OSCAR mRNA expression in BMEC culture (in triplicates) in response to treatment with 5ng/mL TNFα or IL-1β 228
Figure 5.6: OPG (top) and OSCAR mRNA expression (bottom) in BMEC in response to treatment with 5ng/mL TNFα or IL-1β in 3 different experiments.....230
Figure 5.7: Expression of soluble OSCAR by BMEC culture following treatment with TNFα and IL-1β 232
Figure 5.8: Detection of OPG and OSCAR expression by BMEC culture following 48 hours treatment with 5ng/mL of TNFα or IL-1β through immunofluoresence.....234

LIST OF TABLES

Table 1.1: ACR 1987 Revised Criteria for RA.....8
Table 1.2: The 2010 ACR-European League Against Rheumatism (EULAR) classification criteria for RA.....9
Table 1.3: Traditional format of ACR Classification Criteria for OA of the Hip 12
Table 1.4: Alignment of the conserved amino acids sequences of ITAM motifs in DAP12 and FcRγ between species.....45
Table 1.5: Factors regulating OSCAR mRNA expression in osteoclasts.....61
Table 2.1: Demographic details of PO and OA patients for immunohistochemistry study 75
Table 2.2: Secondary and tertiary antibodies as well antigen retrieval buffer corresponding to the primary antibodies used in immunohistochemistry.....77
Table 2.3: Primary antibodies used for immunohistochemistry.....78
Table 2.4: Demographic details of patients in which PO and OA mRNA samples taken from 80
Table 2.5: Primer sequences of each gene investigated 83
Table 2.6: SQA scores for immnostaining of NFATc1 and ITAM-related molecules in PO and OA tissues.....86
Table 2.7: SQA scores for immunostaining of TREM2, OSCAR, cathepsin K and TRAP in PO and OA tissues.....96
Table 2.8: Mean of relative mRNA expression ($-\Delta C_T$) of TREM2, OSCAR, TRAP and cathepsin K in PO and OA tissue groups.....101
Table 4.1: Summary of the demographic information of patients where the synovial tissues obtained from for tissue immunostaining.....167
Table 4.2: Demographic details of active RA and OA patients where synovial fluids were used in OSCAR ELISA. 168
Table 4.3: List of antibodies and antigen retrieval buffer used for paraffin-embedded tissue immunostaining 170
Table 4.4: SQA scores of cathepsin K immunostaining in human active, inactive RA, OA and normal tissues 173

Table 4.5: SQA scores of NFATc1 immunostaining in human active, inactive RA, OA and normal tissues 177
Table 4.6: SQA scores of TREM2 immunostaining in human active, inactive RA, OA and normal tissues 180
Table 4.7: SQA scores of DAP12 immunostaining in human active, inactive RA, OA and normal tissues 182
Table 4.8: SQA scores of OSCAR immunostaining in human active, inactive RA, OA and normal tissues 185
Table 4.9: SQA scores of FcRγ immunostaining in human active, inactive RA, OA and normal tissues 186