

**Isolation and Characterization of Stem Cell
Populations in the Periodontium**

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I dedicate this thesis to my dad. He will be greatly missed by all that had the privilege to know him.

Table of Contents

Table of Figures	i
Table of Tables	i
Declaration.....	i
List of Publications	ii
Acknowledgments	iii
List of Abbreviations	vi
Abstract.....	x
Chapter 1. Introduction.....	1
1.1 Periodontal regeneration	1
1.2 HERS/ERM and the maintenance of periodontium.....	2
1.2.1 HERS and periodontium development.....	2
1.2.1.1 Formation of HERS and the classical theory of periodontium development.....	2
1.2.1.2 Root formation.....	4
1.2.2 The fate of HERS after tooth root development.....	15
1.2.3 The role of ERM in the maintenance of periodontal ligament function.....	16
1.2.3.1 The maintenance of PDL homeostasis.....	17
1.2.3.2 A target during developmental PDL innervation.....	19
1.2.3.3 Cementum repair.....	19
1.3 Limited regenerative capacity of periodontal ligament tissues.....	20
1.4 Stem cells	21
1.4.1 Historical perspective and minimal criteria for defining multipotent mesenchymal stem cells	24
1.4.2 Adult MSC-like cells derived from dental tissues.....	25
1.4.2.1 Stem cells associated with periodontal tissues.....	26
1.4.2.2 The use of stem cells in periodontal tissue regeneration	27
1.4.2.2.1 The use of periodontal ligament progenitor cells in periodontal tissue regeneration.....	27
1.4.3 Surface markers of stem cell populations.....	33
1.4.3.1 Heterogeneity of stem cells.....	33
1.4.3.2 Phenotypic profile of adult mesenchymal stem cells.....	34
1.4.3.3 Characterisation of epithelial stem cells	37

1.5 Summary	38
1.6 Project aims	39
Chapter 2. Materials and Methods	40
2.1 Materials.....	40
2.2 Cell culture	45
2.2.1 Cell culture conditions	45
2.2.2 Isolation of human periodontal ligament stem cells and gingival fibroblasts.....	45
2.2.3 Culture of human PDLSC and GF.....	46
2.2.4 Isolation of ovine ERM and PDLSC	46
2.2.5 Culture of ovine ERM and PDLSC	47
2.2.6 Cell counting and viability testing.....	47
2.2.7 Cryopreservation of cells.....	48
2.2.8 Thawing of cryopreserved cells.....	48
2.3 Cell sorting	48
2.3.1 Immunomagnetic selection.....	48
2.3.2 Colony forming assay	49
2.3.3 Fluorescence-activated cell sorting	50
2.4 Protein analysis	51
2.4.1 Immunohistochemistry	51
2.4.2 Flow cytometric analysis	51
2.4.3 Preparation of protein lysates for western immunoblotting analysis	52
2.4.4 ReduCing agent and Detergent Compatible (RCDC) protein assay.....	53
2.4.5 Western immunoblotting	53
2.5 Proteomics analysis.....	55
2.5.1 Cell surface labelling using CyDye DIGE Fluor minimal dye.....	55
2.5.2 Preparation of cell lysates and membrane protein enrichment.....	56
2.5.3 Multiple membrane fractionations.....	58
2.5.4 Sample preparation for proteomic analysis	58
2.5.5 Protein separation by two-dimensional gel electrophoresis (2-DE).....	58
2.5.5.1 First dimension protein separation: Isoelectric focusing (IEF).....	58
2.5.5.2 Second dimension electrophoresis--SDS PAGE	60
2.5.6 Gel visualisation	60
2.5.7 Flamingo fluorescent staining	61
2.5.8 Automated spot picking.....	61
2.5.9 Protein identification by liquid chromatography-electrospray ionisation ion-	

trap (LC-ESI-IT) mass spectrometry (MS).....	62
2.5.10 Protein characterisation using web-based bioinformatics tools	63
2.6 Gene expression profiling	63
2.6.1 Preparation of total RNA	63
2.6.2 Quantification and purity analysis of RNA	64
2.6.3 Complementary DNA (cDNA) synthesis	64
2.6.4 Real-time PCR.....	65
2.6.5 Gene expression of epithelial-mesenchymal transition markers by real-time PCR.....	65
2.7 Differentiation assays.....	66
2.7.1 Assessment of osteogenic differentiation potential	66
2.7.2 Assessment of adipogenic differentiation potential.....	68
2.7.3 Assessment of chondrogenic differentiation potential	69
2.7.4 Assessment of neurogenic differentiation potential	70
2.8 <i>In vivo</i> transplantation of ERM cells.....	72
2.8.1 Preparation of transplants	72
2.8.2 Transplantation surgery	72
2.8.3 Histological analysis.....	73
2.9 Ovine tooth preparation	73
2.10 Imaging	74
2.11 Statistical analysis	74
Chapter 3. Characterization and purification of ovine ERM cells <i>in vitro</i>	75
3.1 Introduction.....	75
3.1.1 Methods for epithelial cell isolation from the periodontium.....	76
3.1.2 Prospective isolation of keratinocyte stem cells.....	77
3.2 Results.....	79
3.2.1 ERM and PDLF exhibited differential growth pattern.....	79
3.2.2 Differential immunophenotypic profiles of ERM cells and PDLF	80
3.2.2.1 ERM cells expressed both epithelial and mesenchymal markers	80
3.2.2.2 ERM cells expressed epithelial markers <i>in vivo</i>	82
3.2.2.3 Cell surface expression profiles of PDLF <i>in vitro</i>	83
3.2.3 PDLF were unable to undergo mesenchymal epithelial transition when grown under keratinocyte culture conditions	85
3.2.4 Integrin α 6/CD49f can serve as a surface marker to purify ERM cells.....	86
3.3 Discussion	91

Chapter 4. Multilineage differentiation potential of ERM cells <i>in vitro</i> and <i>in vivo</i>	100
4.1 Introduction	100
4.1.1 Mineral-forming capacities of ERM cells <i>in vitro</i> and <i>in vivo</i>	100
4.1.2 Epithelial-mesenchymal-interactions during tooth development and regeneration	101
4.1.3 Epithelial mesenchymal transition.....	102
4.1.4 Multilineage differentiation potential of ERM cells <i>in vitro</i> and <i>in vivo</i> ...	104
4.2 Results	105
4.2.1 Multilineage differentiation potential of ERM cells <i>in vitro</i>	105
4.2.1.1 Mineralization differentiation potential of ERM cells.....	105
4.2.1.2 Adipogenic differentiation potential of ERM cells.....	108
4.2.1.3 Chondrogenic differentiation potential of ERM cells.....	108
4.2.1.4 Multilineage differentiation potential of clonal ERM cell populations in vitro	112
4.2.1.5 Neuronal differentiation potential of ERM cells by exogenous growth factors.....	112
4.2.2 <i>Ex vivo</i> -expanded ERM cells can generate bone, cementum-like tissue and Sharpey's fibre-like structures <i>in vivo</i>	117
4.2.3 ERM undergo epithelial-mesenchymal transition during osteogenic induction	118
4.2.4 Survival of PDLSC in oral keratinocyte media (OKM) and subsequent mesenchymal epithelial transition	118
4.3 Discussion	123
Chapter 5. Development of a protocol for fluorescent cell surface labelling and 2-dimensional gel electrophoresis.....	131
5.1 Introduction	131
5.2 Results	134
5.2.1 Confirmation of cell surface CyDye labelling of human gingival fibroblasts using flow cytometry	134
5.2.2 Comparative 2-DE profiles of CyDye-labelled proteins and total proteins in hydrophobic and hydrophilic protein fractions	135
5.2.3 Membrane protein enrichment step in surface protein sample preparation	138
5.2.4 Protein identification of five randomly picked protein spots demonstrated cytosolic protein contamination in the membrane protein samples.	140
5.2.5 Preparation of membrane proteins using multiple membrane protein	

enrichment steps	142
5.2.5.1 Multiple membrane protein enrichment steps gave rise to similar 2-DE profiles	142
5.2.5.2 Multiple membrane protein enrichment steps diminished but did not eliminate cytosolic protein contamination	142
5.2.6 Collagenase detachment gave rise to better cell viability compared to EDTA detachment.....	144
5.2.7 Collagenase detachment minimised cytosolic protein contamination in the membrane protein preparations	145
5.3 Discussion	146
5.3.1 Studies using CyDye cell surface labelling	147
5.3.2 Challenges in membrane protein investigation	148
5.3.2.1 Inconsistency of stem cell membrane proteome data	148
5.3.2.2 Fractionation and enrichment of membrane proteins	150
5.2.2.3 CyDye cell surface labelling does not exclude intracellular protein labelling.....	152
5.2.2.4 Cell dissociation approaches.....	153
Chapter 6. Investigation of the cell surface proteome of periodontal ligament stem cells (PDLSC) using cell surface labelling	155
6.1 Introduction.....	155
6.2 Results.....	158
6.2.1 Membrane protein expression of <i>ex-vivo</i> expanded human PDLSC.....	158
6.2.2 Protein identification	159
6.2.3 Validation of the expression of 5'-nucleotidase, Thy-1 membrane glycoprotein, Annexin A2 and sphingosine kinase 1	166
6.3 Discussion	174
6.3.1 CD73.....	175
6.3.2 CD90.....	176
6.3.3 Annexin A2.....	178
6.3.4 SPK1	181
6.4 Conclusion	185
Chapter 7. Discussion and concluding remarks.....	186
7.1 Result summary and discussion	186
7.2 Future directions	189
7.2.1 What is the binding subunit of integrin $\alpha 6$ in ERM cells?	189

7.2.2	What keeps ERM cells in mesenchymal surroundings in adulthood?.....	189
7.2.3	Further functional characterisation of ERM cells.....	190
7.2.3.1	Does epithelial mesenchymal transition regulate periodontal ligament homeostasis?	190
7.2.3.2	ERM and neuronal regulation in the periodontium	192
7.2.3.3	Further investigation of mineral formation of ERM cells in a xenogeneic ectopic transplantation model	192
7.2.3.4	Are ERM cells able to contribute to tissue regeneration in periodontal defects?	193
7.2.3.5	Do ERM cells have immunomodulatory properties?.....	193
7.2.4	Functional studies of CD73, CD90, Annexin A2 and SPK1	194
7.2.5	Further assessment of the proteomic data.....	196
7.2.6	Cell surface proteome by non gel-based proteomic technologies	196
7.3	Concluding remarks	197
8.	References.....	200

Table of Figures

Figure 1.1 Schematic representation of root development and fragmentation of epithelial root sheath.....	3
Figure 2.1 The flow chart of cell surface labelling and subsequent proteomic preparation	57
Figure 2.2 The flow chart of multiple membrane fractionation	59
Figure 3.1 The basic structure of integrin [44]	79
Figure 3.2 Representative phase contrast images of ovine periodontal ligament cells	81
Figure 3.3 Representative images of the protein expression profile of Epithelial cell Rests of Malassez (ERM) shown by immunocytochemistry	82
Figure 3.4 Protein expression was confirmed by flow cytometric analysis	83
Figure 3.5 Epithelial cell rests of Malassez (ERM) expressed epithelial cell markers <i>in vivo</i>	84
Figure 3.6 Representative images of the protein expression profile of Periodontal Ligament Fibroblasts (PDLF) shown by immunocytochemistry	85
Figure 3.7 Periodontal ligament stem cells did not under mesenchymal epithelial transition in keratinocyte culture system	87
Figure 3.8 Integrin $\alpha 6$ /CD49f can serve as a surface marker to purify Epithelial cell Rests of Malassez (ERM).....	88
Figure 3.9 Integrin $\alpha 6$ /CD49f was used as a surface marker for the enrichment of Epithelial cell Rests of Malassez (ERM) from three donors.....	90
Figure 4.1 Osteogenic differentiation potential of ERM cells <i>in vitro</i>	107
Figure 4.2 Adipogenic differentiation potential of ERM cells <i>in vitro</i>	110
Figure 4.3 Chondrogenic differentiation potential of ERM cells <i>in vitro</i>	111
Figure 4.4 Multilineage differentiation potential of ERM cells at the clonal level.....	114
Figure 4.5 Morphologically ERM cells became neuron-like cells in neurogenic conditions	115

Figure 4.6 Neurogenic potential of ERM cells <i>in vitro</i>	116
Figure 4.7 Selection of integrin α_6 /CD49f-high fraction of ERM cultures by Fluorescence activated cell sorting (FACS)	119
Figure 4.8 Generation of bone, cementum-like and Sharpey's fibre-like structures <i>in vivo</i> by ERM cells	120
Figure 4.9 ERM cells gave rise to the mineral formation in subcutaneous transplantation	121
Figure 4.10 ERM cells are capable of undergoing epithelial-mesenchymal transition under osteogenic conditions.....	122
Figure 4.11 Periodontal ligament stem cells did not undergo mesenchymal epithelial transition in keratinocyte culture system	123
Figure 5.1 Human gingival fibroblasts were successfully surface labelled with CyDye shown by flow cytometry	136
Figure 5.2 Distinct 2-DE patterns of CyDye-labelled (A) and flamingo-stained (B) hydrophobic proteins of cell surface-labelled human gingival fibroblasts.....	137
Figure 5.3 Distinct 2-DE patterns of CyDye-labelled (A) and flamingo-stained (B) hydrophilic proteins of surface-labelled human gingival fibroblasts	139
Figure 5.4 Membrane protein enrichment step was necessary for surface protein sample preparation	140
Figure 5.5 Protein identification of five random spots demonstrated cytosolic protein contamination in the membrane protein samples	141
Figure 5.6 Multiple membrane protein enrichment steps gave rise to similar 2-DE profiles	143
Figure 5.7 Multiple membrane protein enrichment steps diminished but did not eliminate cytosolic protein contamination.....	144
Figure 5.8 Collagenase detachment minimised cytosolic protein contamination in the membrane protein preparations	146
Figure 6.1 Representative raw 2-DE gels of CyDye labelled proteins of <i>ex vivo</i> expanded	

human periodontal ligament stem cells (PDLSC)	160
Figure 6.2 The location of protein spots identified on the raw 2-DE image	161
Figure 6.3 Validation of cell surface expression of CD73, CD90 and Annexin A2 using flow cytometric analysis in human periodontal ligament stem cells (PDLSC).....	167
Figure 6.4 Validation of cell surface expression of CD73, CD90 and Annexin A2 using flow cytometric analysis in human gingival fibroblasts (HGF)	168
Figure 6.5 Validation of cell surface expression of CD73, CD90 and Annexin A2 using flow cytometric analysis in human keratinocytes.....	169
Figure 6.6 Validation of cell surface expression of CD73, CD90 and Annexin A2 using flow cytometric analysis in ovine periodontal ligament stem cells (PDLSC).....	170
Figure 6.7 Validation of cell surface expression of CD73, CD90 and Annexin A2 using flow cytometric analysis in ovine Epithelial cell Rests of Malassez (ERM).....	171
Figure 6.8 The expression of Annexin A2 and Sphingosine Kinase 1 (SPK1) in various human and ovine cell populations using immunocytochemical analysis	173
Figure 6.9 CD73 is involved in the generation of adenosine.....	176
Figure 6.10 Sphingosine kinase (SPK) plays important roles in regulating cell proliferation and apoptosis.....	183
Figure 7.1 Schematic representations of the stem cell-like properties of ovine ERM cells and putative association of epithelial mesenchymal transition with the maintenance of periodontal ligament homeostasis.....	187

Table of Tables

Table 1.1 Derivation and properties of stem cell populations	23
Table 2.1 Cell Culture Media	40
Table 2.2 Buffers and Solutions	41
Table 2.3 Equipment.....	42
Table 2.4 Antibodies.....	42
Table 2.5 Primer Sequences for RT-PCR.....	44
Table 2.6 Focusing conditions for 11 cm IPG strips (3–10).....	60
Table 2.7 Detection of fluorescence of CyDye DIGE Fluor Minimal Dyes: Laser Excitation Source and Emission Filters [216]	61
Table 4.1 Multilineage differentiation potential of ERM cells at the clonal level	112
Table 5.1 Collagenase detachment gave rise to better cell viability compared to EDTA detachment.....	145
Table 6.1 Membrane-associated proteins on human PDLSC	162
Table 6.2 Cell surface expression of CD73, CD90 and Annexin A2 by flow cytometric analysis. Data represent median %(range).....	172
Table 7.1 The expression of CD73, CD90, Annexin A2 and sphingosine kinase 1	189

Declaration

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 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 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.

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Signed

Jimin Xiong

List of Publications

Epithelial Cell Rests of Malassez contain unique stem cell populations capable of undergoing epithelial-mesenchymal transition. **Xiong J**, Mrozik K, Gronthos S and Bartold PM. *Stem Cells & Development*. In press.

Proteomic characterization of mesenchymal stem cell-like populations derived from various tissue types. Mrozik K*, **Xiong J***, Zilm PS, Gronthos S and Bartold PM. *Stem Cells and Cancer Stem Cells: Therapeutic Applications in Disease and Injury*. In press.
(Mrozik K and Xiong J contributed equally)

Role of epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. **Xiong J**, Gronthos S and Bartold PM. *Periodontology 2000*. In press.

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List of Abbreviations

α -MEM	Minimum essential medium, α modification
x g	Times gravity
μ	Microns
2-DE	Two-dimensional electrophoresis
AAC	Acellular afibrillar cementum
ACN	Acetonitrile
AEFC	Acellular extrinsic fiber cementum
AIFC	Acellular intrinsic fiber cementum
ALP	Alkaline phosphatase
AMP	Adenosine 5'-monophosphate
ASC	Adult stem cells
bFGF	Basic fibroblast growth factor
bHLH	Basic helix-loop-helix
BMP	Bone morphogenic protein
BMSC	Bone marrow stem cells
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
BSP	Bone sialoprotein
CAP	Cementum adhesion protein
cDNA	Complementary deoxyribonucleic acid
CFU-Epi	Colony Forming Unit-Epithelial cells
CFU-F	Colony Forming Unit-Fibroblast
CIFC	Cellular intrinsic fiber cementum
CK	Cytokeratin
CMFC	Cellular mixed stratified fiber cementum
CO ₂	Carbon dioxide
DAPI	4',6-diamidino-2-phenylindole dihydrochloride
DEPC	Diethylpyrocarbonate
DF	Dental follicle
DFPC	Dental follicle progenitor cells
DIGE	Difference gel electrophoresis
Dlx-2	Distal-less gene-2
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl-sulfoxid
DNA	Deoxyribonucleic acid
DNCP	Dentin noncollagenous proteins
DP	Dental pulp
DPSC	Dental pulp stem cells
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
EDX	Energy Dispersive X-ray Analysis

EGF	Epithelial growth factor
EMP	Enamel Matrix Proteins
EMP-1	Epithelial membrane protein 1
EMT	Epithelial to mesenchymal transition
EPMA	Electron Probe Micro-Analysis
ER	Endoplasmic reticulum
ERM	Epithelial Cell Rests of Malassez
ESC	Embryonic stem cells
FA	Formic acid
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
FGF	Fibroblast growth factor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GF	Gingival fibroblasts
GFAP	Glial fibrillary acidic protein
GM-CSF	Granulocyte-macrophage colony stimulating factor
GMSC	Gingiva-derived mesenchymal stem cells
GoPro49	Golgi protein 49kDa
GPI	Glycosyl phosphatidylinositol
GRAVY	Grand average of hydropathicity
H&E	Hematoxylin and eosin
H ₂ O ₂	Hydrogen peroxide
H ₂ SO ₄	Sulphuric acid
HA/TCP	Hydroxyapatite/tricalcium phosphate
HBSS	Hanks balanced salt solution
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HERS	Hertwig's Epithelial Root Sheath
hnRNPC	Heterogeneous nuclear ribonucleoprotein C
HSC	Haematopoietic stem cell
HSP-90β	Heat shock protein-90β
IAA	Iodoacetamide
IBMX	3-Isobutyl-1-methyl-xanthine
ICAT	Isotope coded affinity tag
ICC	Immunocytochemistry
IDO	Indoleamine 2,3-dioxygenase
IEF	Isoelectric focusing
Ig	Immunoglobulin
IHC	Immunohistochemistry
IPG	Immobilised pH gradient
iPSC	Induced pluripotent stem cells
ISCT	International Society for Cellular Therapy
ITSS	Insulin-transferrin-sodium-selenite supplement

KCl	Potassium chloride
kDa	Kilo Dalton
KSC	Keratinocyte stem cells
LC-ESI-IT	Liquid chromatography-electrospray ionisation ion-trap
M	Molar
mA	Milli amps
MACS	Magnetic activated cell sorting
ml/mm/mM	Millilitre/millimetre/millimolar
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MSC	Mesenchymal stem cells
MSX	Muscle segment homeobox gene
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NF	Nuclease free
NF-H	Neurofilament heavy chain
NFI-C	Nuclear factor I family member C
NGF	Nerve growth factor
NH ₄ HCO ₃	Ammonium bicarbonate buffer
NIH	National Institutes of Health
NOD/SCID mice	Non-obese diabetic–severe combined immunodeficient
NSC	Neural stem cells
OKGS	Oral keratinocyte growth supplement
OKM	Oral keratinocyte media
OPG	Osteoprotegerin
OPN	Osteopontin
P	Passage
P/S	Penicillin and streptomycin solution
PAGE	Poly-acrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline + 0.1% Tween-20
PBS-TX	Phosphate buffered saline + 0.3% triton-X 100
PCR	Polymerase chain reaction
PDL	Periodontal ligament
PDLF	Periodontal ligament fibroblasts
PDLP	Periodontal ligament progenitor cells
PDLSC	Periodontal ligament stem cells
PE	Phycoerythrin
PFA	Paraformaldehyde
PGE ₂	Prostaglandin E ₂
PGP 9.5	Protein gene product 9.5
pI	Isoelectric point
PLAP-1	Periodontal ligament associated protein-1

PMD	Post-mitotic differentiating keratinocyte
PPAR	Perioxosome proliferator-activated receptor
RA	Retinoic acid
RCDC	ReduCing agent and Detergent Compatible
RT	Room temperature
RT-PCR	Real time polymerase chain reaction
Runx ₂	Runt-related transcription factor-2
S1P	Sphingosine-1-phosphate
SC	Stem cells
SCAP	Stem cells from root apical papilla
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SHED	Stem cells from human exfoliated deciduous teeth
SHH	Sonic hedgehog
SPARC	Secreted protein acidic and rich in cytokine
SPK	Sphingosine kinase
STRO-1	Early mesenchymal stem cell marker
TA	Transit amplifying keratinocytes
TBP	Tributyl phosphine
TBS	Tris buffered saline
TEMED	N, N, N, N – tetramethyl - ethylenediamine
TGF	Transforming growth factor
Thy-1	Thymocyte differentiation antigen-1
TNSALP	Tissue nonspecific alkaline phosphatase
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VSEL	Very small embryonic-like stem cells
w/v	Weight per volume
Zeb1	Zinc finger E-box-binding homeobox
µg/µl/µM	Microgram/microlitre/micromolar

Abstract

Stem cells represent promising candidates for tissue engineering due to their capacity for self-renewal and their potential for differentiating into multiple cell lineages. The periodontal tissues are composed of various cell types, such as periodontal ligament fibroblasts, osteoblasts, cementoblasts, endothelial cells, the Epithelial Cell Rests of Malassez (ERM). Studies have previously identified periodontal ligament stem cells (PDLSC) within these tissues, which have the capacity to form periodontal ligament, cementum and bone. Another potential source of progenitor cells described in periodontal tissues are ERM, which are the only odontogenic epithelial cells in the adult periodontium. The present study identified that ERM contained a unique multipotential stem cell population with similar properties as described for PDLSC. Furthermore, the present proposal investigated the cell surface protein expression of PDLSC to identify unique markers for the isolation and purification of PDLSC.

The present study demonstrated that ovine Epithelial Cell Rests of Malassez contain a subpopulation of stem cells that could undergo epithelial-mesenchymal transition into mesenchymal stem-like cells with multi lineage potential. *Ex vivo*-expanded ERM expressed both epithelial (cytokeratin-8, E-cadherin and Epithelial Membrane Protein-1) and bone marrow stromal/stem cell markers (CD44, CD29, Heat Shock Protein-90 β). Integrin α_6 /CD49f could be used for the enrichment of clonogenic cell clusters (colony-forming units-epithelial cells [CFU-Epi]) which was weakly expressed by PDLSC. Importantly, ERM demonstrated a capacity to differentiation into bone, fat, cartilage and neural cells *in vitro*, and form bone, cementum-like and Sharpey's fibre-like structures when transplanted into immunocompromised mice. Additionally, gene expression studies showed that osteogenic induction of ERM triggered an epithelial-mesenchymal transition. The present study also examined the cell surface protein expression of human PDLSC

using CyDye cell surface labelling and two-dimensional electrophoresis coupled with liquid chromatography--electrospray-ionization tandem mass spectrometry. In addition to the expression of well known mesenchymal stem cell associated cell surface antigens such as CD73 (ecto-5'-nucleotidase) and CD90 (Thy-1), PDLSC were also found to express two novel cell surface proteins, Annexin A2 and sphingosine kinase 1. Interestingly, previous studies have implicated CD73, CD90, Annexin A2 and sphingosine kinase 1 expression in the maintenance of various stem cell populations. Comparative analyses investigated the expression of CD73, CD90, Annexin A2 and sphingosine kinase-1 in human gingival fibroblasts, human keratinocytes, ovine PDLSC and ovine ERM cells. Importantly, this study found that human skin epithelial cells lacked any cell surface expression for CD73, CD90 and Annexin A2.

In summary, ERM and PDLSC are both important stem cell sources that could play a pivotal role in periodontal homeostasis and regeneration following insult or disease. As periodontal regeneration is essentially a re-enactment of the periodontal tissue development process, it is plausible to suggest that the combination of ERM and PDLSC would hold greater potential for periodontal regeneration compared to established bone marrow-derived mesenchymal stem cells.