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.

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Declaration

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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_2O_2	Hydrogen peroxide
H_2SO_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_4HCO_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	Perixosome 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-90β). 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.