

INVESTIGATIONS ON THE SUITABILITY OF SHEEP AS A MODEL FOR ENDODONTIC REVITALISATION RESEARCH

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in Dentistry

Milad Talib Hamed Al Taii
BDS, MSc (Baghdad)



School of Dentistry
Faculty of Health Sciences
The University of Adelaide
South Australia
AUSTRALIA

2016

Table of Contents

Table of Contents	i
Table of Figures	x
Table of Tables	xvi
Signed statement	xviii
Acknowledgements	xix
List of Abbreviations	xxi
Abstract	xxvi
Chapter 1. Introduction	1
1.1 Introduction	2
Chapter 2. Literature Review	4
2.1 Introduction	5
2.1.1 Human tooth development.....	5
2.1.2 Root formation.....	7
2.1.3 Dentine formation.....	9
2.1.3.1 Primary dentine formation	9
2.1.3.2 Secondary and tertiary dentine formation	10
2.1.4 Cementum formation	11
2.2 Elements for revitalisation	13
2.2.1 Stem cells	13
2.2.1.1 Dental pulp stem cells (DPSCs)	14
2.2.1.2 Stem cells from apical papilla (SCAP).....	15
2.2.1.3 Periodontal ligament stem cells (PDLSCs).....	16

2.2.1.4 Conclusion	17
2.2.2 Signalling molecules	18
2.2.2.1 Extracellular dentine proteins	18
2.2.2.2 Growth factors	19
2.2.2.2.1 Transforming growth factor β	19
2.2.2.2.2 Bone morphogenetic proteins.....	20
2.2.2.2.3 Insulin like growth factor	20
2.2.2.2.4 Vascular endothelial growth factor and fibroblast growth factors ..	21
2.2.2.2.5 Platelet derived growth factor.....	21
2.2.2.2.6 Nerve growth factor	22
2.2.2.3 Effects of signalling molecules on revitalisation.....	22
2.2.3 Scaffold	23
2.2.3.1 Synthetic material scaffolds	24
2.2.3.2 Natural material scaffolds.....	25
2.3 Factors affecting revitalisation outcomes.....	26
2.3.1 Bacterial infection control	27
2.3.1.1 Mechanical debridement of the infection	27
2.3.1.2 Chemical debridement of the infection	28
2.3.1.3 Root canal medication	30
2.3.2 Pulpal and periapical condition before treatment.....	32
2.3.3 Apical diameter of the tooth	33
2.3.4 Orifice barrier materials used in revitalisation	33
2.4 Histological observation of revitalisation outcomes.....	35
2.4.1 Dentine associated mineralised tissue (DAMT).....	36
2.4.2 Intracanal mineralised tissue.....	38

2.4.3 Intracanal soft tissues.....	38
2.5 Animal models for revitalisation research.....	47
2.6 Sheep as a model in dental research.....	48
2.7 Conclusion.....	50
Chapter 3. Standardisation of Sheep as a Suitable Animal Model for Endodontic Revitalisation Research.....	52
3.1 Introduction.....	53
3.2 Part One: Evaluation of the anatomy and the histology of sheep permanent incisor root at the mature age (Full mouth age).....	54
3.2.1 Aims.....	55
3.2.2 Material and methods.....	55
3.2.2.1 Animals.....	55
3.2.2.2 Fixation.....	55
3.2.2.3 Direct measurements.....	56
3.2.2.4 Radiographs.....	58
3.2.2.5 Computer Tomography (CT).....	59
3.2.2.6 Evaluation method reliability.....	60
3.2.2.7 Histology.....	61
3.2.3 Statistics.....	61
3.2.4 Results.....	62
3.2.4.1 Direct measurement results.....	62
3.2.4.2 Radiographic results.....	62
3.2.4.3 CT scan results.....	63
3.2.4.4 Evaluation methods reliability.....	65
3.2.4.5 Histology results.....	67

3.2.5 Discussion	69
3.3 Part Two: A suitable sheep dental age to be utilised for regenerative endodontic research.....	74
3.3.1 Aims	75
3.3.2 Materials and Methods	75
3.3.2.1 Animals	75
3.3.2.2 Direct measurement.....	76
3.3.3 Results.....	78
3.3.3.1 Two-tooth age	78
3.3.3.1.1 Direct measurements	78
3.3.3.1.2 Radiographic measurements.....	78
3.3.3.1.3 CT scan measurements.....	78
3.3.3.1.4 Evaluation methods reliability at two-tooth age	79
3.3.3.1.5 Histology results	80
3.3.3.2 Four-Tooth age.....	81
3.3.3.2.1 Direct measurements	81
3.3.3.2.2 Radiographic measurements.....	82
3.3.3.2.3 CT scans measurements	82
3.3.3.2.4 Evaluation methods reliability at four-tooth age	84
3.3.3.2.5 Histology results	84
3.3.3.3 Six-tooth age	85
3.3.3.3.1 Direct measurements	85
3.3.3.3.2 Radiographic measurements.....	86
3.3.3.3.3 CT scan measurements.....	86
3.3.3.3.4 Evaluation methods reliability at six-tooth age	87

3.3.3.3.5 Histology results	88
3.3.3.4 Incisor teeth CT measurement changes from eruption to maturation age	91
3.3.4 Discussion	91
3.3.5 Conclusion	94
Chapter 4. A Study of an Endodontic Revitalisation Protocol in a Sheep Model.....	95
4.1 Introduction	96
4.2 Aims	97
4.3 Materials and methods	97
4.3.1 Animals.....	97
4.3.2 Surgical procedures.....	97
4.3.2.1 Imaging	97
4.3.2.2 Treatment Protocol.....	101
4.3.3 Computer Tomography (CT) scanning.....	105
4.3.4 Tissue Processing.....	106
4.3.4.1 Fixation	106
4.3.4.2 Decalcification	106
4.3.4.3 Histology	106
4.4 Statistical Analysis	107
4.5 Results	108
4.5.1 Radiographic results	108
4.5.2 Computer Tomography scan analysis	114
4.5.3 Histological observations	119
4.5.3.1.1 First healing region	121
4.5.3.1.2 Second healing region	124

4.5.3.1.3 Third healing region	127
4.5.3.1.3.1 Third healing region (a)	128
4.5.3.1.3.2 Third healing region (b)	132
4.5.3.2 Apical region	136
4.6 Discussion	141
4.6.1 Research findings and future research direction	147
4.7 Conclusion.....	149
 Chapter 5. Simple Protocol for Platelet Rich Plasma Scaffold Preparation and Its	
Effect on Dental Pulp Cells.....	150
5.1 Introduction	151
5.2 Aims	152
5.3 Materials and Methods	152
5.3.1 Ovine dental pulp cells isolation.....	152
5.3.2 Sheep dentine discs preparation.....	153
5.3.3 Platelet rich and platelet poor plasma preparation.....	154
5.3.4 Ovine dental pulp cell proliferation assay.....	154
5.3.5 Migration and distribution assays	155
5.3.5.1 Ovine dental pulp cell migration assay	155
5.3.5.2 Ovine dental pulp cell distribution.....	156
5.3.6 Ovine dental pulp cell differentiation	157
5.3.6.1 Mineralisation detection and quantification	157
5.3.6.2 Alkaline phosphatase detection	159
5.3.7 Ovine dental pulp cell attachment to dentine	160
5.3.8 Statistical analysis.....	160
5.4 Results	160

5.4.1 Ovine dental pulp cell proliferation assay	160
5.4.2 Migration and distribution assays	163
5.4.2.1 Ovine dental pulp cell migration assay	163
5.4.2.2 Ovine dental pulp cell distribution assay.....	165
5.4.3 Ovine dental pulp cell differentiation	167
5.4.3.1 Mineralisation detection and quantification	167
5.4.3.2 Alkaline phosphatase activity	170
5.4.4 Ovine dental pulp cell attachment to dentine	170
5.5 Discussion	173
5.6 Conclusion.....	176
Chapter 6. Conclusions.....	178
6.1 Conclusions	179
Chapter 7. Appendices	181
7.1 Appendix: Pilot studies	182
7.1.1 Chapter 3 related pilot study.....	182
7.1.1.1 Development of a radiograph localizing frame.....	182
7.1.2 Chapter 4 related pilot study.....	184
7.1.2.1 Development of the experimental technique	184
7.1.2.2 Immunohistochemistry (IHC) analysis of expression of DSPP in sheep teeth.....	185
7.1.2.2.1 Aims	185
7.1.2.2.2 Methods	185
7.1.2.2.2.1 Animals	185
7.1.2.2.2.2 Fixation.....	186
7.1.2.2.2.3 Decalcification.....	186

7.1.2.2.2.4 Tissue processing for immunohistochemistry stain.....	186
7.1.2.2.3 Results	187
7.1.2.2.4 Discussion.....	187
7.1.3 Chapter 5 related pilot studies	189
7.1.3.1 Analysis of the suitability of WST-1 colorimetric assay kit for measuring ODPCs proliferation rate	189
7.1.3.1.1 Introduction	189
7.1.3.1.2 Aim	189
7.1.3.1.3 Methods.....	189
7.1.3.1.4 Results	189
7.1.3.1.5 Conclusion.....	190
7.1.3.2 Development of the proliferation assay protocol	191
7.1.3.2.1 Introduction	191
7.1.3.2.2 Aims.....	191
7.1.3.2.3 Method	192
7.1.3.2.4 Statistical analysis	192
7.1.3.2.5 Results	192
7.1.3.2.6 Conclusion.....	193
7.1.3.3 Evaluate the attachment of ODPCs to dentinal walls using SEM.....	194
7.1.3.3.1 Aims	194
7.1.3.3.2 Methods.....	194
7.1.3.3.3 Results	195
7.1.3.3.4 Discussion.....	196
7.1.3.3.5 Conclusion.....	197
7.2 Appendix: Decalcifying solutions.....	198

7.2.1 Laboratory decal solution.....	198
7.2.1.1 Reagents:.....	198
7.2.1.2 Method for making 10 L.	198
7.2.2 EDTA 10% pH 7.4.....	198
7.2.2.1 Preparation.....	198
7.3 Appendix: Tissue dehydration and paraffin embedding protocol	199
7.4 Appendix: Stains for light microscopy protocol.....	200
7.5 Appendix: Immunohistochemistry stain protocols	201
7.6 Appendix: Cell counting protocol.....	204
7.7 Appendix: ODPC cryopreservation and thawing protocols:	205
7.7.1 ODPC cryopreservation and thawing protocols.....	205
7.7.2 Thawing of cryopreserved ODPCs	205
7.8 Appendix: Results related tables	206
7.8.1 Chapter 3 related results.....	206
7.8.1.1 Mature age measurement (mm)	206
7.8.1.2 Two-tooth age measurements (mm).....	209
7.8.1.3 Four-tooth age measurements (mm).....	212
7.8.1.4 Six-tooth age measurements (mm)	214
7.8.2 Chapter 4 related results.....	216
7.8.3 Chapter 5 related results.....	217
7.9 Appendix: Results related figures.....	221
7.9.1 Chapter 3 related figures	221
7.9.2 Chapter 4 related figures	222
7.9.3 Chapter 5 related figures	226
Chapter 8. Bibliography.....	228

Table of Figures

Figure 2.1 Tooth development stages (modified from Li et al. (25)).	6
Figure 2.2 Root formation before eruption (sheep tooth)	8
Figure 2.3 Root formation and developmental after eruption (sheep teeth).	9
Figure 2.4 Dentine types.	11
Figure 2.5 Sheep mandibles	50
Figure 3.1 Sheep anterior segments of mandibles fixed in formalin.	56
Figure 3.2 Direct measurement methods.	57
Figure 3.3 A sheep anterior segment of the mandible placed in the film holder designed for this study.	58
Figure 3.4 Measurements on radiographic image using Image J software.	59
Figure 3.5 Sheep incisors, using the three dimensions CT imaging.	65
Figure 3.6 Comparison between sheep incisor root lengths.	66
Figure 3.7 Comparison between sheep incisors apical root wall thicknesses.	66
Figure 3.8 Comparison between sheep incisor apices.	67
Figure 3.9 High magnification of a section through the pulp.	67
Figure 3.10 Histological sections full mouth sheep teeth.	68
Figure 3.11 Histology sections showing apical area.	69
Figure 3.12 Direct measurement methods.	77
Figure 3.13 Comparison between incisor measurements.	79
Figure 3.14 Two-tooth sheep.	80
Figure 3.15 Two-tooth age.	81
Figure 3.16 Four-tooth age sheep.	83

Figure 3.17 Comparison between incisor teeth measurements of four-tooth age.....	84
Figure 3.18 Four-tooth age histology sections.....	85
Figure 3.19 Comparison between incisor measurements of six-tooth age.....	88
Figure 3.20 Six-tooth sheep model first incisor.....	89
Figure 3.21 Six-tooth age histology sections.....	90
Figure 3.22 Sheep third incisor tooth.....	90
Figure 4.1 Orthodontic round bases on treatment and control teeth as reference points for radiographs.....	99
Figure 4.2 Measurement the root diameters using IMAGE J.....	100
Figure 4.3 Treatment sessions.....	101
Figure 4.4 Buccal abscess associated with an infected tooth. Red arrow points to pus coming out of infected canal.....	103
Figure 4.5 Isolation of infected tooth with a rubber dam. Composite ledge built at the crown cervically to prevent dislodgment of rubber dam clamp	103
Figure 4.6 Canal cleaning with 5.25% NaOCl, 17% EDTA followed by saline.....	104
Figure 4.7 Radiographic images of treatment sessions.....	104
Figure 4.8 Schematic diagram showing regions of the tooth where planes of histological cross sections were taken.....	107
Figure 4.9 Root length measurements from radiograph.....	109
Figure 4.10 Sheep-1 (1) and Sheep-2 (2) radiograph images	110
Figure 4.11 Sheep-3 radiograph images.....	111
Figure 4.12 Sheep-4 radiograph images.....	111
Figure 4.13 Measurements of dentine thickness	113
Figure 4.14 Measurements of apical diameters.....	114

Figure 5.15 CT analysis of the differences between the experimental and the control group at the end of the experiment.....	115
Figure 4.16 3D CT images of Sheep-1, showing coronal and lateral views of experimental and control teeth.	115
Figure 4.17 Sheep-1 CT image.....	116
Figure 4.18 3D CT image of Sheep-2, showing coronal and lateral views of experimental and control teeth.	116
Figure 4.19 Sheep-2 CT images.	117
Figure 4.20 3D CT images of Sheep-3, showing coronal and lateral views of experimental and control teeth.....	117
Figure 4.21 Sheep-3 CT images.	118
Figure 4.22 3D CT Sheep-4, showing coronal and lateral views of experimental and control teeth.....	118
Figure 4.23 Sheep-4 CT images.	119
Figure 4.24 Experimental and control tooth sections.....	120
Figure 4.25 Histology section of the experimental tooth showing all regions.	121
Figure 4.26 Experimental tooth healing regions	122
Figure 4.27 A and B High magnified sections of the first region.....	122
Figure 4.28 First region	123
Figure 4.29 Experimental teeth sections showing the first region.....	124
Figure 4.30 Section showing the second region with its hard tissue matrix structures.	126
Figure 4.31 Enlargements of the second region.	127
Figure 4.32 Section showing the third region (a).....	129
Figure 4.33 Enlarged section of the third region (a).....	130
Figure 4.34 Magnified sections of the third region (a).	130

Figure 4.35 Third region (a).....	131
Figure 4.36 High magnified section of the third region (a) of Sheep-4.....	132
Figure 4.37 Sheep-1, third region (b).	133
Figure 4.38 Third region (b).	134
Figure 4.39 The experimental tooth of Sheep-4.	135
Figure 4.40 The experimental tooth of Sheep-4, third region (b).....	136
Figure 4.41 The apical area of Sheep-1 experimental tooth.	138
Figure 4.42 The apical regions of the experimental teeth	139
Figure 4.43 Sheep-4 apical region.	140
Figure 4.44 Control-tooth.....	141
Figure 5.1 ODPC proliferation assay, experiment design.....	155
Figure 5.2 Migration assay, experiment design.	156
Figure 5.3 Cell distribution assay, experiment design.....	157
Figure 5.4. Differentiation assay, experiment design.	158
Figure 5.5 ODPC proliferation on PRP, PPP scaffolds and control (no scaffold).....	162
Figure 5.6. Scaffold type and root effect on ODPCs migration.....	164
Figure 5.7 ODPCs distribution areas with the highest concentration of cells at day five.....	165
Figure 5.8 ODPCs distribution (300µm) for each scaffold observed by confocal microscope.....	166
Figure 5.9 Qualitative and quantitative analysis of mineralised nodules formation by ODPCs cultured with and without scaffold.....	168
Figure 5.10 Qualitative and quantitative analysis of mineralised matrix formation after adding of mineralisation induction medium to ODPCs.....	169

Figure 5.11. ALP activity of ODPCs cultured on PRP and PPP scaffolds with and without root dentine discs.....	170
Figure 5.12 Histology sections of ODPCs cultured on PRP scaffold.....	171
Figure 5.13 Histology sections of ODPCs cultured on PPP scaffold.....	172
Figure 7.1 Sheep mandibles flat shape (A), human mandible (B), image added for a comparable reason.	182
Figure 7.2 Radiograph localising frame.	183
Figure 7.3 TurboReg plug-in used to correct errors in the image angulations.....	184
Figure 7.4 left incisor with a hollow cavity (A), Right first incisor with apical tissue (B).....	185
Figure 7.5 Negative expression of DSPP by ovine teeth (left side images) and positive expression of DSPP by rat teeth (right side images).....	188
Figure 7.6 Plate reading results of the absorbance rates of different concentrations of ODPCs on PRP scaffold.....	190
Figure 7.7 Chart showing almost linear relation between cell concentration and absorbance rate.....	190
Figure 7.8 ODPCs proliferation after one, five, and seven days of culturing with and without scaffolds.	193
Figure 7.9 image of specimens prepared for SEM.....	195
Figure 7.10 SEM images of ODPCs attach to dentine walls after ten days of culturing on PRP and PPP scaffolds.....	196
Figure 7.11 Histology sections of sheep incisor teeth.....	221
Figure 7.12 Histology sections from Sheep-1 experimental tooth.	222
Figure 7.13 Histology sections from Sheep-3 experimental tooth.	223
Figure 7.14 Histology section showing the second and third healing regions.	224

Figure 7.15 Third region (a).....	224
Figure 7.16 Histology sections at the apex of the experimental tooth.....	225
Figure 7.17 ODPCs proliferation on PRP and PPP scaffolds.....	226
Figure 7.18 ODPCs cultured on PRP and PPP scaffolds in sheep roots.....	227

Table of Tables

Table 2-1 The American Association of Endodontics considerations for endodontic regeneration/revitalisation procedures (275).....	35
Table 2-2 Histology outcomes of revitalisation research in animal models.....	43
Table 2-3 Revitalisation case reports with histology outcomes.	46
Table 3-1 Measurements of incisor teeth of the mature age sheep.	63
Table 3-2 Root lengths of anterior teeth	70
Table 3-3 Dentine thickness of anterior teeth.	71
Table 3-4 Apical diameters of anterior teeth.....	72
Table 3-5 Measurements of permanent first incisors of two-tooth age sheep.....	79
Table 3-6 Incisor teeth measurements of four-tooth sheep.....	82
Table 3-7 Approximate changes in first incisors CT measurements between two-tooth and four-tooth age sheep.	83
Table 3-8 Measurements of incisor teeth at six-tooth age.....	86
Table 3-9 Changes in CT scan measurements of incisor teeth between four-tooth and six-tooth age	87
Table 3-10 CT scan measurement changes of first, second and third incisor from the eruption to the maturation ages. Mature age CT scan measurements were from Part One (Table 3-1).....	91
Table 7-1 Direct measurements of incisor teeth of the mature age sheep (mm).....	206
Table 7-2 Radiographic measurements of incisor teeth of the mature age sheep (mm).....	207
Table 7-3 CT measurements of incisor teeth of the mature age sheep (mm).....	208
Table 7-4 Direct measurements of incisor teeth of two-tooth age sheep (mm).	209

Table 7-5 Radiographic measurements of incisor teeth of two-tooth age sheep (mm).	210
Table 7-6 CT measurements of incisor teeth of two-tooth age sheep (mm).	211
Table 7-7 Direct measurements of incisor teeth of four-tooth age sheep (mm).	212
Table 7-8 Radiographic measurements of incisor teeth of four-tooth age sheep (mm).	212
Table 7-9 CT measurements of incisor teeth of four-tooth age sheep (mm).	213
Table 7-10 Direct measurements of incisor teeth of six-tooth age sheep (mm).	214
Table 7-11 Radiographic measurements of incisor teeth of six-tooth age sheep (mm).	214
Table 7-12 CT measurements of incisor teeth of six-tooth age sheep (mm).	215
Table 7-13 Radiographic (mm) and CT measurements (mm) of the experimental and control teeth before treatment and six months after treatment, (<i>in vivo</i> revitalisation research protocol in a sheep model).	216
Table 7-14 ODPCs proliferation after one day of culturing with and without scaffold.	217
Table 7-15 ODPCs proliferation after five days of culturing with and without scaffold.	218
Table 7-16 ODPCs migration to each scaffold without root dentine addition.	219
Table 7-17 Quantitative analysis of mineralised nodules formation by ODPCs using ARS assay.	219
Table 7-18 Quantitative analysis of ALP activity of ODPCs cultured on PRP and PPP scaffolds with and without root dentine discs.	220

Signed statement

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 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.

I give my consent for this copy of thesis, when deposited in The University of Adelaide Library, to be available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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.

Milad Al Taii

Acknowledgements

Thank you God for given me the strength and the patience to finish my PhD journey.

I would like to thank my supervisors Professor Lindsay Richards, Associated Professor Peter Cathro, and Dr Marita Broberg for their guidance, encouragement, and positive support over the years. Thank you for your understanding of the challenges and difficulties for an international student with a little experience on writing and editing research.

I am grateful to Professor Simon Koblar for allowing me to access his laboratory and his generosity in providing research materials and technical expertise. Thank you to Dr Xenia Kaidonis for sharing her knowledge of stem cells.

Professor Geoffrey Heithersay, Professor Craig Dreyer, and Professor Wayne Sampson, you have a wealth of knowledge. Thank you for generously sharing it.

My gratitude is also directed to Dr Helen James and Dr John Berketa from the Department of Forensic Odontology Unit, for their kindness in allowing me use of their portable x-ray machine. I am grateful to Dr John Berketa, who donated his time to help me with the surgery in the sheep. It was a big relief for me to see you in the theatre helping, supporting and advising.

Thank you to Dr Tim Kuchel and the staff of Gilles Plains LARIF for their support in the surgery, looking after my animals and for ovine tooth collection. Thank you for making me feels comfortable in your facility.

I am grateful for the expert advice on histology techniques from Ms Sandie Hughes and Miss Marjorie Quin.

Thank you to the staff at Adelaide Microscopy Centre and in particular Dr John Terlet, Dr Agatha Labrinidis, Ms Ruth William and Ms Lynn Waterhouse.

Miss Renee Ormsby from the bone cell biology group, thank you for sharing your knowledge of Alizarine red stain, providing the protocol and helping with the method. Chris Leigh from the Anatomy Department at The University of Adelaide, thank you for your help with immuno-staining, and in preparing chemicals for my experiments.

Dr Elizabeth-Ann Farmer and Mrs Lucia Hatch, my friends, thank you for your support and encouragement. Thank you for listening to my problems and helping to solve them.

My thanks and eternal appreciation goes to my husband, Dr Zainulabdeen, my parents and my children for their support over the past few years. Thank you for believing in me and sorry for keeping you worried about me all these years.

I would like to acknowledge the support I received from Iraqi Ministry of Higher Education and Scientific Research, The University of Adelaide, and Babylon University.

I dedicate lovingly this thesis to my country Iraq, the cradle of civilization.

List of Abbreviations

$\mu\text{m}/\mu\text{g}/\mu\text{l}$	Micrometre / Microgram / Microliter
3D	Three Dimensions
AAC	Acellular A fibrillar Cementum
AAE	American Association of Endodontics
AEFC	Acellular Extrinsic Fibre Cementum
ALP	Alkaline Phosphatase
AM	Ameloblast
ARS	Alizarin Red Stain
BC	Blood Clot
BI	Bone-like Island
BMP	Bone Morphogenic Proteins
BMSCs	Bone Mesenchymal Stem Cells
C	Cementum
$\text{Ca}(\text{OH})_2$	Calcium Hydroxide
CaCl_2	Calcium Chloride
Cb	Cementoblasts
CDJ	Cemento-Dentinal Junction
CEJ	Cemento-Enamel Junction
CHX	Chlorhexidine Digluconate
CI	Cementum-like Island

CIFC	Cellular Intrinsic Fibre Cementum
cm	Centimetre
CMFC	Cellular Mixed Fibre Cementum
Coll	Collagen
CPC	Cetylpyridinium Chloride
CT	Computer Tomography
D	Dentine
DAMT	Dentine Associated Mineralised Tissue
DAP	Double Antibiotic Paste
DAPI	4',6-Diamidino-2phenidole
DE	Dental Epithelium
DF	Dental Follicle
DFd	Degrees of Freedom for the Denominator
DFn	Degrees of Freedom for the numerator
DM	Dental Mesenchyme
DMP	Dentine Matrix Proteins
DMSO	Dimethyl-Sulfoxide
DP	Dental Papilla
dpi	Dot per inch
DPP	Dentine Phosphorprotein
DPSCs	Dental Pulp Stem Cells

DSP	Sialoprotein
DSPP	Dentine Sialophosphoprotein
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EK	Enamel Knot
EMSCs	Embryonic Mesenchymal Stem Cells
EO	Enamel Organ
ERM	Epithelial Rest of Malassez
FCS	Foetal Calf Serum
FGF	Fibroblast Growth Factor
G	Group
GIC	Glass-Ionomer Cement
GP	Gutta Percha
H and E	Hematoxylin and eosin
H ₂ O ₂	Hydrogen Peroxide
HA/TCP	Hydroxyapatite / Tricalcium Phosphate
HEPES	(4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid))
HERS	Hertwig's Epithelial Root Sheath
IGFI	Insulin-like Growth Factor
IM	Intra muscular Injection
IMVS	Institute of Medical and Veterinary Science

K	Kilo
L	Litre
LARIF	Large Animal Research and Imaging Facility
M	Molar
MD	Mesio-distal
mg	Milligram
MI	Mineralised Island
MIM	Mineralisation Induction Medium (MIM)
ml/mm/mM	Millilitre/ millimetre/ milliMolar
MTA	Mineral Trioxide Aggregate
mTAP	Modified Triple Antibiotic Paste
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
NCPs	Non - Collagenous Proteins
NGF	Nerve Growth Factor
ns	Non significant
OD	Odontoblast
ODPCs	Ovine Dental Pulp Cells
PDGF	Platelet Derived Growth Factor
PDL	Periodontal Ligament
PDLSCs	Periodontal Ligament Stem Cells

PGA	Poly-Glycolic Acid
PLA	Poly-Lactic Acid
PLGA	Poly-lactic-co-Glycolic Acid
pNPP	p-nitrophenylphosphate
PPP or (PP)	Platelet Poor Plasma
PRP or (PR)	Platelet Rich Plasma
RGD	Arginyl Glycyl Aspartic Tripeptide
ROI	Region Of Interest
SAMRI	South Australia Health and Medical Research Institute
SCAP	Stem Cells from Apical Papilla
SD	Standard Deviation
SEM	Scanning Electron Microscope SEM
TAP	Triple Antibiotic Paste
TGF β	Transforming Growth factor β
U/ml	Unit per millilitre
UV	Ultraviolet
v	Volume
VEGF	Vascular Endothelial Growth Factor
w/v	Weight per volume
x g	Times gravity
α -MEM	Minimum Essential Medium, α modification

Abstract

Revitalisation treatment of infected immature permanent teeth shows promising results by increasing root lengths, dentine wall thicknesses and further narrowing of the open apices. However, previous case reports and animal studies showed variation in the outcomes of revitalisation treatment protocols. In addition, histological studies showed that the healing tissues were different in pulp and dentine tissues. These variations could be due to differences in the type of trauma, infection history, treatment protocol, and animal model. Thus standardisation is required to investigate different aspects of revitalisation protocols to ensure more predictable outcomes.

Thus, the first objective of this project was to develop a standardised model for endodontic revitalisation research by examining the anatomy and histology of sheep teeth at different stages of development to find the most appropriate dental age to use for endodontic revitalisation research. Sheep at two-, four-, six-tooth and mature stages of development were investigated. Histology, standardised radiography and computed tomography have been used to evaluate and measure incisor root length, apical third dentine thickness and apex diameter of each tooth. During development, sheep at all dental ages showed small changes in incisor root lengths and major changes in the apical diameters and the dentinal wall thicknesses from the eruption time to maturation. Sheep appear to be a reliable animal model for endodontic revitalisation research. Each dental age has its advantages and disadvantages. The results of this research found that the two-tooth stage is the most appropriate dental age because permanent incisors are anatomically similar to human immature incisor teeth. In addition, the animals are readily available, small in size, easy to manage, and have a high growth rate.

The second objective of this project was to examine *in vivo* the response of two-tooth age sheep model to commonly used endodontic revitalisation protocol. To achieve this goal,

sheep incisor teeth were infected for four weeks, and then treated with a revitalisation protocol using blood clot as a scaffold. The changes in teeth diameter and the histology of the healing tissue were evaluated six months after treatment. The results showed some further root length development in the experimental teeth, significant increases in dentine wall thickness, and significant narrowing of the root apices of the experimental teeth compared with control teeth. There was also histological evidence of three or four distinct healing regions in the experimental teeth. Less mature tissue was observed coronally and more mature tissue was seen apically, suggesting that repair progressed from the apical to the coronal part of the root.

Revitalisation case reports and animal studies have identified difficulties in the stimulating bleeding during revitalisation treatment. Thus, the third objective of this project was to evaluate the suitability of platelet rich plasma (PRP) scaffold prepared using a simple protocol by examining its effectiveness on stimulating proliferation, migration and differentiation of cultured ovine dental pulp cells (ODPCs). The results showed culturing of ODPCs on both PRP and PPP scaffolds significantly increased the proliferation rate compared to groups without a scaffold. PRP scaffold had a significant stimulation effect on ODPCs proliferation compared to PPP. ODPCs migration rate was higher toward and inside PRP than PPP. Alkaline phosphatase activities of ODPCs cultured on PRP and PPP were significantly higher than the cells cultured without scaffold. ODPCs cultured on PRP scaffolds formed more mineralised nodules than PPP groups with and without the addition of mineralised induction medium. The addition of dentine discs to the scaffolds significantly reduced the activities of the cells. Seeding ODPCs with PRP and PPP in chemically cleaned roots showed migration and attachment of the cells to the dentinal walls with PRP group showing further attachment compared to PPP.

The results of these studies have shown that:

- 1- The sheep is a reliable animal model for endodontic revitalisation research.
- 2- The two-tooth stage is the most appropriate dental age endodontic revitalisation research.
- 3- Endodontic revitalisation treatment using Two-tooth age sheep showed a positive outcome with further development of the experimental teeth, and histological evidence of three or four distinct healing regions.
- 4- PRP scaffold prepared using a simple protocol of blood centrifugation can enhance proliferation, migration, and differentiation of dental pulp cells similar to complicated and long step protocols.