# 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

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# Signed statement

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Milad Al Taii

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

# List of Abbreviations

| | |
|---|---|
| µm/µg/µl | Micrometre / Microgram / Microliter |
| 3D | Three Dimensions |
| AAC | Acellular Afibrillar 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 |
| $Ca(OH)_2$ | 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-2phenlidole |
| 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 |
| $H_2O_2$ | 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 |
| TGFβ | 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.