Effect of growth factors on the osteoinductive potential of Hydroxyapatite β-Tricalcium Phosphate (HA-TCP)

A report submitted to the University of Adelaide in partial fulfilment of the requirements of the Degree of Doctor of Clinical Dentistry (Periodontology)

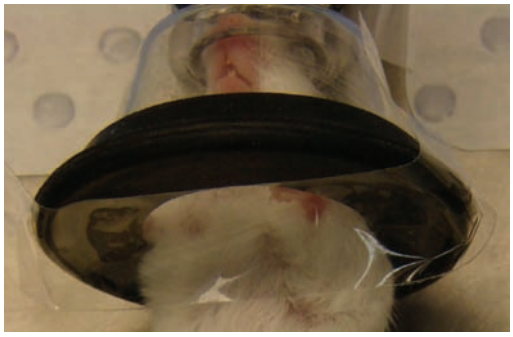
Raymond Chun Wai Chan BDS (Otago)

3. Appendices

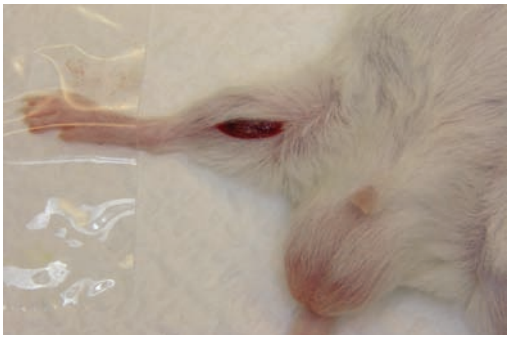
3.1 Abbreviations

ALP	Alkaline Phosphatase
BCP	Biphasic Calcium Phosphate
BMP-	Bone Morphogenetic Protein-
BMP	Bone Morphogenetic Proteins
CaP	Calcium Phosphate
Ca/P ratio	Calcium/Phosphate ratio
CdA	Calcium deficient Apatite
DBBM	Deproteinised Bovine Bone Matrix
DFDBA	Demineralised Freeze Dried Bone Allograft
EMD	Enamel Matrix Protein Derivative
EMP	Enamel Matrix Protein
ePTFE	Expanded Polytetrafluoroethylene
FDBA	Freeze Dried Bone Allograft
FGF	Fibroblast Growth Factor
GBR	Guided Bone Regeneration
HA	Hydroxyapatite
HA-TCP	Hydroxyapatite-Tricalcium Phosphate
ICTP	Carboxyterminal Telopeptide of Type I collagen
IGF-	Insulin-like Growth Factor-
MSCs	Mesenchymal Stem Cells
PDGF-	Platelet Derived Growth Factor-
PDL	Periodontal Ligament
PGA	Propylene Glycol Alginate
rhPDGF-	Recombinant human Platelet Derived Growth Factor-
β-TCP	β-Tricalcium Phosphate
TGF-	Transforming Growth Factor-
VEGF	Vascular Endothelial Growth Factor
vSMC	Vascular Smooth Muscle Cell

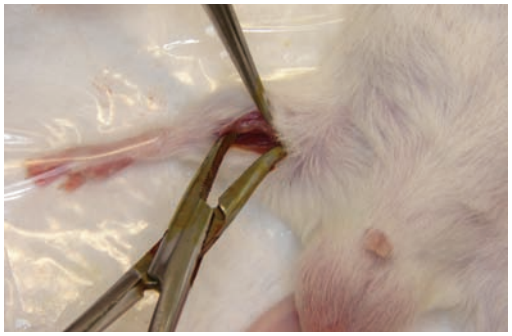
3.2 Appendix One: Surgical Protocol



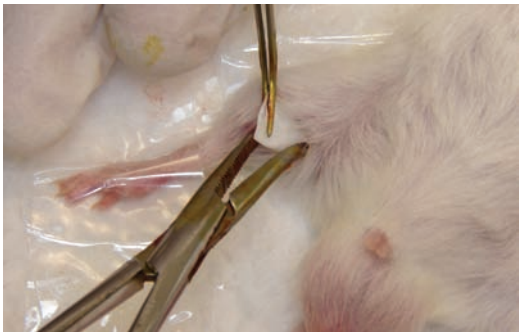
1. Inhalation anaesthesia of the animal was maintained using a nose cone fitted to a rodent stereotactic frame.



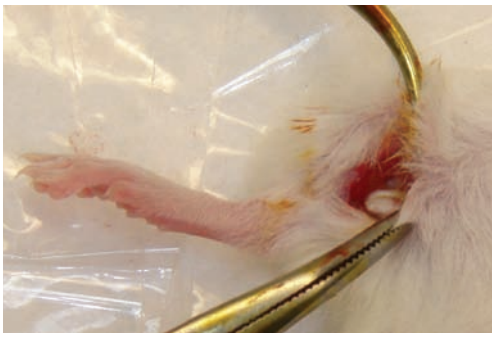
2. After disinfection, a skin incision was made on the medial aspect of both hindlegs.



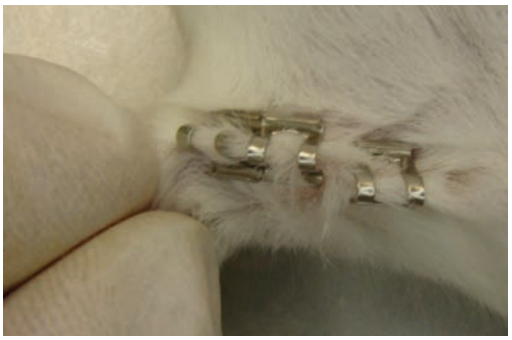
3. An intramuscular pocket was created in the quadriceps muscle using blunt dissection.



4. A half gelatine capsule containing the graft was placed within the intramuscular pocket.

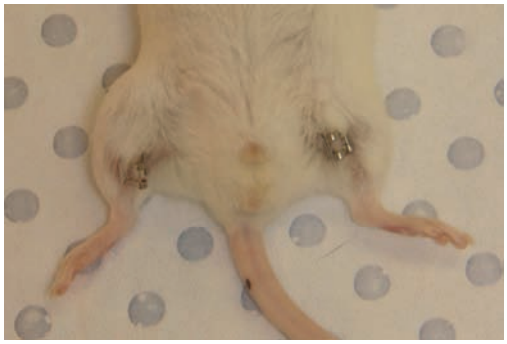


5. The implanted graft was checked to ensure proper placement within the intramuscular pocket.

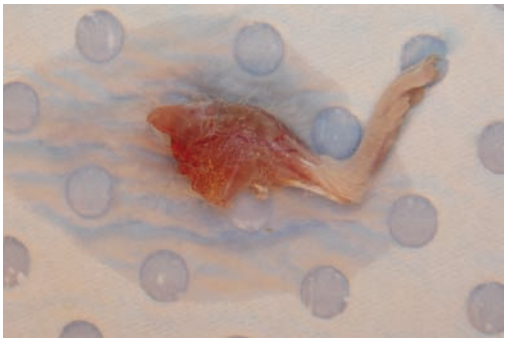


6. After implantation of the graft, the incision was closed with metal staples.

3.3 Appendix Two: Retrieval Protocol

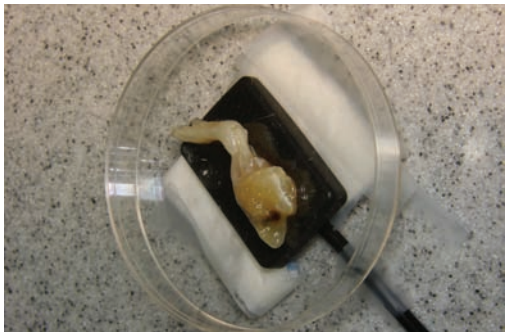


1. At the end of the implantation period, the animal was sacrificed by means of CO_2 asphyxiation.



2. Both hindquarters of each animal were removed followed by removal of skin and fur.

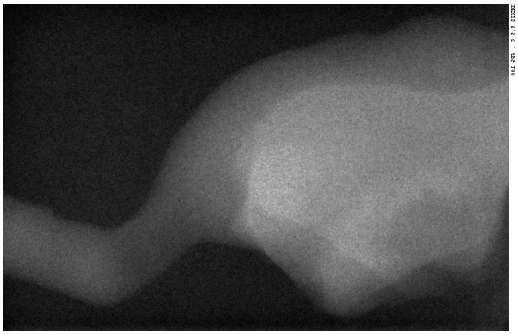
3.4 Appendix Three: Radiographic Protocol



1. The specimen was placed on a device incorporating the dental radiography sensor.

2. The dental radiographic tube was positioned directly above the sensor at a standardised distance for all samples.

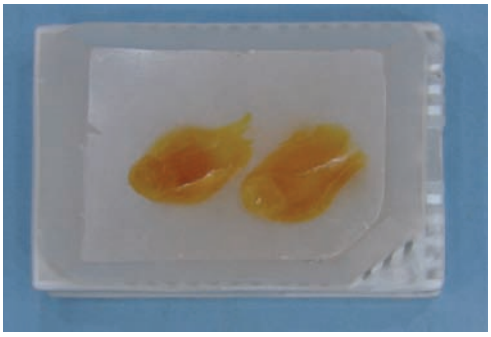
3.5 Appendix Four: Histological Preparation Protocol



1. After decalcification, radiographs were taken of the hindlimbs to ensure complete decalcification of the specimen

2. Prior to embedding, the paw and superior portion of the specimen were excised and discarded. The remaining specimen was sectioned in half according to the location of the graft material as determined by the post retrieval radiographs.

3. The sectioned specimens were oriented with the adjacent cut surfaces facing superiorly.



4. The specimens were paraffin embedded in this orientation.

3.6 Appendix Five: Semi-quantitative analysis of experimental groups at 4 weeks

4 WEEK	Category	HA-TCP	HA-TCP + PDGF	HA-TCP + EMD
Acute Inflammation	0	5	5	10
	1	7	10	5
	2	0	0	0
	3	0	0	0
	4	0	0	0
Chronic Inflammation	0	0	0	0
	1	2	9	3
	2	10	6	11
	3	0	0	1
	4	0	0	0
Resorption/Foreign Body Reaction	0	2	3	4
	1	10	12	11
	2	0	0	0
	3	0	0	0
	4	0	0	0
Fibrosis - Distribution	0	0	0	0
	1	0	2	0
	2	0	2	0
	3	6	4	2
	4	6	7	13
Fibrosis - Density	0	0	0	0
	1	0	2	0
	2	2	8	1
	3	8	5	4
	4	2	0	9
Vascularity - Distribution	0	0	0	0
	1	2	7	6
	2	7	4	5
	3	3	2	4
	4	0	2	0
Vascularity - Area	0	0	0	0
	1	5	5	9
	2	3	4	5
	3	4	1	0
	4	0	5	1
Adipose - Distribution	0	6	5	13
	1	4	3	1
	2	2	1	1
	3	0	6	0
	4	0	0	0
Adipose - Area	0	6	5	13
	1	4	2	1
	2	1	1	0
	3	1	2	1
	4	0	5	0
Lining cell thickness	0	0	0	0
	1	3	10	5
	2	7	5	8
	3	2	0	2
	4	0	0	0
Osteoinduction	0	12	15	15
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0

Numbers represent the number of analysed sections included in each category at 4 weeks

3.7 Appendix Six: Semi-quantitative analysis of experimental groups at 8 weeks

8 WEEK	Category	HA-TCP	HA-TCP + PDGF	HA-TCP + EMD
Acute Inflammation	0	14	12	13
	1	1	1	2
	2	0	0	0
	3	0	0	0
	4	0	0	0
Chronic Inflammation	0	10	3	6
	1	5	10	9
	2	0	0	0
	3	0	0	0
	4	0	0	0
Resorption/Foreign Body Reaction	0	13	10	13
	1	2	3	2
	2	0	0	0
	3	0	0	0
	4	0	0	0
Fibrosis - Distribution	0	0	0	0
	1	0	1	0
	2	0	1	0
	3	4	7	11
	4	11	4	4
Fibrosis - Density	0	0	0	0
	1	3	1	1
	2	3	3	3
	3	7	8	8
	4	2	1	3
Vascularity - Distribution	0	3	0	1
	1	2	2	6
	2	6	3	6
	3	4	8	2
	4	0	0	0
Vascularity - Area	0	3	0	1
	1	5	6	12
	2	7	3	1
	3	0	4	1
	4	0	0	0
Adipose - Distribution	0	11	9	3
	1	3	3	1
	2	0	0	5
	3	1	1	5
	4	0	0	1
Adipose - Area	0	11	9	3
	1	3	3	2
	2	0	0	1
	3	1	0	4
	4	0	1	5
Lining cell thickness	0	0	0	0
	1	12	6	11
	2	1	7	3
	3	2	0	1
	4	0	0	0
Osteoinduction	0	15	13	15
	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0

Numbers represent the number of analysed sections included in each category at 8 weeks