

# **HEALING PATTERNS OF TRANSPLANTED ROOTS COATED WITH AN ALLOGENEIC FIBRIN-FIBRONECTIN CONCENTRATE**

A HISTOLOGICAL STUDY ON THE CHACMA BABOON *PAPIO URSINUS*

Simitha Singh-Rambiritch

A research report submitted to the Faculty of Health Sciences,  
University of the Witwatersrand, Johannesburg,  
in partial fulfilment of the requirements for the degree  
of  
Master of Science

Johannesburg, 2012

## DECLARATION

I hereby declare that this research report is my own unaided work, except where due acknowledgement for assistance received has been made. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been presented before for any degree or examination at this or any other University.

.....

Simitha Singh-Rambiritch

signed this ..... day of ..... 2012

The work reported in this thesis was performed at the Bone Research Unit, School of Physiology, Medical Research Council/University of the Witwatersrand, Johannesburg, South Africa.

To my parents

Dewakpersad and Chandini Singh

## ABSTRACT

This experiment was designed to evaluate whether an allogeneic fibrin-fibronectin protein concentrate (AFFP) can not only prevent ankylosis and root resorption of autotransplanted roots during healing but contribute to regenerate a periodontal attachment as well. In two adult male baboons (*Papio ursinus*), four horizontal alveoli, 2 to 3 mm deep, were prepared bilaterally in the buccal alveolar and basal bone adjacent to the first and second mandibular molars to receive the roots of the adjacent two molars. Following hemisection, the first and second mandibular molars were extracted, the coronal two-thirds of the roots were planed to remove the remnants of the periodontal ligament and cementum and a notch was placed at the junction between the planed and non-planed surfaces. The planed surfaces were demineralised with citric acid at pH 1 for 3 min. Before transplantation, the crowns were resected and the experimental roots and alveoli were coated with the AFFP prepared from pooled fresh-frozen baboon plasma. The animals were killed 55 days after the transplantations. Histometrical evaluation was performed on serial sections cut in a bucco-lingual direction parallel to the long axis of the transplanted roots. An analysis of variance, in relation to the extent of ankylosis and root resorption, revealed minimal differences between the treatments of experimental and control roots both in the planed and non-planed sections. In this primate autotransplantation model, the treatment with AFFP did not prevent ankylosis and root resorption and did not result in the establishment of a new periodontal attachment.

*Key words:* non-human primates, autotransplanted roots, fibrin-fibronectin concentrate.

## ACKNOWLEDGEMENTS

The author wishes to express her sincere gratitude and appreciation to the following persons and organizations whose contributions facilitated the publication of this work:

- ◆ Professor Ugo Ripamonti, Ph.D., Director of the Bone Research Unit, School of Physiology, Medical Research Council/University of the Witwatersrand, for sharing his scientific expertise, inspiring guidance and critical review of the manuscript;
- ◆ Professor Jean-Claude Petit, MDent, Honorary Senior Researcher at the Bone Research Unit, School of Physiology, University of the Witwatersrand, for his guidance, support, and critical review of the manuscript;
- ◆ Mrs Louise Renton, Research Scientist at the Bone Research Unit, School of Physiology, University of the Witwatersrand, for her expert assistance in using the programme for digital histomorphometry;
- ◆ Miss Felicity Meyer, School of Oral Medicine and Periodontology, University of the Witwatersrand; for her assistance
- ◆ Mrs Petra Gaylard at Data Management & Statistical Analysis, for her expert assistance in the analysis of data;
- ◆ My husband Euvir Rambiritch for his constant support and encouragement.

## TABLE OF CONTENTS

DECLARATION .....	ii
DEDICATION .....	iii
ABSTRACT .....	iv
ACKNOWLEDGEMENTS .....	v
TABLE OF CONTENTS .....	vi
LIST OF FIGURES .....	viii
LIST OF TABLES .....	xi
ABBREVIATIONS .....	xii
1 INTRODUCTION .....	1
1.1 Review of the literature .....	2
1.2 Historical background .....	6
1.3 Fibrin-fibronectin protein concentrate .....	7
2 AIM .....	9
2.1 Objectives .....	9
3 MATERIALS AND METHODS .....	11
3.1 Summary of the experimental procedure .....	11
3.2 Statistical analysis .....	12
3.3 Histomorphometry .....	15
4 RESULTS .....	19
4.1 Histomorphological examination .....	19
4.2 Statistical analysis .....	26
5 DISCUSSION .....	35

6	CONCLUSIONS .....	38
7	REFERENCES .....	39
8	ADDENDA .....	45
8.1	Review of the surgical procedure .....	46
8.2	Tables and figures obtained from the statistical analysis .....	54

## LIST OF FIGURES

<i>Figure</i>	<i>Legend</i>	<i>Page</i>
1	Schematic representation of the experimental procedure	6
2	Photomicrograph from the slide number A113-85 (Animal 1) illustrating the mucosal and bony sides and the level of the notch.	19
3	Photomicrograph from slide number A113-85 (Animal 1) showing cementoblasts and new cementum on the root surface apical to the notch on the mucosal side in an AFFP-treated specimen.	20
4	Photomicrograph from slide number A119-85 (Animal 1) of a control specimen showing new attachment on the root surface at the bony interface apical to the notch.	21
5	Magnification of Figure 4 showing new cementum and cementoblasts lining the root surface with collagen fibres inserted perpendicularly into the cementum matrix.	21
6	Photomicrograph of a control specimen from slide number A120-85 (Animal 1) showing new cementum with fibres embedded obliquely at the bony interface apical to the notch.	22
7	Photomicrograph from the slide number A124-85 (Animal 1) of a control specimen showing inserted oblique fibres and non-inserted fibres parallel to the root surface on the mucosal side coronal to the notch.	22



8	Photomicrograph from slide number A136-85 (Animal 2) of an area of root resorption in a control specimen on the bony side coronal to the notch. Resorption lacunae are surfaced by macrophages with enlarged nuclei.	23
9	High power photomicrograph from slide number A83-85 (Animal 2) of an AFFP-treated specimen showing an area of root resorption with absence of macrophages on the bony side coronal to the notch.	24
10	Photomicrograph from slide number A87-85 (Animal 2) of an AFFP-treated specimen showing areas of ankylosis on the bony side coronal to the notch.	25
11	Photomicrograph from slide number A87-85 (Animal 2) of an AFFP-treated specimen showing areas of ankylosis on the bony side coronal to the notch.	26
12	Bland-Altman plot	27
13	A flap is reflected to prepare recipient alveoli in the buccal alveolar and basal bone of the right side of the mandible in animal 1. First and second molars are hemisected.	48
14	Preparation of the recipient alveoli in the right side of the mandible of animal 1. The alveoli are designated as A1PUR, A1PLR, A1AUR, A1ALR (see abbreviations on page x).	49
15	A notch separates the planed from the non-planed root surface.	50
16	Injection of AFFP in the recipient alveoli	51
17	Placement of the resected roots in the recipient alveoli. The experimental roots are further coated with AFFP.	51

18	Resected roots embedded in the alveoli	52
19	Closure of the operated site	52

## LIST OF TABLES

<i>Table</i>	<i>Legend</i>	<i>Page</i>
3.1	Number of histological slides per root suitable for histomorphology and histometry	13
3.2	Number of histological slides analysed per treatment, sites and animal	14
4.1	Intra-observer reliability	26
4.2	Percentage of NA in each of the four regions together with the 95 % confidence interval. Results for the treatment effect for the ANOVA are also shown.	29
4.3	Percentage of RR in each of the four regions together with the 95 % confidence interval. Results for the treatment effect for the ANOVA are also shown.	30
4.4	Percentage of A in each of the four regions together with the 95 % confidence interval. Results for the treatment effect for the ANOVA are also shown.	31
4.5	Percentage of NI in each of the four regions together with the 95 % confidence interval. Results for the treatment effect for the ANOVA are also shown.	33

## ABBREVIATIONS

A	ankylosis
A1PUR	animal 1, posterior upper right
A1PLR	animal 1, posterior lower right
A1AUR	animal 1, anterior upper right
A1ALR	animal 1, anterior lower right
A1PUL	animal 1, posterior upper left
A1PLL	animal 1, posterior lower left
A1AUL	animal 1, anterior upper left
A1ALL	animal 1, posterior lower left
A2PUR	animal 2, posterior upper right
A2PLR	animal 2, posterior lower right
A2AUR	animal 2, anterior upper right
A2ALR	animal 2, anterior lower right
A2PUL	animal 2, posterior upper left
A2PLL	animal 2, posterior lower left
A2AUL	animal 2, anterior upper left
A2ALL	animal 2, anterior lower left
AFFP	allogeneic fibrin-fibronectin protein concentrate
BMP(s)	bone morphogenetic protein(s)
B:N-A	bony interface: from notch to apex
B:N-C	bony interface: from notch to crown
° C	degree Celsius/centigrade
CH <sub>3</sub> COOH	acetic acid
IM	intramuscular

IV	intravenous
KPa	international unit of pressure
M:N-A	mucosal interface: from notch to apex
M:N-C	mucosal interface: from notch to crown
n°	number
NA	new attachment
NI	non-inserted fibres
PBS	phosphate-buffered saline
rpm	rotation per minute
RR	root resorption

#### Statistical abbreviations

A	anterior upper and lower alveoli
C or c	control
DV	dependant variable
Ex	experimental
F	F-ratio
ICC	intraclass correlation coefficient
MS	mean square
p	p-value
P	posterior upper and lower alveoli
propn	proportion
RSD	relative standard deviation
SS	sum of squares
Std Dev	standard deviation
Std Err	standard error