

https://dspace.org/

Healing patterns of transplanted roots coated with an allogeneic fibrin-fibronectin concentrate

Sing-Rambiritch, Simitha

2013-03-18
http://hdl.handle.net/10539/12541

Downloaded from a DSpace repository. https://dspace.org/

3 MATERIALS AND METHODS

All the biochemical and histological preparatory laboratory procedures and the clinical experimental procedures under this heading have been previously accomplished and published by Ripamonti *et al*. (1987, 1989). However, the material described in this research report has neither been evaluated nor reported. The research protocol has been previously submitted to and approved by the Animal Ethics Committee of the University of Witwatersrand under the clearance n° 84/78/04.

3.1 Summary of the experimental procedure

In two adult male baboons (*Papio ursinus*), the first and second mandibular molars were hemisected and extracted. The coronal two-thirds of the roots were planed to remove the remnants of the periodontal ligament and the cementum, and a notch was placed at the junction between the planed and non-planed surfaces. The planed surfaces were demineralised with citric acid and then the crowns were resected. In the meantime, the recipient alveoli beds had been prepared bilaterally in the buccal cortical surfaces of the alveolar and the basal bone of the mandible. In the course of the transplantations, the experimental roots and alveoli were coated with the AFFP concentrate prepared from pooled fresh-frozen baboon plasma. Fibrin and fibronectin were isolated by IMMUNO Ag Vienna, Austria, to produce the adhesive system. The histological slides were prepared from the transplanted roots and the histometrical evaluation was performed on serial sections cut in a bucco-lingual direction parallel to the radicular long axis. The

specimen blocks yielded up to 10 sections taken 100-150 µm apart and were stained with haematoxylin and eosin or picro-Mallory connective tissue stain. Classification of the morphology of the root surfaces were done according to the healing pattern forming at the osseous and mucosal interface respectively: ankylosis, resorption, and newly-deposited cementum and connective tissue fibres. The investigation involved a histological and histometrical analysis followed by a statistical analysis.

3.2 Statistical analysis

The study material consisted of 16 specimens, 8 experimental and 8 control roots. The histological material consisted of 84 slides prepared from the 16 transplanted roots. This sample size was determined after consultation with a statistician. Experimental roots were those transplanted in the right side of the mandible whilst the control roots were those transplanted in the left side. In both sides of the mandible of both animals, the four roots were positioned in two rows and two columns and labelled as follows:

Experimental roots

Animal 1: posterior upper right (A1PUR), posterior lower right (A1PLR), anterior upper right (A1AUR), anterior lower right (A1ALR)

Animal 2: posterior upper right (A2PUR), posterior lower right (A2PLR), anterior upper right (A2AUR), anterior lower right (A2ALR)

Control roots

The labelling was identical and only the letter R was replaced by L for the left hand side of the mandible.

Structure of the main data set

Table 3.1 Number of histological slides per roots suitable for histomorphology and histometry

	Sites	Number of slides available	Number of slides examined
Experimental	A1PUR	4	4
	A1PLR	10	9
	A1AUR	4	3
	A1ALR	7	7
	A2PUR	6	4
	A2PLR	5	2
	A2AUR	7	3
	A2ALR	3	3
Control	A1PUL	6	5
	A1PLL	6	6
	A1AUL	2	2
	A1ALL	2	0
	A2PUL	6	6
	A2PLL	7	6
	A2AUL	3	3
	A2ALL	6	5
Total	16	84	68

There was no data for one of the control units (A1 ALL). The data set was well balanced in terms of the covariates: animal and treatment, but slightly unbalanced in terms of tooth position (posterior vs. anterior). See table below.

Table 3.2 Number of histological slides examined per treatment, sites and animal

	n° of slides examined
Experimental	35
Control	33
Total	**68**
Posterior	42
Anterior	26
Total	**68**
Animal 1	36
Animal 2	32
Total	**68**

The suitable histological material consisted of 68 slides prepared from 15 transplanted roots and measurements were taken from four regions, namely:

1 Mucosal side: notch to the coronal border of the root surface (M:N-C)

2 Mucosal side: notch to apex (M:N-A)

3 Bony side: notch to the coronal border of the root surface (B:N-C)

4 Bony side: notch to apex (B:N-A)

For each region, four measurements were taken:

Percentage of new attachment (NA)

Percentage of non-inserted fibres (NI)

Percentage of ankylosis (A)

Percentage of root resorption (RR)

3.3 Histomorphometry

Out of the 84 slides available, 12 of them were not suitable for evaluation. The reasons were folds in the section preventing precise measurements and loss of notches due to root resorption. The histological slides were scanned and digitalized at a 20-time magnification, 20 pixels, to take the measurements on a computer screen using the Stream Essentials programme with linear distances being measured both on the osseous and mucosal interfaces.

The following linear distances were measured:

1 B:N-C: at the interface of the roots with bone (B), extent of connective tissue from the apical border of the notch (N) in the root surface to the most coronal portion (C) of the root surface. The linear distances related to various healing patterns, i.e. new cementum with inserted (perpendicular orientation) periodontal fibres (NA), non-inserted (parallel orientation) collagen fibres at interface (NI), ankylosis (A) and root resorption (RR) were measured separately.

2 M:N-C: at the interface of the roots with the mucosa (M), extent of connective tissue from the apical border of the notch (N) in the root surface to the most coronal portion (C) of the root surface. The linear distances related to various healing patterns, i.e. new cementum with inserted (perpendicular orientation) periodontal fibres (NA), non-inserted (parallel orientation) collagen fibres at interface (NI), ankylosis (A) and root resorption (RR) were measured separately.

3 B:N-A: at the interface of the roots with bone (B), extent of connective tissue from the apical border of the notch (N) in the root surface to the apex (A) of the root. The linear distances related to various healing patterns, i.e. new cementum with inserted (perpendicular orientation) periodontal fibres (NA), non-inserted (parallel orientation) collagen fibres at interface (NI), ankylosis (A) and root resorption (RR) were measured separately.

4 M:N-A: at the interface of the roots with the mucosa (M), extent of connective tissue from the apical border of the notch (N) in the root surface to the apex (A) of the root. The linear distances related to various healing patterns, i.e. new cementum with inserted (perpendicular orientation) periodontal fibres (NA), non-inserted (parallel orientation) collagen fibres at interface (NI), ankylosis (A) and root resorption (RR) were measured separately.

Each of these measurements was taken in micrometers and was then expressed as a percentage of the total N-C (or N-A) distance, so the total of the four measurements is 100 %.

This method of analysis was planned in consultation with a statistician. To determine the inter-observer reliability (i.e. observer1 or applicant researcher, and observer 2 or supervisor and expert in the technique) of the measurements for ankylosis, ten sections were selected at random and all measurements were repeated ten times on different days,

without reference to the original data. The inter-observer reliability was formally assessed by means of the intraclass correlation coefficient (ICC) derived from a two-way Analysis of Variance (with interaction), with slides as a random effect and observers as a fixed effect. The ICC is defined as the proportion of the overall variance in the measurements attributable to the objects of the measurements (in this case, the slides). The intra-observer (test-retest) reliability was determined by the same method. Results were statistically assessed by means of the paired sample *t*-test. To be unbiased, the observers did all measurements without knowing which roots were in the control or experimental group. Data analysis was carried out using the software Statistica version 10 (Statistica, 2011). The 95 % confidence level was used throughout. Intra-observer reliability was assessed by calculating the relative standard deviation (RSD) for each of the 10 slides measured by each observer (see p. 25 Table 4.1 in Results).

Data were analysed using a linear model procedure for unbalanced multiple analysis of variance (ANOVA). The analysis of variance revealed differences between the experimental and control specimens with regard to the length of new cementum with inserted (perpendicular orientation) periodontal fibres (NA), non-inserted (parallel orientation) collagen fibres at interface (NI), ankylosis (A) and root resorption (RR).

The approach used in this study was to replace the data by the ranks of the observations and then the ANOVA was carried out on the ranks. The results were compared to the ANOVA carried out on the untransformed data.

The ANOVA was carried out with the following factors:

- Treatment (levels: experimental, control)
- Animal (levels: animal 1 (A1), animal 2 (A2))
- Location (levels: posterior, anterior)

All three two-factor and the one three-factor interaction were included as covariates in the model as for a statistical analysis of a previous similar study (Ripamonti, 1985). The data set was quite unbalanced: the number of slides per treatment-animal-location combination ranges from 2 to 13. Post-hoc comparisons were done using the Unequal N HSD test since data were unbalanced.