4 RESULTS

4.1 Histomorphological examination

The histomorphological examination revealed alternating patterns of root resorption, ankylosis, non-inserted fibres and occasional new attachment on both the bony and mucosal sides along the longitudinal surface of most sections (Fig. 2). However, RR, A and NI were the dominant features along the experimental and control roots (see statistical analysis below on pages 28 to 34).

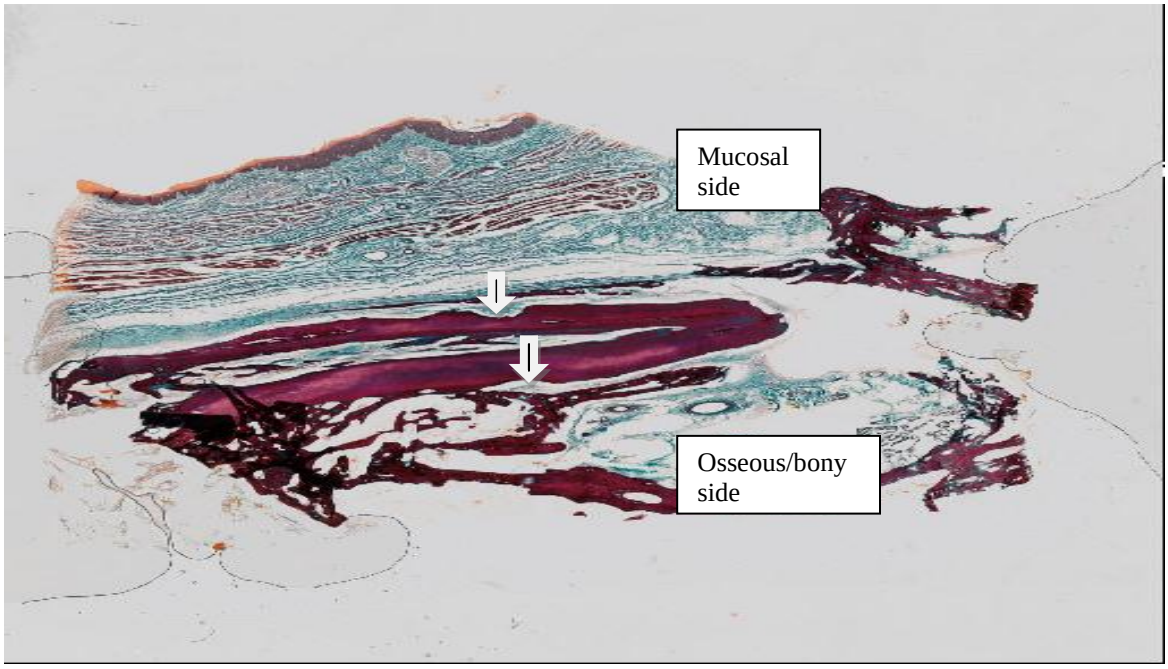

Figure 2 Photomicrograph from the slide number A113-85 (Animal 1) illustrating the mucosal and bony sides and the level of the notch.

NA was occasionally seen in the apical region below the notch (Figs 3).

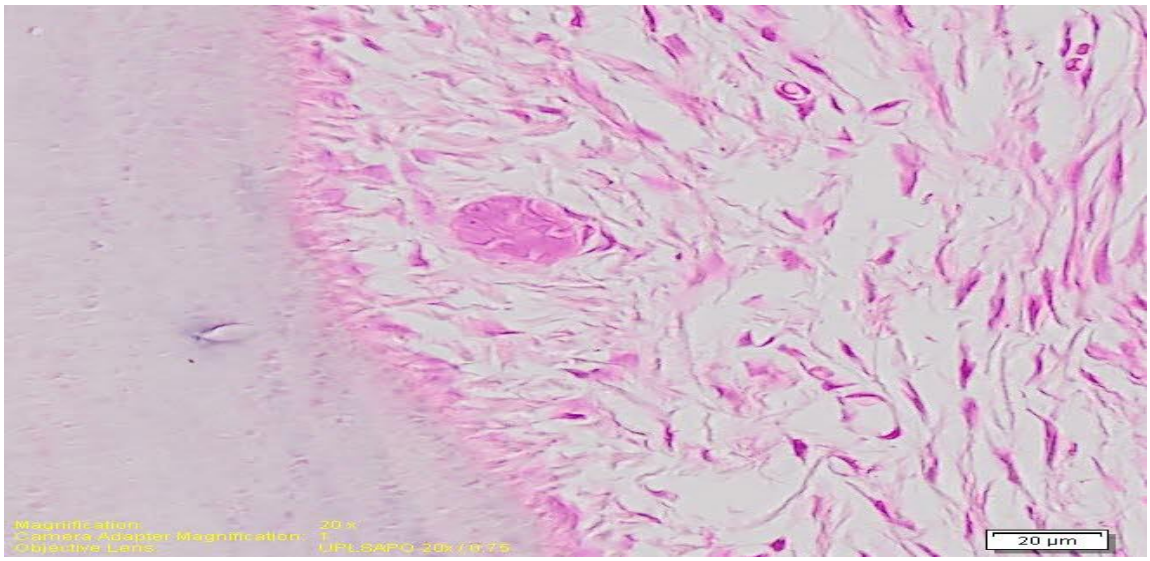

Figure 3 Photomicrograph from slide number A113-85 (Animal 1) showing cementoblasts and new cementum on the root surface apical to the notch on the mucosal side in an AFFP-treated specimen.

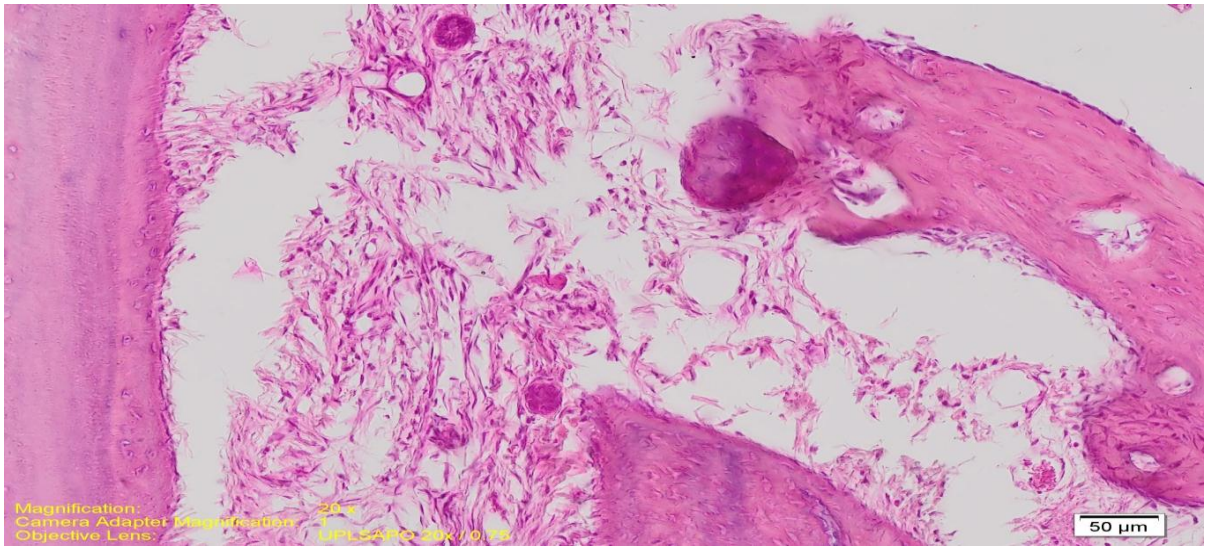

Figure 4 Photomicrograph from slide number A119-85 (Animal 1) of a control specimen showing new attachment on the root surface at the bony interface apical to the notch.

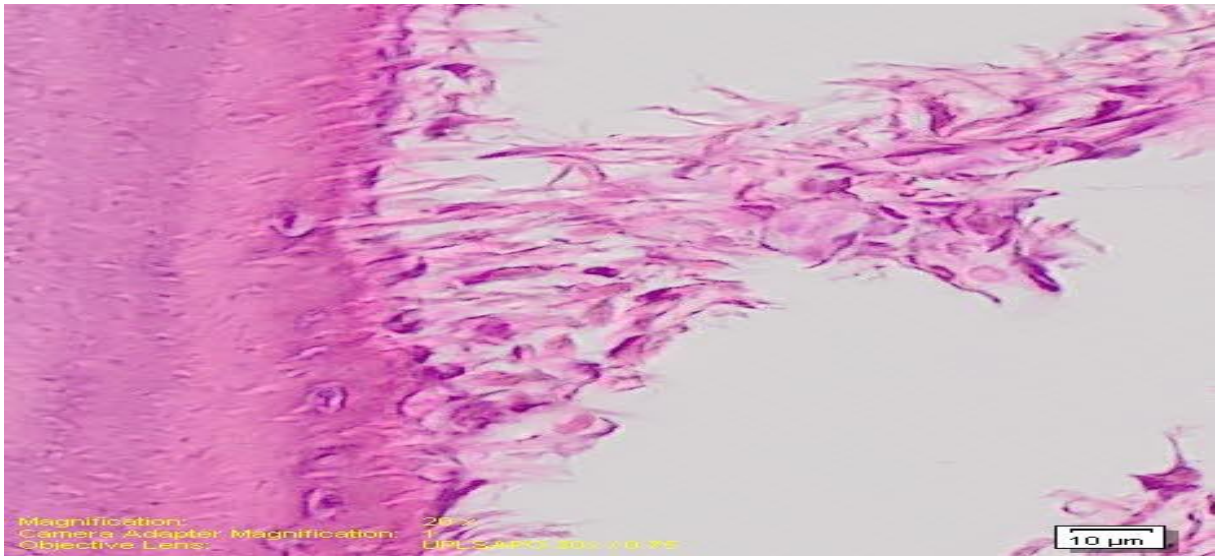

Figure 5 Magnification of Figure 4 showing new cementum and cementoblasts lining the root surface with collagen fibres inserted perpendicularly into the cementum matrix.

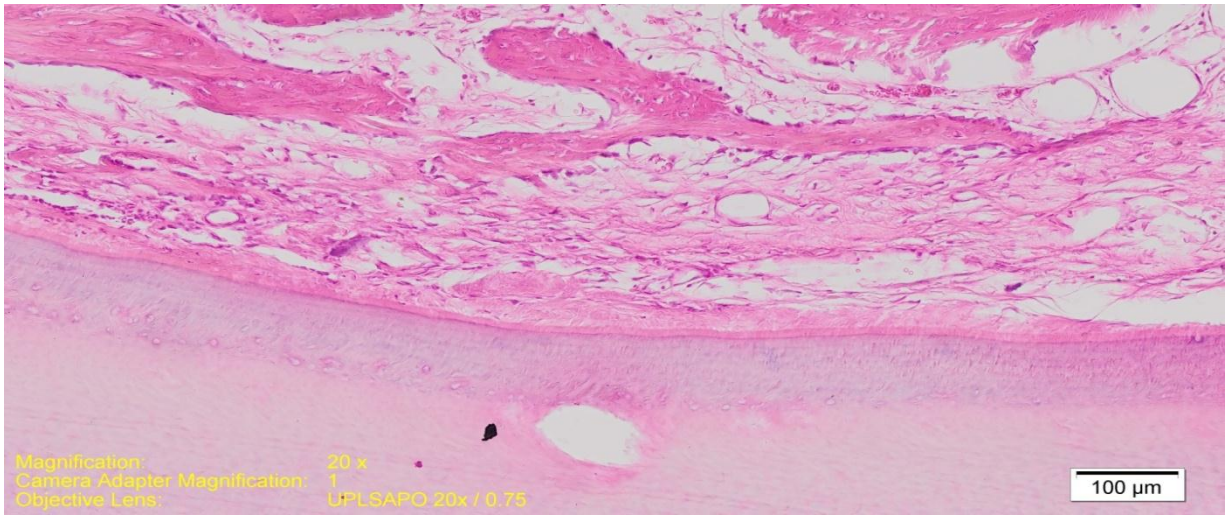

Figure 6 Photomicrograph of a control specimen from slide number A120-85 (Animal 1) showing new cementum with fibres embedded obliquely at the bony interface apical to the notch.

In one specimen, on the mucosal side, fibres appeared to be inserted obliquely into the planed dentine (Fig. 7).

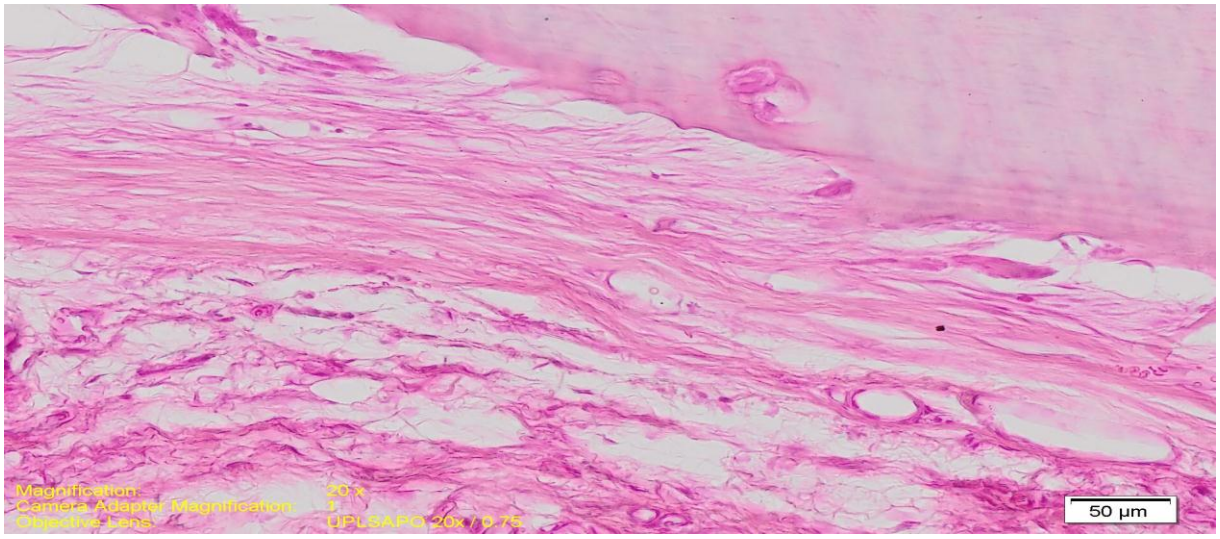

Figure 7 Photomicrograph from the slide number A124-85 (Animal 1) of a control specimen showing inserted oblique fibres and non-inserted fibres parallel to the root surface on the mucosal side coronal to the notch.

From both mucosal and bony sites, RR was characterised by macrophages or osteoclasts with large nuclei forming linear or curved rows (Fig. 8) in contact with the dentinal resorbed surfaces.

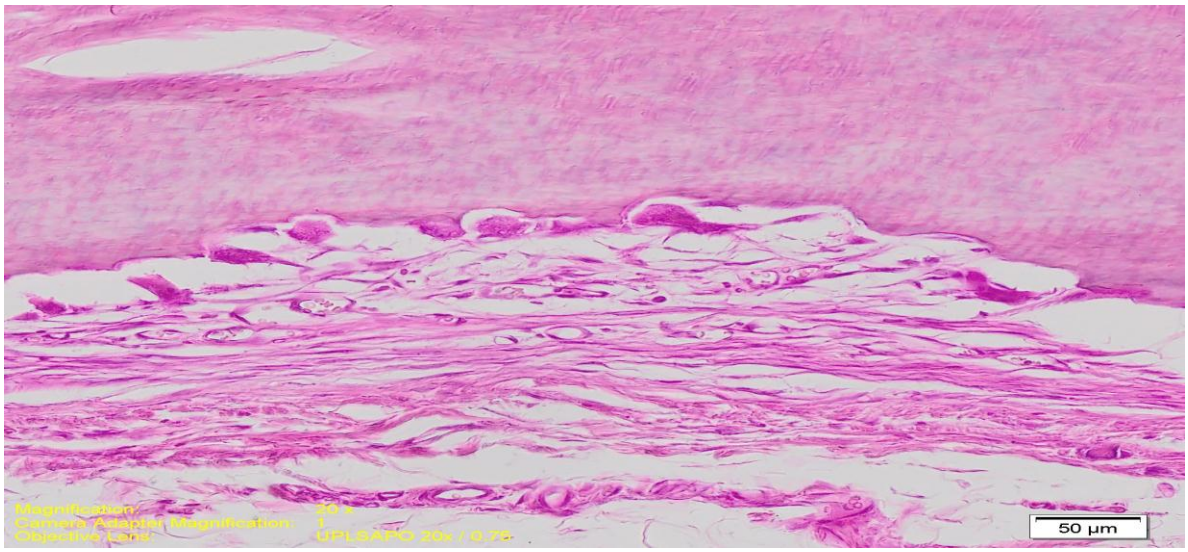

Figure 8 Photomicrograph from slide number A136-85 (Animal 2) of an area of root resorption in a control specimen on the bony side coronal to the notch. Resorption lacunae are surfaced by dentinoclasts with enlarged nuclei.

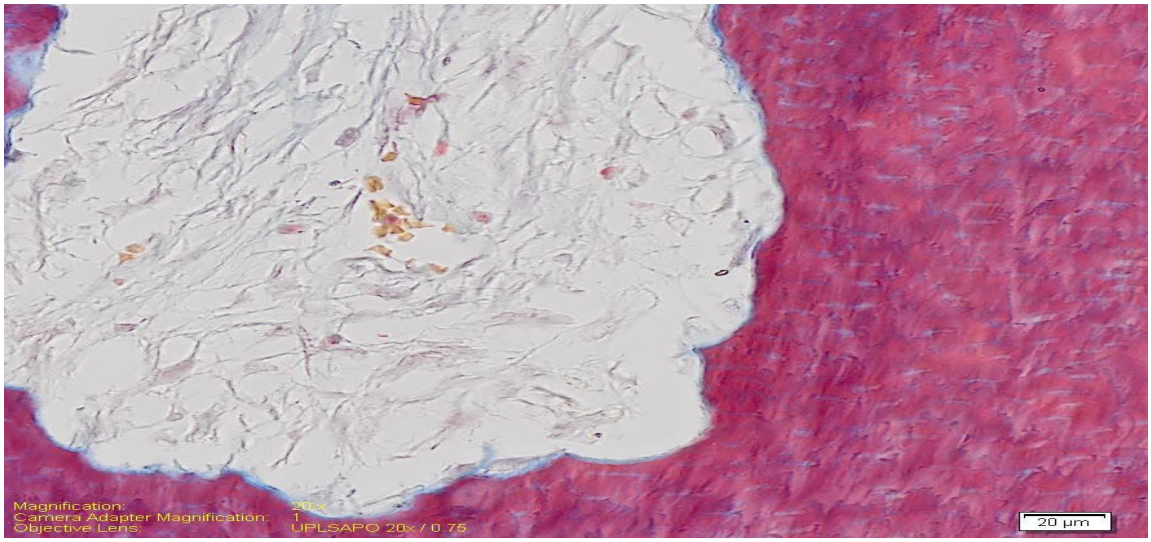

Figure 9 High power photomicrograph from slide number A83-85 (Animal 2) of an AFFP-treated specimen showing an area of root resorption with absence of osteoclasts on the bony side coronal to the notch.

Bridges of dento-alveolar ankylosis were frequently seen with adjacent lacunae apical and coronal to the notch (Fig. 10).

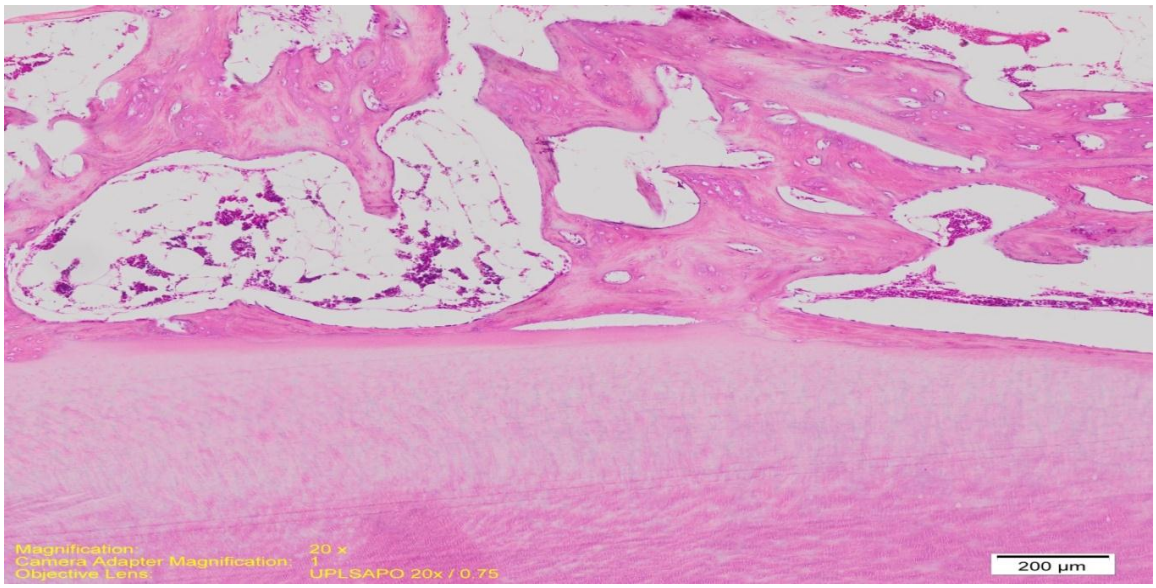

Figure 10 Photomicrograph from slide number A87-85 (Animal 2) of an AFFP-treated specimen showing areas of ankylosis on the bony side coronal to the notch.

Lacunae did not exhibit resorptive areas but contained multiple capillaries surrounded by a loose network of non-inserted connective tissue fibres containing few oval or fusiform cells (Fig. 10). On serial sections, those lacunae appeared to be connected to the marrow spaces of the adjacent bony side.

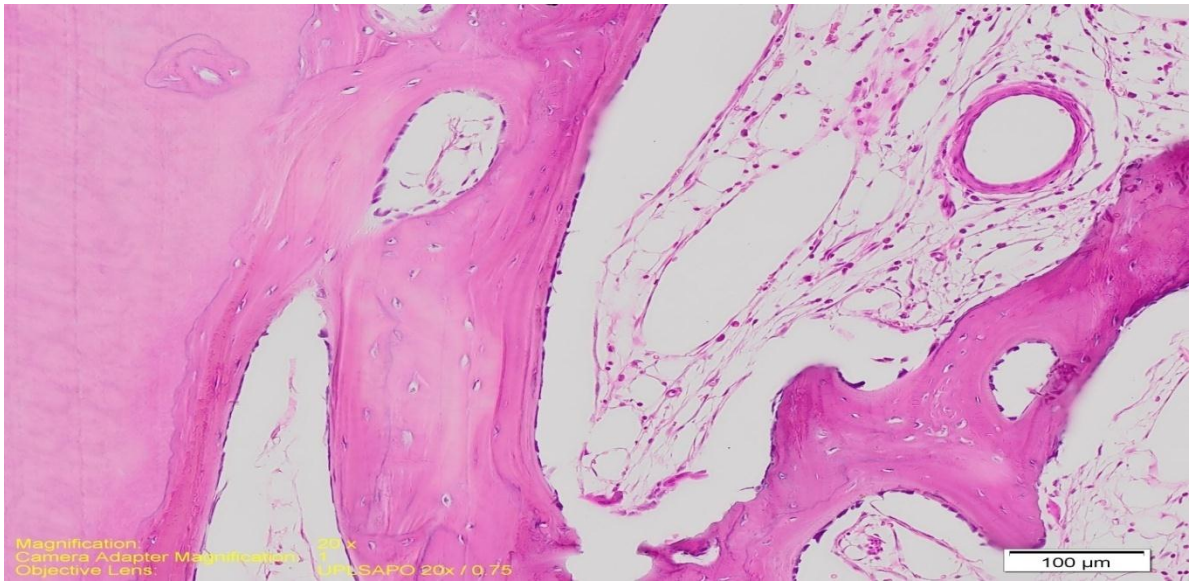

Figure 11 Photomicrograph from slide number A87-85 (Animal 2) of an AFFP-treated specimen showing areas of ankylosis on the bony side coronal to the notch.

4.2 Statistical analysis

Table 4.1 Intra-observer reliability in histological measurements

	Mean (µm)		RSD (%)	
Slide n°	Observer 1	Observer 2	Observer 1	Observer 2
118	369	349	3.88 %	0.37 %
86	483	468	1.52 %	0.15 %
133	1761	1721	1.35 %	0.06 %
83	1831	1856	1.68 %	0.15 %
127	2060	1987	2.99 %	0.04 %
124	2393	2335	1.93 %	0.03 %
87	2493	2455	1.46 %	0.03 %
126	2789	2679	1.60 %	0.02 %
117a	3204	3175	1.38 %	0.01 %
117	3306	3284	1.39 %	0.01 %

The results in the above table indicate that the RSDs for the measurements made by observer 2 (expert) were an order of magnitude lower than those for the measurements made by observer 1 (applicant), presumably as a result of observer 1 recent experience. However, the RSDs for observer 2 are all below 5 %, and mostly below 2 %. Comparing the mean measurements obtained by the two observers for each of the 10 slides, revealed that observer 1 tended to obtain slightly higher results than observer 2 in most cases. The Bland-Altman plot (Fig. 12 below) shows that the bias does not increase with the average magnitude of the measurements (Altman and Bland, 1983; Bland and Altman, 1986).

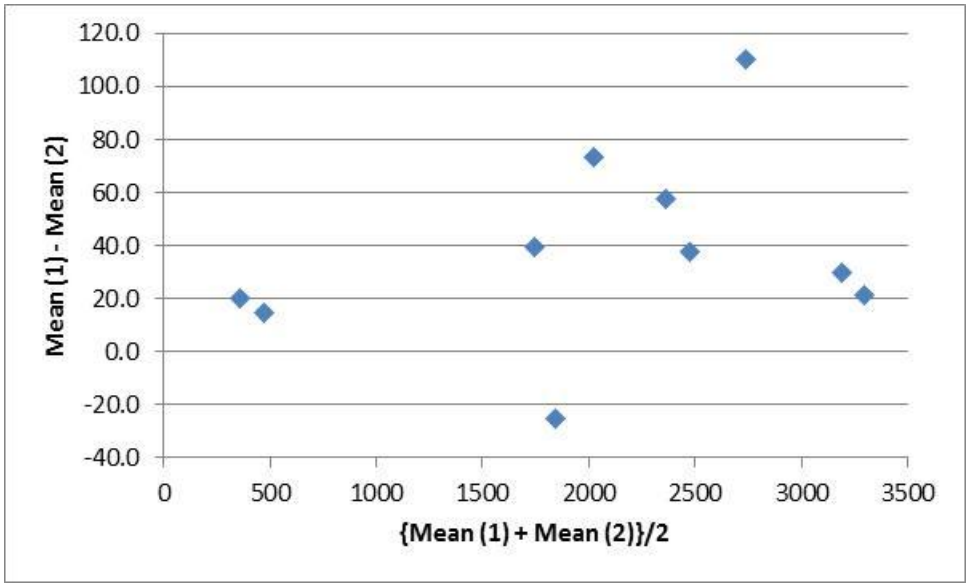

Figure 12 Bland-Altman plot

In this case, the ICC = 0.99 which indicates excellent interobserver agreement for the measurement of ankylosis (Shoukri, 2004).

Analysis of the data: general overview

The frequency distributions of the response variables (see 8.2, pages 54 to 73 in the Addenda) were very positively skewed for RR, NA and A, but negatively skewed for NI as a result of the presence of only NI on many slides. Thus the data have to be transformed for the Analysis of Variance (ANOVA) since this analysis requires the data to be (approximately) normally distributed.

For percentage (proportion) data, there are typically several options for transformation:

- Logit: ln(p/1-p) (where p = proportion): not valid for p = 0
- Arcsin square root: arcsin(sqrt(p)): not valid for p = 0

In both the above cases, zero values for p were replaced by very small values, but in this case this resulted in the retention of the very skew frequency distribution of the data.

The results of the histometrical analysis and the significant probability values are presented in the tables 4.2 to 4.5 below.

New attachment (NA)

Table 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.

	Average percentage of NA with 95 % confidence interval		ANOVA results for difference between treatment means (ranked data)	
Region	Experimental	Control	F	p
M: N-C	5.0 ± 4.9	6.4 ± 6.3	0.66	0.42
B: N-C *	2.8 ± 4.7	8.6 ± 6.6	2.33	0.13
M: N-A	6.9 ± 7.7	10.9 ± 9.6	0.0001	0.99
B: N-A **	10.0 ± 8.3	5.6 ± 6.8	0.12	0.73
Overall ***	6.4 ± 5.5	7.6 ± 3.6	4.21	0.045

* Significant effect for treatment per animal and per location (F = 5.3, p = 0.025)
Control / A1 / Posterior had higher percentage of NA than other combinations
** Significant effect for animal per location (F = 4.72, p = 0.034)

A1 / Anterior had higher percentage of NA than other combinations

*** Significant effect for treatment and location (F = 4.1, p = 0.047)

Control had higher percentage of NA than experimental for posterior only and significant effect of animal (F = 10.6, p = 0.002)

A1 had higher percentage of NA than A2

Thus, there were no significant differences in percentage of NA between the experimental and control groups in any of the four regions.

Overall (all four regions together), the experimental group appeared to have a significantly lower percentage of NA than the control group. The effect size was small (Cohen's d = 0.46). However, bearing in mind that the treatment effect was involved in a higher order effect (treatment and location) which showed that the difference in treatments was significant only for the posterior experimental roots.

Root Resorption (RR)

Table 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.

	Average percentage of root resorption with 95 % confidence interval		ANOVA results for difference between treatment means (ranked data)	
Region	Experimental	Control	F	p
M: N-C *	6.8 ± 5.2	9.5 ± 6.5	0.05	0.83
B: N-C **	2.8 ± 3.8	4.2 ± 3.0	5.87	0.02**
M: N-A ***	7.6 ± 6.8	0.0 ± 0.0	12.4	< 0.001
B: N-A	1.1 ± 1.7	3.0 ± 3.4	0.59	0.44
Overall ****	4.5 ± 2.8	5.0 ± 2.5	0.57	0.45

* Significant effect for treatment per animal and per location (F = 5.4, p = 0.023)
However, post-hoc test (Unequal N HSD) showed no significant differences between groups but significant effect for location (F = 5.6, p = 0.021)
However, post-hoc test (Unequal N HSD) showed no significant differences between groups

** Post-hoc test (Unequal N HSD) showed no significant differences between groups

*** Significant effect for treatment per animal and per location ($F = 12.4$, $p < 0.001$) Experimental / A2 / Posterior had a higher percentage of RR than other combinations

**** Significant effect for treatment per animal and per location ($F = 7.2$, $p = 0.009$) However, post-hoc test (Unequal N HSD) showed no significant differences between groups

There was a significant difference between percentage of RR for the treatment and control groups for the M:N-A measurements. The experimental group appeared to have a higher proportion of RR than the control group. The effect size was moderate (Cohen's d = 0.63). However, it must be accepted that the treatment effect was involved in a higher-order effect (treatment per animal and per location) and that the samples with a high proportion of RR were grouped exclusively in the Experimental / Animal 2 / Posterior group of slides.

There were no significant differences in percentage of RR between the experimental and control groups in any of the other three regions, or overall.

Ankylosis (A)

Table 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.

	Average percentage of ankylosis with 95 % confidence interval		ANOVA results for difference between treatment means (ranked data)	
Region	Experimental	Control	F	p
M: N-C *	9.1 ± 8.5	6.8 ± 5.0	8.07	0.006 **
B: N-C ***	14.1 ± 8.5	7.5 ± 4.2	0.005	0.95
M: N-A ****	9.1 ± 7.4	3.9 ± 4.6	1.11	0.30
B: N-A	3.2 ± 5.2	4.6 ± 5.3	0.06	0.81
Overall *****	9.8 ± 3.7	6.4 ± 2.6	0.96	0.33

* Significant effect for treatment per animal ($F = 6.3$, $p = 0.015$)

Control for A1 had a higher percentage of A than other combinations and significant effect for animal per location ($F = 5.8$, $p = 0.019$)

A1 / Anterior had a higher percentage of A than A2 / Anterior and A2 / Posterior

** Post-hoc test (Unequal N HSD) showed no significant differences between groups

*** Significant effect for location ($F = 11.0$, $p = 0.002$)

Posterior had a higher percentage of A than Anterior

**** Significant effect for animal per location ($F = 20.8$, $p < 0.001$)

A1 / Anterior had a higher percentage of A than other combinations

***** Significant effect for animal and location ($F = 7.1$, $p = 0.01$)

A2 / Anterior had a lower percentage of A than A1 / Anterior and A1 / Posterior

Thus, there were no significant differences in percentage of A between the experimental and control groups in any of the four regions, or overall.

Non-inserted fibres (NI)

Table 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.

	Average percentage of non inserted fibres with 95 % confidence interval		ANOVA results for difference between treatment means (ranked data)	
Region	Experimental	Control	F	p
M: N-C *	79.1 ± 10.3	77.4 ± 9.3	2.59	0.11
B: N-C **	80.2 ± 10.5	79.7 ± 7.9	1.34	0.25
M: N-A ***	76.4 ± 11.5	85.2 ± 10.1	0.27	0.60
B: N-A	85.7 ± 9.7	86.8 ± 8.4	1.06	0.31
Overal ****	79.3 ± 7.3	81.0 ± 5.0	1.49	0.23

* Significant effect for animal per location ($F = 12.0$, $p = 0.001$)

A1 / Anterior had a higher percentage of NI than A2 / Anterior

** Significant effect for location ($F = 4.81$, $p = 0.03$)

Anterior had a higher percentage NI than Posterior

*** Significant effect for animal per location ($F = 22.6$, $p < 0.001$)

A1 / Anterior had a lower percentage of NI than A2 / Anterior and A1 / Posterior

**** Significant effect for animal per location ($F = 7.9$, $p = 0.007$)

A1 / Anterior had a lower percentage of NI than A2 / Anterior

Thus, there were no significant differences in percentage of NI between the experimental and control groups in any of the four regions, or overall.