Chapter 5: Findings

5.1: Materials

PART 1

Histological slides from O'Hara[5] of posterior segments of rat maxillae, comprising three molar teeth were used. These histological slides were prepared from 42 male Sprague-Dawley rats and were examined to investigate the identity of the cells that exhibit changes in levels of nerve growth factor (NGF), NGF receptors (Trk A, Trk B, p75) and calcitonin gene-related peptide (CGRP) expression in the first week after application of orthodontic forces.

5.1.1 Day 3 NGF

At day 3, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M1 and M2), there were inflammation changes detected in bone, i.e. vasodilation of blood vessels, appearance of inflammatory cells. The experimental side also showed generalised staining with increased intensity seen in the PDL (arrow). (Figure 15, 16)

5.1.2 Day 7 NGF

At day 7, the internal control side (left side of the maxillae) showed no discernible change seen in the bone and PDL. On the experimental side (with elastic modules inserted between M1 and M2), increased numbers of inflammatory cells were seen in the bone. Bone morphology appeared to be more trabeculated than Day 3. The experimental side showed intense staining within the pulp, and staining appeared to be concentrated towards the bony 1/3 of the PDL. Also, increased intensity was seen in the PDL (arrow). (Figure 17, 18, 19)

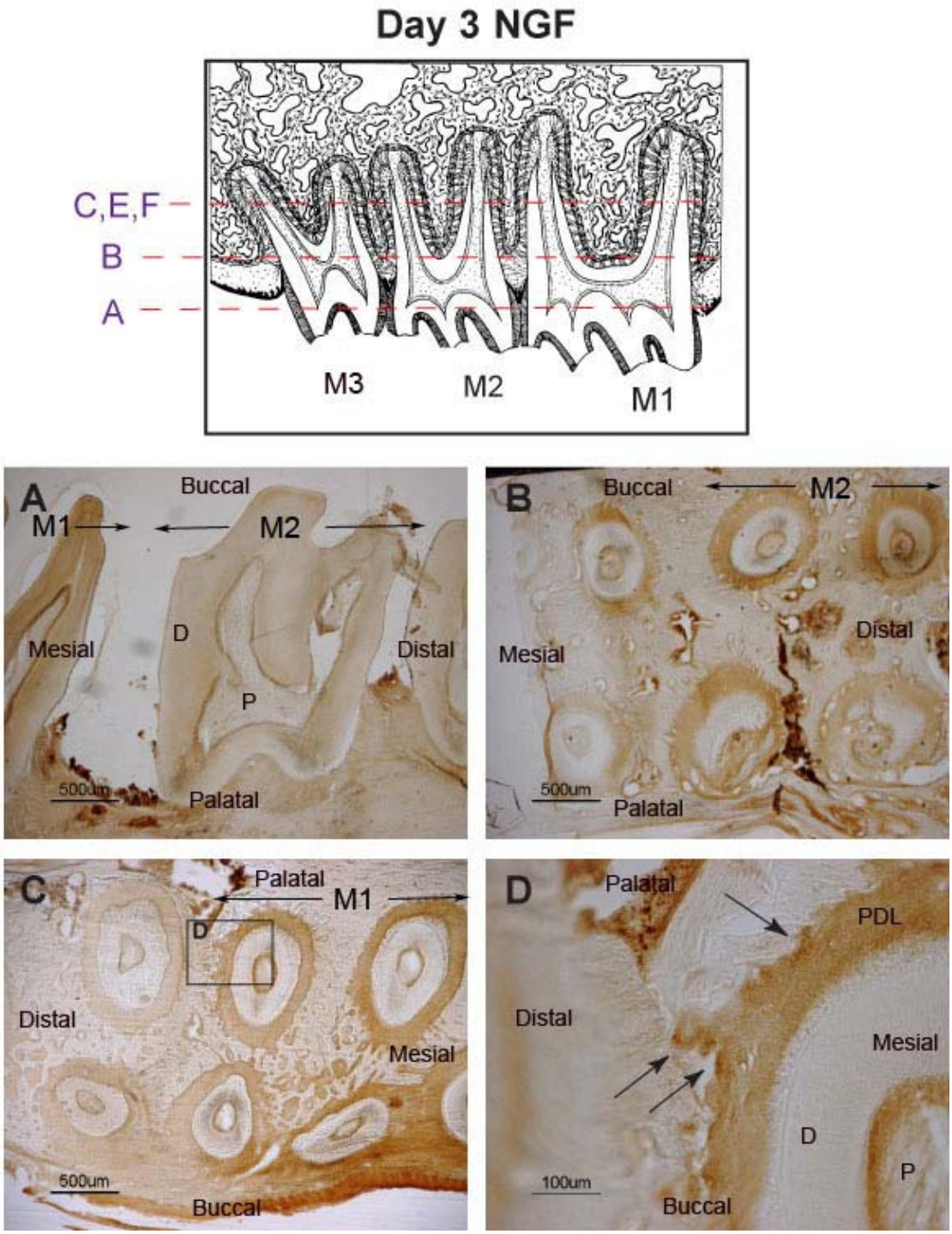

Figure 15: NGF staining, Day 3 after the insertion of elastic modules.

Note increased staining intensity of cells in the PDL of the experimental side. PDL, periodontal ligament. P, dental pulp. D, dentine.

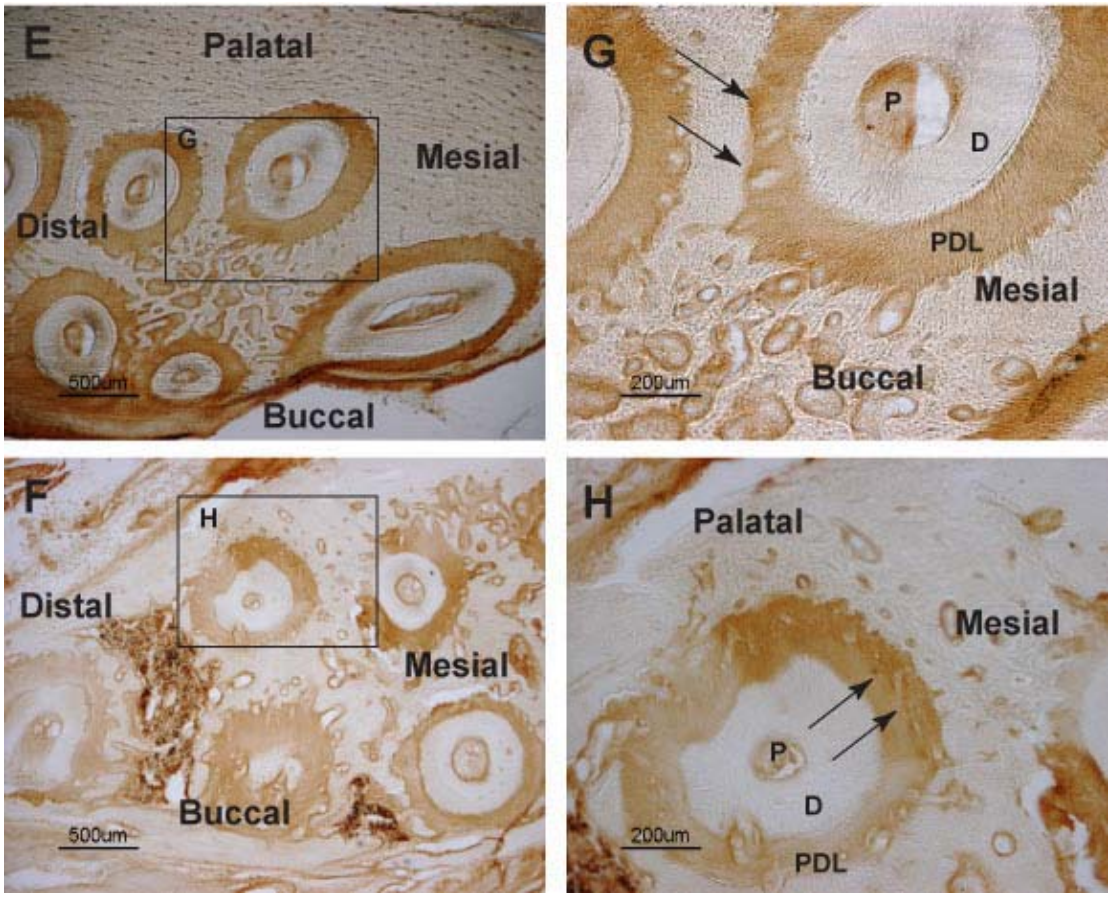

Figure 16: NGF staining, Day 3 after the insertion of elastic modules. PDL, periodontal ligament. P, dental pulp. D, dentine.

Note arrows showing increased staining intensity of cells in the PDL. PDL, periodontal ligament. P, dental pulp. D, dentine.

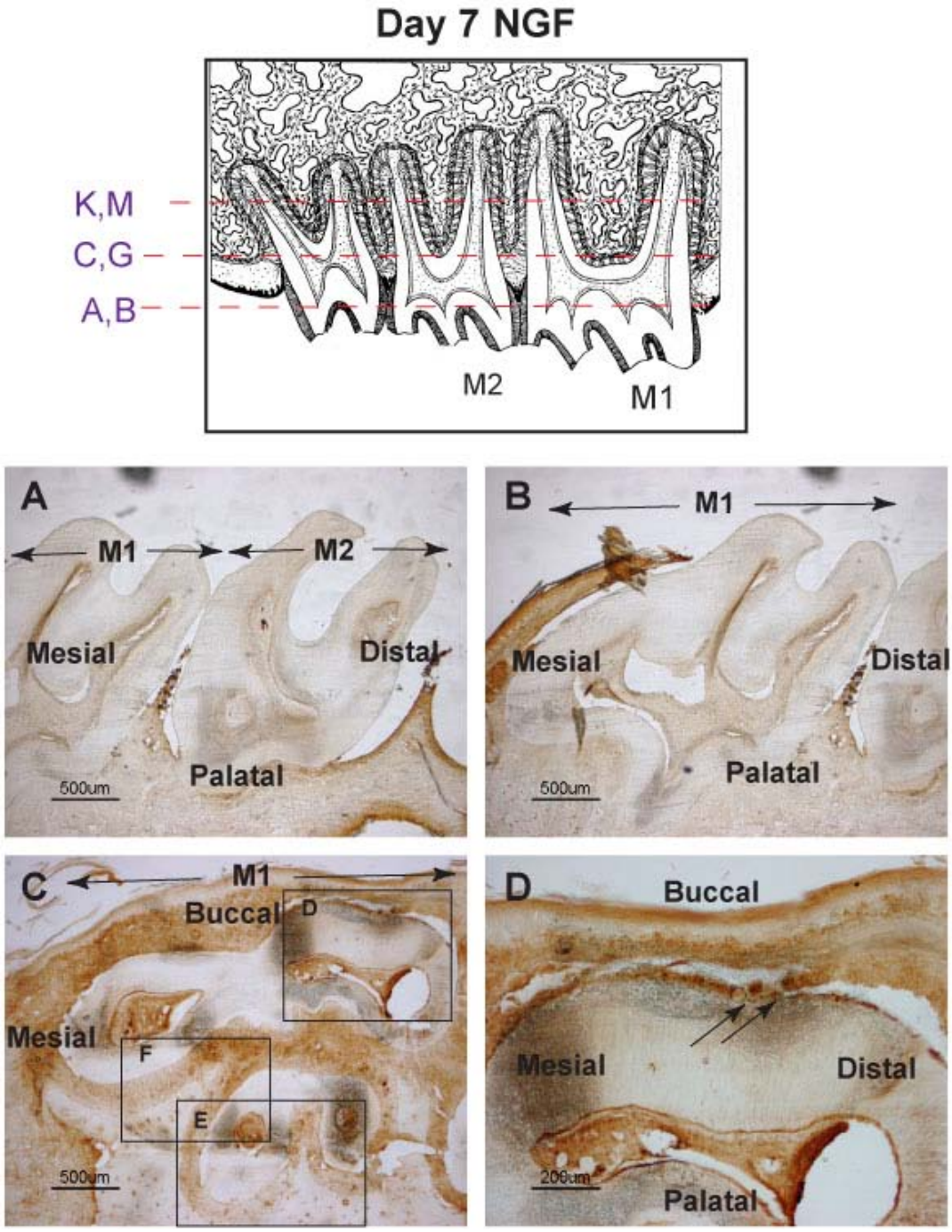

Figure 17: NGF staining, Day 7 after the insertion of elastic modules. Note cells in the PDL showed intense staining of the experimental side.

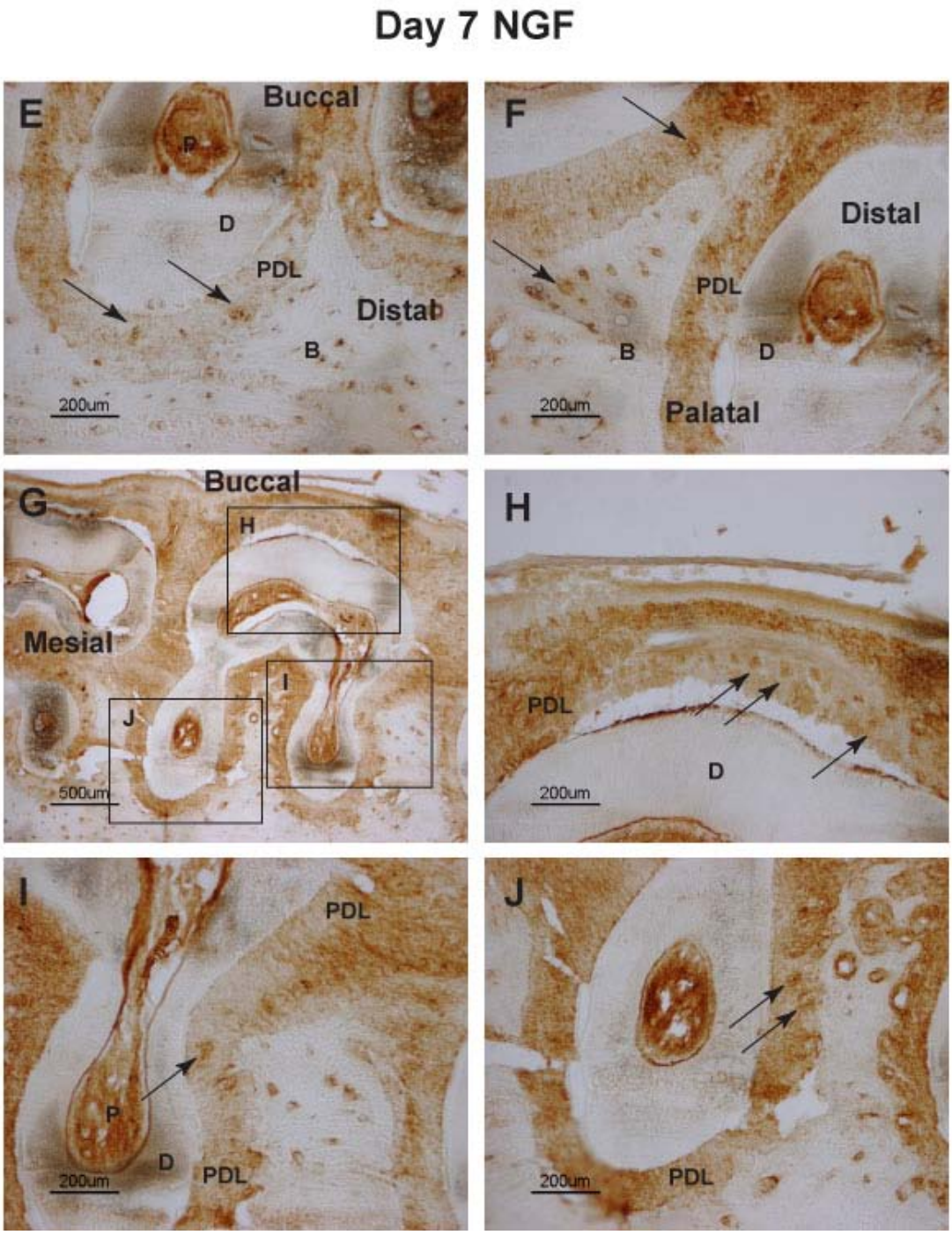

Figure 18: NGF, Day 7. Cells in the PDL showed intense staining.

Note arrows showing increased staining intensity of cells in the PDL of the experimental side. PDL, periodontal ligament. P, dental pulp. D, dentine.

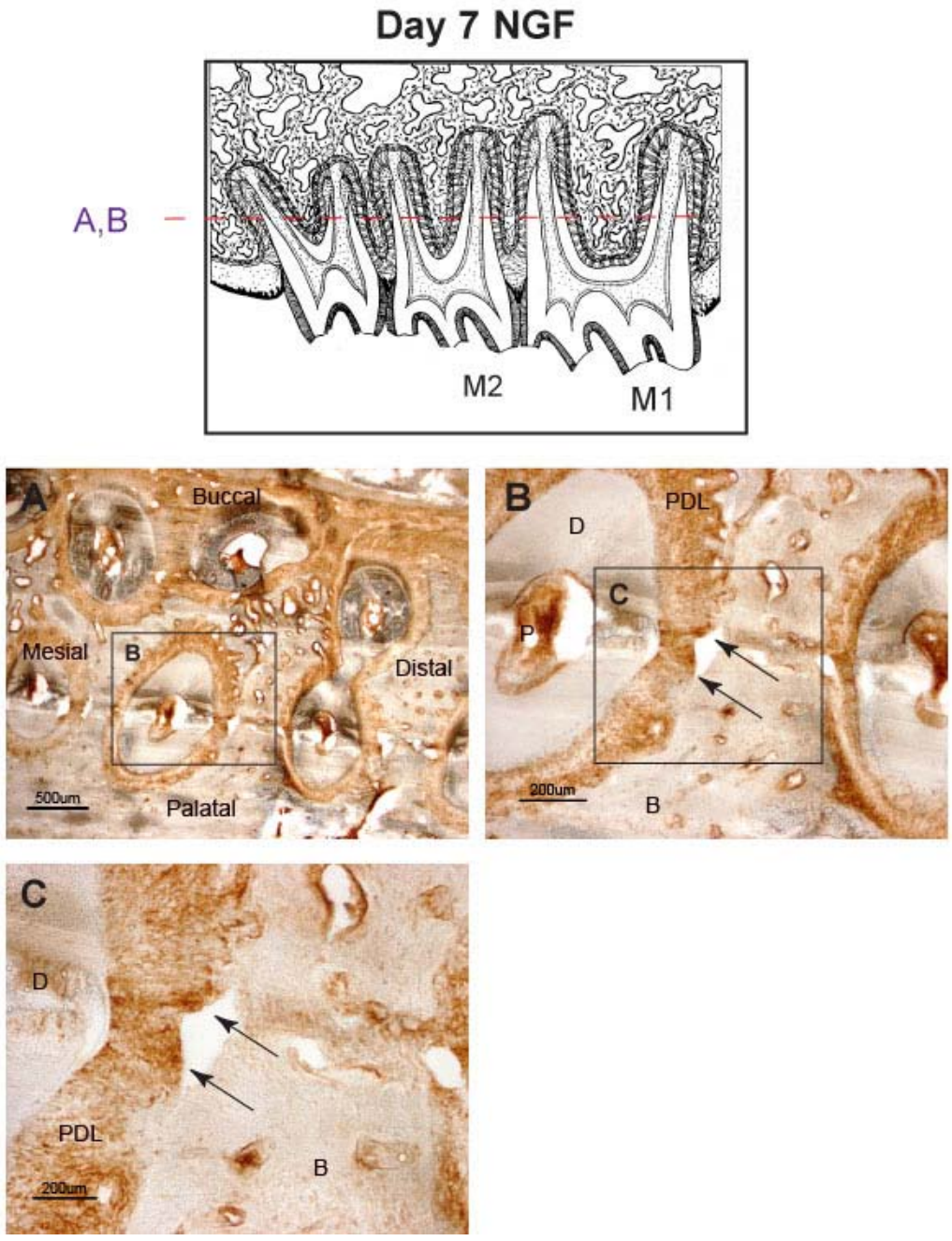

Figure 19: NGF, Day 7 after the insertion of elastic modules.

Note arrows showing osteoclast-like cells in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine.

5.1.3 Day 3 p75

At day 3, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M^1 and M^2), generalised staining was observed at cementum-PDL interface, and mild staining was detected at the PDL-bone interface. Bone trabecula surfaces were also positive for stains.

5.1.3 Day 7 p75

At day 7, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M^1 and M^2), there were inflammation changes detected in inter-radicular bone, i.e. vasodilation of blood vessels, appearance of inflammatory cells. Experimental side also showed intense staining at cementum-PDL interface, PDL bone interface and inter-radicular bone (Figure 20, 21).

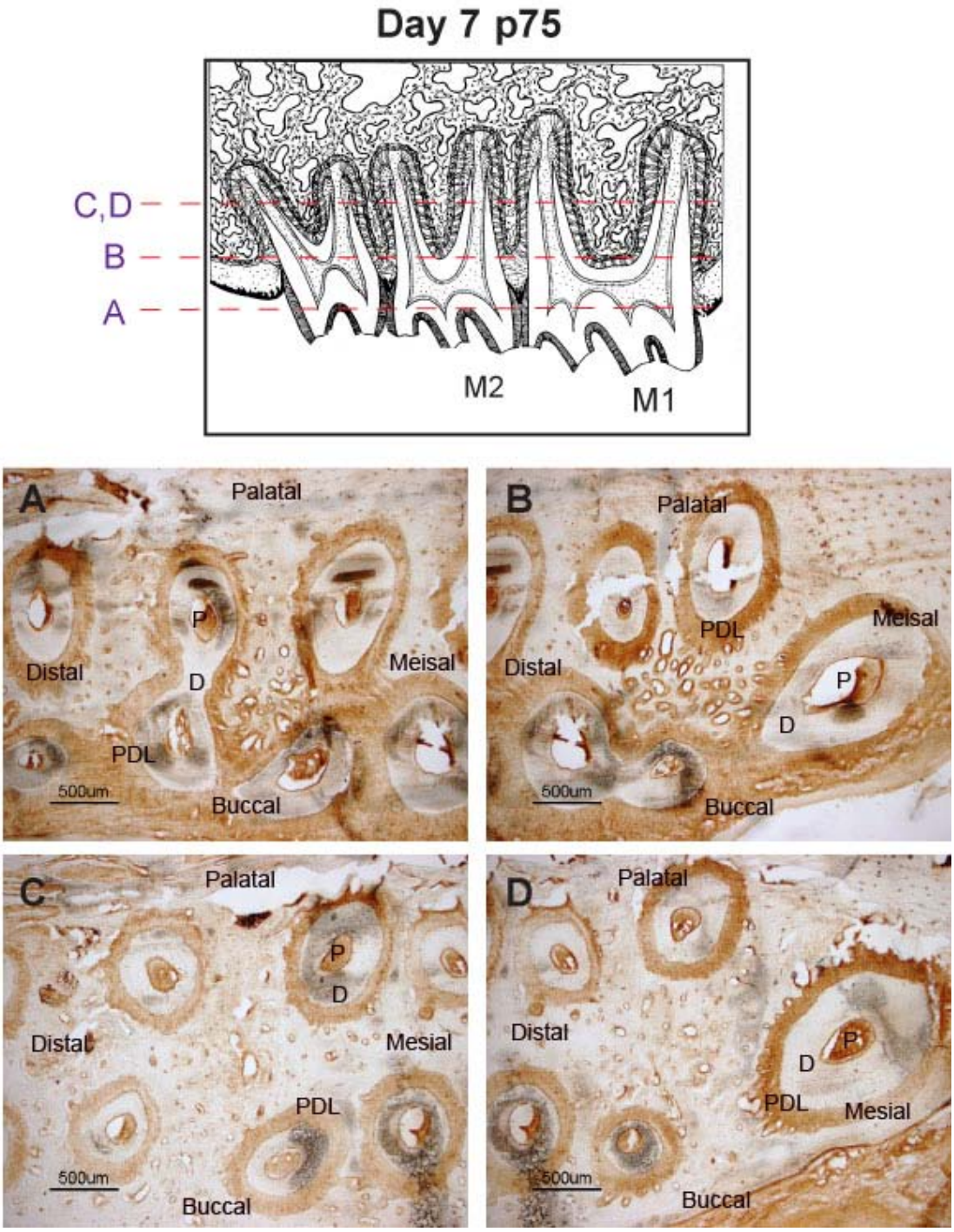

Figure 20: P75, Day 7 after the insertion of elastic modules.

Note areas in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine

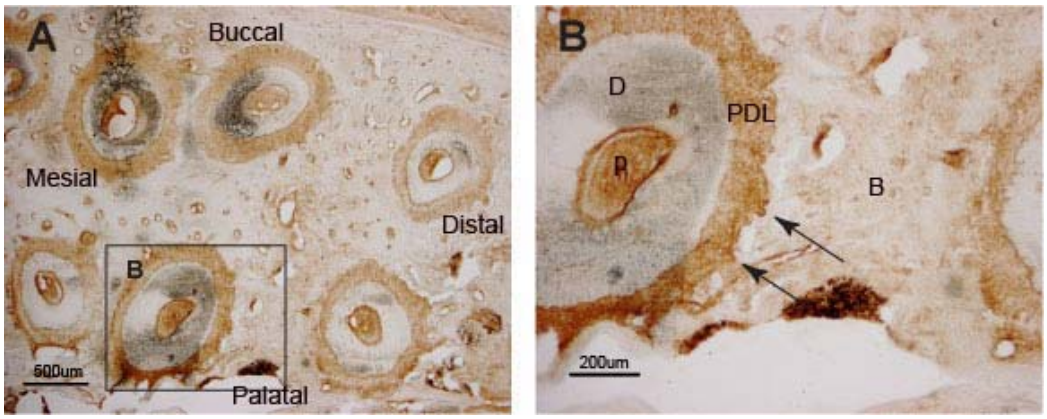

Figure 21: P75, Day 7 after the insertion of elastic modules

Osteoclast-like cells in the PDL of the experimental side showed intense staining (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine.

5.1.4 Day 3 TrkA

At day 3, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M^1 and M^2), there were inflammation changes detected in the bone structure, i.e. vasodilation of blood vessels, appearance of inflammatory cells. Experimental side also showed staining with increased intensity at the cementum-PDL and bone-PDL interfaces. (Figure 22, 23)

5.1.5 Day 7 TrkA

At day 7, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M^1 and M^2), generalised staining observed in the pulp and the PDL with increased intensity when compared to Day 3. Pulp tissue was more intensely stained than the PDL. (Figure 24, 25)

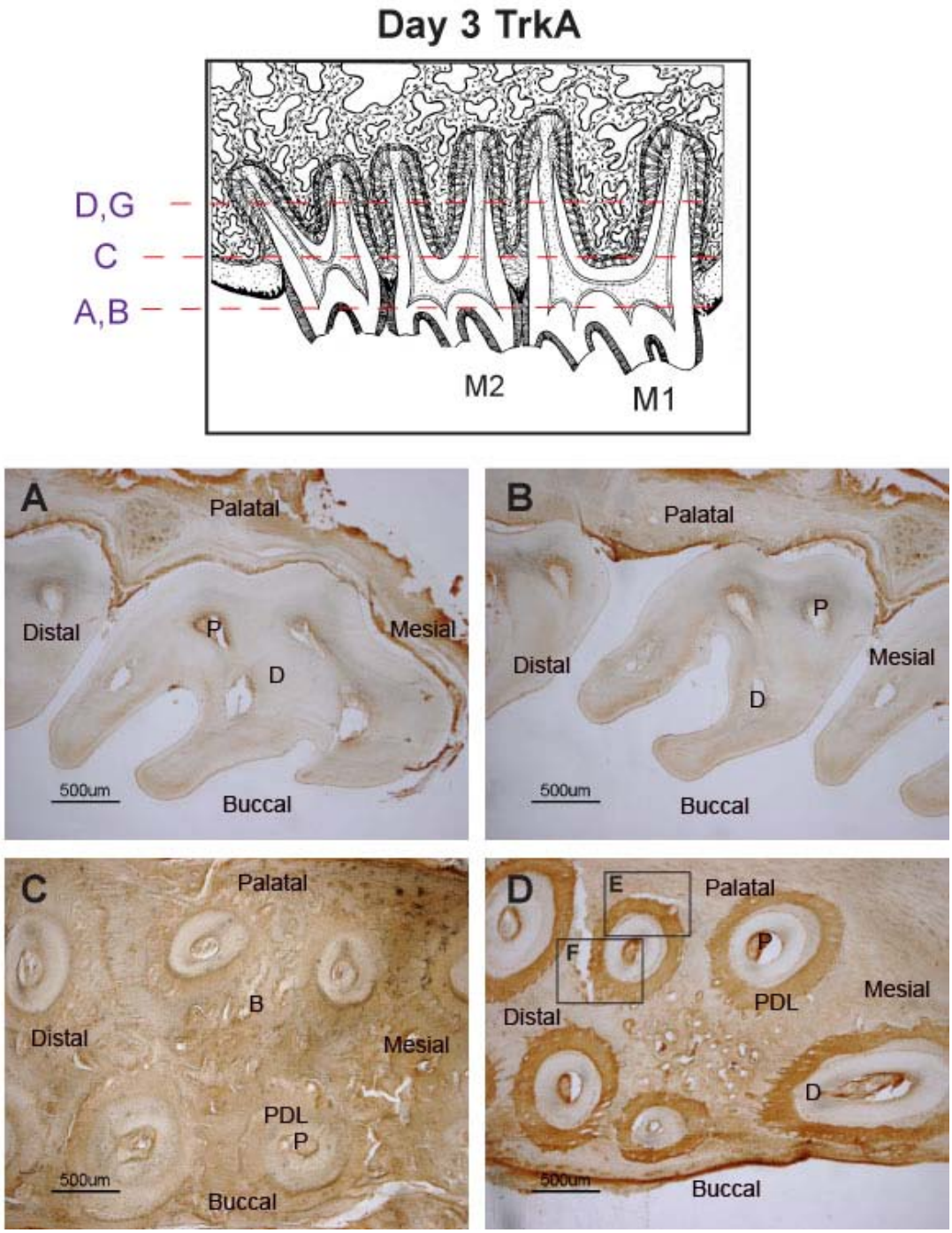

Figure 22: Trk A, Day 3 after the insertion of elastic modules.

Generalised staining detected within the PDL and pulp tissue, mildly in bone of the experimental side. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone

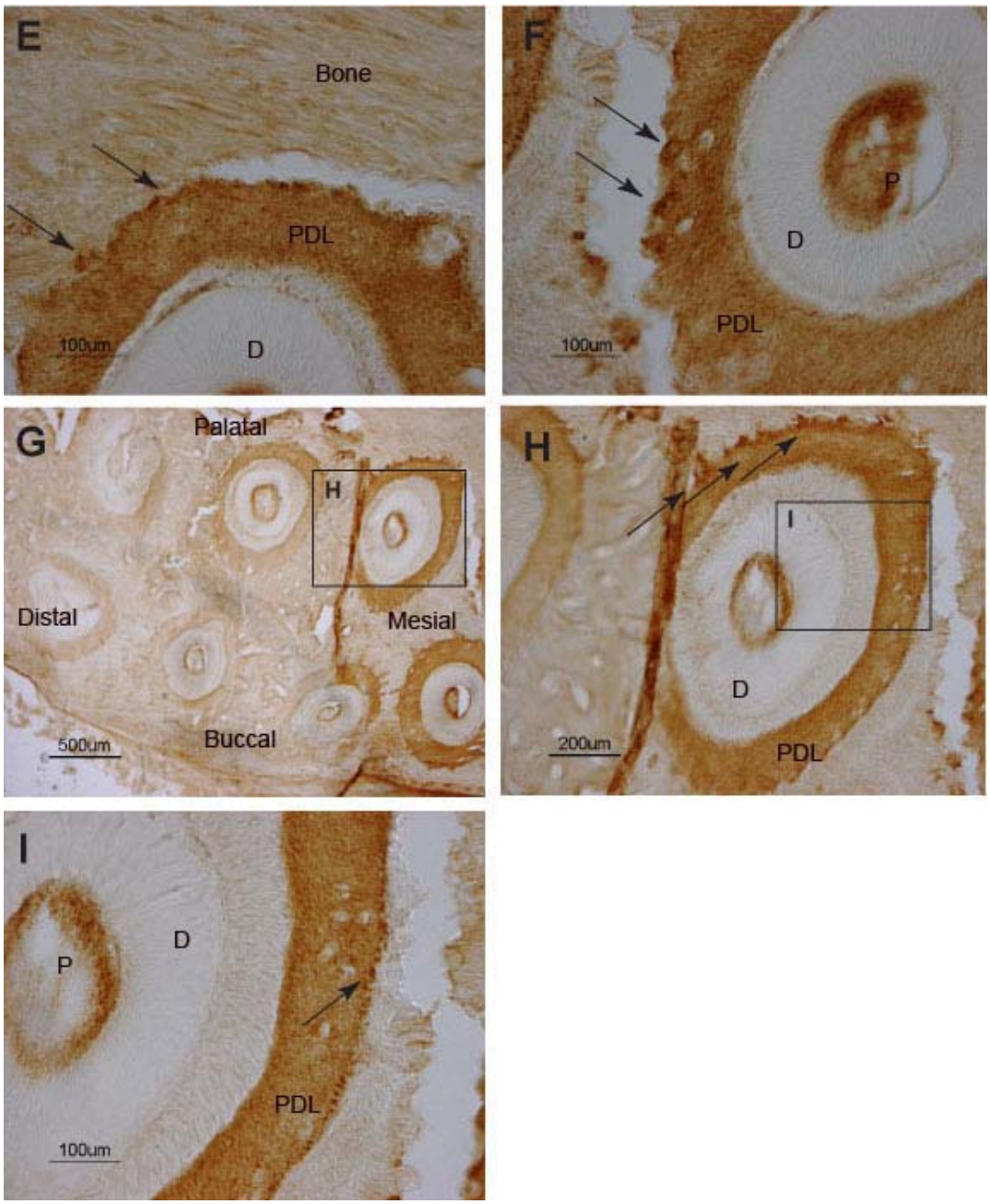

Figure 23: Trk A, Day 3 after the insertion of elastic modules.

Cells (arrow) in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone

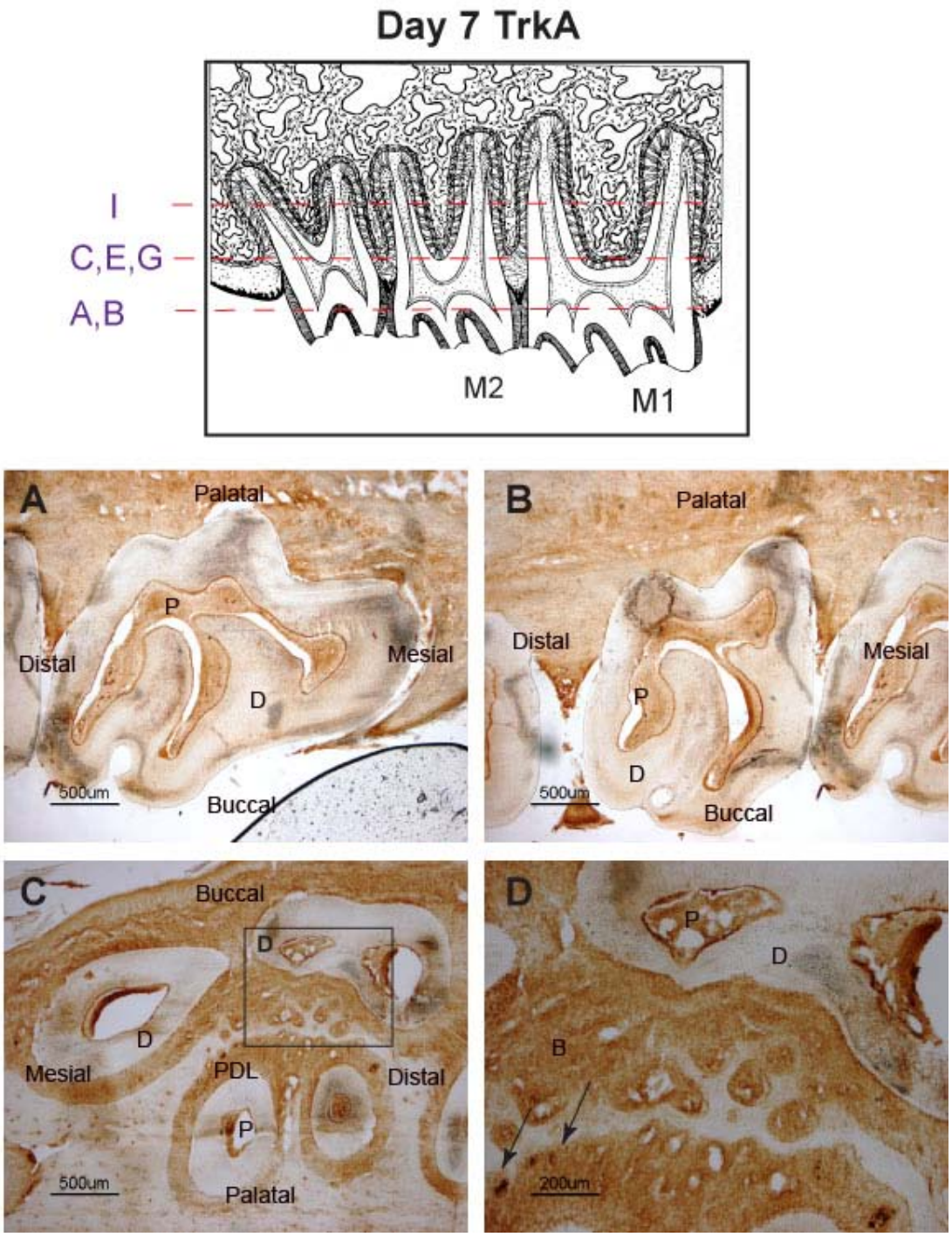

Figure 24: Trk A, Day 7 after the insertion of elastic modules.

Generalised staining observed in the pulp and the PDL of the experimental side. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone

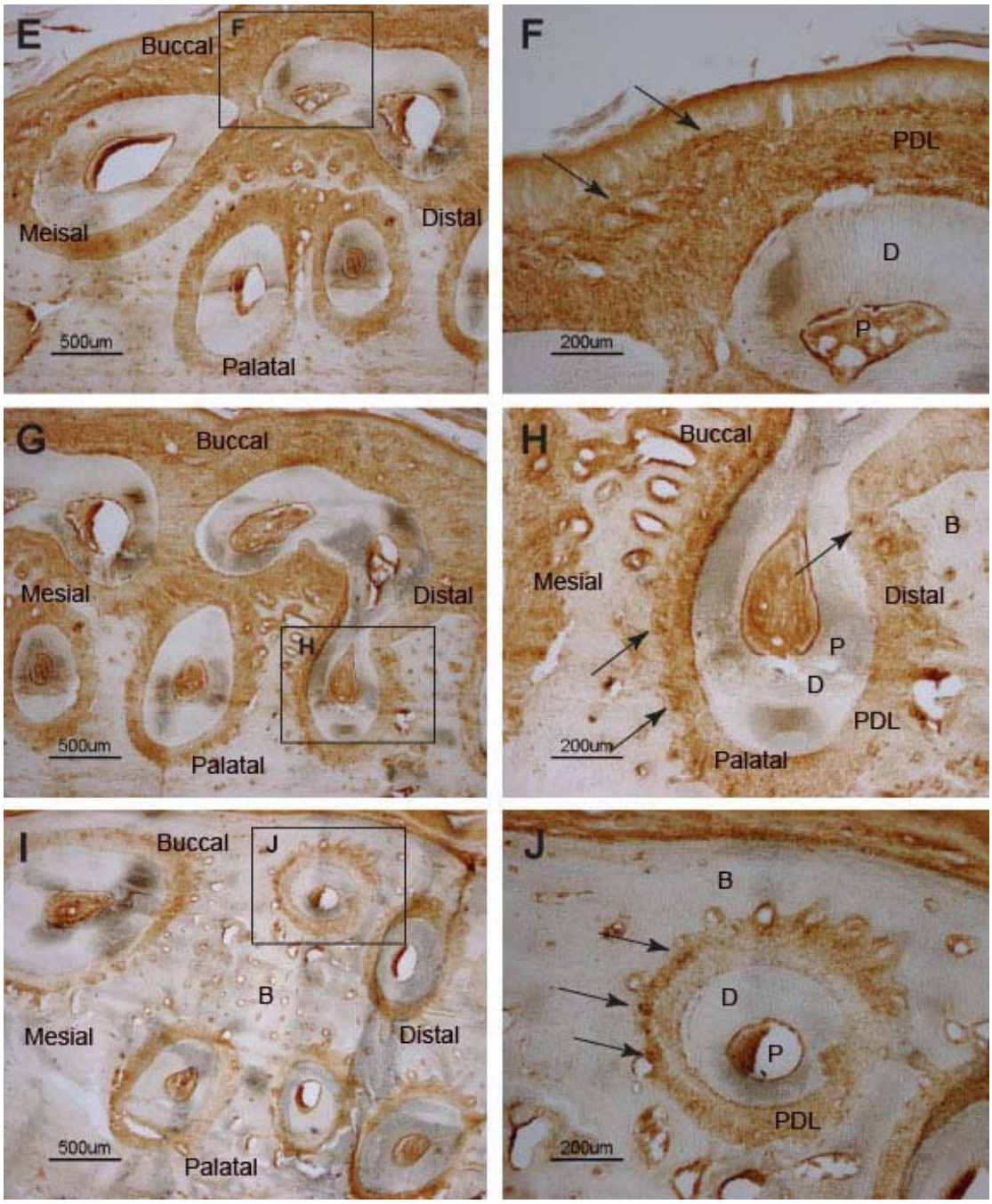

Figure 25: Trk A, Day 7 after the insertion of elastic modules.

Osteoclast-like cells in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

5.1.6 Day 3 CGRP

At day 3, the internal control side (left side of the maxillae) showed that bone and PDL morphology remained unchanged when compared with Day 0. On the experimental side (with elastic modules inserted between M^1 and M^2), there is an increased vasodilation of vessels within bone and fibres were seen within the pulp and near the bone-PDL interface. (Figure 26, 27)

5.1.7 Day 7 CGRP

At day 7, the appearance of fibres on the experimental side (with elastic modules inserted between M^1 and M^2) become more pronounced. Some CGRP immunoreactive fibres were observed at the bone-PDL interface (arrow). Fibres were detected within the PDL and were generally spread out (arrows). In bone, fibres were seen on trabeculae surfaces. (Figure 28, 29, 30)

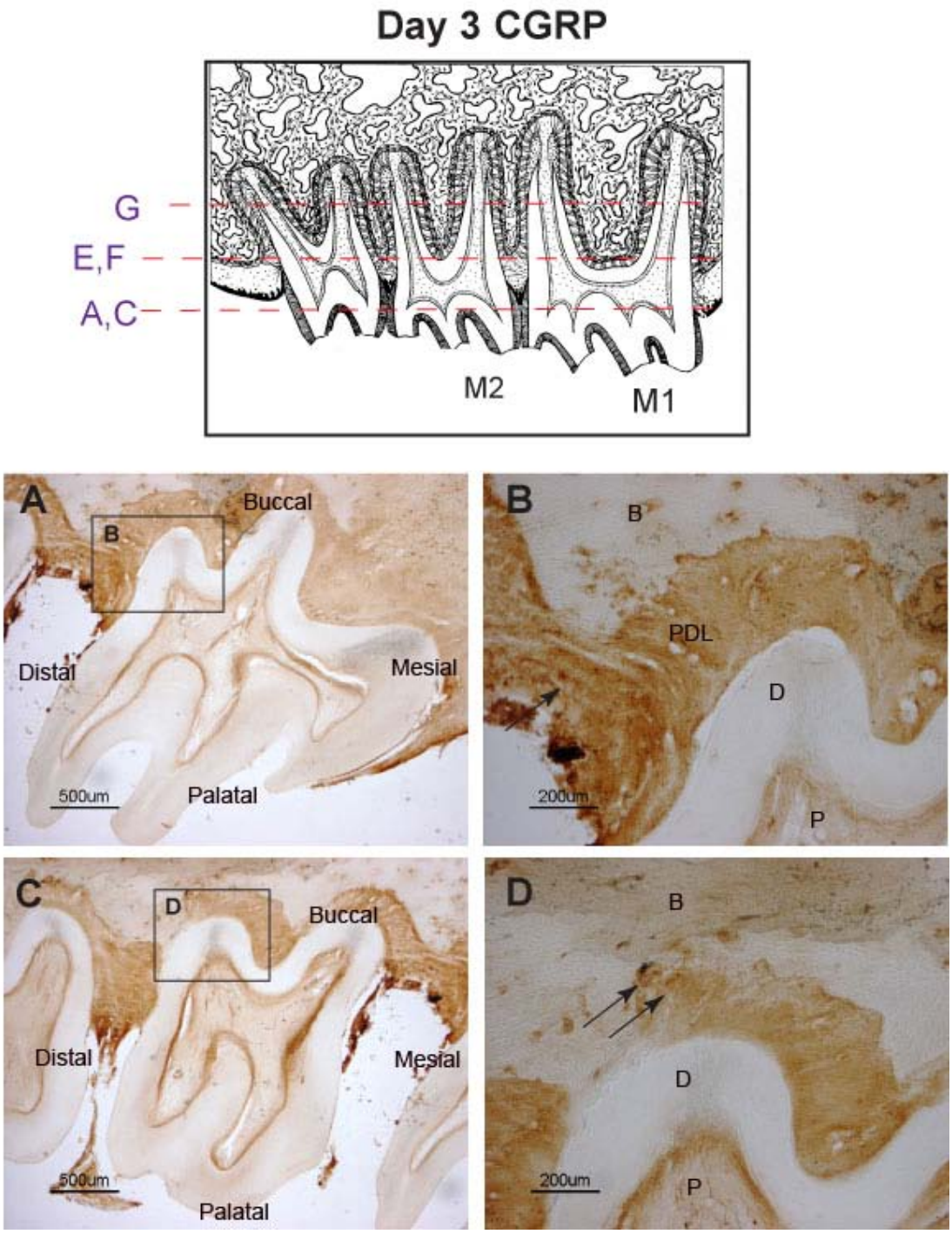

Figure 26: CGRP, Day 3 after the insertion of elastic modules.

Cells (arrows) in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

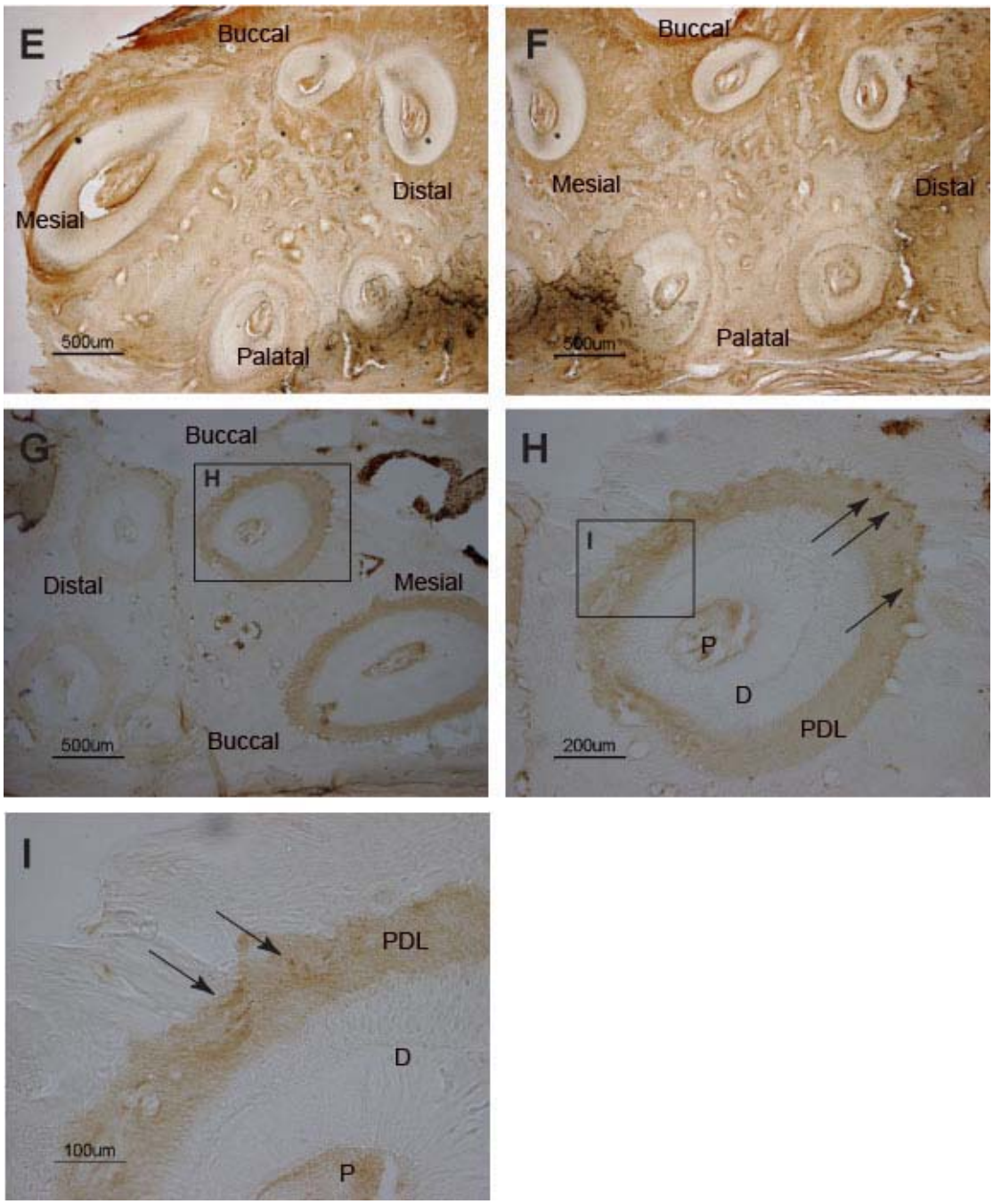

Figure 27: CGRP, Day 3 after the insertion of elastic modules.

Cells (arrow) in the PDL of the experiemental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

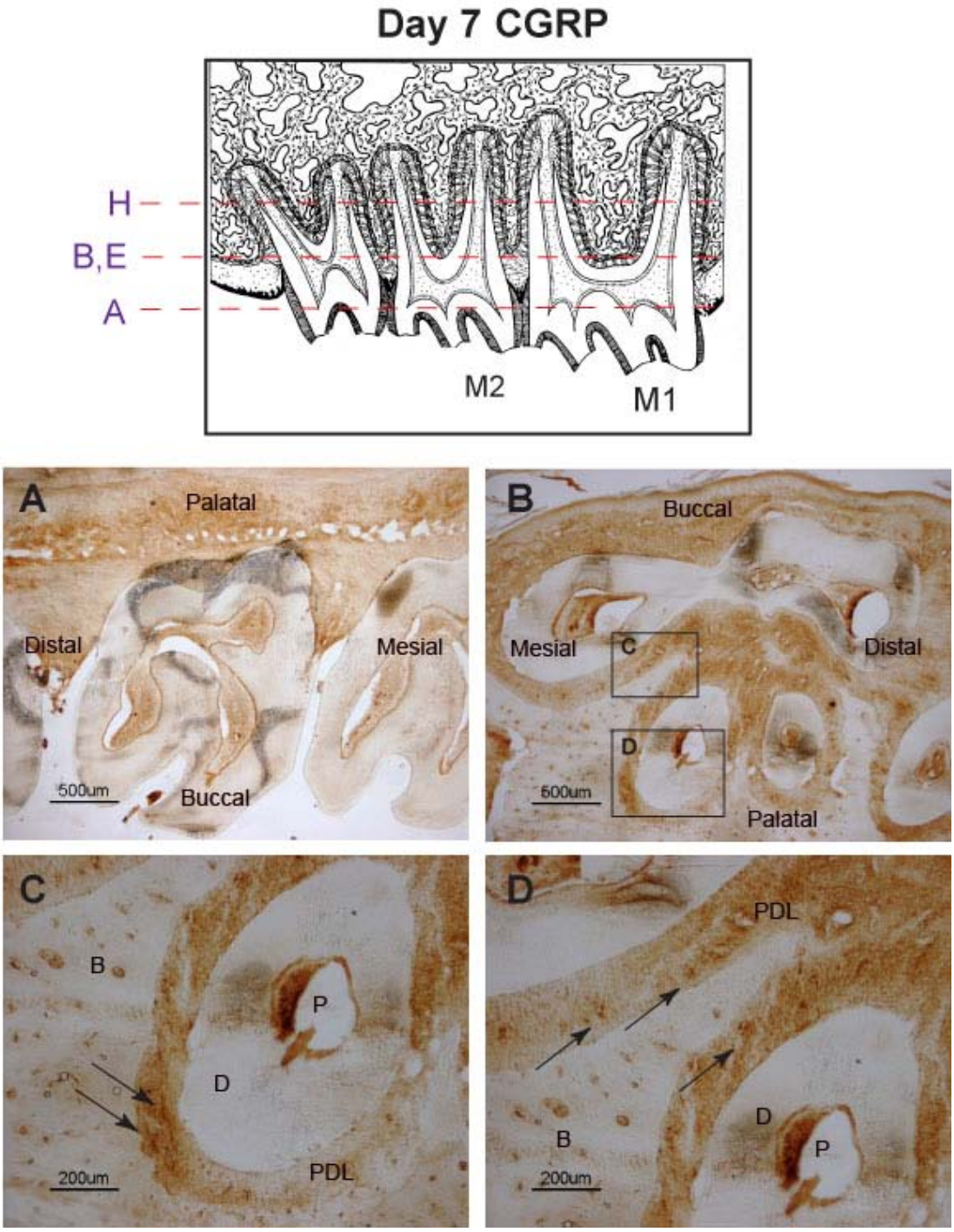

Figure 28: CGRP, Day 7 after the insertion of elastic modules.

CGRP fibres (arrows) in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

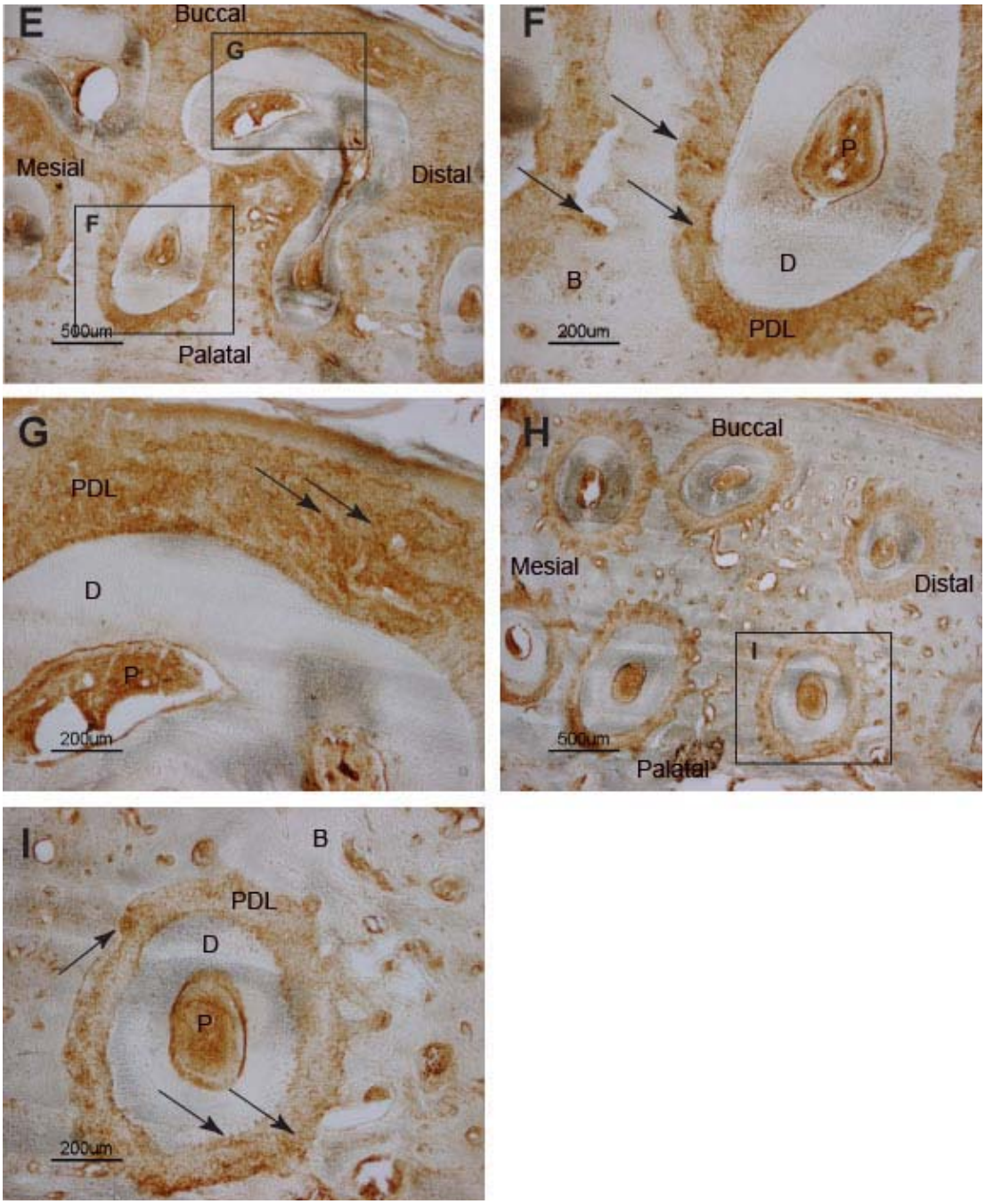

Figure 29: CGRP, Day 7 after the insertion of elastic modules.

CGRP fibres (arrows) in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

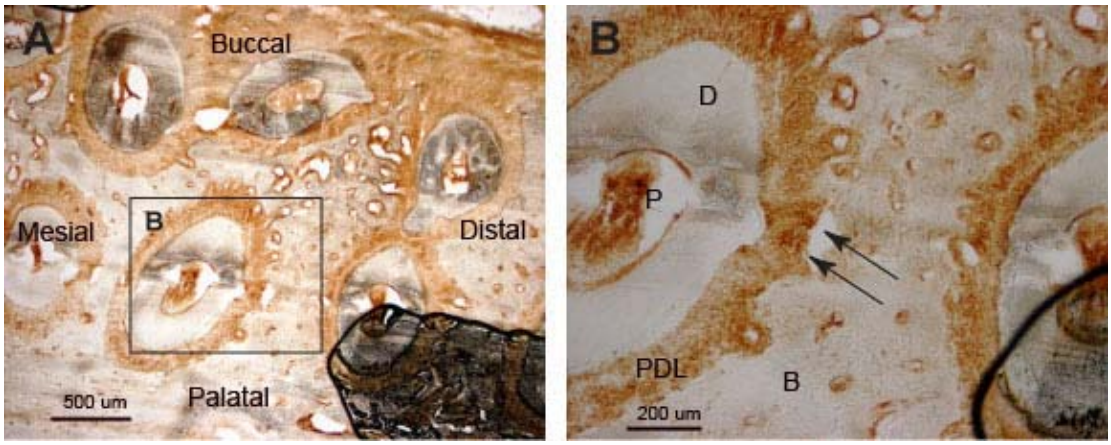

Figure 30: CGRP, Day 7 after the insertion of elastic modules.

Osteoclast-like cells in the PDL of the experimental side showed intense staining. PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

PART 2

An initial pilot study with two animals was conducted to evaluate the appliance design and placement. The main experiment consisted of 28 animals separated into four groups. (Table 1)

Of the 28 experimental male 8 week-old male Sprague-Dawley rats, six animals died during ketamine/xylazine general anaesthetic or the recovery period, whilst five rats were found to have displaced the spring appliance during the experimental period. All remaining animals appeared to suffer no lasting ill effects from the experimental procedures. (Table 4)

Table 4: Coil spring success (* denotes animals which died during experimental procedures.)

Perfusion Day	Group 1 (Control)	S	Group 2 (Control)	S	Group 3 (Test)	S	Group 4 (Test)	S
7	101	*			104	Y		
	102	Y			105	N		
	103	*			106	Y		
	301	Y			305	Y		
	302	Y			306	Y		
	303	Y			307	Y		
	304	Y			308	Y		
14			201	Y			204	*
			202	N			205	N
			203	*			206	*
			401	Y			405	Y
			402	Y			406	N
			403	N			407	Y
			404	*			408	Y

5.2 Anaesthesia

The anaesthetic agent used for the initial experimental procedure was the ketamine/xylazine combination. This agent was acceptable at providing swift surgical anaesthesia with muscle relaxation that permitted experimental procedures to be performed without duress to the animal or the operator. A

single dose of ketamine/xylazine was adequate to maintain anaesthesia during the various procedures which were performed and for a sufficiently lengthy period of time thereafter.

The anaesthetic used at the time of sacrifice was chloral hydrate (5g/100ml H_2O). A single 5ml dosage provided sufficient anaesthesia for the intra-cardiac perfusion procedures to be performed.

5.3 Application of orthodontic force to rat molars

Sentalloy closed coil spring placed between the right maxillary first and second molars provided sufficient force for orthodontic movement of the teeth involved.

Insertion of the spring was difficult at times due to the morphology of the crowns, contact points of the teeth of the animals and the bond strength of the composite to rat enamel. Care was taken not to injure any soft tissue but interdental gingival trauma was inevitable.

The springs had become dislodged in six animals presumably due the effects of masticatory forces and the lack of retention between the stainless steel ligature and the tooth surfaces as the teeth moved apart. Apart from these, moisture control during the composite bonding procedure would affect the bond strength of composite to the tooth surface, which in turn affects the retention of the coil spring.

Ten closed-coil springs were randomly selected and the superelastic properties of the material and the delivered force were tested. A reproducible force of 100 cN was produced over a range of 3 to 6 mm of activation, which was within the activation range used for the test animals. (Figure 31)

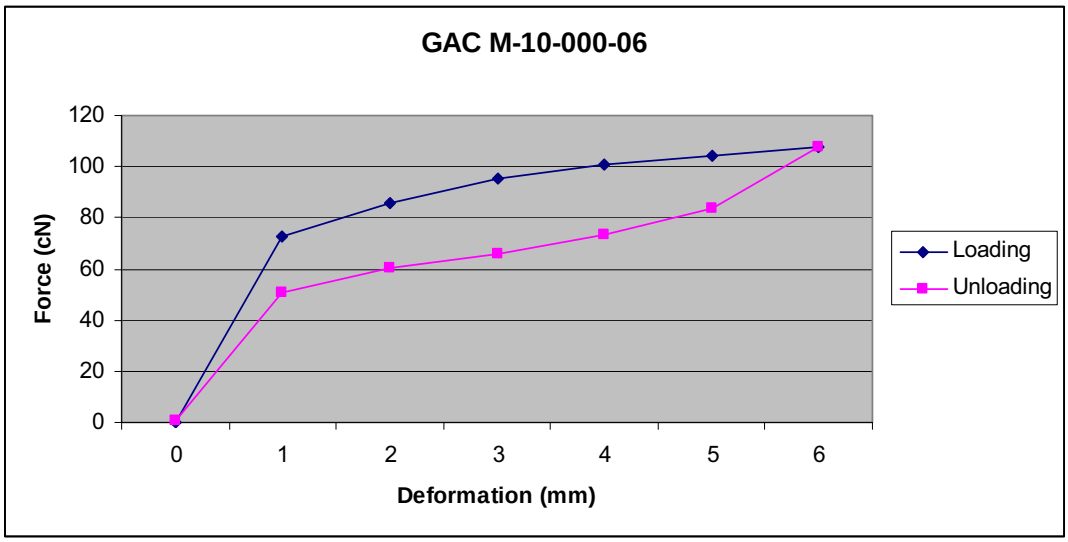

Figure 31: Force deflection diagram of NiTi coil springs used in this study

5.4 Injection of Anti-NGF

Fourteen animals belonging to the experimental group were injected locally with 1.0 μl containing 2μg of anti-NGF into the gingival mucosa between the maxillary right first and second molars using a Hamilton syringe. The volume injected was shown to be enough to affect some reaction in the experimental area.[5]

5.5 Tooth measurement results

All calculations were performed using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

5.5.1 Effects of intervention

To analyse the effects of the injection on measurements of tooth separation, linear mixed effects models were fitted to the data. In the models, the association of treatment group (injection or control) and time (0, 3, 7, 10 and 14 days) were tested.

The results for each of the measurement types are presented below. (*denotes animals which died during experimental procedures, ** denotes animals which had the springs dislodged)

Table 5: Tooth movement measurements of Day 0

(C=control, T= test group with anti-NGF injected, 1st= first direct measurement from incisor to first molar, Molar 1-2=measurement from first and second molars centroids)

Tooth movement measurements of Day 0											
	Day 0-C (direct)				Day 0-T (direct)				Indirect		
rat no.	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean	C	T	Molar 1-2
101	13.74	13.78	13.78	13.77	13.70	13.48	13.61	13.60	*	*	*
102	14.09	13.96	13.92	13.99	13.68	14.00	13.96	13.88	16.80	16.42	1.91
103	13.12	13.17	13.19	13.16	13.38	13.32	13.45	13.38	16.17	16.27	2.09
104	13.89	13.90	13.89	13.89	14.17	14.11	14.09	14.12	16.26	16.72	2.06
105	*	*	*	*	*	*	*	*	*	*	*
106	13.94	13.92	13.99	13.95	13.97	13.97	14.01	13.98	16.65	16.68	2.09
201	12.34	12.40	12.44	12.39	12.49	12.44	12.56	12.50	14.79	15.22	2.30
202	12.37	12.45	12.46	12.43	12.47	12.54	12.52	12.51	14.38	14.95	2.01
203	12.54	12.54	12.54	12.54	12.49	12.57	12.58	12.55	15.22	15.23	2.32
204	*	*	*	*	*	*	*	*	*	*	*
205	12.48	12.49	12.53	12.50	12.52	12.56	12.55	12.54	14.84	15.08	2.16
206	11.60	11.59	11.59	11.59	11.85	11.75	11.86	11.82	*	*	*
301	12.90	12.93	12.92	12.92	12.60	12.62	12.68	12.63	15.93	16.19	2.34
302	13.65	13.64	13.62	13.64	13.40	13.51	13.46	13.46	16.30	16.49	2.33
303	13.33	13.33	13.27	13.31	13.26	13.26	13.24	13.25	15.86	15.96	2.25
304	12.93	12.91	12.94	12.93	13.01	12.94	12.83	12.93	15.92	16.15	2.19
305	13.35	13.29	13.28	13.31	13.34	13.50	13.37	13.40	16.00	16.11	2.05
306	13.08	13.09	13.06	13.08	13.06	13.09	13.05	13.07	16.00	16.06	2.18
307	13.30	13.26	13.30	13.29	13.26	13.38	13.31	13.32	16.19	16.26	2.36
308	13.08	13.03	13.10	13.07	13.35	13.29	13.40	13.35	15.94	16.02	2.51
401	12.83	12.90	12.80	12.84	12.28	12.33	12.22	12.28	15.84	15.82	2.19
402	13.18	13.18	13.21	13.19	13.23	13.27	13.34	13.28	15.94	16.38	2.20
403	12.66	12.79	12.69	12.71	12.63	12.73	12.74	12.70	15.71	15.58	2.32
404	13.25	13.21	13.21	13.22	13.16	13.13	13.20	13.16	16.06	16.16	2.31
405	12.68	12.66	12.69	12.68	12.71	12.76	12.74	12.74	15.41	15.73	2.05
406	13.11	13.14	13.18	13.14	13.06	13.01	13.15	13.07	16.05	15.78	2.38
407	12.41	12.41	12.44	12.42	12.35	12.35	12.28	12.33	15.25	15.05	2.20
408	12.79	12.89	12.81	12.83	12.84	12.79	12.82	12.82	15.69	15.56	2.37

Table 6: Tooth movement measurements of Day 3

Tooth movement measurements of Day 3											
	Day 3-C (direct)				Day 3-T (direct)				Indirect		
rat no.	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean	C	T	Molar 1-2
101	13.46	13.44	13.41	13.44	13.19	13.04	13.38	13.20	*	*	*
102	13.86	13.75	13.86	13.82	12.54	12.88	12.52	12.65	17.06	16.59	2.54
103	13.07	13.17	13.09	13.11	12.21	12.10	12.14	12.15	15.88	15.47	2.36
104	13.21	13.38	13.26	13.28	12.95	12.80	12.81	12.85	16.20	16.04	2.31
105	*	*	*	*	*	*	*	*	*	*	*
106	13.47	13.56	13.39	13.47	13.00	12.89	13.00	12.96	15.63	15.17	2.35
201	12.17	12.18	12.01	12.12	11.35	11.34	11.47	11.39	14.23	14.14	2.32
202	12.06	11.96	11.93	11.98	11.48	11.64	11.63	11.58	14.07	14.11	2.53
203	12.08	12.07	12.12	12.09	11.46	11.49	11.55	11.50	15.20	14.79	2.63
204	*	*	*	*	*	*	*	*	*	*	*
205	11.91	11.90	11.91	11.91	11.75	11.83	11.86	11.81	14.17	14.13	2.21
206	11.22	11.23	11.22	11.22	10.00	10.25	10.16	10.14	*	*	*
301	12.58	12.39	12.52	12.50	11.86	11.98	11.96	11.93	15.22	14.80	2.56
302	13.16	13.14	13.14	13.15	11.98	12.07	11.96	12.00	15.85	15.59	2.51
303	13.29	13.34	13.28	13.30	12.80	12.73	12.86	12.80	16.36	16.00	2.42
304	12.47	12.48	12.52	12.49	11.54	11.67	11.62	11.61	15.28	15.30	2.33
305	12.78	12.77	12.78	12.78	12.03	12.04	11.83	11.97	15.78	15.56	2.47
306	12.23	12.24	12.28	12.25	11.92	11.74	11.91	11.86	15.35	14.93	2.31
307	12.71	12.76	12.79	12.75	11.79	11.77	11.83	11.80	15.76	15.43	2.51
308	12.90	12.89	12.90	12.90	12.18	12.04	11.96	12.06	15.45	15.06	2.65
401	12.53	12.52	12.59	12.55	12.07	11.96	11.80	11.94	15.60	15.31	2.45
402	12.99	12.88	12.87	12.91	11.51	11.68	11.86	11.68	15.30	15.61	2.29
403	12.00	12.05	12.12	12.06	11.65	11.56	11.81	11.67	15.11	14.80	2.68
404	13.18	13.18	13.21	13.19	12.82	12.93	12.97	12.91	15.72	15.36	2.76
405	12.30	12.35	12.38	12.34	11.50	11.55	11.67	11.57	15.11	15.15	2.10
406	12.76	12.77	12.73	12.75	12.49	12.43	12.40	12.44	15.52	15.16	2.58
407	12.10	12.18	12.17	12.15	11.65	11.68	11.71	11.68	14.83	13.91	2.54
408	12.54	12.70	12.63	12.62	11.50	11.35	11.38	11.41	15.54	14.96	2.53

Table 7: Tooth movement measurements of Day 7

Tooth movement measurements of Day 7											
	Day 7-C (direct)				Day 7-T (direct)				Indirect		
rat no.	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean	C	T	Molar 1-2
101	*	*	*	*	*	*	*	*	*	*	*
102	13.54	13.52	13.53	13.53	13.29	13.26	13.28	13.28	16.81	16.22	2.73
103	**	**	**	**	**	**	**	**	**	**	**
104	13.49	13.47	13.48	13.48	13.17	12.99	13.10	13.09	16.33	16.15	2.44
105	*	*	*	*	*	*	*	*	*	*	*
106	13.70	13.75	13.80	13.75	13.07	12.95	13.01	13.01	16.62	15.92	2.67
201	12.04	12.03	11.86	11.98	11.21	11.38	11.23	11.27	14.41	14.24	2.59
202	11.68	11.67	11.70	11.68	11.37	11.33	11.34	11.35	10.91	10.52	2.55
203	12.02	11.96	12.05	12.01	11.17	11.07	11.14	11.13	14.69	14.16	2.71
204	*	*	*	*	*	*	*	*	*	*	*
205	12.30	12.32	12.35	12.32	11.55	11.64	11.60	11.60	15.02	14.51	2.56
206	*	*	*	*	*	*	*	*	*	*	*
301	12.43	12.47	12.45	12.45	11.79	11.82	11.80	11.80	15.35	14.34	2.98
302	13.22	13.36	13.28	13.29	12.69	12.62	12.65	12.65	16.05	15.45	2.64
303	13.00	13.05	13.11	13.05	12.46	12.55	12.48	12.50	15.90	15.15	2.78
304	13.05	13.05	13.12	13.07	12.84	13.02	12.95	12.94	15.54	14.91	2.44
305	12.89	12.77	12.82	12.83	12.40	12.36	12.39	12.38	15.41	14.81	2.54
306	12.86	12.89	12.88	12.88	12.71	12.83	12.70	12.75	15.75	15.33	2.60
307	12.93	12.92	12.65	12.83	12.87	12.89	12.70	12.82	15.98	15.57	2.76
308	12.62	12.54	12.60	12.59	12.18	12.22	12.18	12.19	15.60	14.96	2.69
401	12.47	12.48	12.50	12.48	11.90	11.96	12.00	11.95	15.49	14.82	2.50
402	12.96	13.04	13.08	13.03	12.38	12.42	12.50	12.43	15.95	15.65	2.46
403	12.22	12.25	12.37	12.28	11.24	11.23	11.12	11.20	15.46	14.96	2.70
404	*	*	*	*	*	*	*	*	*	*	*
405	12.28	12.27	12.28	12.28	12.17	12.07	12.18	12.14	15.03	14.72	2.25
406	**	**	**	**	**	**	**	**	**	**	**
407	12.17	12.07	12.09	12.11	11.21	11.25	11.20	11.22	14.77	13.62	2.53
408	12.47	12.37	12.53	12.46	10.46	10.48	10.42	10.45	15.42	14.83	2.63

Table 8: Tooth movement measurements of Day 10

Tooth movement measurements of Day 10											
	Day 10-C				Day 10-T				Indirect		
rat no.	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean	C	T	Molar 1-2
201	11.67	11.62	11.71	11.67	11.24	11.15	11.21	11.20	14.28	13.96	2.71
202	11.64	11.76	11.69	11.70	11.08	11.10	11.03	11.07	14.53	13.49	3.05
203	11.95	11.96	11.89	11.93	10.65	10.73	10.98	10.79	14.86	13.76	2.95
204	*	*	*	*	*	*	*	*	*	*	*
205	12.26	12.28	12.28	12.27	11.56	11.76	11.79	11.70	15.20	14.48	2.67
206	*	*	*	*	*	*	*	*	*	*	*
401	12.28	12.35	12.32	12.32	11.90	11.79	11.93	11.87	15.22	14.45	2.78
402	12.99	13.04	12.96	13.00	11.85	11.95	11.92	11.91	16.12	15.32	2.75
403	**	**	**	**	**	**	**	**	**	**	**
404	*	*	*	*	*	*	*	*	*	*	*
405	12.24	12.25	12.26	12.25	11.46	11.44	11.55	11.48	15.10	14.55	2.38
406	**	**	**	**	**	**	**	**	**	**	**
407	11.95	11.94	11.99	11.96	11.03	10.97	11.00	11.00	14.88	13.65	2.69
408	12.38	12.39	12.39	12.39	11.19	11.19	11.15	11.18	15.20	14.26	2.74

Table 9: Tooth movement measurements of Day 14

Tooth movement measurements of Day 14											
	Day 14-C (direct)				Day 14-T (direct)				Indirect		
rat no.	M1	M2	M3	Mean	M1	M2	M3	Mean	C	T	Molar 1-2
201	11.58	11.60	11.59	11.59	11.06	11.00	11.02	11.03	14.31	13.39	3.01
202	**	**	**	**	**	**	**	**	**	**	**
203	*	*	*	*	*	*	*	*	*	*	*
204	*	*	*	*	*	*	*	*	*	*	*
205	**	**	**	**	**	**	**	**	**	**	**
206	*	*	*	*	*	*	*	*	*	*	*
401	11.89	11.89	11.94	11.91	10.87	10.94	10.90	10.90	14.68	13.54	2.92
402	12.78	12.83	12.76	12.79	11.68	11.70	11.73	11.70	15.72	14.74	2.50
403	**	**	**	**	**	**	**	**	**	**	**
404	*	*	*	*	*	*	*	*	*	*	*
405	12.19	12.19	12.22	12.20	11.73	11.73	11.74	11.73	14.40	13.79	2.54
406	**	**	**	**	**	**	**	**	**	**	**
407	11.73	12.07	11.77	11.86	10.47	10.51	10.50	10.49	14.32	12.60	3.36
408	12.10	12.16	12.12	12.13	11.38	11.45	11.35	11.39	15.38	14.24	2.76

5.5.2 Direct Measurement

Direct measurement was done by measuring the distance between the most mesial point of the maxillary first molar and the most distal point of ipsilateral incisor (distance M-I) at the ginigival level on the experimental and contralateral sides.

All measurements were recorded intraorally prior to appliance insertion, during the experimental period (at day 3, 7, 10, 14) and immediately after sacrifice. All measurements at each time, were repeated 3 times for each side (appliance and non-appliance side) for each side of the maxilla for each animal. The same operator performed all measurements.

Table 10: Type 3 Tests of Fixed Effects (direct measurements)

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	4	54	39.01	<.0001
group	1	54	0.03	0.8537
group*time	4	54	0.47	0.7584

The table “Type 3 Tests of Fixed Effects” shows the significance of fixed effects in the linear mixed effects model. The effects are interpreted as follows:

Time – ignoring group status (Anti-NGF and control), there are changes over time in the outcome measure (tooth movement).
Group – regardless of time, one group has a higher average outcome score than the other.
Group by time interaction – the effect of Anti-NGF depends on the time point of measurement.

In the model, the effect of time on direct measurements was found to be highly significant ($p < 0.0001$). That is, there was a significant change over the 14 day follow-up period in direct measurements across the two groups. In contrast, there was no evidence for a group effect or a group by time interaction effect.

Table 11: Adjusted Means (direct measurements)

Adjusted Means				
Effect	group	time	Estimate	Standard Error
group*time	control	0	-0.06667	0.04429
group*time	control	3	-0.6919	0.09058
group*time	control	7	-0.5749	0.1325
group*time	control	10	-0.7537	0.1377
group*time	control	14	-0.8889	0.2187
group*time	inject	0	0.06750	0.04784
group*time	inject	3	-0.6569	0.09784
group*time	inject	7	-0.5875	0.1390
group*time	inject	10	-0.8663	0.1539
group*time	inject	14	-0.8486	0.2188

Table 11 “Adjusted Means” shows the model estimates for each combination of group (injection or control) and time. The table is useful for describing the nature of relationships within the data. It can often be helpful to present this information in a plot, as shown below.

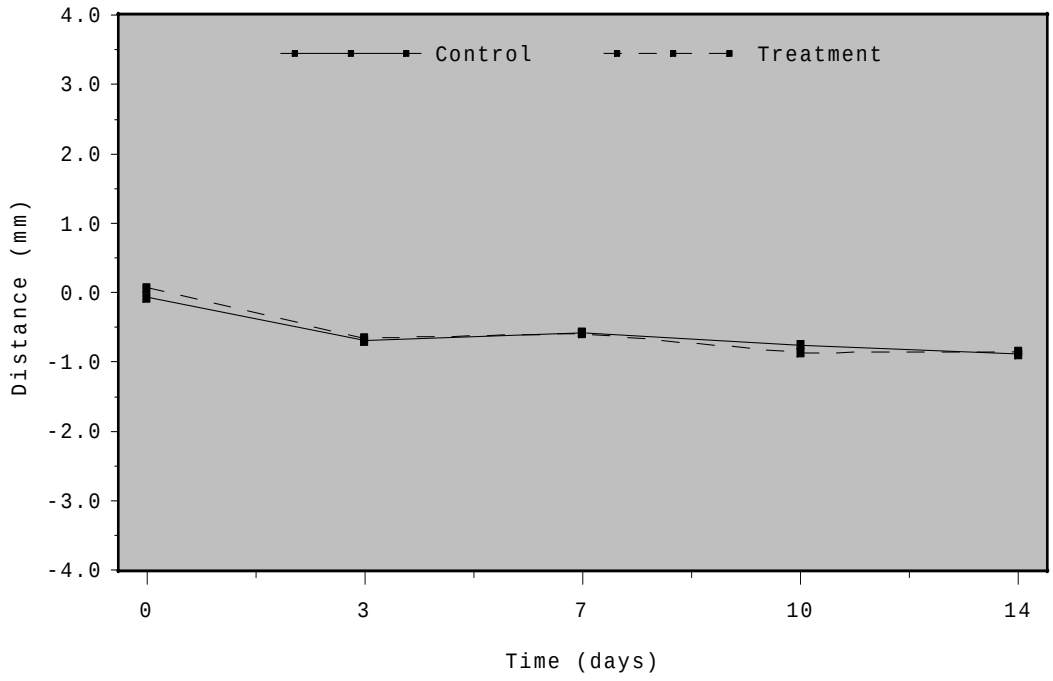

Figure 32: Direct measurements from impressions

As the plot shows, there was almost no difference in direct measurements over time between the two groups. The significant time effect can be explained by the decrease in direct measurements following day 0 (Figure 32).

Note 1: the group by time interaction effect is a test of whether or not the two lines are parallel. A significant interaction effect would provide evidence that the two lines are not parallel.

Conclusion: the injection had no effect on direct measurement.

5.5.3 Indirect Measurement

Indirect measurement was obtained by subtracting the distance between the control teeth (measured indirectly) from the distance between the treatment teeth (measured indirectly).

Table 12: Tests of Fixed Effects (Indirect measurements)

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	4	52	78.23	<.0001
group	1	52	0.05	0.8293
group*time	4	52	1.19	0.3269

Table 13: Adjusted Means (Indirect measurements)

Adjusted Means				
Effect	group	time	Estimate	Standard Error
group*time	control	0	0.1462	0.07024
group*time	control	3	-0.2297	0.07024
group*time	control	7	-0.5771	0.07321
group*time	control	10	-0.9277	0.09378
group*time	control	14	-1.2293	0.1183
group*time	inject	0	0.06890	0.07636
group*time	inject	3	-0.3485	0.07636
group*time	inject	7	-0.5556	0.07815
group*time	inject	10	-0.8078	0.1035
group*time	inject	14	-1.0719	0.1218

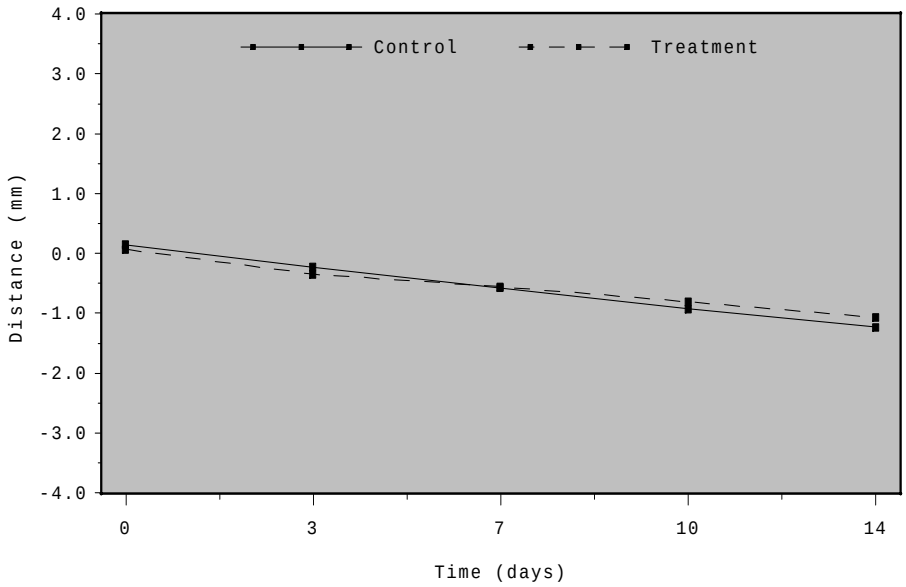

Figure 33: Indirect measurements from impressions

Conclusion: the injection had no effect on indirect measurement.

5.5.4 Molar 1-2 Distance

Table 14: Type 3 Tests of Fixed Effects (M1-M2)

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	4	52	43.92	<.0001
group	1	52	1.22	0.2752
group*time	4	52	1.67	0.1709

Table 15: Adjusted means (M1-M2)

Adjusted Means				
Effect	group	time	Estimate	Standard Error
group*time	control	0	2.2127	0.04742
group*time	control	3	2.4915	0.04742
group*time	control	7	2.6481	0.05034
group*time	control	10	2.8906	0.06902
group*time	control	14	2.8902	0.08792
group*time	inject	0	2.2194	0.05155
group*time	inject	3	2.4158	0.05155
group*time	inject	7	2.5760	0.05332
group*time	inject	10	2.6655	0.07666
group*time	inject	14	2.9256	0.08930

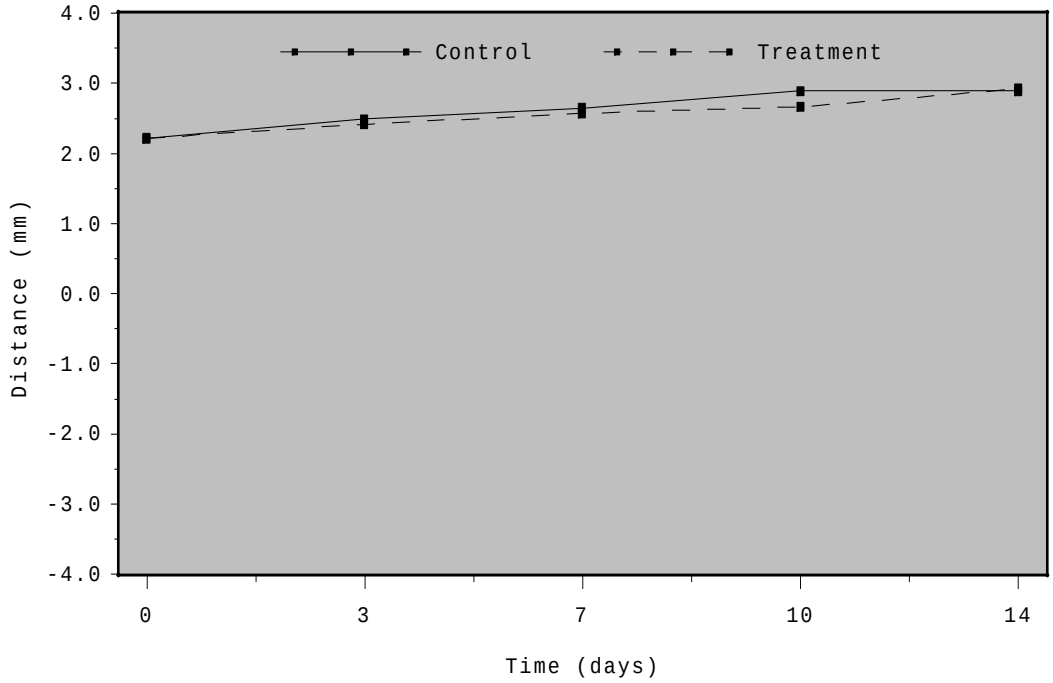

Figure 34: Indirect measurements from right maxillary first molar to second molar

Conclusion: again there is little evidence to show that the injection had any effect on molar 1-2 distance.

5.5.5 Reliability/repeatability of indirect measurements

The repeatability of the indirect measurements was estimated using ***baseline data only***. Data from subsequent time periods were not used so as to avoid the need to incorporate the additional effects of treatment and time into the reliability calculations. Overall the differences between the first and second indirect measurements had a mean of -0.0089mm and a standard deviation of 0.0433mm.

The levels of agreement between the first and second indirect measurement are illustrated in the Bland-Altman plot below. As the plot shows, there was a fairly high degree of agreement between the two measures. The coefficient of repeatability[100] was calculated to be 0.0866, while the upper and lower limits of agreement were given by -0.0955mm and

0.0778mm respectively. Overall these results indicate a high level of repeatability in the indirect measure.

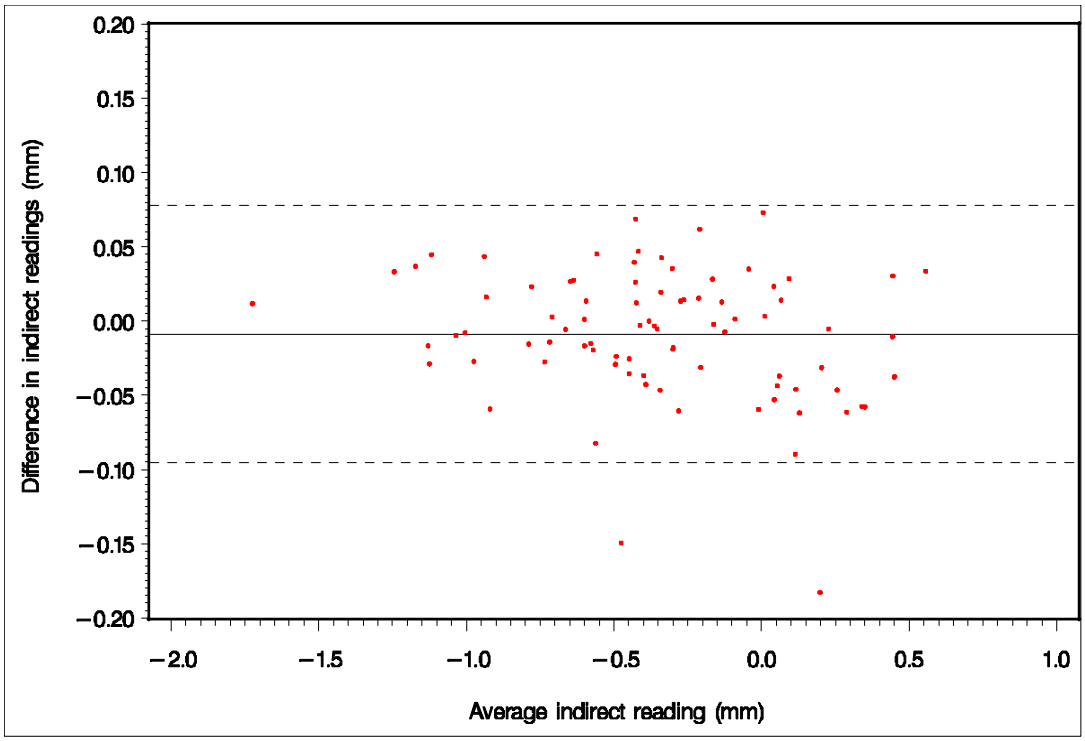

Figure 35: Reliability/repeatability of indirect measurements

5.6 Perfusion of animal

The animals were perfused with a solution containing equal proportions of 0.4% p-benzoquinone and 4% p-formaldehyde. The p-benzoquinone molecules serve as a cross-linking agent between neural tissue and p-formaldehyde molecules, which enhances the fixation of the tissues.

The change in colour of the extremities from pink to brown, and checking for stiffness of the animal carcass indicated the fixation level. Brown discolouration of the extremities and increased stiffness indicated a high level of fixation, whereas yellowish discolouration and flaccidity of the carcass meant that the animal was poorly perfused and, therefore, showed a reduced level of fixation. After gross dissection, the dorsal surface of the cerebral cortex was observed and scored in order to record the success of the perfusion process. The animals were scored 1 – 5 depending on the success

of perfusion/fixation as shown in Table 16. Table 17 indicates that tissue perfusion was achieved to a consistently high level.

Table 16: Tissue perfusion grading

Perfusion Score	Characteristics
1	No brown tissue, poor tissue perfusion
2	Very mild browning of tissues evident, poor tissue perfusion
3	All tissues light brown, mild tissue perfusion
4	All tissues brown, moderate tissue perfusion
5	All tissues dark brown, excellent tissue perfusion

Table 17: Perfusion and coil spring success

(* denotes animals which died during experimental procedures, ** denotes animals which had springs dislodged before they got sacrificed.)

Perfusion Success P(1-6) and Closing coil spring Success S(Y=present N=not present)												
Days	1	P	S	2	P	S	3	P	S	4	P	S
7	101	*	-				104	5	Y			
	102	5	Y				105	**	N			
	103	*	-				106	5	Y			
	301	5	Y				305	4	Y			
	302	4	Y				306	5	Y			
	303	5	Y				307	5	Y			
	304	5	Y				308	5	Y			
14				201	5	Y				204	*	-
				202	**	N				205	**	N
				203	*	-				206	*	-
				401	5	Y				405	5	Y
				402	5	Y				406	**	N
				403	**	N				407	5	Y
				404	*	-				408	4	Y

5.7 Decalcification

All tissues were decalcified according to the protocol using the 4% EDTA solution prepared as per **Apendix 8.3.6**. Radiographic examination of

the tissues revealed no evidence of hard tissue presence after a period of 8 weeks. The specimens were then stored in EDTA and will be processed as part of another future study.

PART 3

5.8 TRAP staining

The modified technique of Goldberg and Barka provided satisfactory TRAP staining of the experimental tissues.[99] The protocol was modified further so as to prevent the melting of the gelatin slide coating and the subsequent loss of adherence between the tissue sections and the experimental tissues as the gelatin was heated. This was achieved by using the solution at 22^0C for a period of 3 hours.

5.9 TRAP activity

Trap-positive cells were detected in anti-NGF and control groups. The intensity of TRAP activity was greatest in areas associated with compression-induced resorption and repair. On the compression side, TRAP activity was observed in the coronal and mid thirds of the root, and along the adjacent margins of the alveolar bone as wells as within the marrow spaces.

TRAP-positive multinucleated cells, however, were not positive for NGF staining.

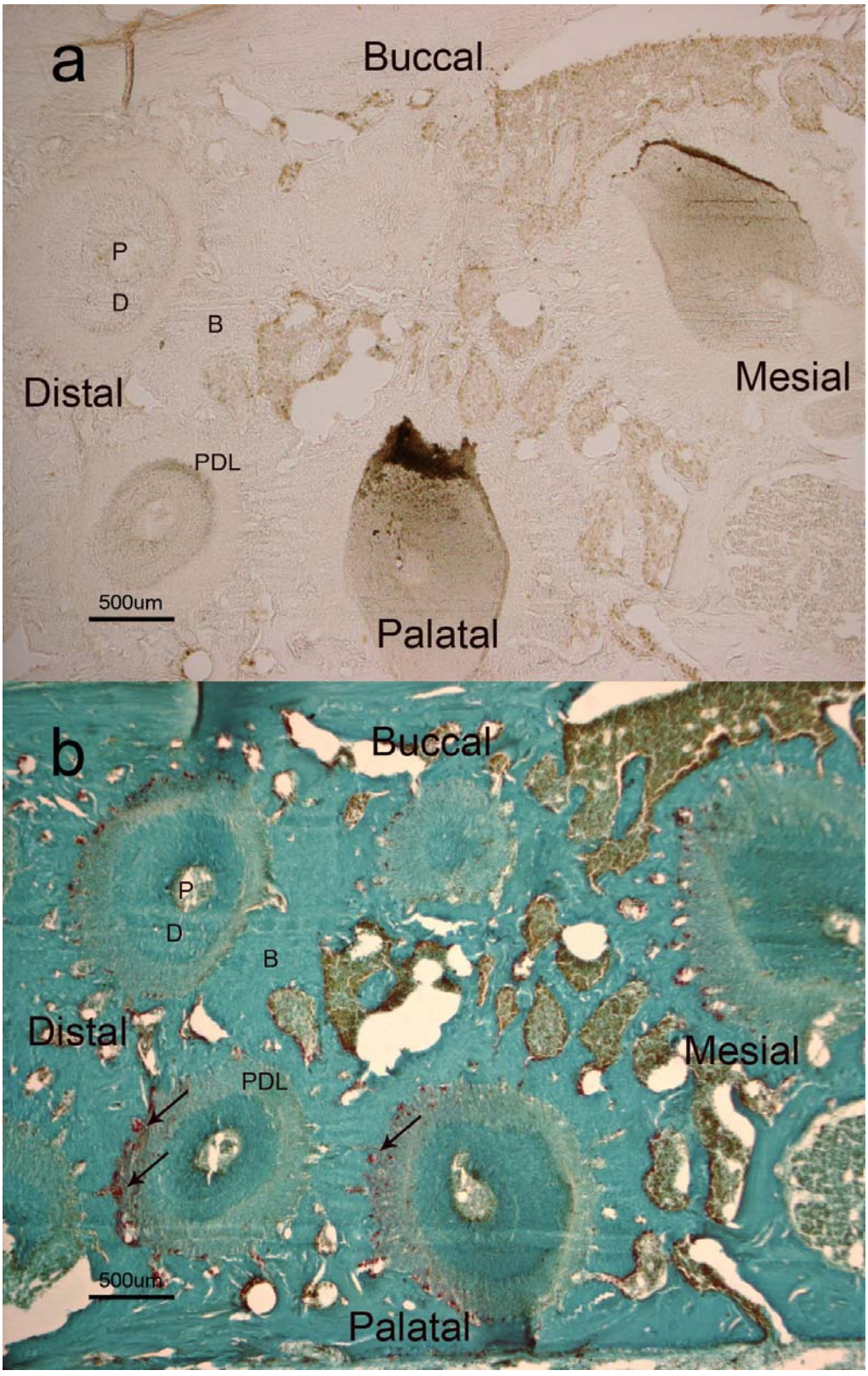

Figure 36: Comparison of anti-NGF (a) and TRAP stained (b) horizontal section at 0 day of M^1 control side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

Note osteoclasts distributed evenly along the distal aspect of the roots.

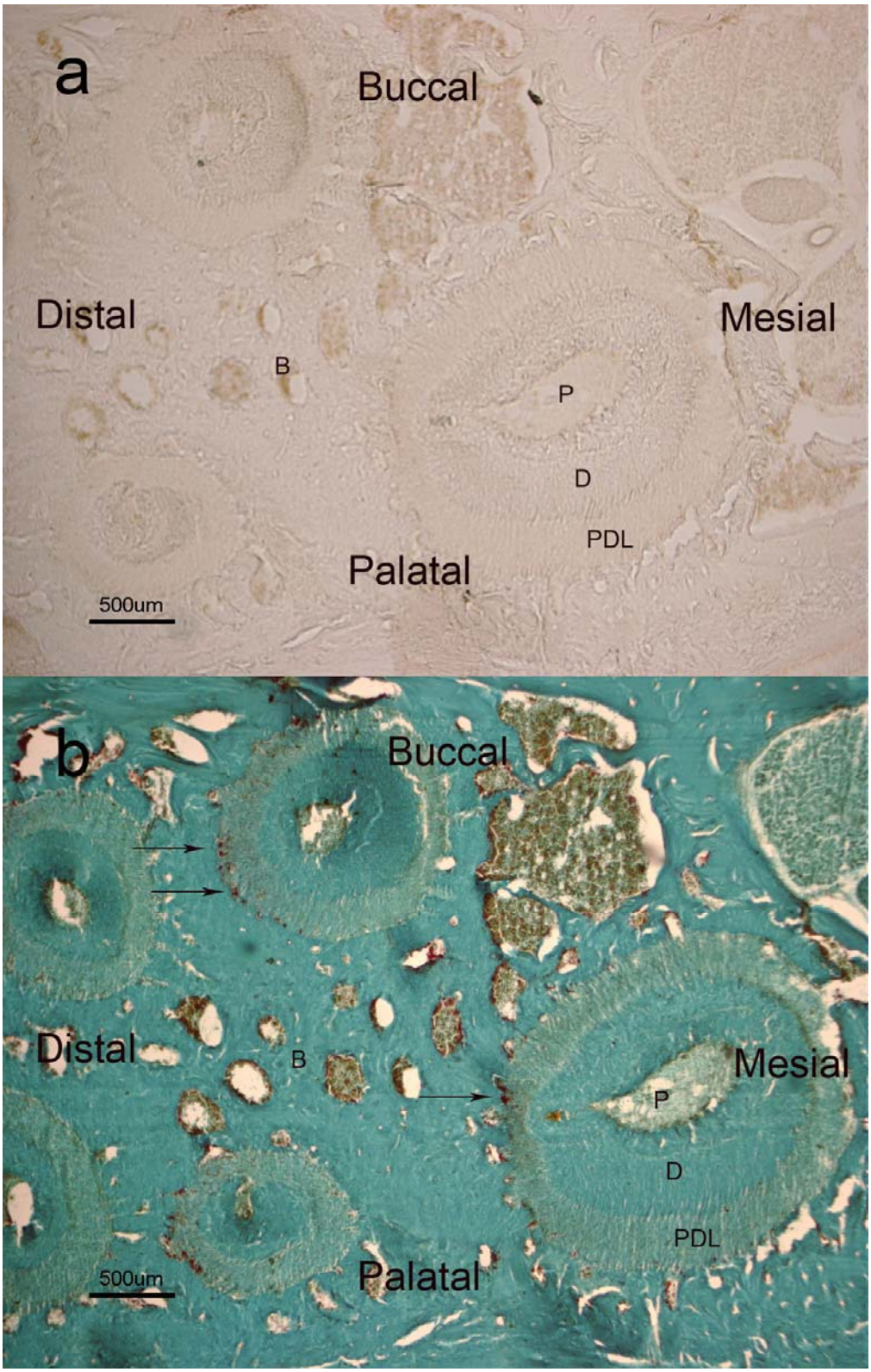

Figure 37: Comparison of NGF staining (a) and TRAP stained (b) horizontal section at 0 day of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

Note osteoclasts distributed on the distal aspect of the roots.

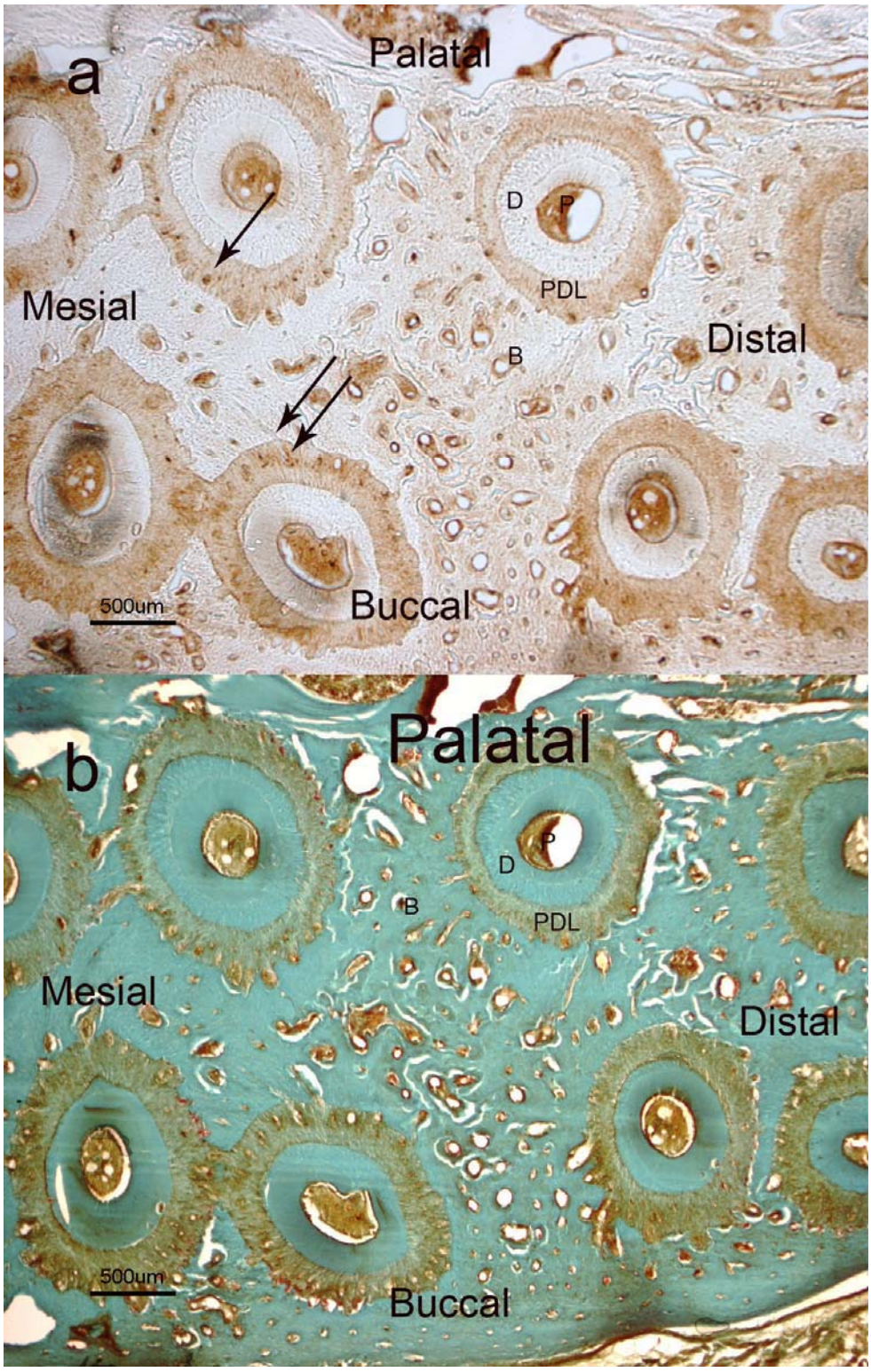

Figure 38: Comparison of NGF staining (a) and TRAP stained (b) horizontal section at 7 days of M^2 control side. NGF stained positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone.

Note NGF staining positive cells are not stained with TRAP.

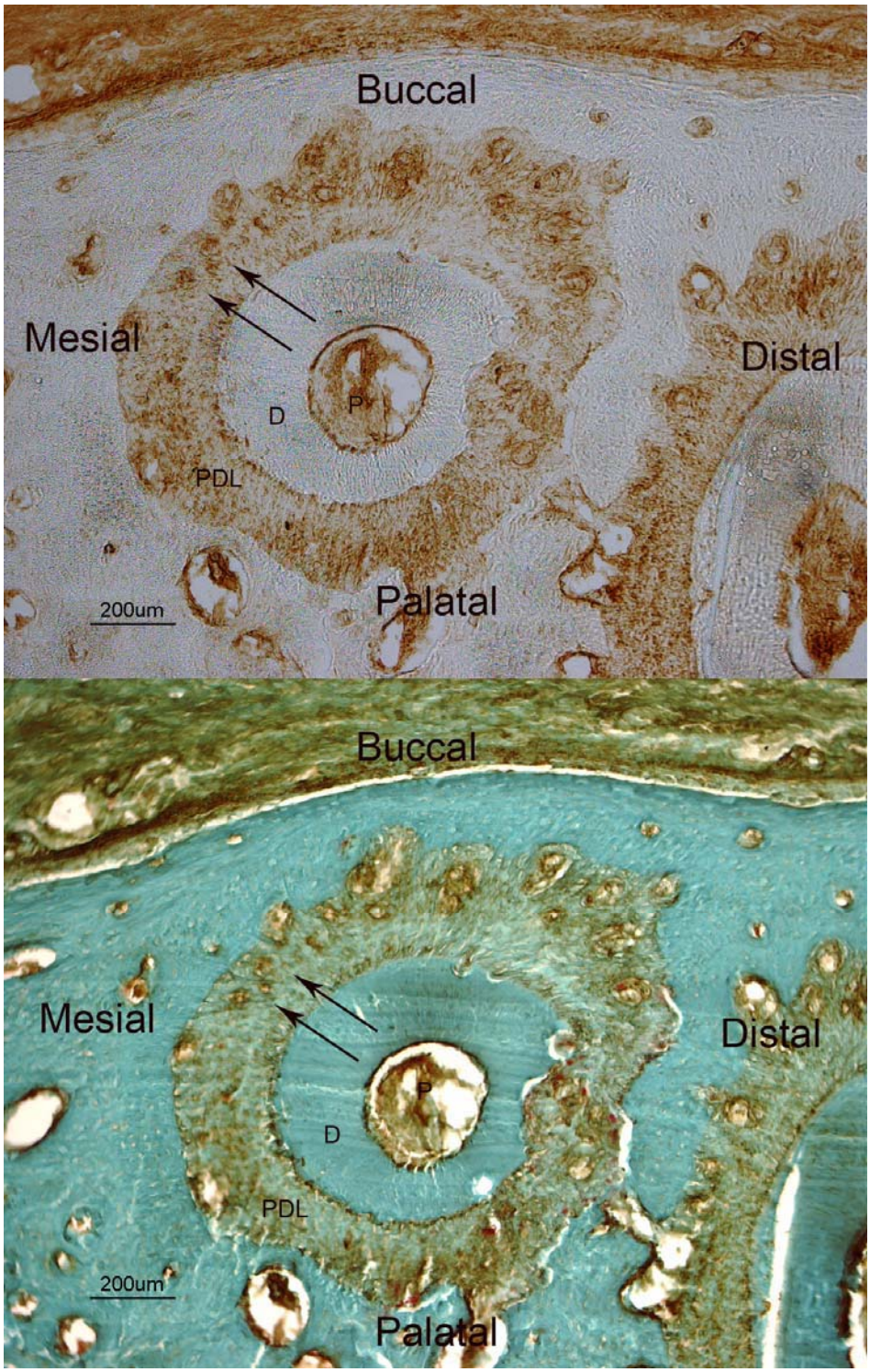

Figure 39: Comparison of NGF and TRAP stained horizontal section at 7 days of M^1 experimental side. NGF staining positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone. Note NGF staining positive cells are not stained with TRAP.

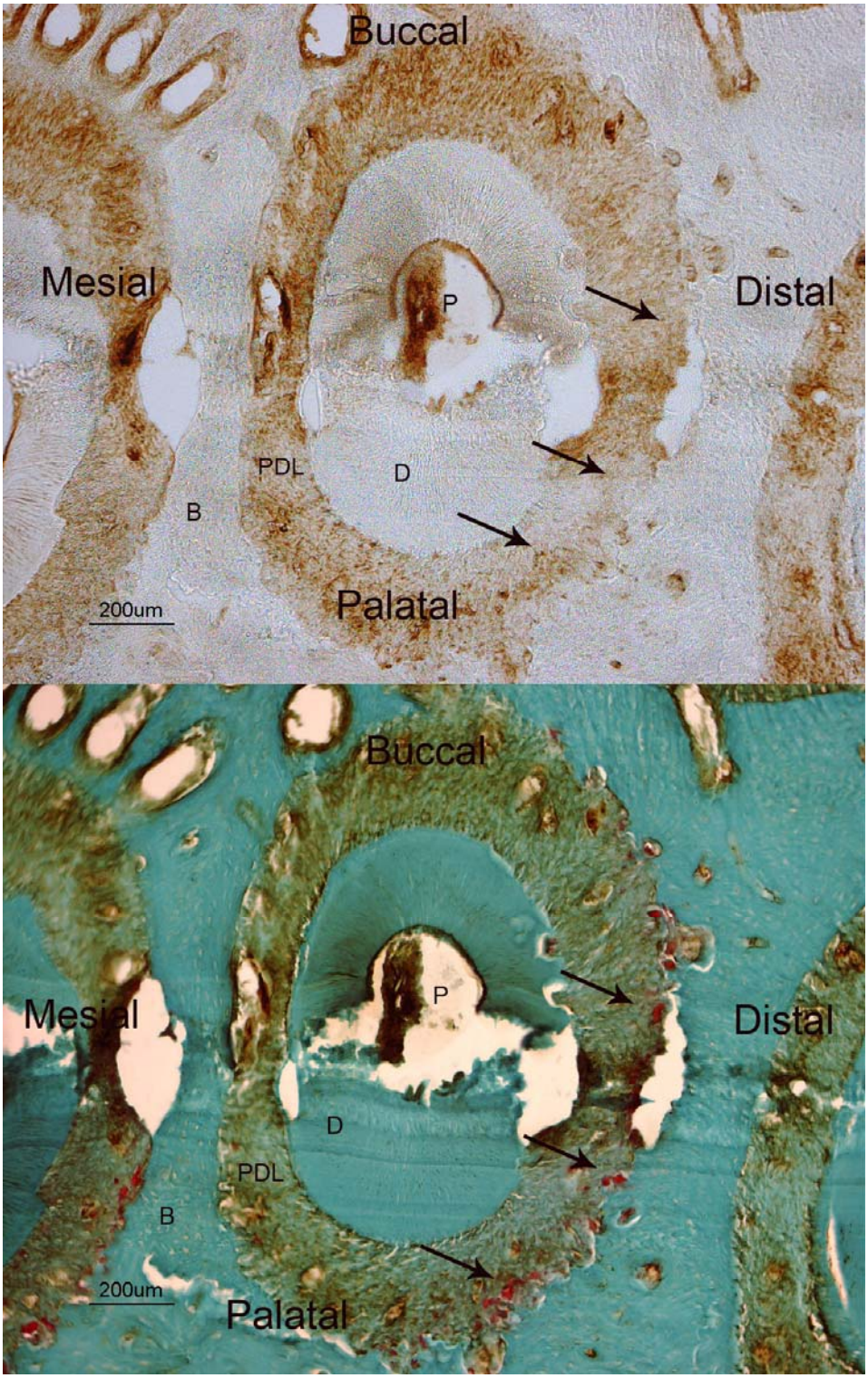

Figure 40: Comparison of NGF and TRAP stained horizontal section at 7 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF immunolabelling.

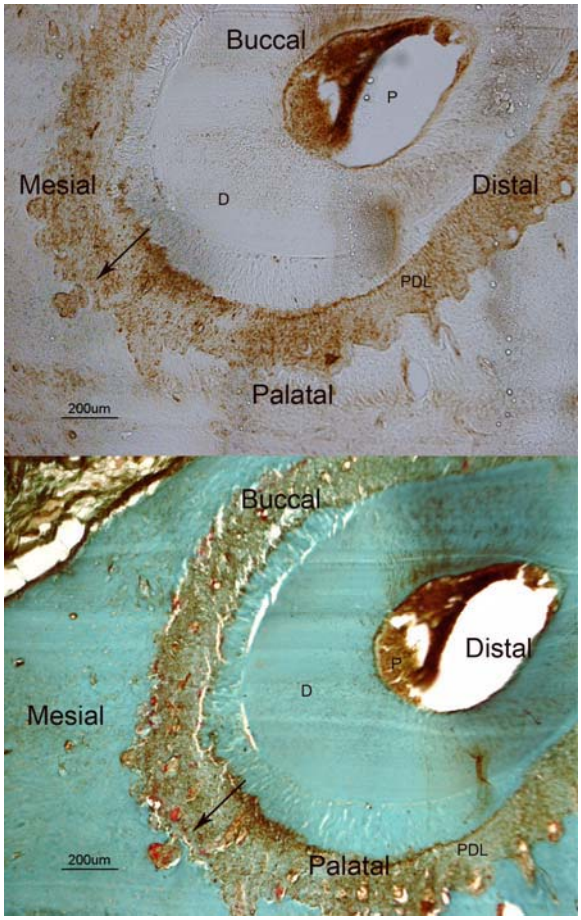

Figure 41: Comparison of NGF and TRAP stained horizontal section at 7 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF immunolabelling.

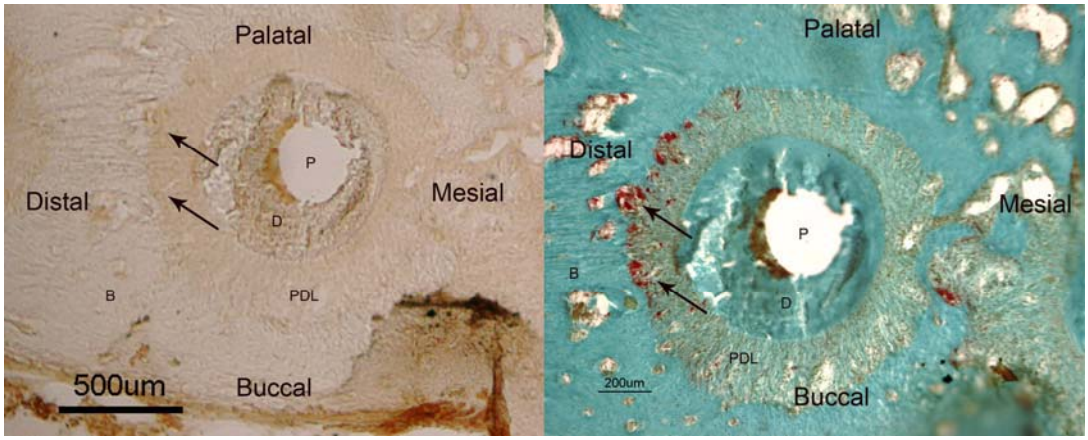

Figure 42: Comparison of anti-NGF and TRAP stained horizontal section at 7 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF.

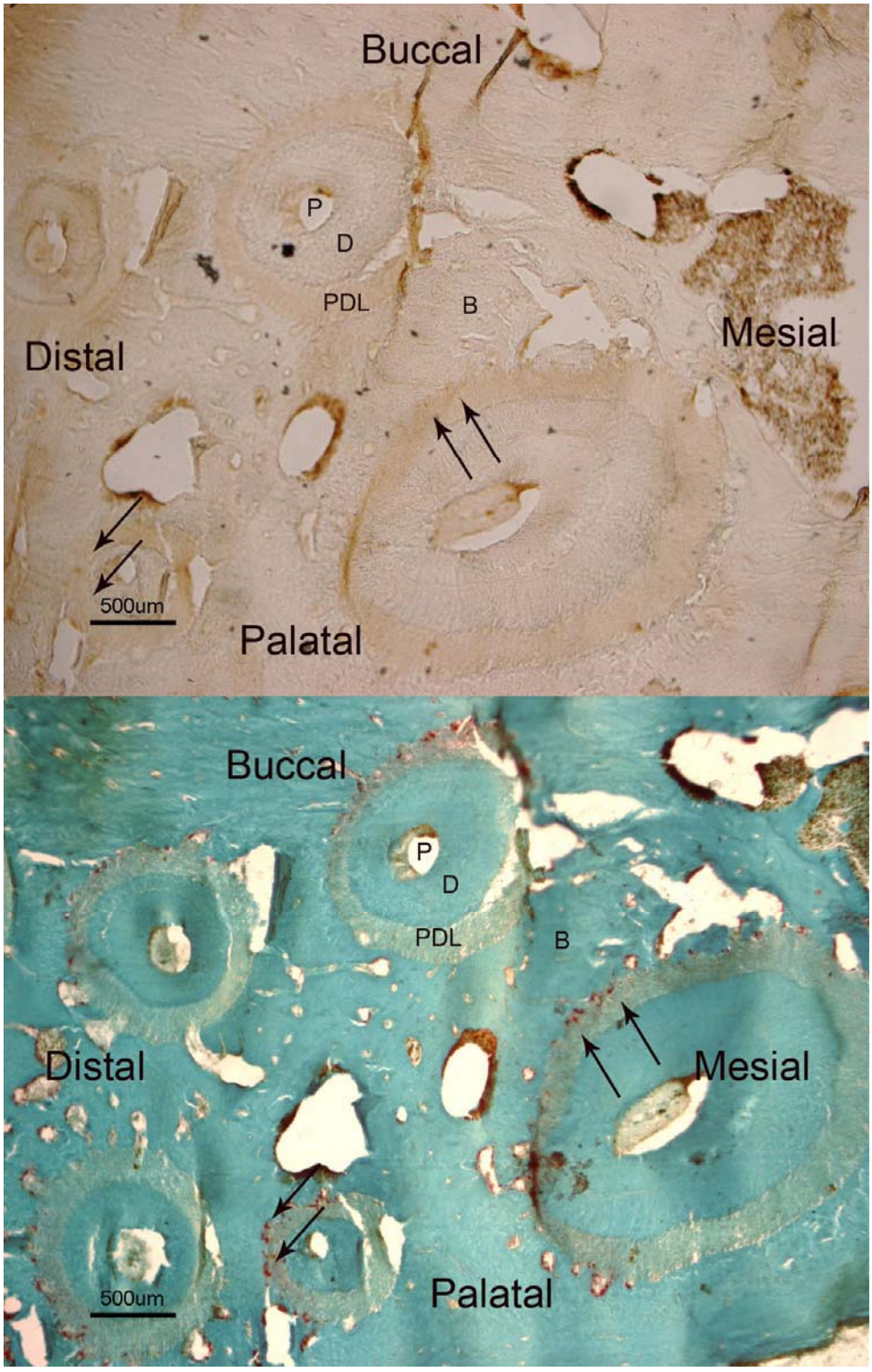

Figure 43: Comparison of anti-NGF and TRAP stained horizontal section at 14 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF immunolabelling.

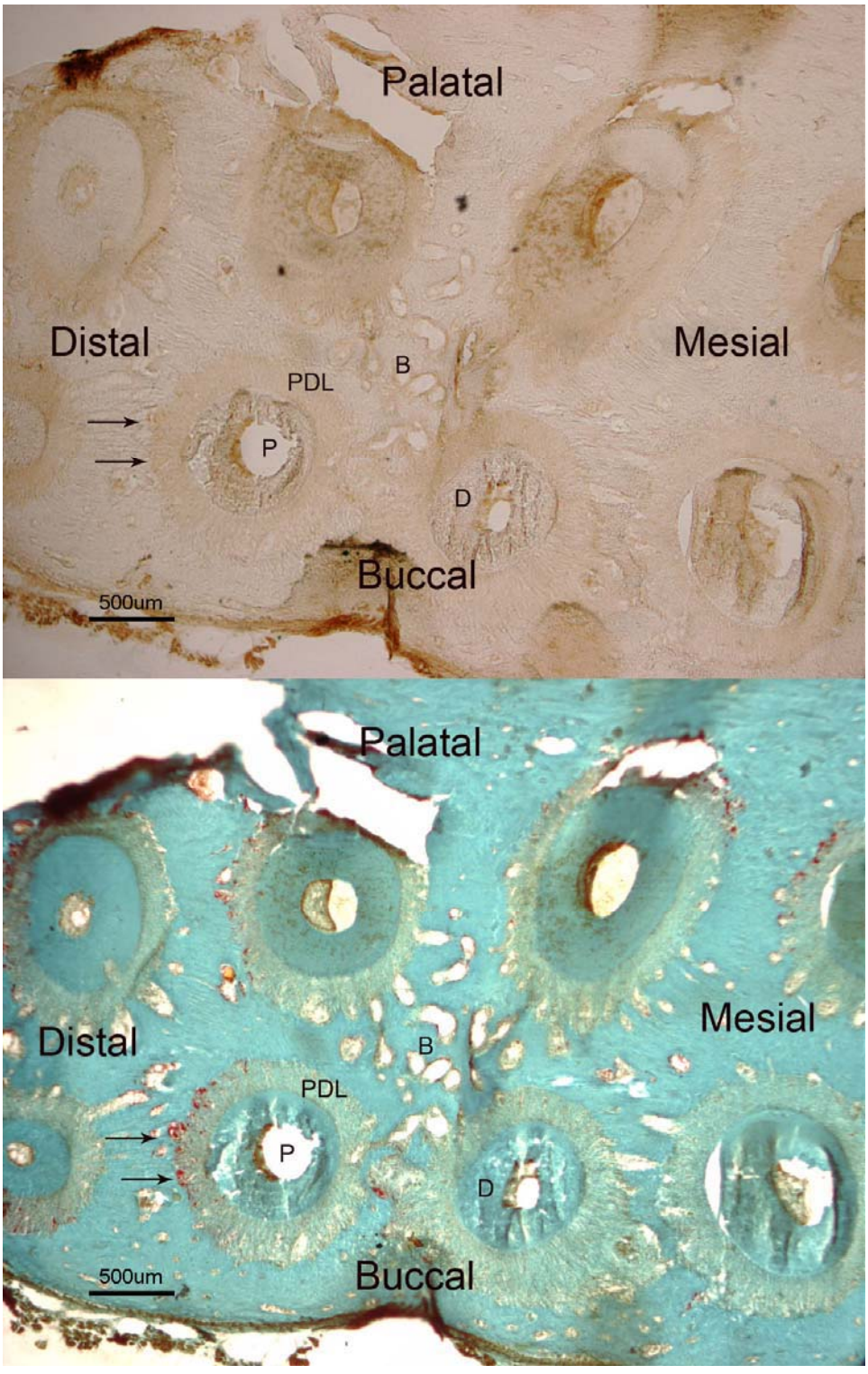

Figure 44: Comparison of NGF and TRAP stained horizontal section at 14 days of M^2 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. B, bone. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF immunolabelling.

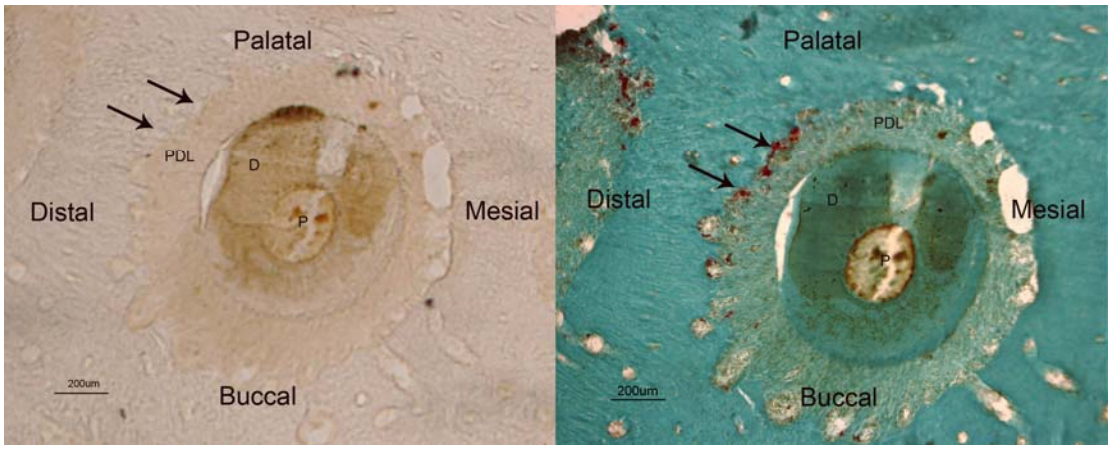

Figure 45: Comparison of NGF and TRAP stained horizontal section at 14 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF.

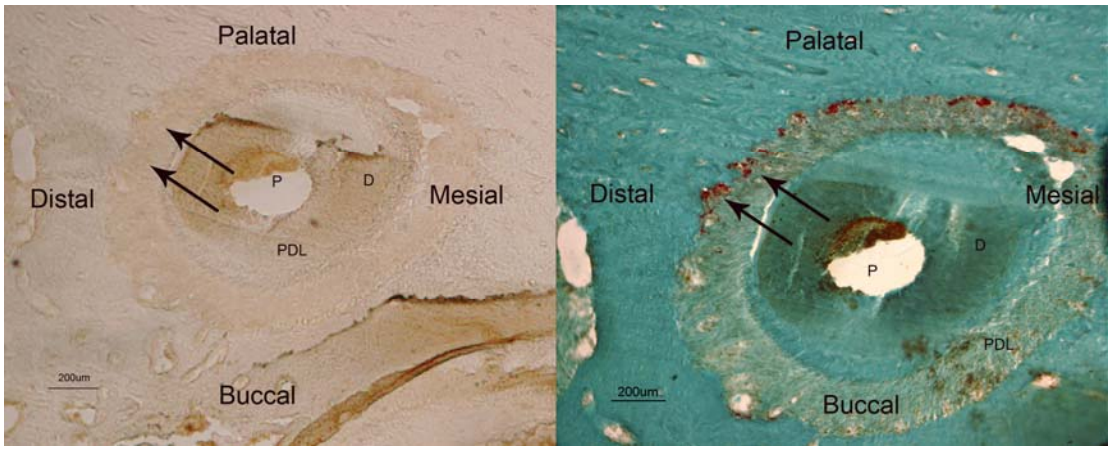

Figure 46: Comparison of anti-NGF and TRAP stained horizontal section at 14 days of M^1 experimental side. TRAP positive (arrows). PDL, periodontal ligament. P, dental pulp. D, dentine. Note osteoclasts distributed more in areas of compression and are not heavily stained with NGF.