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Orthodontic movement after periodontal regeneration of class II furcation: a pilot study in dogs

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Abstract

Purpose: The effect of orthodontic movement on the periodontal tissues of maxillary second pre-molars, after regenerative treatment for class II furcations, was evaluated in four mongrel dogs.

Material and Methods: Class II furcation lesions were created. After 75 days they were treated with bovine bone mineral matrix and guided tissue regeneration with absorbable membrane. After 2 months of daily plaque control, each of the dog's furcation pre-molars was randomly assigned to a test or control group. Orthodontic appliances were placed on both sides of the maxilla using third pre-molars and canines as anchorages. In the test group, bodily orthodontic movement of the second pre-molars was performed in the mesial direction for 3 months while control pre-molars remained unmoved. The dogs were sacrificed for histometric and histologic analyses.

Results: There were no statistically significant differences between the two groups in total bone and biomaterial areas or linear extension of periodontal regeneration on the radicular surfaces. In the test group, however, there was a tendency to a greater quantity of bone and a lesser quantity of biomaterial.

Conclusion: The orthodontic movement was not pre-judicial to the results obtained with the regenerative periodontal treatment.

Key words: biomaterials; guided tissue regeneration; orthodontics; periodontics

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Prognosis of orthodontic movement in patients previously affected by periodontal disease is uncertain because they generally present a reduced periodontium and bone defects such as furcation lesions. Control and elimination of these lesions are difficult resulting in a high percentage of unsuccessful treatment and recurrence of the disease (Hirschfeld & Wasserman 1978, McFall 1982).

Studies have shown that furcation lesions treated by conventional scaling therapies, followed soon by orthodontic movement, remain unaffected or become worse especially when inflammation is present (Roberts et al. 1982, Burch et al. 1992).

Use of regenerative periodontal techniques has improved prognostic of periodontal defects. This is especially true for class II furcation lesions that may be eliminated or reduced to class I lesions which are easier to access for plaque control (Hugoson et al. 1995, Bouchard et al. 1997, Houser et al. 2001).

Therefore, use of regenerative periodontal techniques before orthodontic movement may have the advantage of increasing clinical attachment by significant regeneration of lost periodontal tissues. Although some studies (Ericsson & Thilander 1978, Polson et al. 1984, Boyd et al. 1989, Re et al. 2000) show that a reduced periodontium does not rule out tooth movement, improved attachment and bone support would facilitate better control of subgingival inflammation of infrabony or furcation lesions. For this reason, combined periodontal regenerative treatment and orthodontic movement has been proposed in clinical cases (Nemcovsky et al. 1996, Stelzel & Flores-de-Jacoby 1998, Aguirre-Zorzano et al. 1999, Re et al. 2002).

Studies, including histologic analyses that evaluate orthodontic movement of teeth previously treated by regenerative techniques to verify tissue response and behaviour of biomaterials are important in this context. Tooth movement towards previously grafted extraction sockets (Hossain et al. 1996, Araújo et al. 2001) showed a greater degradation of biomaterial. Also movement of teeth towards alveolar bone defects was a

This work should be attributed to the Department of Periodontology, School of Dentistry at Araraquara, State University of São Paulo – UNESP, Araraquara, SP, Brazil.

stimulating factor for bone apposition, suggesting a beneficial effect of orthodontic movement in regenerative therapy (Vardimon et al. 2001).

However, more studies are needed to evaluate orthodontic movement in periodontal defects, such as furcation lesions, to verify the extent to which orthodontics may improve results obtained with regenerative periodontal treatment.

The objective of this study was to conduct histologic and histometric evaluations, in mongrel dogs, of the effect of orthodontic movement on the periodontal tissues of maxillary second premolars with class II furcation lesions previously treated with bovine bone mineral matrix and guided tissue regeneration using absorbable membrane.

Material and Methods

This study was approved by the Ethics Committee on Animal Experimentation of the School of Dentistry at Araraquara – UNESP. In this pilot study a total of eight teeth, the second upper pre-molars of four dogs, were used. For clinical procedures the dogs, each weighing between 15 and 20 kg, were sedated with pre anaesthetic, levopremazin chloritate (Neozine®, Aventis Pharma Ltd., São Paulo, Brazil) administered intramuscularly 0.2 ml per kg of body weight and anaesthetized with sodium thiobarbiturate (sodium Tiopental®, Abbott Laboratories, São Paulo, Brazil) of a 25 mg/ml concentration in the proportion of 0.5 ml/kg of body weight and kept on intravenous hydration with a physiological solution of 0.9% during surgery. Immediately after each surgery the dogs were given an ampoule of 10 ml of hepatic protector (Frutoplex LN, Marjan e Comércio, São Paulo, Brazil) intravenously and a 2 ml ampoule of dipirone intramuscularly.

The following clinical data were obtained for the second pre-molars at the beginning of the periodontal regenerative treatment and at the beginning and end of the orthodontic movement: probing depth (PD), bleeding on probing (BP), marginal gingival recession (MGR) and clinical attachment level (AL).

Creation and cronification of class II furcation lesions

Initially scaling and tooth prophylaxis with a rubber cup and prophylactic toothpaste were performed in the entire mouth. After a week a class II furcation lesion, 4.00 mm high and 2.00 mm deep, was made on the buccal side of the second upper pre-molars. The cavities were filled with gutta-percha to prevent spontaneous regeneration of the lesions (Cirelli et al. 1997) and allow for lesion cronification (Wikesjö et al. 1991). The first upper pre-molars on both sides were extracted to allow for movement of second upper pre-molars in the mesial direction. The flaps were coronally positioned and sutured. After a week the sutures were removed. The dogs were fed with water-softened ration for 60 days in order to accumulate plaque causing radicular contamination and development of chronic inflammation.

After 60 days of cronification, the gutta-percha was removed and followed by scaling and root planing. To make the orthodontic appliance in the laboratory, the second and third pre-molars were prepared with a high-speed bur to achieve parallel proximal surfaces. Impressions of these teeth were then taken with a silicone-based material.

Periodontal regenerative treatment

Fifteen days after removal of the gutta-percha a mucoperiosteal flap was raised to expose the roots which were scaled with hand instruments. The dimensions of the furcation lesions were measured and found to be 4.25 ± 0.37 mm high, 2.06 ± 0.32 mm deep and 3.00 ± 0.15 mm width. Reference notches were made on the mesial and distal roots at the bone crest level in the furcation region of these second pre-molars.

Regenerative treatment of the furcation lesions with bovine bone mineral matrix (Bio-Oss®, cancellous granules measuring 0.25–1.00 mm, Geistlich Sons Ltd., Wolhausen, Switzerland) and absorbable membrane of glycolide copolymer and lactide (Resolute® XT W.L. Gore & Associates Inc., Flagstaff, AZ, USA) was performed. The bovine bone mineral matrix was gently placed into the defects until a complete filling of the furcation (approximately 0.12 g of Bio-Oss® per defect). Membranes were sutured with a poliglactin 910 no. 4 absorbable suture (Vycril, Ethicon S.A., São Paulo, Brazil) (Figs 1 and 2). The flap was repositioned coronarily with a suspending and inter-proximally interrupted no. 4.0 silk suture (Ethicon, Atraloc, Johnson & Johnson S.A., São Paulo, Brazil) in order to cover the whole membrane and provide more stability to the region (Fig. 3). Thereafter, daily plaque control was begun by applying a 0.2% chlorhexidine gel with a soft brush. During and after this period the dogs continued to be fed with water-softened ration to minimize mechanical trauma.

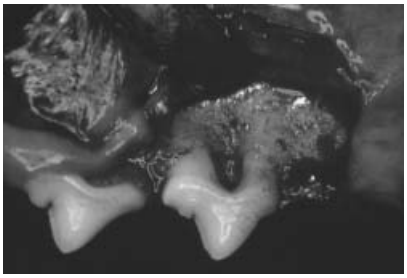

Fig 1. Class II furcation lesion. After the 60 day cronification period, reference notches were made on the mesial and distal roots at the bone crest level in the furcation region.

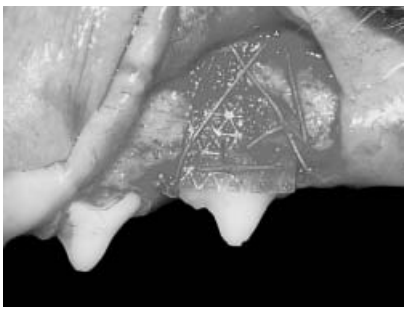

Fig 2. Regenerative treatment. Class II furcation treated with bovine bone mineral matrix and absorbable membrane.

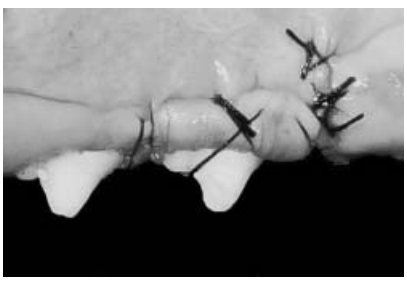

Fig 3. Suture. Flap repositioned and sutured after regenerative therapy.

An antibiotic (Pentabiótico, Fort Dodge®, Campinas, SP, Brazil) of 0.1 ml/kg of body weight was intramuscularly administered to the dogs, immediately after this surgery and repeated 5 days later.

Orthodontic movement

After 60 days, one of these second premolars of each dog was randomly assigned to the test group and the other

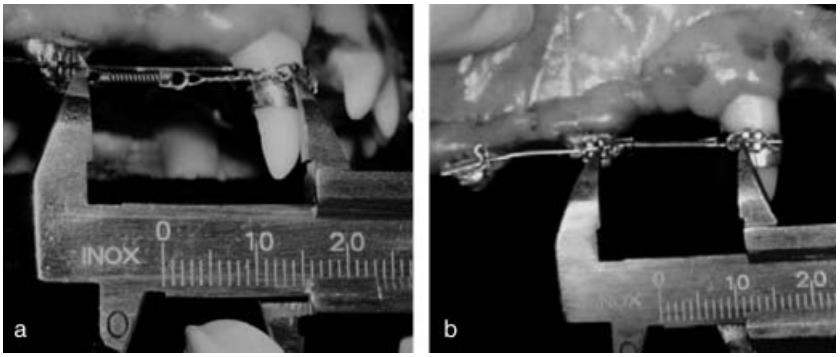

Fig 4. Orthodontic appliance on the maxilla after 60 days of the regenerative treatment. Inter-bracket distance measured with a pachymeter in the test group: a, before: 23 mm; b, after the orthodontic movement: 19 mm.

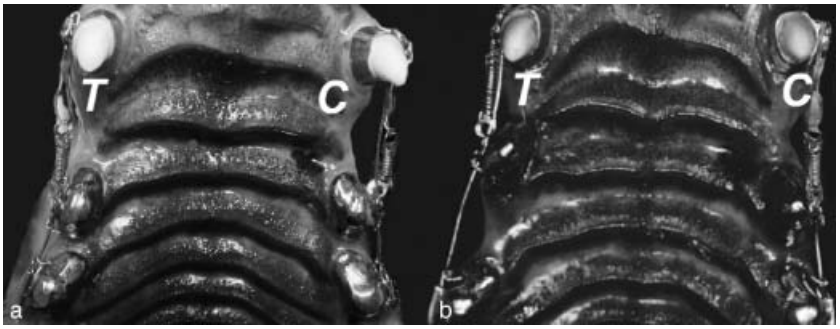

Fig 5. Occlusal view of the maxilla: a, before the orthodontic treatment; b, 90 days after beginning of the orthodontic movement. T, test side; C, control side.

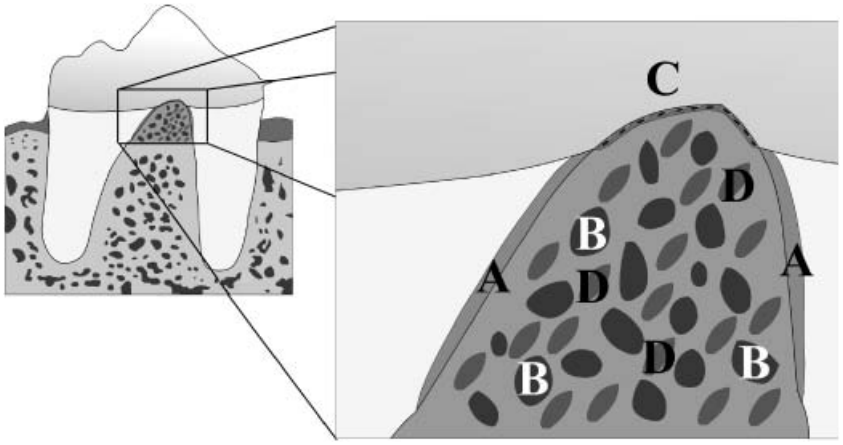

Fig 6. Schematic drawing of histometric analysis of the furcation between the notches mesial (M) and distal (D). **A.** Cementum formation**. B.** Bone filling area. **C.** Epithelial migration. **D.** Biomaterial filling area. The other tissues filling area was the furcation total area except the bone and biomaterial filling area.

to the control group. So each animal had a test and control side resulting in a total of four second pre-molars in each group. Orthodontic appliances were placed on each of the four pre-molars of the test group and the teeth were moved in the mesial direction. Identical appliances were placed on each of the four control group pre-molars, which, however, were never subject to movement (Fig. 4a and b).

After prophylaxis, the nickel–chromium crowns with brackets soldered to the second pre-molar and tubes soldered to the third pre-molar and bands with tubes soldered on the canine teeth were cemented on the respective teeth with adhesive cement (Panavia 21X Dental Adhesive, Kuraray Co. Ltd., Osaka, Japan). Stainless-steel wire of a rectangular cross section of $0.020'' \times 0.025''$ was passed through the tube on the canine and third pre-molar and the bracket on the second pre-molar. Nickel–titanium springs (ORMCO Corporation, Glendora, CA, USA), 9 mm long, providing a light and continuous tension of 100 g, were placed between the second pre-molars and the canine teeth. Activation of the spring was made every 20 days to provide movement in the mesial direction.

The tension of the spring was measured by a tensitometer (ORMCO Corporation) and the length of stretched spring was 11 mm as measured by a pachymeter to obtain 100 g. In the control group, teeth were not moved, although they had the same wire and springs as the test group, however without activation. Orthodontic appliances were used in the control group solely to provide identical conditions for bacterial plaque accumulation and hygiene as in the test group.

After 90 days (Fig. 5) the appliances were removed, the dogs were anaesthetized for sacrifice with an overdose of Thiopental and the second upper pre-molars were removed as a block.

Histologic and histometric analyses

The biopsies were harvested, fixed in 10% formalin and decalcified in Morse solution for routine histologic processing and paraffin embedding.

Histologic sections, 5 μm thick, were cut in a bucco-lingual direction through the entire mesio-distal plane of the whole tooth (Jung Supercut 2065 Leica, Chicago, IL, USA). Five sections were selected for each tooth. The first and last sections showed the reference notches on the root surfaces while three other sections which were equally spaced between the first and last sections, represented the buccal, central and lingual portions of the defects. These sections were stained with haematoxilin and eosin (HE) and Masson trichrome for further descriptive histologic and histometric analyses.

Histometric analysis was performed with image analysis software (Sigma Scan Pro, Jandel Scientific, San Rafael, CA, USA) to obtain the following measurements (Fig. 6):

Table 1. Clinical results according to treatments and measurement time

Dog #	Treatment and time	G	PD (mm)			BP			MGR (mm)			AL (mm)		
			M	B	D	M	B	D	M	B	D	M	B	D
1	Regenerative treatment	C	3	2	3							3	2	3
	Beginning	T	2	1	2							2	1	2
	Orthodontic treatment	C	1	1	2			+		1		1	2	2
	(Initial)	T	1	1	2	+	+			1	1	1	2	3
	Orthodontic-end	C	2	1	2					1		2	2	2
		T	1	1	2					1	1	1	2	3
2	Regenerative treatment	C	1	2	2							1	2	2
	Beginning	T	2	1	2							2	1	2
	Orthodontic treatment	C	2	2	2		+					2	2	2
	(Initial)	T	2	2	2		+					2	2	2
	Orthodontic-end	C	3	2	3							3	2	3
		T	3	2	3							3	2	3
3	Regenerative treatment	C	2	2	2		+					2	2	2
	Beginning	T	2	2	2		+					2	2	2
	Orthodontic treatment	C	2	1	2					1	1.5	2	2	3.5
	(Initial)	T	1	1	1				1	1		2	2	1
	Orthodontic-end	C	2	2	2					1	1.5	2	3	3.5
		T	1	1	2					1		1	2	2
4	Regenerative treatment	C	2	2	2		+					2	2	2
	Beginning	T	2	2	2		+					2	2	2
	Orthodontic treatment	C	1	1	1							1	1	1
	(Initial)	T	1	1	1							1	1	1
	Orthodontic-end	C	2	2	1							2	2	1
		T	2	2	2							2	2	2

PD, probing depth; BP, bleeding on probing; MGR, marginal gingival recession; AL, clinical attachment level; G, group; M, mesial; B, buccal; D, distal; C, control group; T, test group.

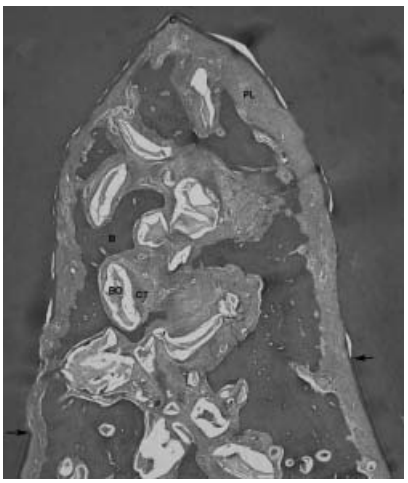

Fig 7. Panoramic view of the furcation lesion of the control group. Healing of the furcation with regeneration of the cementum (C), periodontal ligament (PL) and alveolar bone (B). Biomaterial (BO) associated with the newly formed bone (B) and connective tissue (CT) in partial manner. Arrows indicate the notches. Haematoxylin and eosin, original magnification × 20.

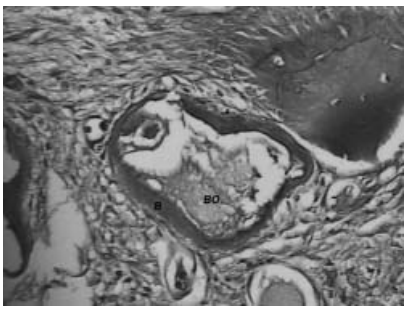

Fig 8. Biomaterial (BO) completely embedded in the newly formed bone (B) of the control group. Haematoxylin and eosin, original magnification × 200.

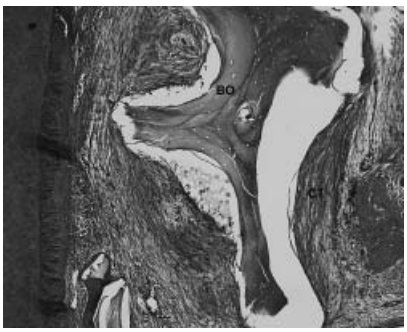

Fig 9. Biomaterial (BO) embedded in the dense connective tissue (CT) of the control group. Masson trichrome, original magnification × 200.

Cementum formation: linear radicular extension, between the notches on the mesial, M, and distal, D, roots of second pre-molars, covered with new cementum.

Epithelial migration: linear radicular extension of the lesion covered with epithelial tissue.

Bone filling area: area of lesion filled with newly formed bone, apically defined by a straight line joining the two radicular notches.

Biomaterial filling area: area of lesion filled with biomaterial.

Area filled with other tissues: area of lesion filled with cementum and non-mineralized tissues.

Statistical analysis of the effect of the orthodontic treatment on the variables of radicular linear extension, in millimetres, and upon the lesion area, in square millimetres, was made using the Wilcoxon test. Each histometric variable was analysed by comparing measurements of the test group to those of the control group, which were considered paired. The significance level of 5% was considered a decision rule for a significant difference.

Results

All sites healed suitably after the surgical procedures. One animal, #1, presented membrane exposure in both the

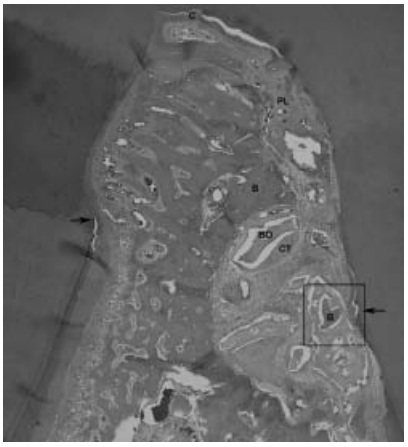

Fig 10. Panoramic view of the furcation lesion of the test group. Healing of the furcation with regeneration of the cementum (C), periodontal ligament (PL) and alveolar bone (B). Biomaterial (BO) embedded in the connective tissue (CT) and with newly formed bone (B) interposed inside of the biomaterial. Arrows indicate the notches and the square indicates the region of Fig. 11. Haematoxylin and eosin, original magnification × 20.

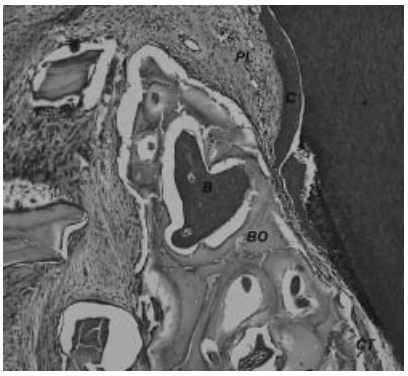

Fig 11. Detail of Fig. 10. Pressure side. New cementum formation (C), bone interposed in the biomaterial (B), periodontal ligament (PL), and biomaterial (BO) associated with the connective tissue (CT). Haematoxylin and eosin, original magnification × 100.

test and control groups during healing. These sites were maintained under reinforced plaque control until resorption of the exposed membrane.

Gingival recession of the second premolars varied from 0 to 1.5 mm. This did not interfere with the furcation closure and was observed in two dogs, #1 and 3, at the beginning of the orthodontic movement and remained unchanged throughout the study. PD for the second pre-molars was 1–3 mm without BP and without loss of attachment after orthodontic movement. Clinical results are in the Table 1. The average amount of tooth movement obtained was 4 mm.

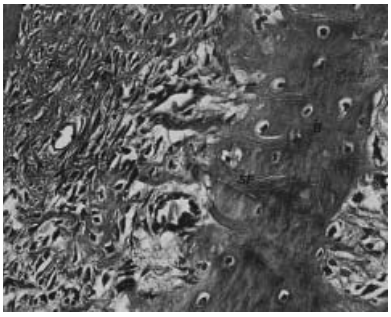

Fig 12. Tension side. Sharpey's fibres (SF) stretched in the direction of orthodontic movement, alveolar bone (B), periodontal ligament (PL). Masson trichrome, original magnification × 400.

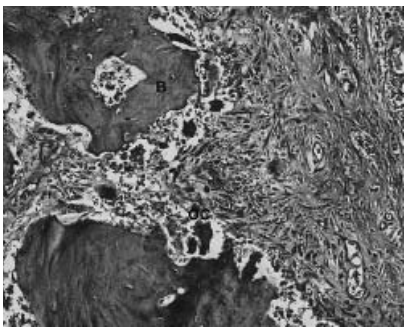

Fig 13. Osteoclasts (OC) around the alveolar bone (B) of the pressure side. Masson trichrome, original magnification × 200.

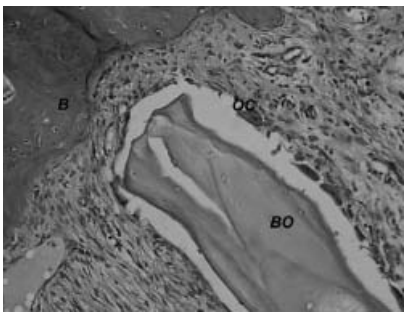

Fig 14. Osteoclasts (OC) around of the biomaterial (BO) of the pressure side, alveolar bone (B). Haematoxylin and eosin, original magnification × 200.

Histologic analysis

Control group

Control teeth presented new cementum formation in the entire furcation region from the radicular notch. The epithelial migration in the roof of the furcation region was present only on the more superficial slices. Inflammatory infiltration, when present, was moderate with sub-epithelial focal exacerbations. There was apposition of new cellular cementum from the radicular notch to the roof of the furcation tending to surface smoothing. Primary trabecular bone completely filled the region (Fig. 7). Osteoclasts interposed among the osteoblasts were common. Generally, the bone graft fragments were covered by fibrous connective tissue or interposed or associated with the newly formed bone in a complete or partial manner (Figs 8 and 9). Bone graft fragments, when found in the periodontal ligament, were not integrated to the cementum.

Test group

Test teeth presented cellular cementum in the entire furcation region from the radicular notch. Radicular resorptions occurred to a greater extent in the pressure areas but with new cementum apposition in the entire resorbed area. Bone tissue in the furcation region was of the primary trabecular type and with a greater tendency to more bone apposition in the test group (Fig. 10). Similar to the control group, the bone graft material was covered by fibrous connective tissue, sometimes also interposed or associated to the newly formed bone in a complete or partial manner and was found present in a lesser quantity than in the control group. Bone graft material tended to displace laterally to the pressure side and down from the radicular notch. Bone graft material, present in the periodontal ligament, also did not appear to be integrated to cementum (Fig. 11).

There was stretching of the collagen fibres with spicular bone arrangement accompanying the tension areas, with the insertion of new periodontal ligament fibres (Sharpey's fibres) in the cementum and in the bone (Fig. 12). There was bone resorption on the pressure side and a presence of osteoclasts around the bone (Fig. 13) and the bone graft material (Fig. 14).

Histometric and statistical analyses

Histometric analysis data of the root linear extension, in millimetres, and of the lesion area, in square millimetres, is presented respectively in Tables 2 and 3. These tables also present the original

Table 2. Histometric evaluations of root linear extensions

Group	Dog#	Linear extension (mm)			Linear extension (%)		
		cemented	epithelial migration	LPR	cemented	epithelial migration	LPR
C	1	5.85	0.53	5.81	91.7	8.3	91.1
	2	4.64	0.00	3.95	10.0	0.0	85.1
	3	6.65	1.35	6.09	83.1	16.9	76.1
	4	4.68	0.00	4.26	100.0	0.0	91.0
T	1	6.29	0.12	3.96	98.1	1.9	61.8
	2	5.30	0.00	5.03	100.0	0.0	94.9
	3	8.10	0.00	5.86	100.0	0.0	72.3
	4	6.44	0.00	2.83	100.0	0.0	43.9

LPR, linear periodontal regeneration.

Table 3. Histometric evaluations of lesion areas

Group	Dog #	Original area (mm^2)			Original area (%)		
		bone	biomaterial	to*	bone	biomaterial	to*
C	1	1.70	0.00	3.15	35.0	0.0	64.9
	2	0.53	0.38	1.95	18.5	13.3	68.2
	3	2.19	0.86	6.02	24.1	9.5	66.4
	4	1.39	0.03	1.36	50.0	1.1	48.9
T	1	1.86	0.01	3.15	37.1	0.2	62.8
	2	1.50	0.26	1.87	41.3	7.2	51.5
	3	2.77	0.35	4.90	34.5	4.4	61.1
	4	2.27	0.06	3.00	42.6	1.1	56.3

*to, other tissues (non-mineralized+cemented).

Table 4. Histometric statistics of root linear extensions, in percentages: mean extensions, standard deviations (SD) and probability *p* values corresponding to the Wilcoxon test

Tissue extensions	Group	Mean	SD	*p*
Cementum Formation	C	93.7	8.067	
	T	99.5	0.936	0.180
Epithelial Migration	C	6.3	8.067	
	T	0.5	0.936	0.180
LPR	C	85.8	7.050	
LPR	T	68.2	21.291	0.273

LPR, linear periodontal regeneration.

Table 5. Histometric statistics of lesion areas in percentages: mean areas, standard deviations (SD) and probability values, *p*, corresponding to the Wilcoxon test

Tissue areas	Group	Mean	SD	*p*
Bone	C	31.9	13.861	
	T	38.9	3.737	0.273
Biomaterial	C	6.0	6.461	
	T	3.2	3.188	0.465
Other tissues*	C	62.1	8.888	
	T	57.9	5.074	0.465

*Non-mineralized+cementum.

measurements that were transformed into percentages values for use in statistical analyses. This transformation was intended to minimize interference of tooth size in the results of the analyses.

Tables 4 and 5 present the probability values, *p,* corresponding to the Wilcoxon test of the data of histometric percentages, respectively, for root linear extension and lesion area. There was no evidence of any significant effect of the orthodontic treatment employed on the histometric variables evaluated at a 5% level of significance.

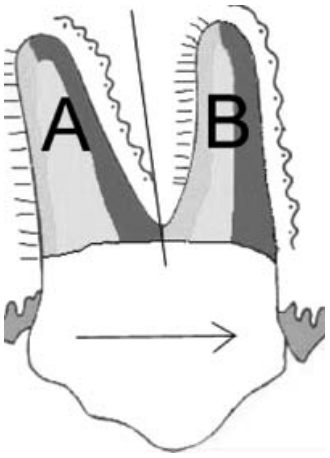

Fig 15. Schematic representation of second pre-molar with bodily movement in the mesial direction (test group). A. Resorption of the alveolar bone of the pressure side. B. Apposition of bone of the tension side.

The inter-radicular area was divided into two halves, the mesial and distal sides and biomaterial areas were calculated, in square millimetres, for both sides as a complementary analysis. In the test group these sides corresponded to pressure (distal) and tension (mesial) sides (Fig. 15). Results are in Table 6.

Comparisons of biomaterial areas were made by the Wilcoxon test at a significance level of 5%. Four comparisons were of interest and are shown in Table 7 together with the probability values, *p*, of the Wilcoxon test. There is no evidence, at a level of significance of 5%, of any difference in the areas of biomaterial between the sides compared.

Table 6 shows a tendency towards smaller values of biomaterial area on the tension side of the inter-radicular space in the test group. However, no statistical difference could be found, possibly because of the small number of dogs involved.

Discussion

This pilot study evaluated tissue reactions in class II furcation lesions on second maxillar pre-molars of dogs after treatment with bovine bone mineral matrix and absorbable membrane followed by orthodontic movement of translation in the mesial direction.

To obtain a tooth movement of translation, a nickel–titanium spring and stainless steel wire of a rectangular cross-section of 0.020″ by 0.025″ were used to provide movement with a control of inclination and without excessive friction.

Movement was begun 2 months after regenerative treatment to prevent mechanical movement from interfering with the healing process of the periodontal tissues and from accelerating membrane absorption, which might be prejudicial to the regenerative results. Studies found did not indicate the best time to begin orthodontic movement after regenerative treatment. The period of 60 days chosen for this study was based upon recent studies that evaluated periodontal regeneration from 60 to 90 days after treatment and that noted advanced healing of the periodontal tissues (Caffesse et al. 1990, Cirelli et al. 1997, Araújo et al. 2001).

Results show a similarity in the characteristics of the periodontal tissues

Table 6. Biomaterial areas on sides of the interadicular space

Dog #	Furcation: mesial (mm^2)	Control: distal (mm^2)	Furcation: tension (mm^2)	Test: pressure (mm^2)
1	0.002	0.000	0.045	0.196
2	1.242	0.479	0.000	0.676
3	0.788	1.233	0.170	1.010
4	0.200	0.000	0.097	0.040
Mean	0.558	0.428	0.078	0.481

Table 7. Probability values, *p,* from the Wilcoxon test for comparison between biomaterial of sides and to the experimental group

Comparison of sides	*p*
Mesial (C) *versus* distal (C)	0.465
Tension (T) *versus* pressure (T)	0.144
Pressure (T) *versus* distal (C)	0.715
Tension (T) *versus* mesial (C)	0.144

C, control group; T, test group.

formed in furcation areas of both groups, which suggests that the orthodontic movement did not interfere with the healing achieved by the regenerative periodontal techniques used. This regeneration was compatible with the results found in literature on treatment of class II furcation lesions (Machtei & Schallhorn 1995, Cirelli et al. 1997, Houser et al. 2001). All furcation lesions were clinically closed and only three teeth (Table 2) presented epithelial migration in the roof of the furcation. The presence of epithelial growth may be associated to membrane exposure and gingival recession clinically observed with these teeth. Lack of membrane adaptation on the external surface of the furcation because of the anatomy of the dog tooth may be another cause (Novaes et al. 2001).

New apposition of cementum occurred on almost all the lesion surfaces in both groups regularizing the radicular resorptions regions in the pressure areas of the teeth moved. Further the linear periodontal regeneration favoured the bone apposition on the tension side. These observations suggest that the earlier periodontal regenerative treatment caused a normal reaction of the tissue to the orthodontic treatment.

Bone tissue formed in the furcation region was of the primary trabecular type and was denser and with smaller medullar spaces in the test group that underwent movement, suggesting greater bone apposition for this group. Although these data were not statistically significant, descriptive analysis emphasized this characteristic. The tendency of a statistically different result could be confirmed with a larger sample.

The bovine bone mineral matrix is a completely deproteinized graft, which maintains the preferential orientation and size of the hydroxyapatite crystals as they are in vivo. Pinholt et al. (1991) considered the mineral structure of Bio-Oss® to be similar to that of the human bone, with an amorphous aspect and lines of increment. It is considered to be an osteoconductive material presenting an interconnecting system of pores that permits the growth of blood vessels, promotes the migration and subsequent attachment of osteogenic cells and supports the deposition of newly formed bone (Wetzel et al. 1995, Berglundh & Lindhe 1997, Valentini et al. 1998, Tapety et al. 2004).

Tapety et al. (2004), when investigating the response of bone cells to this biomaterial, which was grafted in artificial bone defects of rat femurs, found osteoinductive characteristics in Bio-Oss®. After 3 days, the perimeter of Bio-Oss® revealed weak alkaline phosphatase immunoreactivity, a sign of osteoblastic lineage, implying that the Bio-Oss® promoted osteogenic differentiation for the osteoblastic lineage. After 5 days, osteoblasts positive to alkaline phosphatase were found on the Bio-Oss®, secreting bone matrix proteins as well as collagen fibres. Therefore, Bio-Oss® appeared to show a biological affinity for osteogenic cells and a subsequent promotion of their differentiation. However, use of Bio-Oss® alone in other tissues than bone did not induce the formation of ectopic bone (Pinholt et al. 1991, Schwartz et al. 2000). Thus, this biomaterial does not seem to contain a sufficient volume and activity of osteoinductive proteins. Donos et al. (2004) evaluate the effect of guided bone regeneration in combination with bovine bone mineral matrix on the healing of critical-size calvarial defects and observed that the bone mineral particles localized close to the borders of the defect were most of the times surrounded by newly formed bone and that, in the centre of the defect, the biomaterial particles were surrounded by loose fibrous connective tissues with new bone formation around these encapsulated particles. Therefore, the use of this biomaterial could be applied better to bony surfaces from which its osteoconductive properties could be used.

Bio-Oss® is slowly reabsorbed remaining in humans for 3–4 years after its placement (Skoglund et al. 1997, Valentini et al. 1998). Studies with rabbits (Jensen et al 1996) and dogs, including maxillary sinus lift (Wetzel et al. 1995) and alveolar ridge augmentation (Berglundh & Lindhe 1997), also report that the substitution of that biomaterial by newly formed bone occurs slowly.

In this study, Bio-Oss® was also observed to be encapsulated by fibrous connective tissue and that, in other sites the biomaterial was partially or totally covered by bone tissue. Similar results were obtained by other authors that used animals (MacNeill et al. 1999) and humans (Becker et al. 1998, Camelo et al. 1998, Paolantonio et al. 2001). These differences in the histologic findings may be explained by differences in the sites where the Bio-Oss® was placed. The base of the furcation, near the radicular notches, is an area near the remaining alveolar bone and has osteogenic cells, explaining the Bio-Oss® covered by new bone tissue. Also, the orthodontic movement promotes tension and pressure areas and in the latter there is more osteoclastic activity. In this study, the Bio-Oss® remaining on the pressure side was encapsulated by dense connective tissue.

Presence of clastic cells around the biomaterial on the pressure side of the furcation in the test group also was noticed. This may justify the tendency of a smaller quantity of biomaterial in the test group when compared with the control group. Studies with mice and monkeys also showed that Bio-Oss® particles were degraded, probably by osteoclastic activity (Klinge et al. 1992, Hürzeler et al. 1997). Thus, the process of absorption occurred in the Bio-Oss® together with the bone tissue, as part of the remodelling that is constantly taking place in the test group as a function of the orthodontic movement of the teeth. Araújo et al. (2001) carried out the orthodontic movement, in dogs, in the direction of the alveolar ridge grafted with bovine bone mineral matrix and this pro-

moted an increase in the rate of biomaterial degradation. Hossain et al. (1996) observed the great capacity of resorption of β-tricalcium phosphate ceramics under conditions of orthodontic movement when compared with the autogenous bone graft. Donos et al. (2004) observed that the bovine bone mineral matrix resorption process did not follow a specific pattern during the period analysed (4 months) in their study. However, in the present study, the orthodontic force applied could have accelerated the resorption of this biomaterial.

The average amount of tooth movement obtained in this study was 4.0 mm in the mesial direction and the average width of the base of the bone defect in the furcation region, given by the distance between the mesial and distal roots, was 3 mm. Therefore, at the end of the tooth movement, the whole furcation region, with cementum tissues, periodontal ligament and bone, was reshaped and the biomaterial was absorbed or displaced. There was bone apposition on the tension side of the furcation and bone resorption on the pressure side, as the tooth was moved. It is suggested that in the pressure area, the bone tissue and the biomaterial were absorbed but some biomaterial was still present, even when the entire extension of the furcation was moved. This biomaterial that was not totally absorbed was displaced to the mesial side and more concentrated in the pressure (mesial) area because of the new tissues being formed in the tension area.

Presence of biomaterial in the periodontal ligament region, below the radicular notch that is the limit of the area of the class II furcation lesion, was observed in some cases. The probable explanation is that limited extrusion took place during the movement and that the biomaterial remained in place and was then noted below the radicular notch.

Within the limits of our study, we may conclude that there were no differences in relation to the extension of regenerated periodontal tissues in the furcation of the second pre-molars. There was a tendency towards a greater quantity of bone tissue and a lesser quantity of biomaterial in the test group, however not to a statistically significant degree. The biomaterial tended to concentrate on the pressure side, near the mesial surface of the distal root, however not in a statistically significant manner. The previous regenerative periodontal treatment permitted a normal tissue reaction to the orthodontic movement in the pressure and tension areas. Further, the orthodontic treatment did not jeopardize the results achieved with the regenerative periodontal treatment. Additional larger sample studies are necessary to confirm the results of this pilot study and to verify if this association and sequence of these treatments has significant benefit.

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Clinical Relevance

Scientific rationale for study: Periodontal regenerative treatment may improve the prognosis of teeth with previous furcation lesions before orthodontic movement. Insufficient data, however, show the influence of this therapeutic association on the regenerated periodontal tissues.

Principal findings: In this pilot study with dogs, class II furcation lesions were created and treated with periodontal regenerative techniques before orthodontic movement. Regenerated periodontal tissues were maintained after orthodontic movement. There was a tendency of larger bony and smaller biomaterial areas in the furcation when compared with not moved control teeth.

Practical implications: Orthodontic movement may not impair results achieved by periodontal regenerative approach.