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Intrusion of teeth with class III furcation: a clinical, histologic and histometric study in dogs

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Abstract

Aim: To assess orthodontic intrusion effects on periodontal tissues in dogs' pre-molars with class III furcations treated with open flap debridement (OFD) or with guided tissue regeneration (GTR) associated to bone autograft (BA).

Material and Methods: Class III furcations were created in the pre-molars of seven mongrel dogs. After 75 days, teeth were randomly treated with OFD or GTR/BA. After 1 month, metallic crowns were assembled on pre-molars and connected apically to mini-implants by nickel–titanium springs. Teeth were randomly assigned to orthodontic intrusion (OFD+I and GTR/BA+I) groups or no movement (OFD and GTR/BA) groups. Dogs were sacrificed after 3 months of movement and 1 month retention.

Results: All class III furcations were closed or reduced to class II or I in the intrusion groups, while 50% of the lesions in non-moved teeth remained unchanged. Intruded teeth presented higher probing depth and lower gingival marginal level than non-moved teeth ($p<0.01$). Clinical attachment gain was reduced in the intrusion groups by the end of retention ($p<0.05$). OFD+I presented smaller soft tissue area and larger bone tissue area than other groups ($p<0.05$).

Conclusion: Orthodontic intrusion with anchorage via mini-implants improved the healing of class III furcation defects after OFD in dogs. GTR/BA impaired those results.

Key words: furcation defects; periodontal regeneration; surgical periodontal treatment; tooth intrusion

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Furcation lesions have uncertain prognosis and frequently lead to tooth loss (Carnevale et al. 1995, Karring & Cortellini 1999). Regenerative approaches improve the results for furcation treatment; however, unpredictable periodontal regeneration is still obtained for class III furcation defects (Becker 1999, Rossa et al. 2000, Fernandes et al. 2005).

Combined orthodontic and periodontal therapies have demonstrated a viable alternative for advanced periodontal bone defects, increasing clinical attachment gain and bone defect filling (Melsen et al. 1988, 1989, Corrente et al. 2003). In the absence of periodontal disease combined with adequate plaque control, reduced periodontium and periodontal defects are not a limitation for orthodontic movement (Ericsson & Thilander 1978, Polson et al. 1984, Boyd et al. 1989, Re et al. 2000). Recently, the use of periodontal regenerative techniques before orthodontic treatment was proposed to improve the prognosis of tooth movement in order to facilitate the control of subgingival inflammation and stress distribution (Hossain et al. 1996, Nemcovsky et al. 1996, Stelzel & Flores-de-Jacoby 1998, Aguirre-Zorzano et al. 1999, Araújo et al. 2001, Re et al. 2002). On the other hand, orthodontic movement performed before regenerative treatment facilitated a reduction of periodontal defects and an increase of clinical attachment gain, because of teeth repositioning in alveolar bone (Stelzel & Flores-de-Jacoby 1998, Cirelli et al. 2003) and increased cellular activity (Roberts & Chase 1981, Melsen 2001). After orthodontic treatment, regenerative treatment can still be performed in 'on' or 'for'? reduced periodontal defects, improving prognosis (Stelzel & Flores-de-Jacoby 1998).

Conflict of interest and source of funding statement

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Teeth movement toward alveolar bone defects is a stimulating factor for bone apposition, indicating a beneficial effect of orthodontic movement on regenerative therapy (Vardimon et al. 2001, Nemcovsky et al. 2004). Knippenberg et al. (2007) demonstrated the differentiation of mesenchymal stem cells into bone cells after mechanical loading in vitro. The combination of body movement after GTR and bovine-derived xenograft in the treatment of class II furcation lesions in dogs has shown a tendency toward larger, new-formed bone area compared with non-moved teeth (da Silva et al. 2006). Thus, orthodontic movement performed after regenerative treatment could stimulate periodontal and bone healing, improving the prognosis of periodontal lesions.

Favourable results have also been demonstrated with orthodontic intrusion. New connective tissue attachment and bone defect filling were observed during intrusion of periodontally involved teeth in the absence of gingival inflammation (Melsen et al. 1988, 1989, Cardaropoli et al. 2001, Corrente et al. 2003, Re et al. 2004). Nevertheless, intrusion is considered a critical movement, as it requires fixed orthodontic appliances in several teeth to anchorage reinforcement (Alessandri & Giunta 1996). The results are unpredictable and may lead to root resorption and extrusion and inclination of the anchorage teeth (Alessandri & Giunta 1996). Besides, patients previously affected by periodontal diseases have reduced periodontium that is also difficult in conventional orthodontics. Recently, to avoid undesirable effects, mini-implants have been used as anchorage to obtain pure intrusion of molars (Ohmae et al. 2001, Chang et al. 2004, Yao et al. 2004).

So far, it is unknown whether an association of regenerative periodontal treatment with intrusion can improve the healing of class III furcation lesions. The aims of this study were to assess, clinically and histologically, the effects of orthodontic intrusion, obtained with mini-implants, on class III furcation pre-molars in dogs treated with open flap debridement (OFD), and whether guided tissue regeneration (GTR) associated to bone autograft (BA) previous to orthodontic intrusion could improve the outcomes.

Material and Methods

This study was approved by the Ethics Committee on Animal Experimentation of the School of Dentistry of Araraquara – UNESP. A total of 28 pre-molars (third and fourth lower) of seven mongrel dogs (weighing between 15 and 20 kg) were used. For all clinical procedures, dogs were pre-anaesthetized using levopremazin chloritate (Neozine®, Aventis Pharma Ltd., SP, Brazil), administered intramuscularly at 0.2 ml/kg of body weight, and further anaesthetized intravenously with 0.5 ml/kg of body weight of sodium thiobarbiturate 25 mg/ml (sodium Tiopental®, Abbott Laboratories, SP, Brazil) and kept on intravenous hydration with a 0.9% physiological solution during surgery. Immediately after each surgery, dogs were given 10 ml of a hepatic protector (Frutoplex LN, Marjan e Comércio, São Paulo, Brazil) intravenously and 2 ml of dipirone intramuscularly.

Teeth were randomly divided into four groups as follows:

OFD: teeth with class III furcation defect treated with OFD.

OFD plus intrusion (OFD+I): teeth with class III furcation defect treated with OFD, followed by intrusion movement.

GTR and BA (GTR/BA): teeth with class III furcation defect treated with OFD associated with GTR and BA.

GTR and BA plus intrusion (GTR/BA+I): teeth with class III furcation defect treated with OFD associated with GTR and BA, followed by intrusion movement.

Seven pre-molars were assigned per group in such a way that each animal had one tooth in each group. Randomization was therefore performed, for each dog, by drawing two identified cards: one regarding the group and another regarding the tooth.

Clinical measurements were performed by a calibrated examiner at the designated time points: before creation and cronification of furcation lesions (C), immediately before the periodontal regenerative treatment (R), at the beginning (BI – before intrusion) and the end of orthodontic movement (AI – after intrusion) as well as at the end of retention (F – final). The following clinical data were obtained from the lower third and fourth pre-molars: presence of visible plaque (Pl), presence of marginal gingival bleeding (GB), probing depth (PD), bleeding on probing (BP), marginal gingival level (MGL) [distance between gingival margin and cement enamel junction (CEJ)] and clinical attachment level (CAL) referred to CEJ. MGL was considered negative if marginal gingival level was positioned above the CEJ and zero if MGL was on the same level as CEJ. CAL was obtained using the formula PD plus MGL. Horizontal classification of furcation lesions (Hamp et al. 1975) was measured using a Nabers probe (HF, Hu-Friedy, Chicago, IL, USA) at the R and F periods.

Creation and cronification of class III furcation lesions

Scaling and tooth prophylaxis using a rubber cup and prophylactic toothpaste were initially performed in the entire mouth. After a week, a class III furcation lesion, 4 mm high, was created in the third and fourth lower pre-molars. The cavities were filled with gutta-percha to prevent spontaneous regeneration of the lesions (Cirelli et al. 1997) and to allow lesion cronification (Wikesjo et al. 1991). Flaps were sutured in a coronal position. Sutures were removed after 1 week. During a 60-day cronification period, dogs were fed with water-softened feed in order to accumulate plaque and to promote radicular contamination and the development of chronic inflammation.

Periodontal treatment

After furcation cronification, gutta-percha was removed and teeth were scaled and root planed. The crowns of the third and fourth lower pre-molars were prepared using a high-speed bur to achieve parallel proximal surfaces. Impressions were taken with a silicone-based material (Xantopren® & Optosil®, Heraeus Kulzer South America, São Paulo, SP, Brazil). Silver metallic crowns were made with orthodontic hooks at the buccal and lingual surfaces.

After 15 days, a mucoperiosteal flap was raised and the roots were scaled with hand instruments (Fig. 1a). The dimensions of the furcation lesions were measured and found to be, on average, 4.05 ± 0.95 mm high, 4.46 ± 0.94 mm deep and 2.96 ± 0.72 mm wide, with no statistical differences among them ($p = 0.451$, 1-way ANOVA). Reference notches using low-speed bur no 1/2 (KG Sorensen, São Paulo, SP, Brazil) were made on the mesial and distal roots at the bone crest level in the furcation region to orientate the histologic and histometric analyses.

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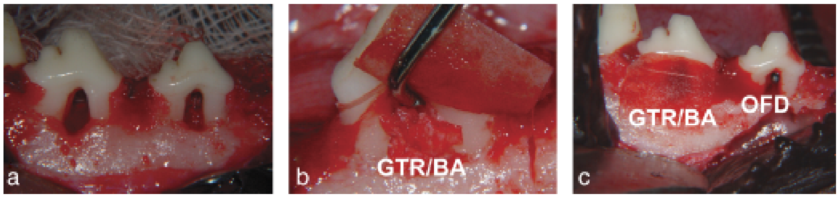

Fig. 1. Periodontal treatment of class III furcation lesions. (a) Surgical access of class III furcation lesions after the cronification time point. (b) Treatment with guided tissue regeneration using absorbable membrane and bone autograft (GTR/BA group). (c) Adjacent pre-molars from the GTR/BA and OFD groups.

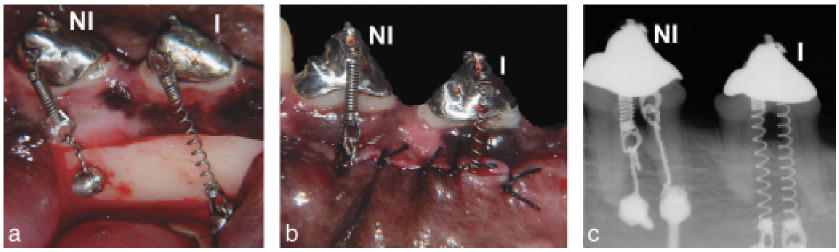

Fig. 2. Orthodontic intrusion after periodontal treatment. (a) Mini-implants placed in the apical cortical bone of the mandible and connected to metallic crowns by nickel–titanium springs. The mini-implants were active on the teeth of the intrusion groups (I) and passive in the non-intrusion groups (NI). (b) Suture after mini-implant installation. (c) Radiographic exam taken immediately after mini-implant installation.

Regenerative treatment of furcation lesions using BA and an absorbable copolymer membrane of glycolide and lactide (Resolut ® Adapt W.L. Gore & Associates Inc., Flagstaff, AZ, USA) was performed at the buccal and lingual side (Figs. 1b and c). The BA was obtained by scraping the buccal apical surface of the lower first molar. Membranes were sutured with a 4-0 poliglactin 910 absorbable suture (Vycril, Ethicon S.A., SP, Brazil). The flap was repositioned coronally with a suspended and inter-proximally interrupted 4-0 silk suture (Ethicon, Atraloc, Johnson & Johnson S.A., SP, Brazil) in order to cover the whole membrane and improve the region's stability. Sutures were removed after 10 days. Benzyl penicillin and streptomycin (Pentabiótico, Fort Dodge®, Campinas, SP, Brazil) were administered IM (0.1 ml/kg of body weight) immediately after surgery and 5 days later.

Daily plaque control was performed by applying a 0.2% chlorhexidine gel with a soft brush until the end of the study. During and after this period, the dogs continued to be fed with water-softened feed to minimize mechanical trauma.

Installation of mini-implants and orthodontic movement

After 30 days, tooth prophylaxis was performed and the metallic crowns were cemented with an adhesive material (Panavia 21X Dental Adhesive, Kuraray Co. Ltda, Osaka, Japan). For orthodontic anchorage, 7-mm-long and 1.3-mm-diameter titanium mini-implants (Neodent® Implante Osteointegrável, Curitiba, PR, Brazil) were installed 3 mm apically to a line joining the root apexes, at the middle distance between the roots of each treated pre-molar (verified by periapical radiographs) in both buccal and lingual cortical plates. A mucoperiosteal flap was opened 2 mm above the muco-gingival junction to avoid contact with the furcation region, and the alveolar bone was denuded. The cortical bone was drilled with a 1.1 mm round bur (Neodent®) under saline irrigation. The mini-implants were installed in the apical direction with a 45° angulation to the bone surface, using a miniature screwdriver up to 10 N, verified with a torque-meter (Neodent®). This angulation was applied to avoid contact between mini-implants and root surfaces. Then, two nickel–titanium springs (GAC International, Inc., Bohemia, NY, USA), 7 mm long, providing a light and continuous tension of 25 g, were installed, connecting the mini-implants to the hook on the metallic crown of each tooth, followed by a periapical radiograph (Fig. 2). The final intrusive force in the test teeth was 50 g. Inactive identical appliances were placed on control pre-molars, to provide identical conditions for bacterial plaque accumulation and hygiene as in the test groups. The mini-implant was covered with the sutured flap. Sutures were removed after 7 days, and tooth prophylaxis was performed every 20 days. The distance between mini-implants and the hooks's base on the metallic crown on the buccal and lingual sides was measured at the beginning (BI) and end (AI) of orthodontic movement to obtain the amount of tooth intrusion.

After 90 days, teeth stabilization was carried out for 1 month. Retention was prepared using a stainless steel wire of 0.25 mm (MORELLI, Sorocaba, SP, Brazil) that passes through the hole of the mini-implant's head and over the hook of the metallic crown without causing any force to the tooth. A new periapical radiograph exam was taken.

Horizontal classification of furcation lesions after retention (F) was performed, using as reference the mean bucco-lingual extension of the furcations measured immediately before the regenerative periodontal treatment (R), which was 4.46 ± 0.94 mm. Defects with horizontal probing at depths up to 1.5 mm, which corresponds to 1/3 of furcation, were classified as class I furcation lesions. Defects with horizontal probing at depths more than 1.6 mm and less than the total furcation were classified as class II furcation lesions, and through-through defects were classified as class III furcation lesions (Hamp et al. 1975).

After the retention period, the appliances were removed and the dogs were sacrificed with an overdose of thiopental. The third and fourth lower pre-molars were removed in block.

Histologic and histometric analyses

The biopsies were fixed in 10% formalin for 48 h and decalcified in Morse solution for 4 months for routine histological processing and paraffin embedding. The mini-implants were removed carefully before paraffin embedding.

Histological semi-serial sections, 5 μm thick, were cut in the sagittal plane through the entire bucco-lingual extension of the tooth (Jung Supercut 2065 Leica, Chicago, IL, USA). Five sections were selected for each tooth: the two most external sections showing the reference notches on both root surfaces (buccal and lingual sections) and three other sections that were equally spaced between the external sections, represent-

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ing the central and internal portions of the defects. Haematoxylin and eosin (HE) and Masson trichrome staining were used.

Histometric analysis was performed with image analysis software (Sigma Scan Pro, Jandel Scientific, San Rafael, CA, USA) to obtain the following measurements (Fig. 3):

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

Connective tissue (CT): linear radicular extension of the defect in direct contact with connective tissue.

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

Linear periodontal regeneration (LPR): linear radicular extension of the lesion with new cementum, bone and periodontal ligament regeneration.

Bone filling area (Bo): lesion area filled with newly formed bone, including medullar and trabecular bone, apically defined by a straight line joining the two radicular notches.

Bone autograft filling area (BA): sum of BA particle areas inside the lesion area.

Soft tissue area (ST): lesion area filled with epithelium, connective tissue and periodontal ligament. The total lesion area in the furcation region delimited by two radicular notches was first obtained. Afterwards, the bone filling and BA filling areas were measured and subtracted from the total area to obtain the soft tissue area.

Root resorption was measured as previously described (Konoo et al. 2001) and calculated as a percent of the root surface with cratering. First, the entire root surface adjacent to alveolar bone was measured, including craters (RS). Next, the surface with cratering was measured (CS). Percent root resorption = CS/RS × 100.

Histometric linear and volumetric data were presented as a percentage of total root linear extension and lesion area. Percentages were used to minimize the interference of tooth size in the analyses results.

Statistical analysis of clinical and histometric data

Analysis of variance followed by Tukey's post hoc test was used to measure PD, MGL and CAL. Friedman's test was applied to analyse Pl, GB and BP, followed by the non-parametrical multiples comparisons test. The *t*-test compared the amount of intrusion and root resorption between intrusion groups, and Wilcoxon's test compared horizontal classification of the furcation lesion between R and F periods. Statistical analysis of the effect of the treatments on the histometric linear measurements, in millimetres, and upon the area measurements, in square millimetres, was made using Friedman's test, followed by the non-parametrical multiples comparisons test. A level of 5% was considered a significant difference.

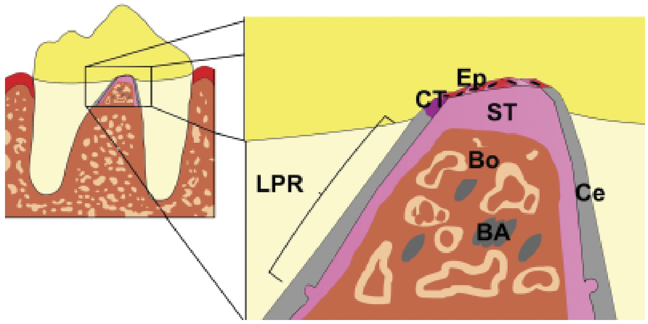

Fig. 3. Schematic representation of linear and area variables evaluated in the histometric analysis of the furcation defects. Ce, cementum formation; CT, connective tissue; LPR, linear periodontal regeneration; Ep, epithelium; Bo, bone tissue area; ST, soft tissue area; BA, bone autograft area.

Table 1. Horizontal furcation classification in the lower third and fourth pre-molars in dogs, according to groups and time points

Groups	Cl III – R	Cl III – F	Cl I/II – F	0 – F
OFD	7	4	0	3
OFD+I	7	0*	4	3
GTR/BA	7	3	2	2
GTR/BA+I	7	0*	6	1

Data are expressed as the number of teeth with the presence of class I/II, III or absence (0) of furcation lesion at the regeneration (R) and end of retention (F) time point. Mean furcation depth in the regenerative phase was 4.46 ± 0.94 mm. Defects with horizontal probing depths up to 1.5 mm, which corresponds to 1/3 of furcation, were classified as class I furcation lesions. Defects with horizontal probing depths of >1.6 mm and less than the total furcation were classified as class II furcation lesion s, and through–through defects were classified as class III furcation lesions.
*Significant effect of intrusion in the reduction of class III furcation lesions, between R and F time points, $p < 0.05$ ($n = 7$).
OFD, open flap debridement; GTR, guided tissue regeneration; BA, bone autograft.

Results

Clinical analysis

The average amount of tooth intrusion observed was 4.4 ± 1.9 mm in the OFD+I group and 4.7 ± 2.5 mm in the GTR/BA+I group, with no significant difference between groups ($p > 0.05$). Also, there were no significant differences among groups for Pl, GB and BP, demonstrating that similar plaque control was obtained in all groups (data not shown).

The furcation lesions were re-evaluated and clinically re-classified at the end of the retention period (Table 1). Class III furcations were still present in four teeth in the OFD group and in three teeth in the GTR/BA. Neither intrusion group presented any class III furcation lesion. Instead, the initial lesions were closed or reduced to class II or I furcations ($p < 0.05$).

PD significantly increased in the intrusion groups at the end of the intrusion and retention periods, compared with previous time points and with non-intrusion groups at the same time points (Table 2, $p < 0.01$).

Intrusion groups presented significant variation of MGL, which was coronally positioned at the end of intrusion and the end of retention when compared with previous time points and with non-intrusion groups (Table 2, $p < 0.01$).

Although in the OFD+I group, a clinical attachment gain after the intrusion and retention period was observed

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Table 2. Mean percentage (M) ± SE and 95% confidence interval (CI) of the probing depth (PD), marginal gingival level (MGL) and clinical attachment level (CAL), according to groups and time points

Groups	C		R		BI		AI		F	
	$M \pm$ SE	95% CI	$M \pm$ SE	95% CI	$M \pm$ SE	95% CI	$M \pm$ SE	95% CI	$M \pm$ SE	95%CI
OFD										
PD	1.90 ± 0.05	1.5;2.3	1.90 ± 0.2	1.5;2.3	1.86 ± 0.2	1.4;2.3	2.12 ± 0.1	1.7;2.6	1.69 ± 0.3	1.2;2.1
MGL	0	0	0.14 ± 0.1	−0.4;0.3	0.06 ± 0.1	−0.5;0.6	0.12 ± 0.1	−0.4;0.7	0.07 ± 0.2	−0.5;0.6
CAL	1.90 ± 0.04	1.5;2.3	2.05 ± 0.2	1.6;2.5	1.92 ± 0.2	1.5;2.3	2.24 ± 0.1	1.8;2.7	1.76 ± 0.3	1.3;2.2
OFD+I										
PD	2.02 ± 0.1	1.6;2.5	2.02 ± 0.1	1.6;2.5	1.55 ± 0.2	1.1–2.0	3.55 ± 0.4*,#	3.1;4.0	2.90 ± 0.2*,#	2.5;3.3
MGL	0	0	0.24 ± 0.1	−0.3;0.8	−0.07 ± 0.1	−0.6;0.5	−2.00 ± 0.6*,#	−2.6; −1.4	−1.43 ± 0.3*,#	−2.0; −0.9
CAL	2.02 ± 0.1	1.6;2.5	2.26 ± 0.2	1.8;2.7	1.48 ± 0.2†	1.0;1.9	1.55 ± 0.3†	1.1;2.0	1.48 ± 0.2†	1.0;1.9
GTR/BA										
PD	1.83 ± 0.1	1.4;2.3	1.95 ± 0.1	1.5;2.4	2.10 ± 0.1	1.6;2.5	2.07 ± 0.1	1.6;2.5	2.00 ± 0.1	1.6;2.4
MGL	0	0	0.05 ± 0.1	−0.5;0.6	0.02 ± 0.1	−0.5;0.6	0.21 ± 0.1	−0.4;0.8	0.19 ± 0.1	−0.4;0.8
CAL	1.83 ± 0.1	1.4;2.3	2.0 ± 0.1	1.6;2.4	2.12 ± 0.2	1.7;2.5	2.29 ± 0.1	1.9;2.7	2.19 ± 0.1	1.8;2.6
GTR/BA+I										
PD	1.86 ± 0.1	1.4;2.3	2.07 ± 0.2	1.6;2.5	1.98 ± 0.1	1.5;2.4	3.83 ± 0.5*,#	3.4;4.3	3.07 ± 0.3*,#	2.6;3.5
MGL	0	0	0.14 ± 0.1	−0.4;0.7	−0.02 ± 0.1	−0.6;0.5	−2.05 ± 0.7*,#	−2.6; −1.5	−1.55 ± 0.5*,#	−2.1; −1.0
CAL	1.86 ± 0.1	1.4;2.3	2.21 ± 0.3	1.8;2.6	1.95 ± 0.1	1.5;2.4	1.79 ± 0.3	1.4;2.2	1.52 ± 0.3	1.1;1.9

*$p < 0.01$ – significantly different from C, R and BI time points (intra-treatment comparison);
#$p < 0.01$ – significantly different from OFD or GTR/BA groups in the same time point (inter-treatment comparison);
†$p < 0.05$ – significantly different from C and R time points (intra-treatment comparison) ($n = 7$).
C, cronification; R, immediately before the periodontal regenerative treatment; BI, before intrusion; AI, after intrusion; F, final of retention; OFD, open flap debridement; OFD+I, open flap debridement plus intrusion; GTR/BA, guided tissue regeneration and bone autograft; GTR/BA+I, guided tissue regeneration and bone autograft plus intrusion.

(Table 2, $p < 0.05$), these values were not significantly different from the OFD group. The GTR/BA+I group tended to present a clinical attachment gain after the intrusion and retention time points. This tendency was also observed when the GTR/BA+I group was compared with the GTR/BA group at the end of retention (Table 2, $p = 0.07$).

Histologic and histometric analysis

The majority of the samples presented epithelial migration at the roof of the furcation region, frequently on the external cuts. A light lymphoplasmocytary inflammatory infiltration with subepithelial exacerbations was frequently observed. Teeth presented new cementum formation in the furcation region from radicular notches up to the epithelial tissue. There was an? apposition of new cellular cementum filling the radicular resorptions in the furcation and in the lateral radicular surfaces of teeth tending to surface smoothing. A fibrous connective tissue with collagenous fibres in a regular disposition and a primary trabecular with newly formed bone filled the furcation defects (Figs 4–7). There was no evidence of any significant difference among groups on the linear extension of cementum formation, epithelial migration and periodontal regeneration (Fig. 8).

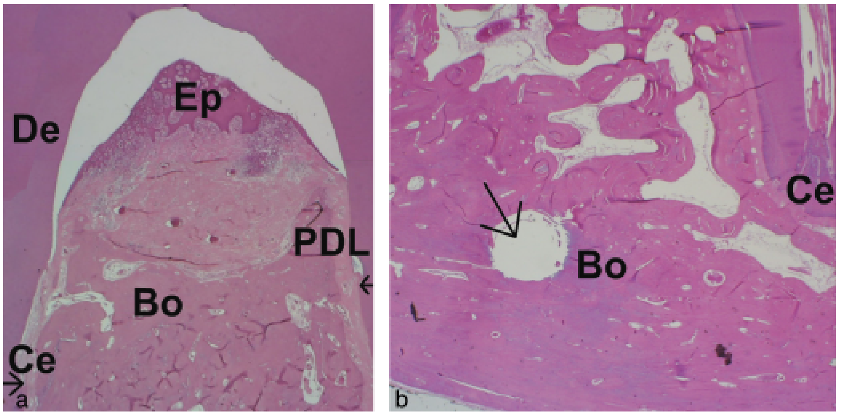

Fig. 4. Panoramic view of the furcation region and mini-implant site in the OFD group. (a) Healing of the furcation area with epithelial migration (Ep), new cementum (Ce), periodontal ligament (PDL) and alveolar bone formation (Bo). Arrows indicate notches. Dentin (De). (b) Arrow indicates mini-implant site evolved by bone (Bo). Haematoxylin and eosin, original magnification × 20.

A significant effect of orthodontic treatment on the furcation defect filling was observed in the OFD+I group, which presented a smaller soft tissue area and a larger bone area in the furcation region compared with the OFD and GTR/BA groups (Figs 5 and 9, $p < 0.05$).

A complete regeneration of the furcation lesion with newly formed cementum, periodontal ligament and bone tissues was observed in one dog from the OFD group, two dogs from the GTR/BA group, and one dog from the GTR/BA+I group. BA evolved by newly formed bone or connective tissue was observed in both the GTR/BA (Fig. 6b) and GTR/BA+I groups.

There were areas of active resorption with osteoclasts in the Howship lacunas at the apical and furcation region in one dog from the OFD+I group (Fig. 5b) and three dogs from the GTR/BA+I group. Mean root resorption was 1.83 ± 2.4% in the GTR/BA+I group and 0.23 ± 0.6% in the OFD+I group ($p = 0.09$). Mini-implants were evolved by bone tissue in all groups, as shown in the OFD group (4b).

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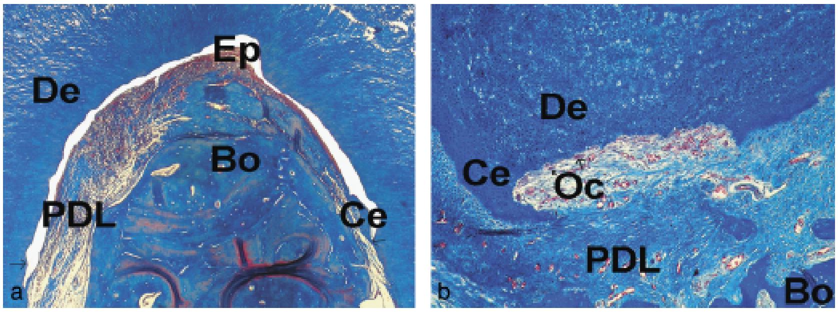

Fig. 5. Panoramic view of the furcation region and apical root resorption in the OFD+I group. (a) Healing of the furcation area with epithelial migration (Ep), new cementum (Ce), periodontal ligament (PDL) and alveolar bone formation (Bo). Dentin (De). Arrows indicate notches. Masson trichrome, original magnification ×20. (b) Active apical root resorption. Arrows indicate osteoclasts (Oc). Masson trichrome, original magnification ×100.

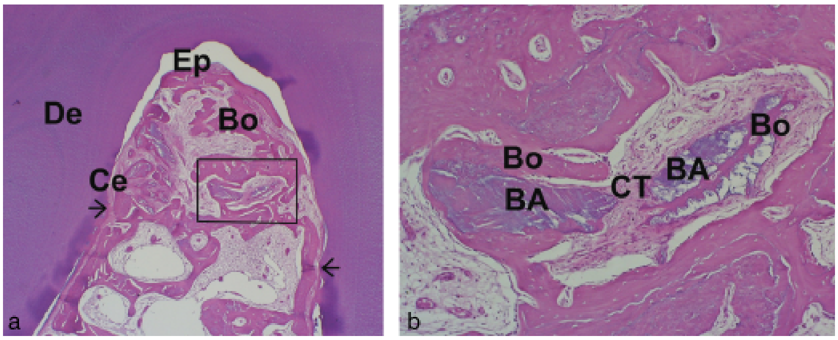

Fig. 6. Panoramic view of the furcation area and bone autograft in the GTR/BA group. (a) Healing of the furcation area with epithelial migration (Ep), new cementum (Ce), periodontal ligament (PDL) and alveolar bone formation (Bo). Dentin (De). Arrows indicate notches and the rectangle indicates (b). Haematoxylin and eosin, original magnification ×20. (b) Bone autograft (BA) integrated to newly formed bone (Bo) and evolved by connective tissue (CT). BA is being substituted by newly formed bone (Bo). Haematoxylin and eosin, original magnification ×100.

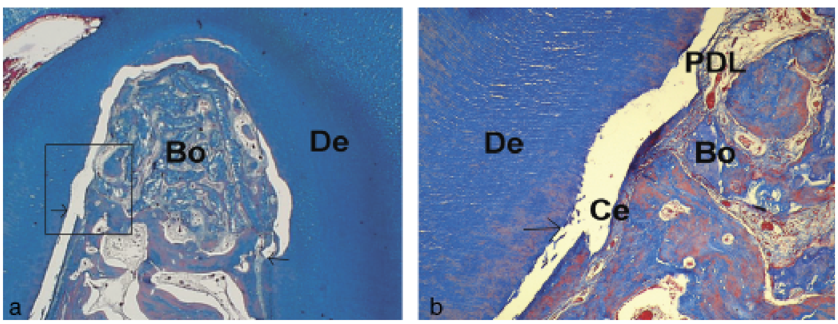

Fig. 7. Panoramic view of the furcation region in the GTR/BA+I group. (a) Healing of the furcation area with new cementum (Ce), periodontal ligament (PDL) and alveolar bone formation (Bo). Dentin (De). Arrows indicate notches and the square indicates (b). Masson trichrome, original magnification ×20. (b) Detail of the square in (a) with new cementum formation (Ce), periodontal ligament (PDL) and alveolar bone (Bo) above the notch. Dentin (De). Masson trichrome, original magnification ×100.

Discussion

This study evaluated the effects of intrusion orthodontic movement on periodontal tissue healing in teeth with class III furcation lesions previously treated with OFD or regenerative techniques (GTR/BA) in dogs.

To obtain the intrusion movement, mini-implants were inserted apically in both buccal and lingual bone plates of each tooth in the same level in order to prevent tooth inclination and lead to pure intrusion (Yao et al. 2005). Both intrusion groups obtained more than 4 mm of total apical movement without significant differences between them. These values were also observed by other authors using mini-implants (Ohmae et al. 2001, Carrilo et al. 2007) and were higher than in studies using the anchorage provided by teeth (Diedrich et al. 1992, Ng et al. 2005, 2006). The primary stability obtained was sufficient to apply orthodontic force on the same day of mini-implant installation, without failures during the intrusion period (Favero et al. 2002, Kyung et al. 2003, Miyawaki et al. 2003, Liou et al. 2004). Histological, loading and unloading mini-implants were involved by bone, as observed previously (Ohmae et al. 2001, Salina et al. 2006).

Orthodontic force was initiated in the early healing phase (30 days after surgery) (Matsuura et al. 1995, Araújo et al. 1997) to increase the mitotic activity of periodontal cells (Roberts & Chase 1981, Bumann et al. 1997, Melsen 2001), as used in a previous study (Diedrich et al. 1992, 2003). Besides cell activation in the periodontal ligament, mechanical loading in bone tissues results in the flow of interstitial fluid in the canalicular non-mineralized matrix, resulting in bone apposition (Klein-Nulend et al. 1995a, b). Currently, adult mesenchymal stem cells with multilineage potential (Pittenger et al. 1999) and bone marrow stromal cells have been investigated for their use in generating bone tissue after in vitro loading (Thomas & el Haj 1996, Meinel et al. 2004).

Clinically, a significant reduction of furcation lesions was observed in both intrusion groups ($p < 0.05$). At the end of retention, class III furcations were present only in the non-intrusion groups (Table 1). Although the results of periodontal treatment of class III furcation lesions tended to be inconsistent and unpredictable (Palioto et al. 2003; Fernandes et al. 2005, Roriz et al. 2006), the interaction of periodontal and orthodontic treatments led to significant clinical closure of class III furcation lesions in this study. The intrusion groups presented an increased PD and decreased MGL, as observed in other studies (Bondevik 1980; Murakami et al. 1989). No sample from any group presented clinical attachment loss. The OFD+I and GTR/BA+I groups presented a clinical attachment gain of 34.73% and 31.18%, respectively, at the end of retention (Table 2). These results are in line with other studies (Amiri-Jezeh et al. 2004) and

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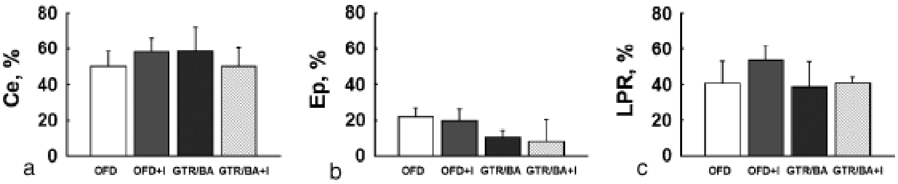

Fig. 8. Histometric analysis of root linear measurements in the furcation region between notches. (a) Ce, cementum formation; (b) Ep, epithelial migration; (c) LPR, linear periodontal regeneration. There were no significant differences between groups. Values are expressed as mean percentage $\pm$ SD ($n = 7$). OFD, open flap debridement; OFD+I, open flap debridement plus intrusion; GTR/BA, guided tissue regeneration and bone autograft; GTR/BA+I, guided tissue regeneration and bone autograft plus intrusion.

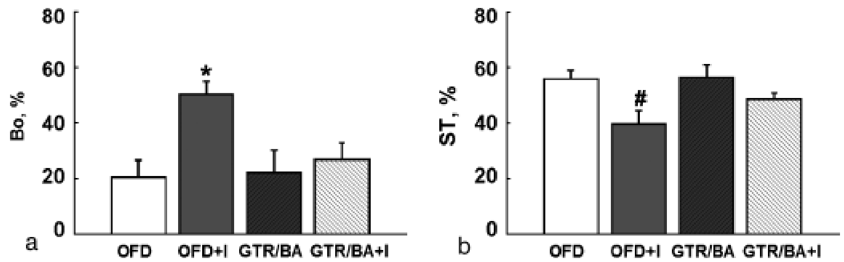

Fig. 9. Histometric analysis of area measurements in the furcation region between notches. (a, b) The OFD group presented a smaller area of soft tissue and a larger area of bone tissue ($p < 0.05$). Values are expressed as mean percentage $\pm$ SD ($n = 7$). OFD, open flap debridement; OFD+I, open flap debridement plus intrusion; GTR/BA, guided tissue regeneration and bone autograft; GTR/BA+I, guided tissue regeneration and bone autograft plus intrusion; Bo, bone tissue area; ST, soft tissue area. $^*p < 0.05$, significant difference from OFD and GTR/BA; $^\#p < 0.05$, significant difference from OFD.

demonstrate that periodontal attachment was maintained or improved during intrusion. It is hypothesized that the increased mitotic activity induced by the orthodontic stimulus (Roberts & Jee 1974, Melsen et al. 1988, Melsen & Agerbaek 1991) results in an improved prognosis for a new attachment.

The dimensions of the furcation lesions measured after the cronification period were 4.05 ± 0.95 mm high, without any significant difference among groups. An incomplete regeneration of class III furcation lesions was observed in more than 50% of the non-intrusion groups, which has been demonstrated by other studies (Anderegg et al. 1991, Park et al. 1995, Mehlbauer et al. 2000, Rossa et al. 2000, Rupprecht et al. 2001, Palioto et al. 2003, Fernandes et al. 2005). Class III furcation lesions are critical defects, especially when the vertical opening is larger than 3 mm (Pontoriero et al. 1989). In such defects, osteogenic cells are supplied only from the bottom of the furcation region (Polson et al. 1984; Mehlbauer et al. 2000), which may explain the incomplete regeneration observed.

In the intrusion groups, at the end of tooth movement the whole furcation defect was moved apically to bone crest level. It would be expected that a complete, newly formed bone would be found at all furcation regions after intrusion, but it was not observed in all cases. In the OFD+I group, higher bone filling area and lower soft tissue area were present when compared with the non-intrusion groups ($p < 0.05$), but these results were not observed in the GTR/BA+I group. In addition, the OFD+I group demonstrated a tendency toward a higher LPR (53.45%), i.e., presence of new cementum, periodontal ligament and bone. However, GTR/BA treatment was expected to favour the migration of periodontal and bone cells into the furcation region, once GTR promotes the exclusion of epithelial and gingival cells from the periodontal defects, favouring periodontal regeneration (Nyman et al. 1982). Besides, GTR plus BA can improve the prognostic of periodontal regeneration in furcation lesions (Reynolds et al. 2003). Also, BA is considered to be the gold standard material for any regeneration procedure because of its osteogenetic, osteoinductive and osteoconductive properties (Simion & Fontana 2004). An explanation for the obtained results in the GTR/BA+I group is that the application of orthodontic movement 30 days after the regenerative treatment may have raised the expected inflammatory process due to membrane and BA absorption, accelerating this process and interfering with tissue regeneration. An increase in macrophage and leukocyte migration (Gianelly 1969, Krishnan & Davidovitch 2006) and osteoclast activation (Noxon et al. 2001) is usually observed during orthodontic movement. Therefore, our initial hypothesis, that regenerative treatment prior to orthodontic intrusion could improve the results when compared with the OFD+I group, was rejected. We observed that this association impaired clinical and histological outcomes. Diedrich et al. (2003) also observed an unfavourable interaction of periodontal regeneration, orthodontic remodeling and membrane degradation in the treatment of three-wall bony defects in dogs.

Intrusion is a critical orthodontic movement regarding for external root resorption, because pressure is highly concentrated at the conical apex and furcation region (Parker & Harris 1998, Jeon et al. 1999, Han et al. 2005, Harris et al. 2006). However, several reports indicate that 50 g, which was used in our study, is an adequate load to promote pre-molar intrusion and avoid severe root resorption (Dellinger 1967, Cooke & Bedi 1985, Diedrich et al. 1992, Southard et al. 1995, Faltin et al. 2001). In this study, a new apposition of cellular cementum regularized areas of radicular resorption, when present. No significant difference was observed in the linear root resorption extension between intrusion groups ($p > 0.05$). However, some areas with active root resorption were observed in both intrusion groups, either at the apex or the furcation region, which might be due to failure of dentin-protecting cementoblastic layer formation on the radicular area, as observed in another study (Katzhendler & Steigman 1999).

Within the limits of this study, it was possible to conclude that mechanical

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loading, applied in an early healing phase after surgical periodontal treatment on teeth with a class III furcation defect, promoted clinical elimination or reduction of the class III defects and improved bone filling in the defect area treated with OFD. Intrusion after 1 month of the regenerative periodontal treatment with membrane and BA did not improve bone formation in the defect area, suggesting that interaction between orthodontic movement and biomaterial degradation must be analysed in future research.

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Clinical Relevance

Scientific rationale for the study: Orthodontic treatment has been associated with periodontal regenerative techniques to improve prognosis for patients affected by advanced periodontal disease. In this study, dogs' teeth with class III furcations were intruded after OFD, associated or not with BA and GTR.

Principal findings: Clinical elimination or reduction of furcations was obtained after periodontal treatment and intrusion. Larger bone area was obtained after OFD and intrusion in the furcation region.

Practical implications: Orthodontic intrusion can be associated with surgical periodontal treatment to improve the prognosis of class III furcations.

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