Autogenous Bone Graft With or Without a Calcium Sulfate Barrier in the Treatment of Class II Furcation Defects: A Histologic and Histometric Study in Dogs

Tatiana M. Deliberador,* Maria J.H. Nagata,* Flávia A.C. Furlaneto,* Luiz G.N. Melo,* Tetuo Okamoto,* Maria L.M.M. Sundefeld,† and Stephen E. Fucini‡

Background: The purpose of this study was to histologically evaluate the healing of surgically created Class II furcation defects treated using an autogenous bone (AB) graft with or without a calcium sulfate (CS) barrier.

Methods: The second, third, and fourth mandibular premolars (P2, P3, and P4) of six mongrel dogs were used in this study. Class II furcation defects (5 mm in height × 2 mm in depth) were surgically created and immediately treated. Teeth were randomly divided into three groups: group C (control), in which the defect was filled with blood clot; group AB, in which the defect was filled with AB graft; and group AB/CS, in which the defect was filled with AB graft and covered by a CS barrier. Flaps were repositioned to cover all defects. The animals were euthanized 90 days post-surgery. Mesio-distal serial sections were obtained and stained with either hematoxylin and eosin or Masson's trichrome. Histometric, using image-analysis software, and histologic analyses were performed. Linear and area measurements of periodontal healing were evaluated and calculated as a percentage of the original defect. Percentage data were transformed into arccosine for statistical analysis (analysis of variance; $P<0.05$).

Results: Periodontal regeneration in the three groups was similar. Regeneration of bone and connective tissue in the furcation defects was incomplete in most of the specimens. Statistically significant differences were not found in any of the evaluated parameters among the groups.

Conclusion: Periodontal healing was similar using surgical debridement alone, AB graft, or AB graft with a CS barrier in the treatment of Class II furcation defects. *J Periodontol 2006; 77:780-789.*

KEY WORDS

Animal research; bone grafting; calcium sulfate/therapeutic use; guided tissue regeneration; periodontal diseases/therapy.

* Department of Surgery and Integrated Clinic, Dental School of Araçatuba, University of the State of São Paulo "Júlio de Mesquita Filho," Araçatuba, São Paulo, Brazil.
† Department of Preventive Dentistry, Dental School of Araçatuba, University of the State of São Paulo "Júlio de Mesquita Filho."
‡ Private practice, Hanover, NH.

The treatment of furcation defects is a complex and difficult task. It often compromises the success of periodontal therapy.[1,2] The ultimate goal of periodontal therapy includes not only the arrest of progressive periodontal disease but also the predictable regeneration of the periodontium at the site of previous periodontal breakdown. The regeneration of Class II furcation defects, although possible, is not considered a predictable procedure, especially in terms of complete bone fill.[3] Numerous surgical modalities have been tested in an attempt to achieve regeneration of these defects,[4] including guided tissue regeneration (GTR). Early human[5,6] and animal[7] studies have shown that predictable regeneration of the attachment apparatus can be accomplished by treatment based on the principle of GTR.

According to Machtei and Schallhorn,[4] the GTR procedure is the treatment of choice for Class II furcation defects. The use of augmentation materials in addition to a physical barrier may enhance the regenerative outcome in the treatment of furcation defects treated with GTR.[8] Therefore, bone grafts have been combined with GTR

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procedures to enhance the potential for bone regeneration. The rationale behind the use of bone grafts or alloplastic materials is the assumption that the material may contain bone-forming cells (osteogenesis), serve as a scaffold for bone formation (osteoconduction), or that the matrix of the bone grafts contains bone-inducing substances (osteoinduction) that would stimulate both the regrowth of alveolar bone and the formation of new attachment.[9] Traditionally, the gold standard for osseous regeneration has been autogenous bone (AB). It is an organic material that forms bone by osteogenesis, osteoinduction, and osteoconduction.[10]

Clinical[11-14] and animal[15,16] studies evaluating the combined use of the GTR procedure with diverse graft materials and bone substitutes in the treatment of Class II furcation defects have yielded contradictory results. Some clinical studies have demonstrated better bone fill of the defect with combined procedures than with GTR alone.[11,13] Other studies have not found any significant difference in the results.[12,14] In a histologic study, Caffesse et al.[15] concluded that adjunctive bone grafting did not appear to enhance regeneration. However, other authors have reported better bone fill of furcation defects when a bone substitute was used in combination with GTR.[16]

Several types of alloplastic materials have been used to treat periodontal defects. Calcium sulfate (CS) has a long history of safe use in both dentistry and medicine. It is totally bioabsorbable, osteoconductive, and biocompatible.[17] In 1992, Sottosanti[18] introduced the use of CS as a barrier in combination with demineralized freeze-dried bone allograft. According to Sottosanti, the CS barrier retards epithelial and connective tissue ingrowth to allow a predictable bone regenerative response. However, he emphasized that histologic evidence of new cementum and connective tissue fiber insertion into a previously diseased root surface needed to be documented.[18] Clinical studies[19,20] have demonstrated favorable results using bone grafts with a CS barrier to treat diverse types of periodontal defects. Histologic evidence of new bone formation and periodontal regeneration also has been reported.[21]

The combination of AB graft with a CS barrier has been shown to be a promising technique. However, there is a lack of histologic studies evaluating the healing of periodontal defects treated with these materials. The purpose of this study was to histologically evaluate the healing of surgically created Class II furcation defects treated using an AB graft with or without a CS barrier.

MATERIALS AND METHODS

This research protocol was approved by the University of the State of São Paulo "Júlio de Mesquita Filho" (UNESP) Dental School of Araçatuba Animal Research Care Committee. All guidelines regarding the care of animal research subjects were strictly followed. Six adult male mongrel dogs, weighing 15 to 20 kg, were used in this study at the Animal Facility of the Dental School of Araçatuba, São Paulo State University "Júlio de Mesquita Filho." The animals were in good systemic and oral health. They were given a thorough intraoral examination that included periodontal probing and radiographs.

The teeth selected for the creation of Class II furcation defects were the second, third, and fourth mandibular premolars (P2, P3, and P4) on both sides. Following block randomization, they were assigned to one of three groups: group C (control), in which the defect was filled with blood clot; group AB, in which the defect was filled with AB graft; and group AB/CS, in which the defect was filled with AB graft and covered by a CS barrier (calcium sulfate paste).§

Prior to all experimental procedures, the animals received atropine sulfate‖ (0.04 mg/kg body weight intramuscularly [IM]) as a preanesthetic medication and were then anesthetized using xylazine¶ (1 mg/kg body weight IM) and a combination of tiletamine hydrochloride with zolazepam hydrochloride# (50 mg/kg body weight IM).

Supragingival and subgingival scaling of all teeth with Gracey curets and ultrasonic instruments, followed by coronal polishing, was done 1 week prior to the surgical procedure. Plaque control was maintained by daily topical application of 0.2% solution of chlorhexidine gluconate.

Surgical Procedure

All surgeries were performed by the same surgeon. Surgical sites were locally infiltrated with 2% mepivacaine containing epinephrine (1:100,000) to reduce hemorrhaging. Intrasulcular incisions were made from the mesial of the canine to the distal of the second molar, and mucoperiosteal flaps were elevated to expose both buccal and lingual bone plates. Class II furcation defects measuring 5 mm in height and 2 mm deep horizontally were surgically created on the buccal surface of teeth P2, P3, and P4.[22] The defects were created using sterile carbide round burs in a low-speed handpiece under continuous sterile saline irrigation, along with Oschenbein and Rhodes microchisels. The dimensions of the defects were verified with a periodontal probe.** The root surfaces were carefully scaled and root planed with rotary instruments and curets to remove all cementum. Reference

§ Biomet, Warsaw, IN.
‖ Atropion, Ariston Indústrias Químicas e Farmacêuticas, São Paulo, Brazil.
¶ Coopazine, Coopers Brasil, São Paulo, Brazil.
# Zoletil 50, Virbac, São Paulo, Brazil.
** PCPUNC15BR, Hu-Friedy do Brasil, Rio de Janeiro, Brazil.

notches were placed in the roots at the level of the alveolar crest using a number 1/2 round bur. These notches started on the buccal aspects of the roots and extended into the furcation areas as deep as the Class II furcation defect permitted.

In groups AB and AB/CS, bone was harvested from the buccal aspect of the surgical site in the molar area using a disposable cortical bone collector[††] and then immediately placed to fill the defect completely. In group AB/CS, the CS was prepared according to the manufacturer's instructions and then placed over the AB graft. The CS barrier covering the graft was 1.5 to 2.0 mm thick and extended ~2 to 3 mm on to the surrounding bone. In group C, the furcation defects were filled with blood clot only (Fig. 1). The surgical flaps were coronally positioned and sutured. Modified vertical mattress sutures were made in the interproximal areas. In addition, horizontal mattress sutures, each of which engaged the base of both the buccal and lingual flaps, were placed circumferentially around the tooth to further stabilize the soft tissues. The animals were given amoxicillin (33.3 mg/kg body weight) orally twice a day for 7 days starting 2 hours before surgery.

Post-Surgical Procedures

All animals were given an oral analgesic (ketorolac, 5 mg/kg body weight) once a day for 3 days post-surgery. They were placed on a soft diet until euthanasia. Plaque control was maintained throughout the experimental period by topical application of 0.2% solution of chlorhexidine gluconate five times weekly. Sutures were removed 10 days post-surgery. The dogs were sedated 2 weeks after surgery using a combination of 0.2% acepromazine maleate[‡‡] (0.1 mg/kg body weight IM) and ketamine hydrochloride[§§] (6.66 mg/kg body weight IM) for careful prophylaxis with ultrasonic instruments and polishing. This procedure was repeated every 7 days until euthanasia. At each post-surgery procedure, the surgical areas were carefully examined to make sure that the graft materials had not become exposed and subsequently lost. At 90 days post-surgery, the animals were euthanized by perfusion under sedation with xylazine[‖‖] (2 mg/kg body weight IM) and general anesthesia with thiopental[¶¶] (12.5 mg/kg body weight intravenously [IV]).

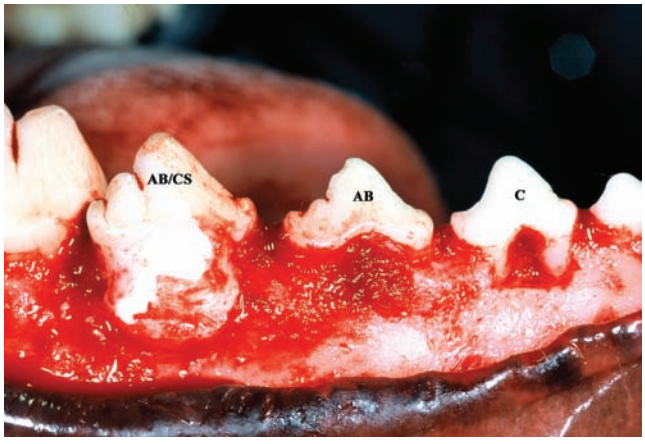

Figure 1.
Buccal view showing the Class II furcation defects after treatment (groups AB/CS, AB, and C).

Tissue Processing (Laboratory Procedures)

Blocks containing one tooth and the surrounding tissues were removed and fixed in 10% neutral formalin. Each specimen (tooth) was decalcified in Morse solution (50% formic acid and 20% sodium citrate in a 1:1 ratio) and embedded in paraffin following routine processing.

Serial sections (6 μm thick) were cut in a mesiodistal direction and stained with either hematoxylin and eosin (H&E) or Masson's trichrome for analysis by light microscopy.

Histologic and Histometric Analyses

Five sections per tooth were selected for microscopic analysis, corresponding to the first and the last sections containing notches in both roots and to three equidistant intermediate sections. Thus, representative sections of the initial (external most), intermediate, and final (internal most) portions of the defects, in a bucco-lingual direction, were obtained.[23]

The descriptive histologic evaluation assessed the presence or absence of root resorption and/or dentoalveolar ankylosis, the location of new cementum and bone formation, the direction of periodontal fibers, the degree of epithelial migration, and the degree of tissue inflammation. The furcation defect was considered to have total bone fill when newly formed bone was seen throughout the previously created defect in all five histologic sections. For the histometric analysis, images of the histologic sections were captured by a digital camera[##] connected to a light microscope[***] with a ×1.25/0.04 objective and then transferred to a computer. Measurements were made using image-analysis software.[†††] All measurements were made by the same investigator, different from the one who performed the surgeries. The investigator underwent a rigorous initial calibration process. A second calibration process was conducted, in which measurements were made three times for each histologic section evaluated. A mean was calculated from these

†† Koop, Indústria e Comércio de Produtos para Odontologia, Curitiba, Brazil.
‡‡ Acepran, Univet Indústria Veterinária, São Paulo, Brazil.
§§ Vetaset, Fort Dodge Animal Health, Fort Dodge, IA.
‖‖ Coopazine, Coopers Brasil.
¶¶ Thiopentax, Cristália Produtos Químicos Farmacêuticos, Itaparica, Brazil.
Olympus DP 10, Olympus Optical, Tokyo, Japan.
*** Olympus BX 50 F4, Olympus Optical.
††† SigmaScan Pro version 2.0, Jandel, San Rafael, CA.

three values. The measurements considered in this study were compared to the mean values obtained in the second calibration using split-plot analysis of variance (ANOVA). No statistically significant differences were found ($P = 0.9596$). This confirms that the investigator was well calibrated; therefore, there was no significant error in the measurements used in this study.

The following linear and area measurements were obtained by histometric analysis (Fig. 2).

Linear measurements. 1) Root surface of the defect (L-RSD): linear radicular extension between the apical limits of the notches made in the mesial and distal roots; 2) cementum formation (L-CF): sum of the linear radicular extensions of the defect covered with new cementum; 3) connective tissue (L-CT): sum of the linear radicular extensions of the defect covered with new connective tissue; 4) epithelial migration (L-EM): sum of the linear radicular extensions of the defect covered with epithelial tissue; 5) ankylosis (L-A): sum of the linear radicular extensions of the defect in direct contact with bone tissue; 6) free surface (L-FS): sum of the linear radicular extensions of the defect either not covered by any type of tissue or covered with bacterial plaque; 7) periodontal regeneration (L-PR): sum of the linear radicular extensions of the defect covered with new cementum adjacent to the new bone; and 8) bacterial plaque (L-BP): sum of the linear radicular extensions of the defect covered with bacterial plaque.

Area measurements. 1) Total defect area (A-TD): furcation area apically delimited by a straight line joining the apical limits of the two radicular notches; 2) newly formed bone area (A-NFB): portion of the total area of the defect filled with newly formed bone; 3) other tissue area (A-OT): portion of the total area of the defect filled with cementum and non-mineralized tissues; and 4) empty region area (A-ER): portion of the total area of the defect without any other tissue or filled with bacterial plaque.

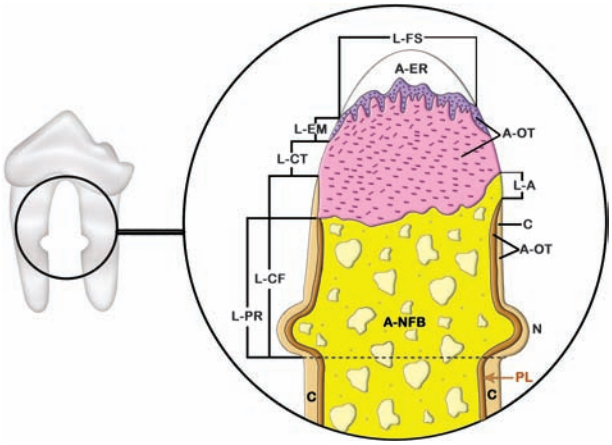

Figure 2.
Schematic illustration representing the histometric measurements performed in the furcation area. Area measurements (described in text) are as follows: A-NFB, A-OT, and A-ER (A-TD = A-NFB + A-OT + A-ER). Linear measurements (described in text) are as follows: L-CF, L-A, L-CT, L-EM, L-FS (L-RSD = L-CF + L-A + L-CT + L-EM + L-FS), and L-PR. PL = periodontal ligament; N = notch; C = cementum.

Statistical Analysis

Evaluation for each tooth corresponded to an average value calculated from measurements obtained from five sections of each tooth. The linear measurements (L-CF, L-CT, L-EM, L-A, and L-FS) were calculated as a percentage of the L-RSD. The linear extensions of these variables did not overlap. Thus, the sum of these variables corresponded to 100% of the L-RSD. The variables L-PR and L-BP also were evaluated separately as a percentage of L-RSD. The values of L-BP were practically zero. Therefore, it was not possible to analyze these data statistically. The area measurements (A-NFB, A-OT, and A-ER) were calculated as a percentage of the A-TD.

The animal was used as the statistical unit ($N = 6$). The normality of the data was confirmed, and the data variances were shown to be similar. Percentage data were transformed into arccosine for statistical analysis. The significance of differences among groups was determined by ANOVA. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Clinical Observations

All animals tolerated the surgical procedures well. Healing was uneventful, with all three groups having a similar clinical presentation characterized by a mild inflammatory response. Minimal gingival recession was observed in two teeth of group C, two teeth of group AB, and three teeth of group AB/CS.

Histologic Analysis

Most specimens of groups C, AB, and AB/CS did not have total bone fill of the furcation defect. The newly formed cementum varied in thickness, either partially or totally covering the root surfaces of the defect. Most specimens had partial coverage of the root surfaces with cementum. The newly formed periodontal ligament was well organized, very cellular, and highly vascularized, with fibers oriented perpendicular or oblique to the root surface that were clearly inserted into the new bone and new cementum (Fig. 3). Areas of dentoalveolar ankylosis were seen in some specimens of all groups.

Group C. The newly formed bone was seen restricted to the notch areas, extending slightly coronal to the notch areas, or partially filling the mid-portion of the furcation defect (Fig. 4). The remaining areas of the furcation were filled with fibrous connective

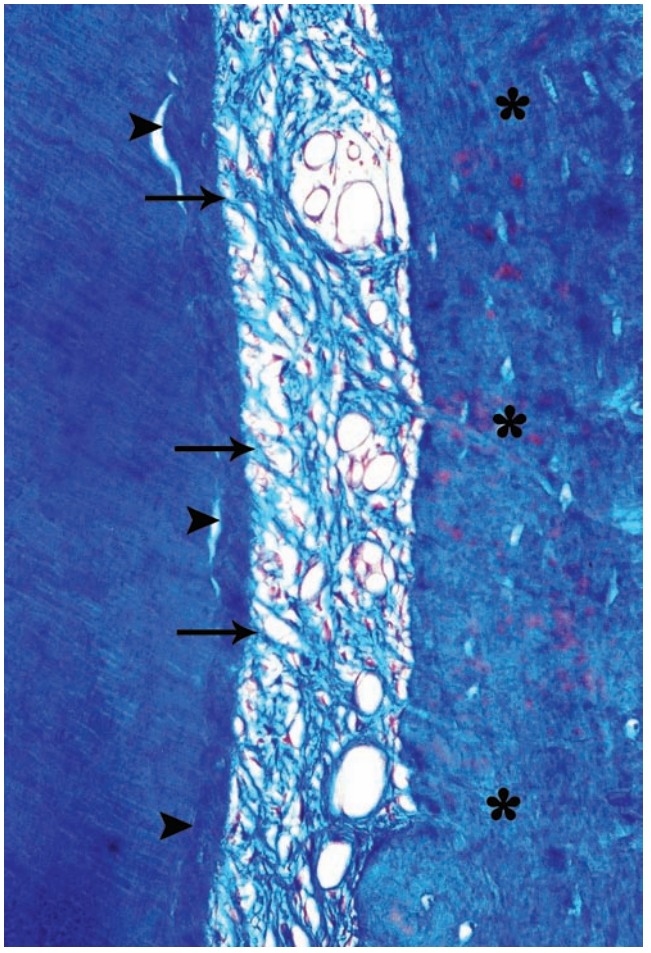

Figure 3.
Group AB. Shown is the newly formed periodontal ligament with fibers, oriented perpendicular or oblique to the root surface (arrows), inserted into the new bone (asterisks) and the new cementum (arrowheads) (Masson's trichrome; original magnification ×160).

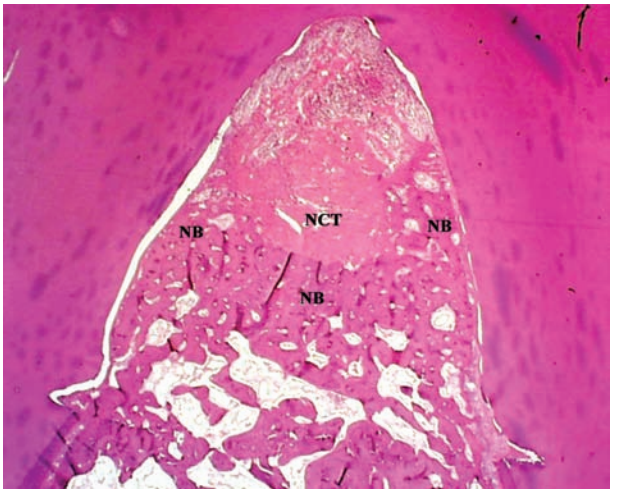

Figure 4.
Group C. Shown is a panoramic view of the furcation area, with new alveolar bone (NB) and new connective tissue (NCT) in the mid-portion of the furcation defect (H&E; original magnification ×12.5).

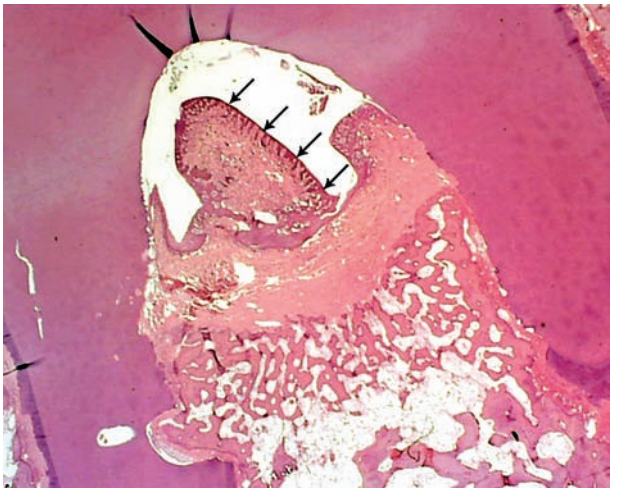

Figure 5.
Group C. Shown is a panoramic view of the furcation area, with epithelial migration (arrows) and gingival recession in the coronal and mid-portions of the furcation defect (H&E; original magnification ×12.5).

tissue containing a moderate to intense chronic inflammatory infiltrate. Total bone fill of the furcation was observed in only one specimen. In most specimens, the initial histologic sections (buccal side) showed epithelial migration into the coronal portion of the furcation. In some cases, epithelial projections were observed in the subjacent connective tissue that had an intense chronic inflammatory infiltrate. In two specimens, gingival recession was observed in the coronal and the mid-portions of the furcation (Fig. 5). Although root resorption lacunae were observed in some specimens, they had repaired and no areas of active resorption remained.

Group AB. In some specimens, newly formed bone was observed in the area between the notches and extending to the mid-portion of the furcation. In others, newly formed bone was seen filling the area between the notches, whereas in the mid-portion of the defect, it was restricted to the lateral areas close to the root surfaces. The center of the mid-portion was filled with dense connective tissue with a moderate chronic inflammatory infiltrate (Fig. 6). The coronal portion of the furcation was filled with fibrous connective tissue with a moderate to intense chronic inflammatory infiltrate. Total bone fill of the furcation was observed in only two specimens (Fig. 7). In half of the specimens, the initial histologic sections presented epithelial migration in the coronal portion of the furcation, with a moderate to intense chronic inflammatory infiltrate in the subjacent connective tissue. Repaired root resorption lacunae were observed without areas of active resorption.

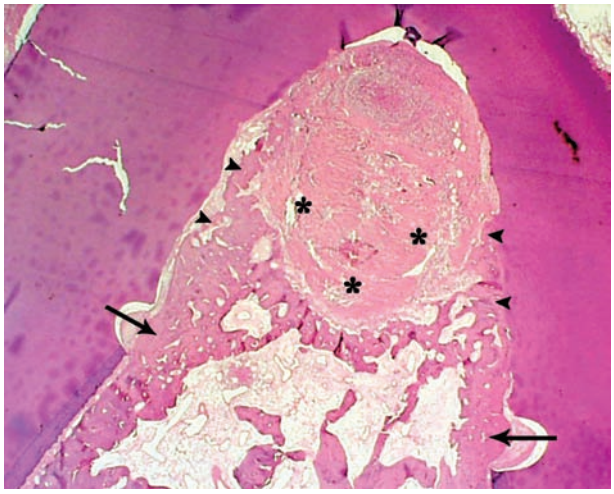

Figure 6.
Group AB. Shown is a panoramic view of the furcation area, with newly formed bone in the area between the notches (arrows). In the mid-portion of the defect, the new bone is restricted to the areas close to the roots (arrowheads). The central portion is filled with dense connective tissue (asterisks) (H&E; original magnification ×12.5).

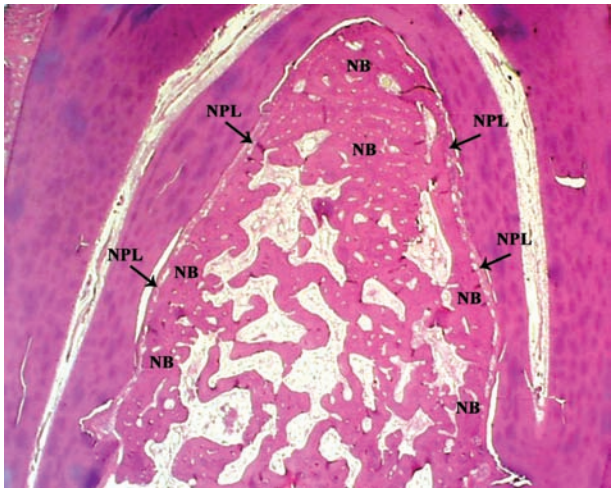

Figure 7.
Group AB. Shown is a panoramic view of the furcation area, with total bone fill of the furcation defect (H&E; original magnification ×12.5). NPL = newly formed periodontal ligament.

Group AB/CS. Newly formed bone was observed in the area between the notches. In some specimens, the new bone filled the entire mid-portion of the furcation (Fig. 8). In other specimens, the new bone was restricted to the areas close to the roots in the mid-portion of the furcation, whereas the central area was filled with fibrous connective tissue. In general, the coronal portion of the furcations had a very cellular and highly vascularized connective tissue with a light to moderate chronic inflammatory infiltrate. Total bone fill of the furcation was seen in three specimens (Fig. 9). Epithelial migration was observed in the coronal portion of the initial histologic sections of some specimens. In some cases, epithelial projections were seen in the subjacent connective tissue associated with a light chronic inflammatory infiltrate. Gingival recession and bacterial plaque were observed in two specimens. Most specimens had repaired root resorption lacunae without areas of active resorption.

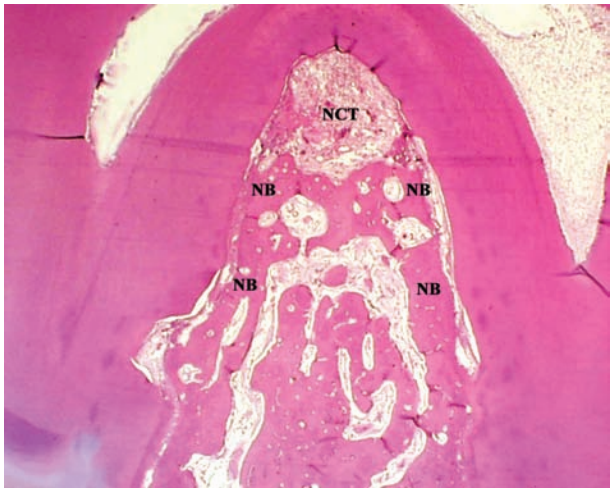

Figure 8.
Group AB/CS. Shown is a panoramic view of the furcation area. New bone fills the mid-portion of the furcation defect, whereas new connective tissue is seen in the coronal portion (H&E; original magnification ×12.5).

Histometric and Statistical Analyses

The mean percentage values and SDs of the linear measurements, with comparison among the groups, are reported in Table 1 and Figure 10. The mean percentage values and SDs of the area measurements, with comparison among the groups, are shown in Table 2. A-TD of all groups, in square millimeters, was compared using ANOVA. No statistically significant differences were found between groups, with $F = 0.95$ and $P = 0.4195$.

DISCUSSION

The purpose of this study was to histologically evaluate the healing of surgically created Class II furcation defects treated using an AB graft with or without a CS barrier. In this study, Class II furcation defects were surgically created and immediately treated; that is, an acute experimental model was used. It has been demonstrated that conventional root debridement eliminates most, if not all, of the cementum in natural disease or chronic defects, resulting in root surface conditions similar to those of acute defects.[24,25]

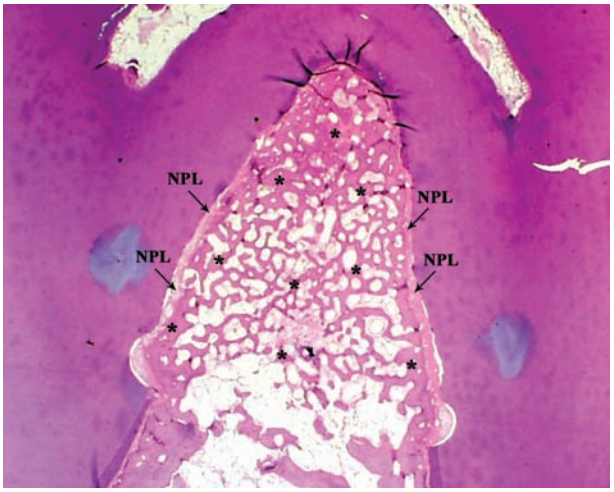

Figure 9.
Group AB/CS. Shown is a panoramic view of the furcation area, with total bone fill of the furcation defect. New bone (asterisks) and the newly formed periodontal ligament are visible (H&E; original magnification ×12.5).

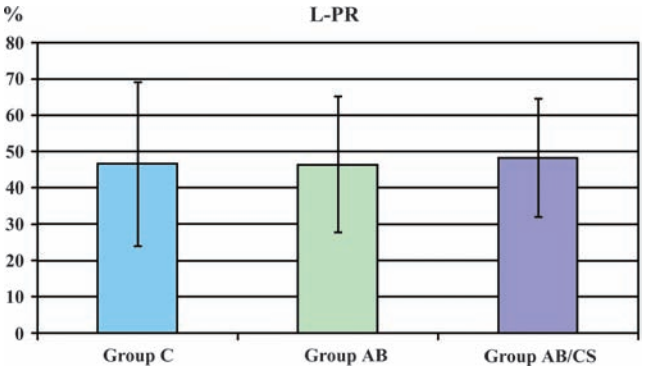

Figure 10.
Mean percentage values of L-PR for groups C, AB, and AB/CS.

According to Novaes et al.,[22] the acute experimental model is valid as long as critical dimensions are established with the aim of impeding the occurrence of spontaneous regeneration. The authors stated that the dimensions of the bone defect created in their study (5 mm in height × 2 mm in depth) limited the occurrence of spontaneous regeneration. Their control specimens showed signs of minimal regeneration in the most apical part of the defect, with migration of the junctional epithelium observed apical to the furcation roof. However, even though the present study used Class II furcation defects with the same dimensions used by Novaes et al.,[22] a considerable amount of new bone formation was observed in some specimens, and total bone fill of the furcation defect was seen in one specimen of group C. The mean percentage of bone formation for this group was 61.86% (Table 2). Similar results were obtained by Plotzke et al.,[26] who conducted a study in dogs in which they histologically evaluated acute Class II furcation defects treated with a polymeric composite. Even though the authors did not state the dimensions of the furcation defect or the percentage of bone formation, a considerable amount of new bone also was observed in the specimens of their control group treated by surgical debridement only. Because total bone fill of the furcation defect was observed in only one specimen of group C in the present study, the possibility of biologic variability should also be considered. This biologic variability may be due to the erratic behavior of genetic, biochemical, physiological, or immunological host factors.[27]

In the present study, serial mesio-distal histologic sections were made to obtain a panoramic view of the furcation area. Recently, the value of such histologic sections in the analysis of Class II furcation defects has been questioned.[27,28] However, by following specific guidelines to select five mesio-distal sections from each specimen, the initial (buccal),

Table 1.

Mean Percentage Values and SDs of the Linear Measurements With Comparison Among the Groups

	Group C		Group AB		Group AB/CS		ANOVA	
Measurement	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	F	*P*
L-CF	56.75	23.06	58.74	17.52	62.27	15.86	0.16	0.8506
L-CT	35.19	17.68	32.95	17.11	26.48	10.28	0.56	0.5866
L-EM	5.04	8.05	6.41	7.67	10.05	9.56	1.39	0.2938
L-A	1.29	3.17	1.89	2.17	1.81	3.57	0.39	0.6897
L-FS	1.54	3.18	0.00	0.00	2.87	6.23	0.88	0.4447

Table 2.

Mean Percentage Values and SDs of the Area Measurements With Comparison Among the Groups

	Group C		Group AB		Group AB/CS		ANOVA	
Measurement	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	F	*P*
A-NFB	61.86	9.27	64.95	15.75	59.85	20.90	0.38	0.6943
A-OT	37.50	9.08	35.05	15.75	39.15	19.19	0.24	0.7932
A-ER	0.62	1.17	0.00	0.00	1.74	4.26	0.58	0.5790

intermediate, and final (lingual) portions of the defect were accurately represented.[23] Therefore, valid measurements of the healing process of the entire furcation defect were obtained.

In this study, two specimens of group AB had total bone fill of the furcation defect, with insertion of functional periodontal ligament fibers into newly formed cementum and newly formed alveolar bone as well. These results corroborate the findings of other histologic studies that have demonstrated complete periodontal regeneration when AB graft was used to treat intrabony and furcation defects.[29,30] New bone formation in group AB (64.95%) was similar to group C (61.86%) and group AB/CS (59.85%; Table 2). Even though different models were used, these results are similar to those of Nilvéus et al.,[31] who did not observe any difference in bone formation in Class III furcation defects in dogs treated with AB compared to control. However, the results of the present study are contradictory to those of other studies that showed a greater amount of bone fill in the sites treated with bone graft than in sites without.[30,32]

Cortical autograft was used in this study. Because only a few cells can survive in this type of graft, it is not appreciably osteogenic but does provide an osteoconductive substrate for host bone formation.[33] According to Misch and Dietsh,[10] cortical grafts may act as a barrier to soft tissue invasion. However, epithelial migration was observed in this study in the coronal portion of the furcation in half of the specimens analyzed, demonstrating that cortical grafts do not always act as a barrier to soft tissue invasion.

In group AB/CS, AB graft was used in combination with a CS barrier. One of the functions of a graft material used in GTR is to act as a scaffold to provide and maintain space by preventing collapse of the membrane.[15] Space maintenance is a particularly important aspect of GTR surgeries.[3] Therefore, in this study, the AB graft was used to support the CS barrier and maintain the subjacent space.

When used in GTR procedures, bioabsorbable barriers must effectively exclude gingival epithelium and connective tissue to permit the selective repopulation of the root surface and adjacent alveolar defect area by periodontal ligament and/or alveolar bone cells. Once this process is completed, the barrier should be resorbed and replaced by the periodontal connective tissue without exerting adverse effects on the healing process.[34] If bioabsorbable membranes disintegrate too early, they may not prevent migration of the gingival epithelium along the root surface throughout the entire healing period. Karring et al.[35] reported that apical migration of epithelium tended to occur within 2 weeks after surgery. Therefore, it may be necessary to maintain the membrane structure in vivo for at least 3 to 4 weeks.[36] No remnants of CS barrier were observed in the present study 3 months post-surgery. Even though three specimens of group AB/CS presented complete periodontal regeneration of the furcation defect, the mean periodontal regeneration in group AB/CS was similar to that observed in groups C and AB (Fig. 10). Also, epithelial migration occurred similarly in all three groups (Table 1). Therefore, it seems that the CS barrier was not as effective at excluding epithelium and gingival connective tissue or in promoting periodontal regeneration, as demonstrated by Kim et al.[21] in surgically created intrabony defects in dogs. Some possible hypotheses may explain the absence of significant differences in periodontal regeneration observed in group AB/CS in relation to groups C and AB. First, Sottosanti[18] stated that a CS barrier resorbs completely in 2 to 3 weeks. If resorption of the CS in this study caused it to lose its barrier function too early, it would have been unable to prevent apical migration of the epithelium,[35] thus compromising periodontal regeneration. In this context, it should be noted that there is a lack of histologic studies documenting the exact amount of time before resorption of CS causes it to lose its ability to function as a barrier. Another hypothesis would be the possibility of fractures occurring in the CS in the initial post-operative period that would also compromise its function as a barrier. According to Sottosanti,[37] it is important to

work rapidly so that the flaps can be sutured before the plaster sets to minimize the chance of fracturing the barrier. The author has also emphasized that, once the barrier has hardened, any pressure placed on it during suturing can crack the barrier, making its replacement necessary.[18] Therefore, there is a possibility that the CS barrier may have cracked during the initial post-operative healing because of flap movement when the animals were eating or drinking. These fractures could have allowed the epithelial ingrowth in the furcation area. Both hypotheses are supported by the results obtained in this study, in which the occurrence of epithelial migration showed a similar behavior in groups AB/CS, AB, and C.

A similar, minimal amount of gingival recession was observed in some specimens of all groups. Therefore, the use of a CS barrier did not provoke more gingival recession compared to the other groups.

Root resorption has been frequently observed in animal and human studies after the use of AB grafts.[29,30,38] In this study, root resorption also was seen frequently in specimens of groups AB and AB/CS. Animals studies[15,30] have shown frequent dentoalveolar ankylosis when bone grafts are used to treat furcation defects. However, in the present study, the occurrence of dentoalveolar ankylosis was similar in the three groups (Table 1).

CONCLUSIONS

Contradictory results have been reported when bone grafts or bone substitutes were used with or without GTR to treat Class II furcation defects.[11,14,15] No statistically significant differences were found in any of the evaluated parameters among the groups in this study. Therefore, it is clear the use of regenerative techniques to treat Class II furcation defects gives highly variable results; thus, doubts still exist about their real benefit. Clearly, more histologic studies are needed. Periodontal healing was similar whether using surgical debridement alone, AB graft, or AB graft with a CS barrier in the treatment of Class II furcation defects.

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Correspondence: Dr. Maria J.H. Nagata, Department of Surgery and Integrated Clinic, Dental School of Araçatuba, University of the State of São Paulo "Júlio de Mesquita Filho," Rua José Bonifácio 1193, 16015-050 Araçatuba, São Paulo, Brazil. E-mail: mjnagata@uol.com.br.

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