The periodontal tissues in healed degree III furcation defects

An experimental study in dogs


Abstract. The aim of the present study was (i) to describe the periodontal tissue that formed in degree III furcation defects in mandibular molars of dogs following GTR therapy and (ii) to compare this healed periodontium to the corresponding tissue of pristine furcations. The study was performed in 10 mongrels dogs. In 6 of the dogs (group A), the 2nd and 4th premolars in both sides of the mandible were extracted 2 months prior to the start of the study. The 4 remaining dogs (group B) were used as normal untreated controls. In the dogs of group A, a furcation defect was produced in the 3rd mandibular premolars and reconstructive surgery was later performed in accordance with the GTR technique. 5 months after reconstructive surgery, all 10 animals (groups A+B) were sacrificed and perfused with a solution of 10% neutral buffered formalin through the carotid arteries. Tissue blocks containing the experimental teeth were excised, demineralized in EDTA, embedded in paraaffin. Serial sections were cut in the mesiodistal plane and parallel with the long axis of the roots. The microtome was set at 7 μm. The sections were stained in hematoxyline and eosin or Van Gieson’s connective tissue staining. From each biopsy, 3 sections, 14 μm apart, and representing the central part of the furcation, were selected for light microscopic examination. In the healed furcation sites, histometric and morphometric measurements were performed at different levels (zones), either at the mesial or distal root surfaces; (zone 1) immediately apical of the notch; (zone 2) coronal to the notch where the newly formed alveolar bone was in continuity with the reduced bone crest; (zone 3) coronal to the notch; representing the most coronally positioned area of new bone formation; (zone 4) coronal to the notch; representing areas with no alveolar bone present. In the pristine furcation sites, the measurements were made at zones which corresponded to the location of the zones in the healed furcations. The present data demonstrated that all furcation sites in group A after 5 months of healing exhibited comprehensive de novo cementum formation in the previously exposed parts of the intraradicular root surfaces, and that collagen fibers invested in the newly formed cementum. Comparisons between the pristine and the healed furcations disclosed that the periodontal ligament of the healed furcations was poorly organised, and that bone formation was frequently incomplete.

Key words: periodontal healing; new attachment; guided tissue regeneration; degree III furcation involvement; dogs

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Periodontal regeneration means restoration of the various components of the periodontium lost in disease in their appropriate locations, amounts and relationships to each other (Aukhil 1991). Thus, while clinical measurements may document wound and defect closure following therapy, periodontal regeneration can only be evaluated by histological means.

Different regenerative therapies, including the use of bone graft or bone substitutes, acid conditioning of the root surface, the application of growth factors, occlusive membranes, etc., have been advocated to accomplish regeneration of periodontal attachment lost in destructive periodontal disease (for review, see Gottlow (1994)). Findings from several animal studies have documented that guided tissue regeneration (GTR) may promote new attachment formation (Aukhil et al. 1983, 1986, Caffesse et al. 1990a, Gottlow et al.)
radicular surfaces of the 2 roots. Each notch was placed in the root at the level of the reduced bone crest. In one side of the mandible, a biodegradable membrane (Resolut®, Regenerative material, Gore-Tex, W. L. Gore Associates, Inc., Flagstaff, Arizona, USA) was adjusted to cover the buccal and lingual aspects of the furcation entrance, and 2–3 mm of the alveolar bone crest. The membranes were secured in position with single sutures. The periosteum, at the base of the mucoperiostal flaps, was incised and the soft tissue was positioned to ensure that the membranes were completely covered. The flaps were secured in the desired position with vertical mattress sutures which were placed in the edentulous ridge areas.

In the other side of the mandible, the 3rd premolar was treated in the same manner but with the placement of an expanded polytetrafluoroethylene membrane (e-PTFE; Periodontal Material®, Gore-Tex, W. L. Gore Associates, Inc., Flagstaff, Arizona, USA). The e-PTFE membranes were surgically removed 30 days after the reconstructive surgery (for details, see Pontoriero et al. (1992)).

Starting the day of surgery and continuing during the subsequent 2 weeks, the animals received, twice daily, 500 mg of amoxicillin (Amimox®, Tika, Lund, Sweden).

The 3rd mandibular premolars of the remaining 4 dogs (group B), were during the experiment period exposed to metacilous plaque control and served as controls (pristine furcations).

5 months after reconstructive surgery, all 10 animals (groups A+B) were sacrificed with an overdose of sodium pentobarbital. They were perfused with a solution of 10% neutral, buffered formalin through the carotid arteries. The jaws were removed and placed in a fixative. Tissue blocks containing the experimental teeth were excised and de-mineralized in EDTA. The blocks were subsequently washed, dehydrated in ethanol, infiltrated and embedded in paraffin. Serial sections were cut in the mesio-distal plane and parallel with the long axis of the roots. The microtome was set at 7 μm. The sections were stained in hematoxyline and eosin or Van Gieson's connective tissue stain. From each biopsy, 3 sections, about 14 μm apart, and representing the central part of the furcation, were selected for light microscopic examination.

In the healed furcation sites, an Or-
Fig. 1. Schematic drawing illustrating the different levels (zones) of the healed furcation in which the histometric and morphometric measurements were performed.

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Fig. 2. Mesial-distal section of the central and coronal portion of the pristine furcation area. B = bone, C = cementum, P = periodontal ligament. The delineated areas (a, b) are shown in higher magnification in Figs. 4a, b. Van Gieson staining: original magnification x 50.

Fig. 3. Histogram describing the proportions of the pristine and healed furcation sites that were comprised of periodontal ligament tissue (PDL), mineralized bone, bone marrow and bone marrow-like tissue (*), and residual tissue (R).

Arahelplan® (Leitz, Germany) microscope equipped with a Microlab® (Leitz, Germany) unit was used to determine the quality and quantity of the newly formed tissue. Thus, histometric and morphometric measurements were performed to describe the composition of the tissue in different levels (zones) of the previous defect; (zone 1; Fig. 1) immediately apical of the notch; (zone 2) coronal to the notch where the newly formed alveolar bone was in continuity with the surgically reduced bone crest; (zone 3) coronal to the notch; representing the most coronally positioned area of new bone formation; and (zone 4) coronal to the notch; representing areas with no alveolar bone present. In the pristine furcation sites, the histometric and morphometric measurements were performed in locations corresponding to those used in the healed furcation sites.

In each zone, the following distances were determined (×40): (1) the width of the cementum (width-C); (2) the width of the periodontal ligament (width-PDL); (3) the number of collagen fibers (per 100 µm of linear root length) inserting in the cementum (no. fibers-C); (4) the number of collagen fibers per 100 µm of linear root length inserting in the bone (no. fibers-B). The measurements describing width-PDL and no. fibers-B were not performed in zone 4.

Morphometric measurements (magn. ×400 and a lattice comprising 100 points; modified from Schroeder & Münzel-Pedrazzoli (1973)) were performed in the different zones to determine the proportions of the periodontal ligament tissue representing: (i) collagen fibers (Cf), (ii) vascular structures (V), (iii) cells and (iv) residual tissue (R). In addition, the overall proportions of organized periodontal ligament, mineralized bone, bone marrow, bone marrow-like tissue and residual tissue were assessed in a stereomicroscope (Olympus®, Japan) connected to an IBM computer (Compaq®, UK) by outlining the structures with a mouse curser. The periodontal tissues were also examined
in a light microscope (Leitz DM RBE®, Leica, Germany) using polarized light and interference contrast.

A mean value for each dog was determined for each of the parameters assessed. A Student t-test for paired and unpaired samples was used to analyse the differences between the various parameters in the different zones within

**Fig. 4.** High power magnification of the delineated areas in Fig. 2. (a) Area close to the furcation forix. Arrows indicate the peripheral dentine located between the granular layer of Tomes and the cementum. (b) Periodontal ligament, cementum and bone demonstrating a high density of well organized collagen fibers. CD=circumpulpal dentine, GLT=granular layer of Tomes, PD=peripheral dentine, C=cementum, P=periodontal ligament, B=bone. Van Giessen staining: original magnification ×400.

**Fig. 5.** Mesial-distal section of the central and coronal portions of the healed furcation area. B=new bone, NC=new cementum, P=periodontal ligament, arrows=apical border of the notches. H&E staining: original magnification ×100.

**Fig. 6.** High-power magnification of zone 4 (Fig. 1) of the healed furcation. Note the cellular nature of the new cementum. NC=new cementum, CD=circumpulpal dentine. H&E staining: original magnification ×400.
Fig. 7. Healed furcation. High power magnification of the new cementum disclosing the predominance of intrinsic fibers (arrowheads) over the extrinsic fibers (small arrows). Small areas of resorption (larger arrows) at the border between the new cementum and the circumpulpal dentine. NC=new cementum, CD=circumpulpal dentine. H&E staining: original magnification×400.

Fig. 8. Zone 3 (Fig. 1) of the healed furcation. Early stage of bone formation with a thin layer of bone (B) lined on both sides (arrows) by osteoblasts. NC=new cementum, P=new periodontal ligament, BM=bone matrix. H&E staining: original magnification×400.

Fig. 9. Zone 2 (Fig. 2) of the healed furcation illustrating a high density of collagen fibers in the newly formed periodontal ligament tissue. NC=new cementum, CD=circumpulpal dentine, P=new periodontal ligament, B=bone. H&E staining: original magnification×400.

Fig. 10. Zone 1 (Fig. 1) of the healed furcation. Note new cementum formation apical of the notch. NC=new cementum, OC=original cementum, D=circumpulpal dentine, P=new periodontal ligament, B=bone, E=epithelial remnants of Malassez, N=apical border of the notch. H&E staining: original magnification×400.

and between healed and pristine furcations sites.

Results

During healing, 1 premolar in 1 of the 6 dogs of group A developed a marginal abscess and was excluded from the study. A clinical examination performed immediately before sacrifice, disclosed that the gingival margin of all remaining furcation sites in groups A and B was clinically normal, and positioned coronal to the fornix of the buccal and lingual furcations.

Gross histological observations

The area of the pristine furcation (group B) was occupied by cementum,
periodontal ligament and bone (Fig. 2). The pristine furcation site was comprised of 29.6% periodontal ligament tissue, 63.8% mineralized bone, 6.7% bone marrow (Fig. 3). The cementum which was continuous with the peripheral dentine was of either acellular or cellular type. The acellular cementum was comprised mainly of extrinsic collagen fibers, while the cellular cementum included both extrinsic and intrinsic fibers (Figs. 4a, b). Frequently, the cementum layers included both types of cementum. The periodontal ligament was densely packed with collagen fibers bundles, which extended from the cementum to the bone. The connective tissue of the periodontal ligament also included several large vascular structures which seemed to be located closer to the bone than to the cementum surface. Epithelial remnants of Mallassez were observed close to the root surfaces. The centre of the furcation site was occupied by alveolar bone. Large bone marrow spaces were observed in the most apical portions of the furcation site (Fig. 2).

The overall characteristics of the healed furcation (group A) differed markedly from the pristine sites. The space of the healed furcation was occupied by cementum, periodontal ligament and bone in various stages of maturation and level of organisation (Fig. 5). The healed furcation site contained smaller amount of periodontal ligament tissue (15.9% vs 29.6%), and bone tissue (27.9% vs 63.8%) than the pristine furcation but, 40.8% bone marrow-like tissue, and 15.8% residual tissue (Fig. 3). Newly formed cementum with inserting collagen fibers (i) was observed to cover the entire surface of all previously exposed areas of the furcation, (ii) was continuous with the circumpulpal dentine (Ten Cate 1980), the surface of which exhibited discrete but abundant areas of arrested resorption (Fig. 6). A layer of peripheral dentine (Torencek 1980) could not be observed. The new cementum was of cellular, extrinsic and intrinsic fiber type, although the intrinsic fibers dominated (Fig. 7).

Different stages of bone regrowth and periodontal ligament organisation could be observed in the healed furcation sites. Bone formation was frequently incomplete and, as a rule, a large bone marrow space occupied the centre of the defect (Fig. 5). The organisation of the collagen fiber bundles

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*Fig. 11.* Histogram describing the overall width of the cementum (width-C), the width of the periodontal ligament (width-PDL), the number of fibers inserting in the cementum (no. fibers-C), and in the bone (no. fibers-B) in the pristine and healed furcation sites.

*Fig. 12.* Histogram describing the width of the cementum in different zones of the pristine and healed furcation sites.

*Fig. 13.* Histogram describing the width of the periodontal ligament (width-PDL), the number of collagen fibers inserting in the cementum (no. fibers-C) and the bone (no. fibers-B) in different zones of the pristine and healed furcation sites.
within the periodontal ligament appeared to be closely related to the presence or absence of newly formed alveolar bone. Thus, in zone 4 (Figs. 1, 6), the collagen bundles of the periodontal ligament were mainly comprised of thin fibers running more or less parallel to the cementum surface. In zone 3, poorly organised fiber bundles were observed (Fig. 8), but the degree of organisation increased consistently through zones 2 and 1 (Figs. 9, 10). No epithelial remnants of Malassez were observed in the newly-formed periodontal ligament.

**Histometric measurements**

The width of the cementum was in the pristine furcation sites found to be significantly smaller than in the healed furcations (41.3 versus 67.7 μm; p<0.05), while the width of the established periodontal ligament (width-PDL) was about 138 μm in both groups of furcations (Fig. 11). In the pristine furcations, the numbers of collagen fibers inserting in the cementum and in the bone (11.7 and 7.9/100 μm) were significantly larger than in the healed furcations (5.6 and 4.9/100 μm).

The results of the histometric measurements made in the different zones of the pristine and healed furcations are described in Figs. 12, 13. The width of cementum was about 40 μm in the 4 different zones of the pristine furcations, and was significantly wider in the healed furcations (about 66 μm; Fig. 12). In zone 1, the old cementum was 51.9 μm wide and covered by a layer of 17 μm new cementum. The width of the periodontal ligament did not differ significantly among zones 1–3 in the pristine and healed furcations (Fig. 13). The number of collagen fibers inserting in the cementum and in the bone was about 12 and 8, respectively (per 100 μm) in all 4 zones of the pristine furcations (Fig. 13). In the healed furcations, the density of the fibers inserting in cementum decreased gradually from 9.6 (per 100 μm) in zone 1, to 2.5 in zone 4, while the number of collagen fibers inserting in the bone was about 6 (per 100 μm) in zones 1 and 2, but only 2.7 in zone 3.

**Morphometric measurements**

The periodontal ligament of the pristine furcation was comprised of 77.7% collagen, 9.2% vascular structures, 11.1% cells, and about 2.0% residual tissue (Fig. 14). In the healed furcations the periodontal ligament contained significantly less collagen (46.9% versus 77.7%; p<0.05), but more vascular structures (18% versus 9.2%), cells (18.7% versus 11.1%), and residual tissue (16.4% versus 2.0%).

In the pristine furcations, the collagen, vascular structures, cell, and residual tissue densities (Fig. 15) were similar in all 4 zones (about 78%, 9%, 11% and 2%, respectively). In the healed furcations, the periodontal ligament in the different zones had a markedly different composition. The volume that was occupied by collagen decreased from 65% and 55% (zones 1 and 2) to 43.2% (zone 3) and 24.4% (zone 4). The density of vascular structures was about 12% in zones 1 and 2 but was considerably larger in zones 3 and 4 (19.1% and 29%). The density of cell was about 14% in zone 1 and about 20% in zones 2, 3 and 4. The volume occupied by residual tissue increased from 9.2% and 12.3% (zones 1 and 2) to 17.6% (zone 3) and 26.6% (zone 4).

**Discussion**

The present investigation demonstrated that all furcation sites in group A after 5 months of healing exhibited comprehensive de novo cementum formation in the previously exposed parts of the intraradicular root surfaces, and that collagen fibers invested in the newly formed cementum. The analyses, however, also documented that the periodontal ligament was poorly organised and that bone formation consistently was incomplete. Comparisons between the healed and the pristine furcation
sites disclosed that (i) the newly formed cementum was of cellular, intrinsic fiber character, (ii) the periodontal ligament was in several zones poor in collagen but rich in vascular structures, cells and matrix elements, and (iii) bone fill in the defect space was incomplete.

The new cementum in the healed furcations appeared to be reparative in character as indicated by features such as (i) the enhanced thickness, (ii) the greater cellularity; (iii) the predominance of intrinsic rather than extrinsic collagen fibers, and (iv) the investment of sparse, extrinsic Sharpey's fiber groups. In this respect the present findings are in accordance with studies by, e.g., Listgarten (1972), Nalbadian & Frank (1980) which demonstrated that the cementum which forms on surgically denuded root surfaces is a relatively thick, cellular cementum with a predominance of intrinsic collagen fibers running parallel with the root surface.

The present observations further documented that while in the pristine sites, a thin layer of peripheral dentine (also called Hopewell-Smith layer or intermediate cementum; for details, see Schroeder (1986)) was consistently present in the cementum-dentine junction (Fig. 4), no such layer could be identified in the healed furcations (Figs. 6, 8, 9). In contrast, in the healed furcations, the new cementum had formed directly over the circumpulpal dentine, the surface of which exhibited minute signs of resorption. Such transient, superficial resorption of root dentine frequently occurs in periodontal wound healing (Frank et al. 1974, Nalbadian & Frank 1980, Aukhil et al. 1983, 1986). It has been suggested that the peripheral dentin may play a role for the attachment between the original cementum and the dentine (Tornneck 1980) and that new cementum during periodontal wound healing may be deposited directly on circumpulpal dentine (Schüpbach et al. 1993). The lack of peripheral dentine may in such a situation compromise the stability of the dentine/cementum interface and favor the establishment of a split, during histologic preparation, between the newly formed cementum and the previously denuded dentine (Schroeder 1992). Despite the lack of a typical layer of peripheral dentine, however, the new cementum in most furcation sites of group A appeared to be in intimate contact with the circumpulpal dentine and was during processing not separated from the dentine by an artifactual split. There are reasons to suggest that the presence of resorption areas in the circumpulpal dentine may have accounted for the absence of such a split (Egelberg 1987).

The model used in the present study established conditions which allowed the formation of a uniformly thick cementum in the entire periphery of the furcation defect, i.e., also in areas remote from the existing cementum and periodontal ligament. This observation is at variance with findings previously reported, which indicated that newly formed cementum in regenerative therapy is consistently thicker in its apical than in more coronal portions (Nyman et al. 1982, Gottlow et al. 1984, 1990, Choi et al. 1993). In the studies referred to, it was suggested that new cementum was formed by cells which migrated from an existing layer of cementum or periodontal ligament. Hence, the dimensional differences, in apico-coronal direction, of the neocementum could be explained by the location of the original cementum (Nyman et al. 1982), and by the presence of a supportive blood supply in the periodontal ligament of the apical portion of a defect (Choi et al. 1993). The main difference between the present study and the data previously reported rests on the fact that the central portions of the furcations of group A, consistently healed with cementum formation in the entire circumference of the defect. There are reasons to suggest therefore, that when epithelial cells are excluded from the coagulum of a degree III furcation defect, the formation of cellular, intrinsic fiber cementum which follows initial, minute root resorption is predictable.

The width of periodontal ligament (width-PDL) in zones 1 through 3 of the healed furcations was similar to the width-PDL in the pristine furcations. This implies that during healing of the furcation defect, the dimensions of the PDL were restored and that the presence of this layer of connective tissue may have prevented bone from growing into contact with the root, causing ankylosis and root resorption (Karring et al. 1980, Nyman et al. 1980). It has been suggested that the epithelial remnants of Malassez may play an important role in maintaining a PDL of a certain "functional" width (Löe & Waerhaug 1961, Hodges 1969, Lindskog et al. 1988). In the present analysis, however, no epithelial remnants were observed in newly formed periodontal ligament. Hence, the role of this embryonic structure in the regenerated or mature PDL seems unclear. This conclusion is in agreement with Wesselin & Beertsen (1993) who from an experiment in the mouse stated that it is unlikely that the rests of Malassez are a prerequisite for the repair and maintenance of the periodontal ligament.

The overall composition of the periodontal ligament in the healed furcation was in several important aspects different from the corresponding tissue of the pristine furcation. Thus, the PDL of zones 1 through 3 in the healed furcations harbored less collagen, but more vascular structures, cells, and matrix elements than the pristine furcations. This may suggest that at the time of biopsy, healing of the furcation defect in group A was not completed and that with time a more mature ligament tissue would result. Such a conclusion is supported by data describing the degree of new bone formation. Thus, while in the pristine furcation sites about 63.8% of the volume was occupied by bone, in the healed furcations the corresponding figure was 27.9%. On the other hand, the possibility cannot be ruled out that the furcation defect established at surgery in the present study may have been too large to become fully restored during healing.

Zusammenfassung

Die parodontalen Gewebe in geheilten Furkationsgrad-III-Defekten. Eine experimentelle Studie an Hunden

Das Ziel der vorliegenden Studie war es 1) das parodontale Gewebe, das sich in Grad-III-Furkationsdefekten der Unterkiefermolaren von Hunden nach GTR-Therapie gebildet hat, zu beschreiben und 2) dieses geheilte Parodontium mit dem entsprechenden Gewebe von unberührten Furkationen zu vergleichen. Die Studie wurde an 10 Mischlingshunden ausgeführt. Bei 6 Hunden (Gruppe A) wurden der zweite und vierte Prämolar auf beiden Seiten des Unterkiefers zwei Monate vor dem Start der Studie extrahiert. Die 4 restlichen Hunde (Gruppe B) wurden als normale unbehandelte Kontrollen benutzt. Bei den Hunden von Gruppe A wurde am dritten Unterkieferprämolar eine Furkationsdefekt erzeugt und später wurde die rekonstruktive Chirurgie entsprechend der GTR-Technik durchgeführt. 5 Monate nach der rekonstruktiven Chirurgie, wurden alle 10 Tiere (Gruppe A+B) mit einer Lösung von 10% neutralen, gepufferten Formalin durch die Carotisarterien perfundiert. Gewebe-Blöcke, die experimentelle Zähne beinhalt-
Résumé

Les tissus parodontaux dans les lésions de la furcation de degré III après cicatrisation. Etude expérimentale chez le chien

Le but de la présente étude était (i) de décrire le tissu parodontal qui se formait dans les lésions de la furcation de degré III dans des molaires inférieures de chiens après traitement par GTR et (ii) de comparer ce parodontal cicatrisé avec le tissu correspondant de furcations à l'état initial. L'étude a été pratiquée sur 10 chiens mâles. Chez 6 des chiens (groupe A), les 2èmes et 4èmes prémolaires ont été extraites des 2 côtes de la mandibule 2 mois avant le début de l'étude. Les 4 autres chiens (groupe B) ont servi de témoins normaux non traités. Dans les chiens du groupe A, une lésion de la furcation a été produite au niveau des 3èmes prémolaires inférieures et on a plus tard pratiqué, avec préparation d'encoches repères dans les racines au niveau de la crête osseuse, une reconstruction chirurgicale suivant la technique GTR. Les 10 animaux (groupe A+B) ont tous été sacrifiés 5 mois après la reconstruction chirurgicale et ont reçu par les artères carotides une perfusion avec une solution à 10% de formaline neutre tamponnée. Des blocs de tissu contenant les dents expérimentales ont été excisés, décalcifiés à l'EDTA et inclus dans la paraffine. Des coupes en série ont été pratiquées dans le plan mésio-distal et parallèlement à l'axe longitudinal des racines. Le microtomé était réglé à 7 μm. Les coupes ont été colorées à l'hématoxyline et éosine ou par la coloration du tissu conjonctif de Van Gieson.

Pour chaque biopsie, 3 coupes distantes de 14 μm et représentant la partie centrale de la furcation ont été sélectionnées pour l'examen au microscope optique. Dans les furcations cicatrisées, les sites ont fait l'objet de mesures histométriques et morphométriques à différents niveaux (zones), soit sur les surfaces radiculaires mésiales soit sur les surfaces distales; (zone 1) immédiatement en apical de l'encoche; (zone 2) en coronaire de l'encoche, là où l'os alvéolaire nouvellement formé était en continuité avec la crête osseuse réduite; (zone 3) en coronaire de l'encoche; représentant la partie la plus coronaire de la néoformation osseuse; et (zone 4) en coronaire de l'encoche, représentant les zones où il n'y a pas d'os alvéolaire. Dans les sites furcataires à l'état initial, les mesures ont été pratiquées au niveau de zones correspondant à la position des zones des furcations cicatrisées. Les données obtenues ont montré que tous les sites furcataires du groupe A présentaient après 5 mois de cicatrisation une importante formation de néoépithélium dans les parties auparavant dénudées des surfaces radiculaires des racines, et que des fibres collagènes se couvraient de cément nouvellement formé. La comparaison entre les furcations à l'état initial et les furcations cicatrisées a révélé que le desmodonte des furcations cicatrisées était mal structuré et que la formation osseuse était souvent incomplète.

Références


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