J Periodont Res 2006; 41; 535–540

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00902.x

Effect of two different approaches for root decontamination on new cementum formation following guided tissue regeneration: a histomorphometric study in dogs

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Gonçalves PF, Gurgel BCV, Pimentel SP, Sallum EA, Sallum AW, Casati MZ, Nociti Jr FH. Effect of two different approaches for root decontamination on new cementum formation following guided tissue regeneration: a histomorphometric study in dogs. J Periodont Res 2006; 41: 535–540.

Background and Objective: The aim of the present study was to evaluate comparatively the effect of two different approaches for root decontamination on new cementum formation following guided tissue regeneration (GTR).

Material and Methods: Nine mongrel dogs were used to obtain bilateral chronic class III furcation defects by placing cotton ligatures around both third mandibular premolars. The teeth were randomly assigned to receive one of the following treatments: scaling and root planing, by means of hand and rotatory instruments, in order to remove soft and hard deposits as well as all root cementum (group A); or removal of only soft microbial deposits, by polishing the root surface with rubber cups and polishing paste, aiming for maximum root cementum preservation (group B). Both groups were treated with GTR, with the use of resorbable polyglycolic-lactic acid membranes (RESOLUT XT®).

Results: Four months later, data analysis showed that a superior length (mm) (3.59 ± 1.67 and 6.20 ± 2.26 for groups A and B, respectively; $p = 0.004$) and a thicker layer (μm) (18.89 ± 9.47 and 52.29 ± 22.48 for groups A and B, respectively; $p = 0.001$) of new cementum was achieved by keeping the root cementum in place during root decontamination (group B). Regardless of the treatment modality, the new cementum was predominantly of a reparative, cellular extrinsic and intrinsic fiber type.

Conclusion: Within the limits of the present study, it may be concluded that root cementum preservation may affect the new cementum formation following GTR in class III furcation defects, and the treatment modality did not influence the type of newly formed cementum.

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Key words: dogs; periodontal regeneration; root cementum

Accepted for publication March 14, 2006

The major challenge in periodontal therapy is to restore the structure and function of the dental attachment apparatus lost during the course of the disease. While there has been some success in periodontal regeneration techniques, including the use of barrier membranes for guided tissue regeneration (GTR), growth factors and enamel matrix proteins, these approaches have not resulted in predictable outcomes, especially regarding new cementum formation and attachment (1).

Cementum is a thin mineralized tissue covering the root surface of teeth that provides an interface through which the periodontal ligament anchors the tooth to the alveolar socket. There is accumulating histological evidence that cementum is critical for appropriate maturation of the periodontium, during development as well as during the regeneration of periodontal tissues (2). Concern regarding cementum exposuse to bacterial endotoxins has led to the use of extensive scaling and root planing as a treatment for periodontal disease, in an attempt to remove the contaminated part of the root cementum (3–6).

On the other hand, there is evidence to demonstrate that bacterial endotoxins do not penetrate deeply into the exposed cementum (7,8), but rather form a loosely attached layer on its surface (9,10). Thus, a gentler treatment approach to the root surface has been proposed, because it has been observed, both in dogs and in humans, that periodontal health can be achieved with simple polishing of the root surface (11,12), meaning that the removal of the root cementum for the purpose of eliminating such endotoxins may not be necessary.

Preservation of the root cementum may be an important factor for avoiding root structure loss, dentin hypersensitivity (13) and to prevent root resorption (14). It may also favor periodontal regeneration, providing a suitable microenvironment for new cementum formation to occur, because the cementum matrix is a rich source of growth factors that influence the activities of various periodontal cell types (2,15,16). To the best of the author's knowledge, there are no data available on the impact of preserving the 'diseased' root cementum on the nature and amount of new cementum formation following GTR. Thus, it was the objective of the present study to investigate histometrically, in dogs, the effect of two different approaches for decontamination of the root surface on the new cementum formation following GTR.

Materials and methods

Animals

Nine adult male mongrel dogs (mean weight: 15.50 ± 3.10 kg) were included in this blinded split-mouth study. All surgical procedures were performed under general anesthesia with intravenous injection of a 3% sodium pentobarbital solution (30 mg/kg). This study was approved by the Institutional Committee on Animal Research.

Teeth extraction and periodontitis induction

Two months prior to the start of the experiment, the 2nd and 4th mandibular premolars (P_2/P_4) were bilaterally extracted, and both 3rd premolars (P_3) were assigned as experimental teeth. Cotton ligatures were then bilaterally placed around P_3, and the animals were fed with a soft diet in order to induce periodontitis and to produce chronic class III furcation defects. Clinical and radiographic examinations were carried out periodically to monitor the course of the disease. When bone loss achieved half of the root length and 'through and through' furcation defects were produced (4–5 mo), the ligatures were removed and a 2-wk period of supragingival plaque control was initiated, including daily tooth brushing and topical application of 0.12% chlorhexidine.

Treatment procedure

At the end of the plaque control period, crestal and sulcular incisions were made, buccal and lingual mucoperiosteal flaps were raised, and a notch was placed at the alveolar bone crest level on the mesial and distal roots of both P_3, in the furcation area. The teeth were randomly assigned to receive one of the following treatments.

(1) The root surface was carefully scaled and planed with curettes and diamond coated flame-formed burs, and polished with the use of rubber cups and a polishing paste, in order to remove all plaque and calculus, in an effort to eliminate all cementum previously exposed to the bacterial biofilm (group A).
(2) The root surface was not scaled but only polished, as described above, so that soft bacterial deposits, but not the 'diseased' root cementum, were removed (group B).

All the defects were treated by the GTR technique, using resorbable membranes (RESOLUT XT™; Regenerative material, Gore-tex; Gore Associates, Flagstaff, AZ, USA). Two membranes were trimmed and adapted to cover the buccal and lingual aspects of the furcation defect, both extending to the surrounding bone (2–3 mm) and sutured around the tooth with resorbable sutures. The flaps were coronally positioned to cover the membranes and expanded polytetrafluoroethylene-interrupted (e-PTFE-interrupted) sutures were applied (Gore-tex sutures; Gore Associates). After surgery, the animals were given an intramuscular injection of penicillin (1 : 50,000 IU), and the same dose was repeated after 5 d. Postoperatively, plaque control was performed by daily topical application of 0.12% chlorexidine solution until the end of the experiment, and the sutures were removed after 14 d of healing.

Histology procedures

Four months after surgery, the animals were killed with an overdose of anesthesic solution, the jaws were dissected, and biopsies from the P_3 regions were harvested and fixed in a 4% neutral-buffered formalin solution for 1 wk. Specimens were decalcified in a solution of equal parts of 50% formic acid and 20% sodium citrate for 4 mo at room temperature. The decalcified specimens were washed in running water, dehydrated and embedded in paraffin. Serial mesio-distal sections (7 μm thick) were obtained and stained with hematoxylin and eosin.

Histometric analysis

Ten sections, representing the middle portion of the furcation defect (30 μm apart) were used to obtain the mean for each parameter in each dog, measured by a calibrated and blinded examiner using an image-analysis system (Image Pro Plus 3.0; Media Cybernetics, Silver Spring, MD, USA). The following linear measurements were obtained in the mesial and distal roots under the furcation:

- new cementum extension (NCE): extension (mm) of the root surface covered by new cementum; and
- new cementum width (NCW): measured (in μm) in both mesial and distal roots, at a point located 1500 μm coronally to the notch.

Statistical analysis

The data were averaged and the hypothesis that there was no difference between the groups regarding the evaluated parameters was tested by intergroup analysis (e.g. group A vs. group B) using the paired *t*-test ($\alpha = 0.05$).

Results

Clinical observations

The healing was uneventful; no suppuration or abscess formation was observed. Some sites presented gingival recession (1–2 mm), with exposure of the coronal part of the membrane (three and two sites in groups A and B, respectively). In these cases, plaque control was performed with topical application of 1% chlorhexidine gel during the whole experimental period, and they showed no further complications. At the end of the experiment, the soft tissues had totally healed and exhibited no clinical signs of inflammation.

Histomorphologic analysis

Histological analysis showed that the healed furcation sites were occupied by epithelium, connective tissue, cementum, periodontal ligament and bone. Different stages of bone regrowth and periodontal ligament organization were observed. The newly formed alveolar bone presented large bone marrow spaces in the central area of the defect, while mineralized bone occurred in the periphery of the bone tissue. The periodontal ligament presented densely packed collagen fiber bundles oriented parallel to the root surface or inserted into the new cementum, and the presence of functionally oriented collagen fibers appeared to be closely related to the presence or absence of newly formed alveolar bone adjacent to new cementum.

The newly formed cementum was of the cellular type, with extrinsic and intrinsic fibers, irrespective of the treatment. In the healed sites that presented a complete filling of the furcation, cementum with inserting collagen fibers had formed along the entire root surface of the defect. When cementum was absent, a soft tissue adaptation was observed directly in contact with peripheral dentin. In these cases, resorption lacunae containing multinucleated giant cells were occasionally found on the dentin surface (Fig. 1). Ankylosis was not detected in any section. It was observed that a uniformly thick new cementum layer was deposited over the pre-existent cementum, forming a continuous layer along the root surfaces on the teeth of group B (Figs 2 and 3). Resorption lacunae were not found along the root surfaces that were only polished, which presented a smooth profile.

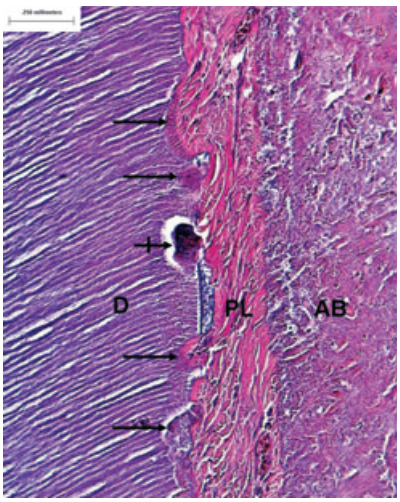

Fig. 1. Photomicrography illustrating the presence of irregularities (thin arrows) and a resorption lacuna containing a multinucleated giant cell (crossed arrow) on the dentin surface for a group A section. AB, alveolar bone; D, dentin; PL, periodontal ligament. Original magnification ×100, hematoxylin and eosin (H&E) staining.

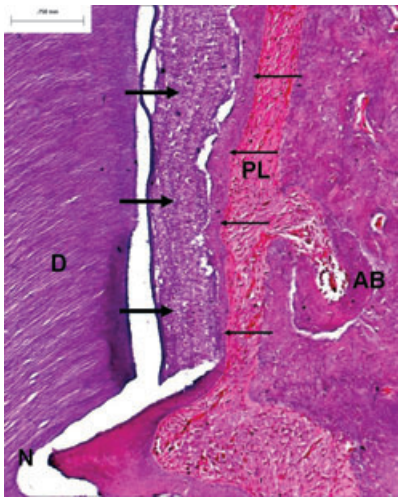

Fig. 2. Mesio-distal section showing the apical area of the furcation defect where the 'diseased' root cementum was kept in place (group B). Observe that a continuous layer of new cementum of the cellular type (thin arrows) was present over the pre-existent cementum (thick arrows). AB, alveolar bone; D, dentin; N, notch; PL, periodontal ligament. Original magnification ×25, hematoxylin and eosin (H&E) staining.

Histometric analysis

Intergroup analysis demonstrated that the pattern of new cementum formation was affected by the presence of cementum on the root surface previously exposed to dental biofilm. A longer extension (3.59 mm ± 1.67 and 6.20 mm ± 2.26 for groups A and B, respectively; $p = 0.004$) and a thicker layer (18.89 μm ± 9.47 and 52.29 μm ± 22.48 for groups A and B, respectively; $p = 0.001$) of new cementum were observed for group B. The histometric results are presented in Fig. 4.

Discussion

The results of the present study showed that, after GTR, cementum formation

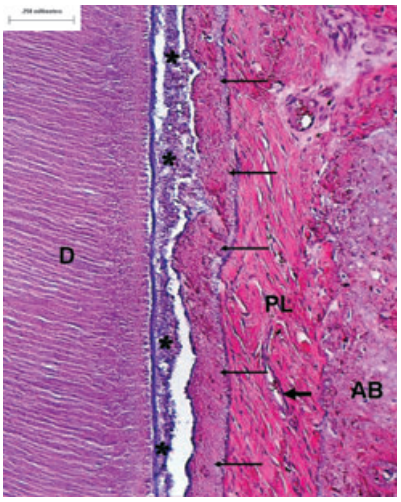

Fig. 3. High magnification of a root surface where the 'diseased' root cementum was kept in place after root decontamination (group B), showing a thick and uniform layer of new cementum (thin arrows) over the pre-existent cementum (*). Note that a gap is present between the new cementum and the old one. The periodontal ligament contains blood vessels (thick arrow) and collagen fibers inserted into the new cementum and bone. Original magnification ×50, hematoxylin and eosin (H&E) staining.

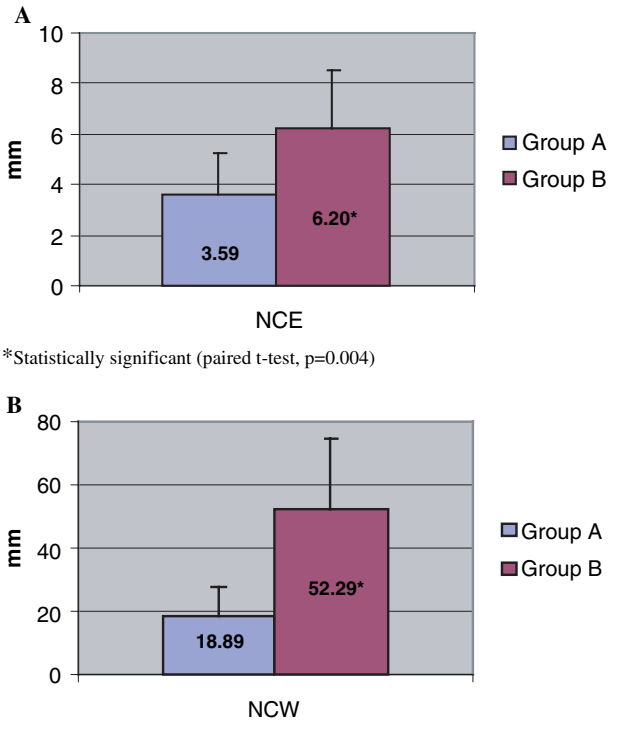

Fig. 4. Graphic representation of the newly formed cementum following guided tissue regeneration in groups A and B. The mean and standard deviation are shown for the evaluated parameters. (A) New cementum extension (NCE); (B) new cementum width (NCW). *Statistically significant (paired *t*-test, $p = 0.001$).

was influenced by the presence of the pre-existent cementum, even if previously exposed to dental biofilm. These findings add a new perspective to the idea of a gentler treatment approach to the root surface, which leaves most of the cementum in place but, at the same time, removes and disturbs the attached bacterial biofilm. Additionally, the present findings support previous reports (11,12) showing that cementum removal, with aggressive scaling and root planing, may not be required for resolving inflammation, but that the elimination of soft bacterial deposits, rather than the removal of the cementum, is essential for accomplishing periodontal health following therapy.

Although not measured histometrically (for methodological reasons), it was observed in the present study that the newly formed cementum, following GTR, was consistently thicker in its apical than in its more coronal portions, which is in agreement with findings previously reported regarding the dimensional aspects of the new cementum in the apico–coronal direction (17,18). This would be explained by the location of the original cementum and by the presence of a supportive blood supply in the periodontal ligament of the apical portion of the defect (17). Furthermore, the consistent formation of new cementum over the original cementum in group B, also in areas remote from the existing periodontal ligament, may indicate a role for the original cementum in modulating new cementum formation.

Similarly to previous reports in dogs and humans (18–20), the present findings indicate that acellular extrinsic fiber cementum (AEFC) does not seem to form predictably after GTR, and that once the process of periodontal healing had been initiated the resulting cementum was usually of a reparative, cellular, extrinsic and intrinsic fiber type. The findings of the present study additionally indicate that a cellular, extrinsic and intrinsic fiber type of cementum formed irrespective of the presence of the 'diseased' cementum or its removal. At this point in time, the AEFC has only been shown in regenerated sites treated with enamel matrix derivative proteins (EMD) (20,21), but the extent to which the AEFC is essential for a functional periodontium remains to be investigated (22).

Root cementum formation is an important factor in recovery of the periodontal ligament attachment to the tooth, but very little is known about the influence of pre-existent cementum on the healing process following periodontal disease. Various biomaterials and growth factors have been investigated for their ability to induce cementogenesis, but in fact cementogenesis appears to be limited when compared with the extent of bone regeneration (15). The present findings indicate that pre-existent root cementum may favor new cementum formation *in vivo*, and further studies should be considered in order to investigate the mechanisms involved.

To the authors' knowledge, there is no evidence to explain the mechanisms by which the old cementum would favor new cementum formation; however, some aspects of preserving root cementum should be considered. Surface cementum appears to present a high chemotatic activity to human fibroblasts when compared with dentin or deep cementum (23). Moreover, the cementum matrix is a rich source of growth factors, which influence the activities of various periodontal cell types (15). Recent studies have additionally suggested that dentin-derived bioactive factors, when released at the healing site, present pro-inflammatory properties that may alter the forming periodontal tissues (24,25), and the presence of root cementum may play a protective role in this regard. The availability of animal and human cementoblastic cells in culture (26,27) has enabled us to investigate further various aspects of cementogenesis. Characterization studies have shown that these cementoblasts retain the expression of genes (such as bone sialoprotein and osteocalcin) associated with mineralized tissues, promote mineralization and respond to growth factors *in vitro* (28,29). Additionally, *in vivo* studies have demonstrated that cementoblasts have a marked ability to induce mineralization in periodontal wounds when delivered via polymer sponges (30,31).

The results of the present study do not signify that scaling and root planing should be avoided before GTR therapy. For instance, removal of hard deposits, such as calculus and mineralized surface coatings, may not be performed without the removal of some cementum. However, considering the present evidence that root cementum may modulate periodontal regeneration, further studies should be considered in order to investigate the factors and mechanisms involved, and also to elucidate the clinical predictability of this gentler treatment approach of the root surface, on a long-term basis.

Conclusions

Within the limits of the present study, it can be concluded that a gentler treatment approach to the root surface, allowing the preservation of old cementum, may affect the formation of new cementum following GTR. In addition, the treatment modality did not influence the type of newly formed cementum.

Acknowledgements

This research was supported by State of São Paulo Research Foundation – FAPESP, São Paulo, Brazil(02/09244–5 and 02/09245–1). We thank Mariana Piovezan Fugolin Lazarin for the preparation of the histologic material.

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