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Susceptibility of GTR-regenerated periodontal attachment to ligature-induced periodontitis

An experiment in the monkey

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Abstract

Aim: This study aimed to compare the susceptibility of guided tissue regeneration (GTR)-regenerated periodontal attachment to ligature-induced periodontitis with that of the pristine periodontium.

Methods: Periodontal breakdown was produced in four monkeys by the placement of orthodontic elastics around experimental teeth (test teeth). During a flap operation, the root surfaces were scaled and planed, and a notch indicating the apical termination of scaling and root planing was made in the root surface. Following resection of the crowns and endodontic treatment, an e-PTFE membrane was adapted over the roots. Subsequently, the flaps were sutured to complete closure of the wound (submerged). At membrane removal after 5 weeks, the crowns of the contralateral teeth serving as controls were resected, and the roots treated endodontically during a flap operation. Artificial composite crowns were then placed on both test and control roots. After 3 months of tooth cleaning, cotton floss ligatures were placed passively around both test and control teeth for a period of 6 months. Two weeks later the animals were sacrificed.

Results: Histological analysis demonstrated that the instrumented root surfaces of the test teeth were covered by newly formed cementum of the reparative, cellular, extrinsic and intrinsic fiber type, while the cementum on the controls was mainly acellular extrinsic fiber cementum. Histometric assessments demonstrated that similar attachment loss had occurred on test (1.0 ± 0.5 mm) and control roots (1.0 ± 0.4 mm) during the 6 months of ligature-induced plaque accumulation.

Conclusion: The results indicate that teeth with a periodontal attachment apparatus formed by GTR is not more susceptible to periodontitis than those with a pristine periodontium.

Key words: animals; GTR; new attachment; periodontal regeneration; periodontitis; root cementum

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Introduction

The clinical application of guided tissue regeneration (GTR) in periodontal therapy involves the placement of a physical barrier to ensure that the previously periodontitis-affected root surfaces become repopulated with cells from the periodontal ligament, while at the same time gingival connective tissue and oral epithelium are prevented from contacting the root surface during healing (Karring et al. 1993). Results from animal and human histological studies have shown that following GTR therapy, new cementum with inserting collagen fibers is formed on the previously periodontitis-affected root surfaces and that varying amounts of bone are regenerated (Nyman et al. 1982, Gottlow et al. 1984, 1986). The new cementum formed on the root surface following GTR treatment differs from the pristine root cementum regarding structure, thickness, and the number of inserting collagen fibers. While pristine cementum is predominantly of the acellular extrinsic fiber type, it is reparative cellular extrinsic/intrinsic fiber cementum that is formed following GTR treatment (Schüpbach 1993, Araújo et al. 1996, Bosshardt & Selvig 1997, Sculean et al. 1999, 2000).

The long-term stability of the treatment outcomes following GTR treatment of intrabony defects and furcation involvements has been evaluated in a few clinical trials. The results indicate that attachment gain obtained following treatment can be maintained on a long-term basis (Gottlow et al. 1992, Becker & Becker 1993, McClain & Schallhorn 1993, Machtei et al. 1996). However, the stability of the treated sites is dependent upon the participation of the patients into a recall program, and upon the absence of bacterial plaque, bleeding on probing, and re-infection of the sites with periodontal pathogens (Cortellini et al. 1994). In addition, it was demonstrated in a controlled clinical trial, comparing the susceptibility to disease recurrence at sites treated with GTR and sites treated with root planing, that patient factors such as compliance with oral hygiene, smoking habits, and susceptibility to disease progression rather than the employed treatment modality were the major determinants of stability of the treated sites (Cortellini et al. 1996).

The response of regenerated periodontal tissues to plaque was evaluated in a study in monkeys (Ling et al. 1994). The results indicated that plaque-induced inflammation was less at sites treated with GTR when compared to originally clinically healthy sites, and that more osteoclastic activity occurred at GTR-treated sites. However, whether the qualitative difference between regenerated and pristine cementum may influence the susceptibility of new attachment gained by GTR to plaque-induced periodontal breakdown has not been evaluated histologically. Therefore, the aim of the present study was to examine the susceptibility of periodontal regeneration produced by GTR to ligature-induced periodontitis as compared with that of the pristine periodontium.

Materials and Methods

Four monkeys (*Macaca fascicularis*) were used in this study. During operative procedures, the animals were anesthetized with Ketalar® Vet. (Parker-Davis, Barcelona, Spain). In each monkey, a first maxillary premolar and molar, and a mandibular lateral incisor were selected as test teeth and subjected to periodontal breakdown by placing orthodontic elastics around them. A new elastic was placed on top of the other every second week. After 2–3 months, when about 50% bone loss was recorded radiographically, the elastics were removed and tooth cleaning with topical application of chlorhexidine 0.5% once a week was instituted. After 1 month, reverse bevel incisions were made at the buccal and oral aspect of the experimental teeth, and mucoperiosteal flaps were elevated. All granulation tissue was removed and the exposed root surfaces were scaled and planed using a diamond bur rotating at slow speed, and under irrigation with sterile saline. A notch was then prepared in the root surface at the level of the apical extension of the intrabony defect. Subsequently, the crowns of the teeth were cut off at the level of the cemento-enamel junction. The pulp tissue was extirpated and the root canal was instrumented and filled with calcium hydroxide root filling material. Regarding the maxillary multi-rooted teeth (molars and premolars), the two buccal roots were cut free and extracted, and only the palatal root was retained. An e-PTFE membrane (Goretex Periodontal Material®, Gore & Associates, Flagstaff, AZ, USA) was adapted over each root, and was finally covered by a coronally displaced flap in such a way that the membrane and the root were completely submerged. Five weeks later, the operated areas were reopened by a flap procedure, and the membrane was removed (Fig. 1). The flaps were then sutured with their margin positioned at the coronal level of the cut root so that the tissue formed under the membrane was completely covered. At this time point, a flap operation was also performed on the contralateral control teeth. The crowns of these teeth were cut off at the level of the cemento-enamel junction, the pulp tissue was extirpated, and the prepared root canals were filled with calcium hydroxide. The two buccal roots of the maxillary multi-rooted teeth were extracted like in the test side. The flaps were then sutured back to their former position. After 15 days, the coronal portion of the filling material in the root canal of all experimental teeth was removed with a No. 40 endodontic file, and class II cavities

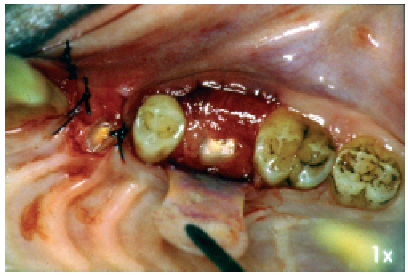

Fig. 1. Test root at membrane removal showing that new periodontal tissues have formed under the membrane to the coronal level of the cut root.

Fig. 2. Experimental tooth with an artificial composite crown during the period of ligature-induced periodontitis.

were prepared in the adjacent teeth of the experimental molars and premolars while circumfirential grooves were prepared in the teeth adjacent to the experimental incisors. Finally, impressions were taken and individual casts of the experimental teeth were poured. Artificial crowns were prepared by means of light cured composite with parts of endodontic files No. 40 as posts for anchoring in the root canal. The crowns were fixed in the root canal with cementum and splinted to the adjacent teeth with orthodontic wire and light cured composite. After a 3-month period of tooth cleaning twice a week, the cleaning was abolished, and cotton floss ligatures were placed passively around both control and test teeth in order to facilitate plaque accumulation (Fig. 2). After 6 months of ligature-induced plaque accumulation, the ligatures were removed and 2 weeks later, the animals were sacrificed with an overdose of Ketalar® Vet. They were immediately perfused with 10% neutral buffered formalin through the carotid arteries. The jaws were removed and placed in fixative. After fixation, tissue blocks containing the experimental or control teeth with surrounding tissues were dissected free and demineralized in EDTA and formic acid, and embedded in paraffin. Serial sections, 8 μm

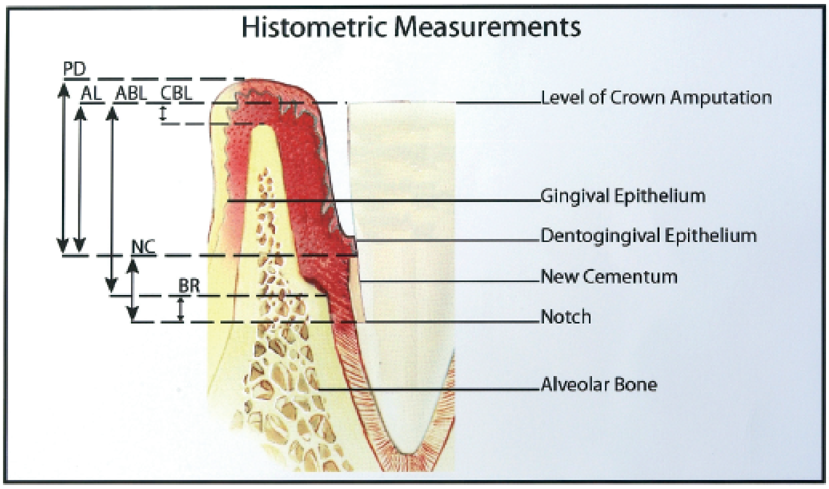

Fig. 3. Schematic illustration of histometric assessments.

Fig. 4. Microphotograph of a test specimen with a connective tissue attachment regenerated by GTR. After 6 months of ligature-induced periodontitis, loss of attachment has occurred from the level of root amputation to the level indicated by the arrow (Heidenhain's azan variant stain, magnification × 30).

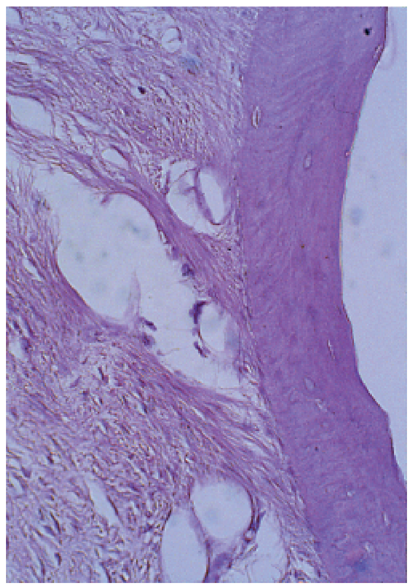

Fig. 5. High magnification of new cementum of the cellular, extrinsic and intrinsic fiber type generated by GTR (Heidenhain's azan variant stain, magnification × 190).

thick, were cut in the mesio-distal direction through the experimental and control teeth, parallel to their long axis. The sections were then stained with haematoxylin–eosin or Heidenhain's azan variant stain, and examined in the light microscope.

Five sections from the mid-portion of the root, 80 µm apart, were used for histometric assessments. The following linear distances were measured (Fig. 3):

(1) Free gingival margin to the apical termination of the dentogingival epithelium (PD); i.e. pocket depth.
(2) Level of crown amputation to the apical termination of the dentogingival epithelium (AL); i.e. loss of connective tissue attachment.
(3) Level of crown amputation to the crest of the bone defect (CBL); i.e. the coronal bone level.
(4) Level of crown amputation to the bottom of the bone defect (ABL); i.e. the apical bone level.
(5) Apical border of the notch in the root surface to the coronal level of newly formed cementum (NC); i.e. the amount of newly formed attachment (only on test roots).
(6) Apical border of the notch to the coronal level of newly formed bone (BR); i.e. the amount of newly formed bone (only on test roots).

The results were analysed with the Wilcoxon test for paired observations using each experimental site as the statistical unit ($N = 24$). This statistical unit was chosen because, for ethical reasons, as few primates (four monkeys) as possible should be used and because comparisons could be made between identical locations within the same animal (split mouth design).

Results

Twelve control and 12 test roots were available for histologic analysis providing a total of 24 control and 24 test surfaces for histometric assessments.

Except for the cementum on the root surface, the control and test roots exhibited similar histologic features (Figs. 4 and 6). Periodontal pockets of varying depth had formed and an inflammatory cell infiltrate could be seen in the connective tissue adjacent to the dentogingival epithelium, but a zone of non-infiltrated connective tissue was always interposed between the apical termination of the epithelium and the crest of the alveolar bone.

New cementum of the cellular, extrinsic and intrinsic fiber type was present on all test roots from the apical border of the notch to the apical termination of the dentogingival epithelium (Figs. 4 and 5). New cementum was not encountered coronal to the attachment level. The new cementum had formed on the root dentin but also on old cementum left on the surface. It was in continuity with the cementum apically to the notch and became gradually thinner in the coronal direction. The new cementum in the apical portion was considerably thicker than the pristine cementum in the control specimens, and frequently a split was present between the new cementum and the root surface. Occasionally, a split was located just at the level of connective tissue attachment, but epithelium was never seen between the detached cementum and the root surface. Regrowth of alveolar bone had occurred in all test specimens.

The pristine cementum on the control roots was mainly acellular, extrinsic

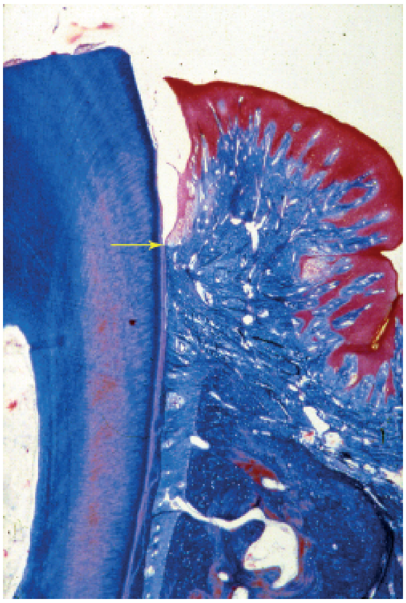

Fig. 6. Microphotograph of a control specimen with a pristine periodontium. After 6 months of ligature-induced periodontitis, loss of attachment has occurred from the level of root amputation to the level indicated by the arrow (Heidenhain's azan variant stain, magnification × 30).

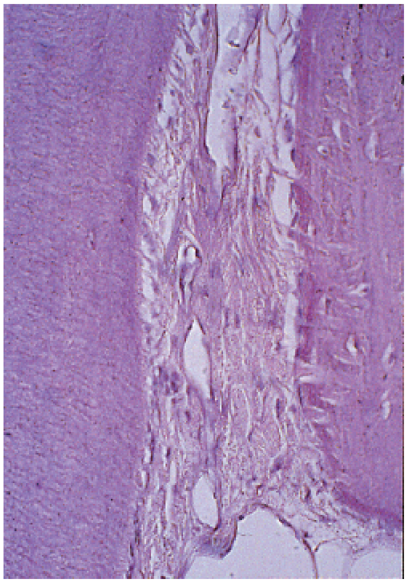

Fig. 7. High magnification of pristine, acellular, extrinsic fiber cementum on the control root (Heidenhain's azan variant stain, magnification × 190).

fiber cementum although areas of cellular, extrinsic and intrinsic fiber types were also seen (Figs. 6 and 7).

The results of the histometric assessments are presented in Table 1. The average pocket depth was 2.6 ± 0.6 mm on the controls and 2.0 ± 0.8 mm on the test roots. The attachment loss was on average 1.0 ± 0.4 mm on the controls and 1.0 ± 0.5 mm on the test roots. The coronal and apical bone level was 1.5 ± 0.6 mm and 1.5 ± 0.5 mm, respectively, in the controls while it was 1.6 ± 0.7 mm and 1.7 ± 0.7 mm in the test roots. The length of new cementum on the test roots was on average 2.5 ± 0.9 mm, which corresponded to 72.8% of the length of the detached root surface. The average bone regrowth in the test roots was 1.8 ± 0.9 mm, which corresponded to 51.0% of the length of the detached root surface. Only the difference in pocket depth between test and control roots was statistically significant ($P < 0.05$).

Table 1. Histometric assessments in roots with a pristine (control) and regenerated periodontium (test)

	Mean	SD	Minimum	Maximum	*N*
Control					
PD	2.6	0.6	1.6	3.6	24
AL	1.0	0.4	0.6	2.0	24
CBL	1.5	0.6	0.8	2.8	24
ABL	1.5	0.5	0.8	2.8	24
Test					
PD	2.0	0.8	1.0	3.6	24
AL	1.0	0.5	0.0	1.6	24
CBL	1.6	0.7	0.4	2.4	24
ABL	1.7	0.7	0.4	2.4	24
NC	2.5	0.9	1.0	4.0	24
BR	1.8	0.9	0.1	3.2	24

SD, standard deviation; PD, pocket depth; AL, attachment loss; CBL, coronal bone level; ABL, apical bone level; NC, new cementum; BR = bone regrowth.

Discussion

The present study demonstrated that ligature-induced periodontitis produced similar attachment loss on teeth with a pristine periodontium as on those with a periodontium regenerated by GTR.

New cementum was identified on all test roots coronal to the notch in the root surface and below the attachment level, covering about 2/3 of the entire length of the instrumented surface. This new cementum was of the reparative, cellular, extrinsic and intrinsic fiber type like that described by other investigators following regenerative therapy (Araújo et al. 1996).

It has been shown that on previously periodontitis-affected surfaces of roots that are crown-resected, covered with a membrane and kept submerged during healing, new cementum formation occurs almost predictably to or close to the level of crown amputation (Gottlow et al. 1984, Sander & Karring 1995). Therefore, it seems reasonable to anticipate that at the start of ligature-induced plaque accumulation, the regenerated periodontal attachment extended to or close to the level of crown amputation, thereby providing a baseline situation comparable to that on the control roots with a pristine periodontium. However, it cannot be excluded that the regeneration of cementum has failed to reach the level of crown amputation on all test roots. This situation, on the other hand, would suggest that the regenerated periodontium is less susceptible to periodontitis than the pristine periodontium when considering the finding in the present study that periodontal breakdown is approximately 1 mm on both test and control sites. Another shortcoming in defining the baseline situation on test and control roots is that the surgical/endodontic/restorative and hygiene manipulations on the experimental sites may have had a detrimental effect on the attachment level prior to the start of ligature-induced periodontitis. It is unlikely, however, that this may have caused major changes, and, in addition, such changes would most likely have been similar on the test and control sites.

The reason that regenerated cementum was not always identified all the way to the level of crown amputation on the test roots is most likely due to the fact that the newly formed cementum coronal to the attachment level has been lost during the histologic preparation because of the artifactual split which was observed almost consistently in the test specimens. This split formation which regularly occurs between the reparative cementum and the root surface in histologic specimens (Nalbandian & Frank 1980, Luder & Zappa 1998, Sculean et al. 1999, 2000) has been suggested to express poor quality of the regenerated cementum. The findings in the present study, however, where no difference was observed between the rate of periodontal destruction on the test and control roots, indicate that the qualitative difference between new cementum (cellular, extrinsic and intrinsic fiber type) and pristine cementum (acellular, extrinsic fiber type) has no significant effect in vivo, and that the split formation between the new cementum and the root surface is an artifact associated with histologic preparation, most likely related to demineralization of the tissue blocks as suggested by Listgarten (1972). This view is supported by the results of a study of Schüpbach et al. (1993) showing that the new cementum formed following GTR treatment was linked to the root surface by mineralized collagen fibrils.

Regrowth of alveolar bone coronally to the level of the notch in the root surface was observed on all test roots but to a varying degree (range 0.1–3.2 mm). In contrast to the level of attachment on the test roots, there is not a basis of estimating whether or not the bone level was comparable to that of the controls at the start of ligature-induced periodontitis.

The reason that the control roots presented significantly deeper pockets than the test roots can only be speculated on, but it may be related to variations in the position of the gingival margin at baseline, caused by the differences in tissue manipulation at the test and control sites.

The results of the present study indicate that teeth with an attachment apparatus regenerated following GTR treatment are not more susceptible to periodontitis than teeth with a pristine periodontium. This finding supports the results of several clinical trials showing that attachment gain obtained following GTR treatment can be maintained on a long-term basis (Gottlow et al. 1992, Becker & Becker 1993, McClain & Schallhorn 1993, Cortellini et al. 1994, Machtei et al. 1996), and in particular the results of a controlled clinical trial reported by Cortellini et al. (1996), showing a similar susceptibility to disease recurrence at sites treated with GTR and sites treated with root planing.

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