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The structure of periodontal tissues formed following guided tissue regeneration therapy of intra-bony defects in the monkey

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Abstract

Objective: To describe the periodontal tissues formed following guided tissue regeneration (GTR) therapy of intra-bony defects (IBD).

Methods: Eight adult *Macaca fascicularis* monkeys were used. Proximal IBD were created at the mandibular second pre-molars and second molars. After 3 months, GTR surgery was performed. The animals were euthanized at 6 months and 2 years after surgery. Block biopsies were harvested, and prepared for histological analysis.

Results: At 6 months the defect had healed with new cementum (NC), periodontal ligament (PDL) and bone. The NC seemed to be firmly anchored to the dentin. Supra-crestally, the NC consisted of a 10 μm thick layer of acellular extrinsic fibre cementum (AEFC). Sub-crestally, the NC was considerably thicker and consisted of an inner layer of AEFC and an outer thicker layer of cellular mixed fibre cementum (CMFC). The extrinsic fibre density amounted to about 10 fibres per 100 μm. The PDL was wider than the pristine PDL and widened in coronal direction. After 2 years of healing, the thickness of the NC in the sub-crestal compartment had increased by about 20 μm and the fibre density had increased by about 50%.

Conclusion: After 2 years of healing the structure of the regenerated tissues resembled that of pristine periodontal tissues.

Key words: animal study; GTR; intra-bony defects; occlusal membranes; periodontal regeneration

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Guided tissue regeneration (GTR) is a treatment modality by which it is possible to promote regeneration of periodontal tissues lost through periodontitis (e.g. Nyman et al. 1982a,b, Gottlow et al. 1984, Isidor et al. 1985, Aukhil et al. 1986, Karring et al. 1993). Periodontal regeneration has been defined as the process by which architecture and function of the periodontal tissues are completely renewed (The American Academy of Periodontology 1992) and includes the formation of a new connective tissue attachment (NA) new cementum (NC) and supporting bone (Karring et al. 1993). It is not conclusive, however, whether the ensuing healing following GTR represents a ''restitutio ad integrum'' or a healing by repair (Egelberg 1987, MacNeil & Somerman 1999). NC in contrast to pristine (original) cementum, has been characterized as a cellular cementum that contains a varying density of inserting fibres, and is poorly attached to the dentin surface (Gottlow et al. 1984, Gottlow et al 1990, Lindskog & Blomlof 1992, Blomlof & Lindskog 1994, Cochran et al. 2003, Donos et al. 2003, Kostopoulos & Karring 2004). It has further been suggested that new ''reparative cementum'' has osteoblast origin, and, hence, resembles bone tissue (Lindskog & Blomlof 1992, 1994 and Blomlof & Lindskog 1994).

In a series of experimental studies in dogs, the periodontal tissues formed after GTR were characterized and compared with pristine periodontal tissues in mandibular pre-molars (Araujo et al. 1996, 1997, 1998). The NC differed from the pristine cementum and it was suggested that the healing results following GTR had the characteristics of ''reparative'' rather then regenerative.

On the other hand Schüpbach et al. (1993) in a study on periodontal wound healing in class III furcation defects in the dog suggested that the healing result should be considered as regenerative. It was reported that NC resembled old cementum as newly formed periodontal

−3 months	−2 months	−1 month	Day 0	6 months	2 years
Defect preparation	Steel wires removed	Plaque control initiated	Regenerative surgery	6 monkeys sacrificed	2 monkeys sacrificed

Fig. 1. Study outline.

ligament (PDL) fibres were integrated and that the NC and the root surface were linked by mineralized collagen fibrils. In a recent study Graziani et al. (2005) evaluated periodontal healing following GTR therapy of dehiscence type defects in the monkey. Healing periods from 6 weeks to 2 years were analysed and it was suggested that healing resulted in a periodontium the structure of which resembled that of the pristine tissues. Thus in specimens representing 2 years of healing, the NC opposite new bone consisted of a narrow zone of acellular extrinsic fibre cementum (AEFC) anchored to the circumpulpal dentin and covered by a thicker cellular mixed fibre cementum (CMFC).

The aim of the present study was to evaluate the periodontal tissues formed following GTR therapy of intra-bony defects (IBD) and long-term healing in the monkey.

Material and Method

Eight 5–7 years old monkeys (*Macaca Fascicularis*) were used. The study protocol was approved by the Regional Ethics Committee for Animal Research, Sweden. Surgical procedures, were performed under general anaesthesia (Ketalar® 10 mg/kg bodyweight; Pfeitzer Inc. NY, USA) and local anaesthesia (Xylocain®-Adrenalin (5 mg/ml; Astra, Sweden). The first pre-molars as well as the first and third molars in the mandible were extracted 2 months before the surgical preparation of IBD (Gottlow et al. 1984). The outline of the experiment is depicted in Fig. 1.

Experimental periodontitis and preparation of IBD

Experimental periodontal IBD were produced in the mandibular second premolars and second molars largely according to the methods described by Ellegaard et al. (1974).

Following intra-crevicular incisions buccal and lingual full thickness flaps were raised around the experimental teeth. Three-wall IBD that were 4 mm deep and 2 mm wide and extended from the buccal to the lingual line angle were produced at the proximal aspects of the experimental teeth using a low-speed bur and a bone chisel. No root surface instrumentation was carried out. Orthodontic steel wires were twisted around the neck of the teeth and about 10 mm of the steel wire extended to the base of the bony defect. The flaps were re-positioned and sutured. The sutures were removed after 2 weeks and the steel wires were removed after 1 month. While no tooth cleaning procedures were performed during the first 2 months of healing, plaque control consisting of polishing with rubber cups and pumis and irrigation with 0.2% chlorhexidine solution was carried out twice weekly for the following month.

Periodontal surgery including GTR

Periodontal surgery was performed at the experimental teeth 3 months after defect preparation. Buccal and lingual full thickness flaps were raised and the granulation tissue was removed. The root surfaces were scaled and planed using rotating diamond stones in order to remove the cementum layer and planed with sonic scalers. A total of 32 teeth with 64 defects were treated

GTR barriers were attached around the neck of the teeth to cover the defect and 2–3 mm of the surrounding bone. The experimental teeth were randomly assigned to receive either a bio-resorbable poly-lactic acid (PLA) barrier, (Guidor Matrix Barrier, Guidor AB, Huddinge, Sweden) or a non-resorbable ePTFE barrier, (Gore-Tex Periodontal Material, W.L. Gore & Associates, Flagstaff AZ, USA). There were 16 teeth with 32 defects in each treatment category. The flaps were coronally displaced and sutured to cover the barriers. All animals received intra-muscularly injected Medicyklin® vet. (5 mg/kg body weight; Norbrook Lab Ltd., Northern Ireland, UK) once a day for 3 days.

The ePTFE barriers were surgically removed after 4 weeks. Mucosal flap was raised and the membranes were dissected free and removed. The flaps were adjusted and coronally displaced to cover the wound area and sutured. The sutures were removed after 2 weeks. Plaque control procedures that included polishing and topical application of a 0.2% chlorhexidine digluconate solution were performed twice weekly for 6 month after surgery.

Six animals were euthanized after 6 months by i.v. injection of a pentothal–sodium solution. The remaining two animals were euthanized after 2 years.

Biopsy, histological processing and analysis

The mandibles were removed and tissue blocks containing the experimental teeth and their periodontal tissues were dissected free and placed in 10% buffered formalin. The specimens were decalcified in Trifluoracetic acid, dehydrated and subsequently embedded in paraffin. Mesial-distal sections were produced with the microtome set at 3–5 μm and stained in hematoxylin and eosin (H&E).

Histological analysis

Histological analysis was carried out using a Leitz DM RBE® microscope (Leica, Wetzlar, Germany) set for light microscopy added by polarized light and interference contrast. Histometric and morphometric measurements were performed using an image system Q-500MC® (Leica) connected to the DM RBE microscope.

From each root, three sections, about 50 μm apart and representing the mid-proximal portion of the defect, were selected for the analysis.

The following linear measurements were made at × 25 magnification:

The height of the defect, i.e. the distance from cemento–enamel junction (CEJ) to the apical extension of root instrumentation (aRI), the amount of NA i.e. the distance from the apical termination of the aJE (apical extension of junctional epithelium identical to coronal extension of new attachment), to the aRI, the amount of NC and the height and area of new bone.

Comparisons were made between tissue formed in the defect compartments and corresponding pristine tissue areas and the analyses were confined to the following (Fig. 2):

Position I: the supra-crestal area of the defect.

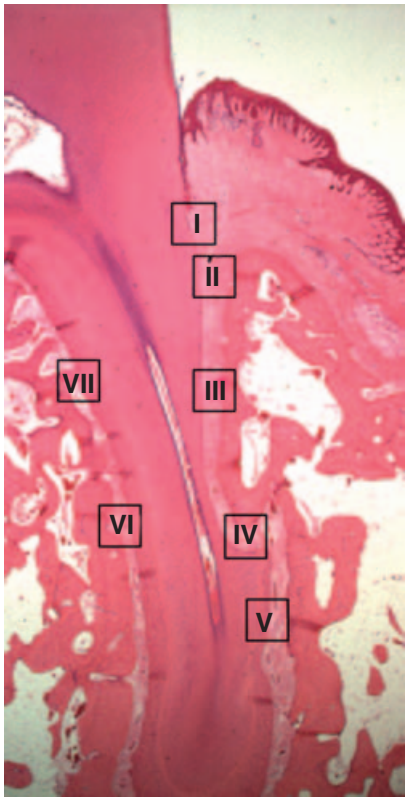

Fig. 2. Mesio-distal section of a regenerated specimen depicting positions I–VII at which histometric assessment were made. Hematoxylin and eosin staining. Original magnification × 16.

Position II: the level of the crest of the newly formed bone.
Position III: a sub-crestal compartment, at the mid level of the newly formed bone.
Position IV: at the aRI.

Pristine areas:

Position V: 300 µm apical to the aRI.
Position VI: an area corresponding to the level of position IV.
Position VII: and area corresponding to the level of position II.

In all positions the thickness (µm) of the cementum (× 400), the numeric fibre density, i.e. the number of extrinsic fibres within a 100 µm zone (× 400) and the width (µm) of the PDL (× 200) were assessed.

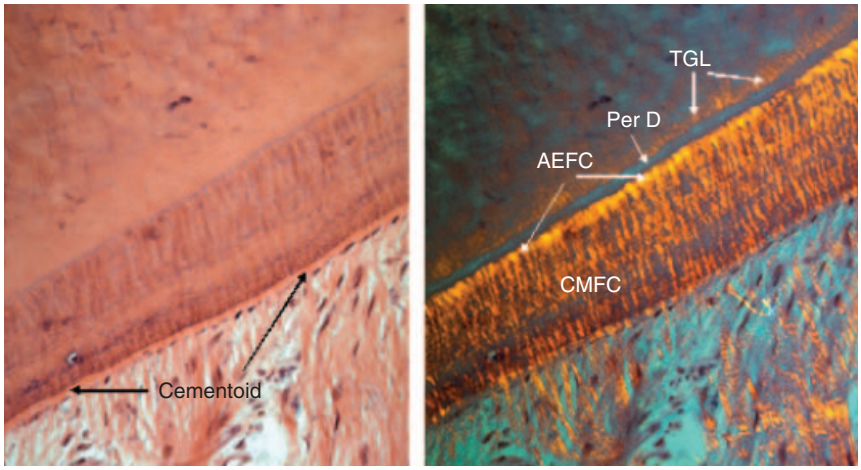

Fig. 3. Pristine cementum. Normal light (left) and polarized light (right). Per D, peripheral dentin; TGL, Tomes granular layer; AEFC, acellular extrinsic fibre cementum; CMFC, cellular mixed fibre cementum. Hematoxylin and eosin staining. Original magnification × 400.

Morphometric analysis

Morphometric assessments of the composition of the connective tissue within the PDL were performed in positions II, III, IV, V, VI and VII. The proportions of the PDL tissue representing collagen fibres (Co) vascular structures (V), fibroblasts (C) and residual tissue (R) were determined using a point counting procedure (Schroeder & Munzel-Pedrazzoli 1973). A lattice comprising 100 points was superimposed over the tissue at magnification × 400. Mean values ware calculated from the assessments made in positions II, III and IV (regenerated PDL) and in positions V, VI and VII (pristine PDL), respectively

Data analysis

Mean values for each animal were determined for each of the variables. Group mean values and standard devia-

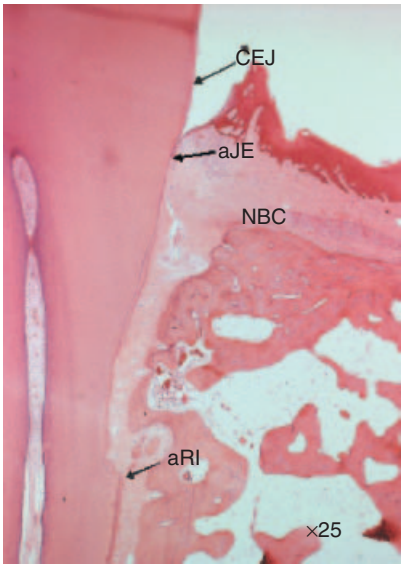

Fig. 4. Mesio-distal section of a 6 month specimen showing landmarks for linear measurements. CEJ, cemento–enamel junction; aJE, apical extension of junctional epithelium identical to coronal extension of new attachment; NBC, newbone crest; aRI, apical extension of root instrumentation, i.e. bottom of the defect. Hematoxylin and eosin staining. Original magnification × 25.

Table 1. Defect height (CEJ to apical extension of root instrumentation –(aRI)), bone defect (from crest to aRI) in mm and mm^2, bone fill in mm and mm^2 and % of defect area, new attachment in mm and % of defect height and new cementum in mm, % of defect height and % of new attachment (Mean values and SD)

	CEJ-aRI	Bone defect		Bone fill			New attachment		New cementum		
	mm	mm	mm^2	mm	mm^2	%	mm	%	mm	% defect	% NA
6 months	3.94 ± 0.4	2.81 ± 0.2	5.1 ± 0.4	2.63 ± 0.3	4.6 ± 0.5	89 ± 7	2.77 ± 0.3	71 ± 3	2.45 ± 0.3	65 ± 3	92 ± 5
2 years	3.82 ± 0.2	2.69 ± 0.1	4.9 ± 0.6	2.67 ± 0.2	5.1 ± 0.9	104 ± 6	2.67 ± 0.2	70 ± 7	2.51 ± 0.1	66 ± 6	94 ± 1

CEJ, cemento–enamel junction.

tions were calculated for the different healing periods (6 months, 2 years).

Results

Clinical observations

Healing was usually uneventful. Gingival inflammation, recession and membrane exposure occurred but were rare. Exposed material of resorbable membranes had disappeared spontaneously after 4 to 6 weeks. Exposed parts of non-resorbable ePTFE membranes were left untouched until membrane removal after 4 weeks.

Histological observations

Pristine periodontium

An area in position VI of pristine periodontium is shown in Fig. 3. The cementum layer in this zone was about 130 μm thick and consisted of a narrow

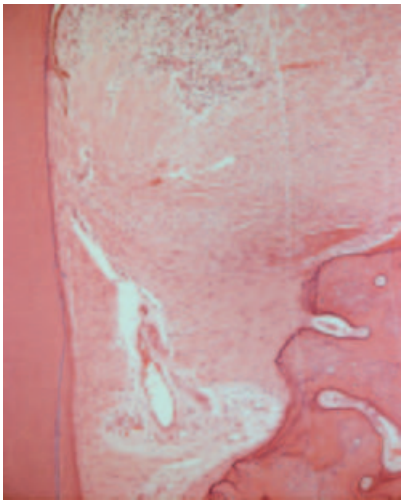

Fig. 5. Positions I and II of Fig. 4. Note the thin cementum in the supra-crestal compartment becoming gradually wider in apical direction from the bone-crest level. Hematoxylin and eosin staining. Original magnification × 200.

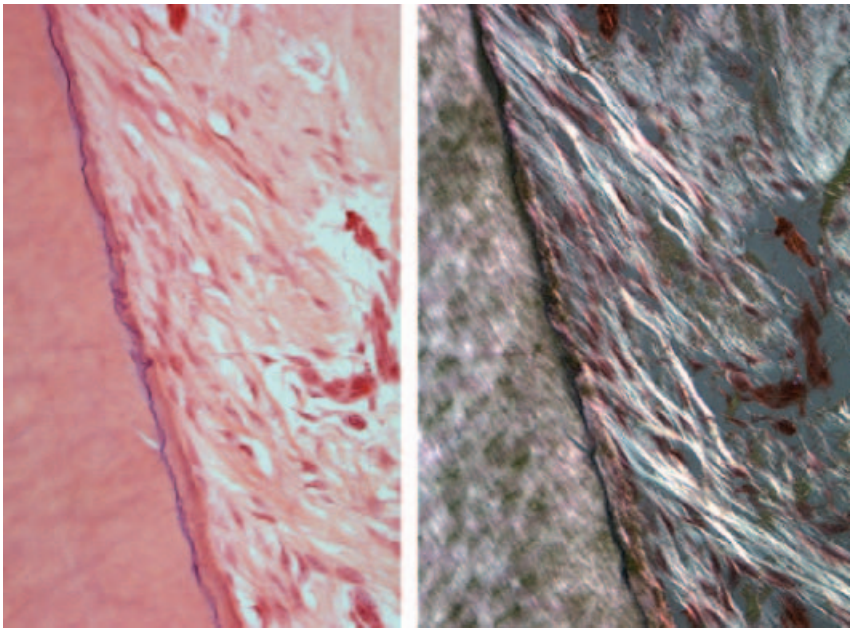

Fig. 7. Six month specimen of regenerated acellular extrinsic fibre cementum formed on the circum-pulpal dentin in position I. Note the extrinsic fibres extending into the surface of the circum-pulpal dentin. Normal (left) and polarized (right) light. Hematoxylin and eosin staining. Original magnification × 400.

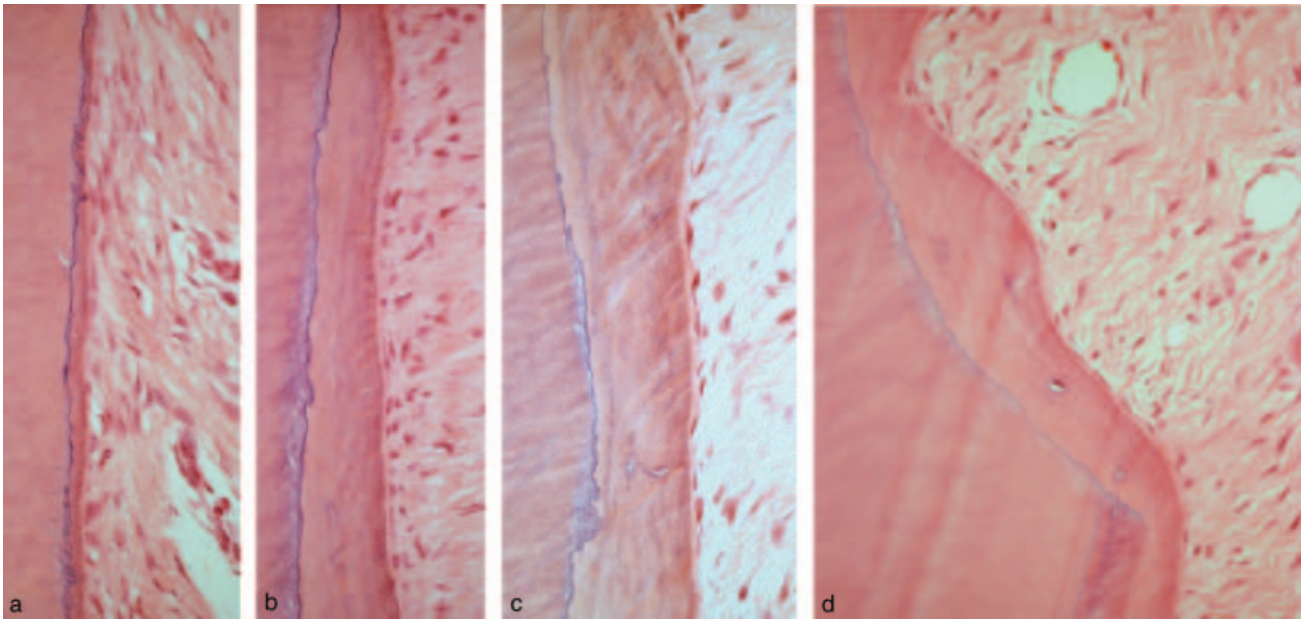

Fig. 6. Six month specimen of regenerated cementum. A, position I; B, position II; C, position III; and D, position IV. Note the gradual thickening of the regenerated cementum. Note also the close adaptation of the cementum to the underlying dentin and the bluish zone in the interface between the circum-pulpal dentin and the regenerated cementum indicating an early root resorption. Hematoxylin and eosin staining. Original magnification × 400.

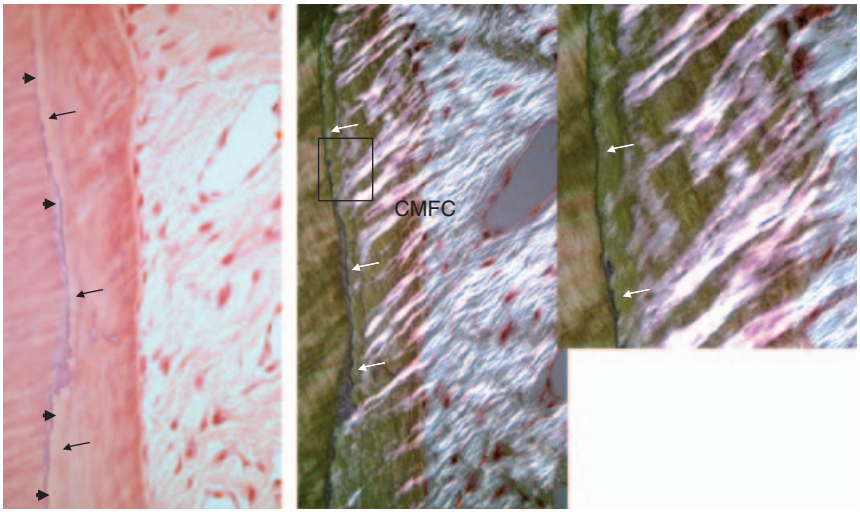

Fig. 8. Six month specimen showing regenerated cementum in normal (left) and polarized (mid and right) light. The new cementum consists of a narrow layer of acellular extrinsic fibre cementum (small arrows) anchored to the underlying circum-pulpal dentin, on top of which is seen a thicker layer of cellular mixed fibre cementum. Arrow heads denote the ruffled dentin surface and the close adaptation of the cementum to the circum-pulpal dentin. Right figure shows delineated area in higher magnification. Hematoxylin and eosin staining. Original magnification × 400.

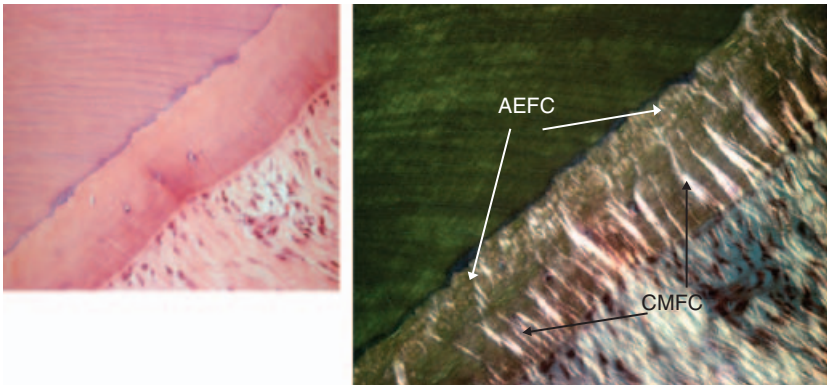

Fig. 9. Two-year specimen showing regenerated cementum in normal (left) and polarized (right) light. The new cementum consists of a layer of acellular extrinsic fibre cementum (AEFC) anchored to the underlying circum-pulpal dentin, on top of which is a thicker layer of cellular mixed fibre cementum (CMFC). Both the AEFC and the CMFC demonstrate a high density of extrinsic fibres, some of which can be followed from the periodontal ligament to the dentin.

zone of AEFC lateral to the peripheral dentin and a wider zone of CMFC containing both extrinsic and intrinsic collagen fibres.

Defect areas (Table 1 and Figs 4–9)

There were only small differences between the specimens representing 6 months and 2 years healing time with respect to defect height, IBD depth and defect area (Table 1).

NA including NC, PDL and bone had formed within the defect compartments (Fig. 4). The NC extended to a distance of 300–400 μm apical to the aRI and was laid down on top of the pristine cementum (Fig. 6d).

New bone had the appearance of lamellar bone with wide marrow spaces (Figs 2 and 4). The amount of new bone was 2.63 ± 0.3 mm at the 6 month specimens and 2.67 ± 0.2 mm at the 2 year specimens (Table 1). This corresponded to a defect resolution of 89 ± 7% at 6 months and 104 ± 6% at 2 years.

The extension of the NA amounted to 2.77 ± 0.30 mm and 2.67 ± 0.16 mm at the 6 months and 2 years specimens respectively, corresponding to 71 ± 3% and 70 ± 6% of the defect height (Table 1). Most of this new attachment also showed clearly identified NC (92 ± 5% and 94 ± 1%, Table 1). In some specimens the coronal extension of the NC did not reach the aJE. In the gap between to coronal extension of the NC and the aJE collagen fibres seemed to attach directly to the dentin surface in an angulated fashion. The NA terminated either coronal to the new bone crest (Figs 4 and 5) or at the level of or slightly apical to the bone crest. The NC was in direct contact with the circum-pulpal dentin, the ruffled surface of which indicated transient root resorption (Fig. 6).

In the supra-crestal compartment the NC was very thin (Figs 5 and 6a) and had the appearance of AEFC (Fig. 7). At the level of the bone crest and regions further apical, the cementum was considerably thicker than in the supra-crestal compartment (Figs 5 and 6). In the areas apical to the bone crest the NC consisted of two layers. A thin layer of AEFC which was continuous with the supra-crestal AEFC was covered by a thicker layer of CMFC that contained extrinsic and intrinsic collagen fibres (Figs 8 and 9). Extrinsic fibre bundles extended from the circum-pulpal dentin through the NC and continued into the new PDL (Figs 8 and 9).

Histometric assessment

Cementum thickness (Fig. 10).

The thickness of new and pristine cementum at the various positions and healing times is presented in Fig. 10. After 6 months of healing, the NC was thinner than the pristine cementum in all positions except in the most apical part of the defect (position IV). The cementum thickness at 6 months measured 10.7 ± 6.4 μm in position I, 20.2 ± 9.5 μm in position II, 38 ± 21 μm in position III and 100.2 ± 23.1 μm in position IV. After 2 years of healing the new sub-crestal cementum was about 20 μm thicker than in the 6 months specimens. In position I, i.e. within the supra-crestal compartment, however, the NC remained almost as thin as after 6 months of healing.

Extrinsic fibre density (Fig. 11)

After 6 months of healing the numerical fibre density (in the following referred to as extrinsic fibre density) of the NC varied between 9.0 ± 1.3 in position I to

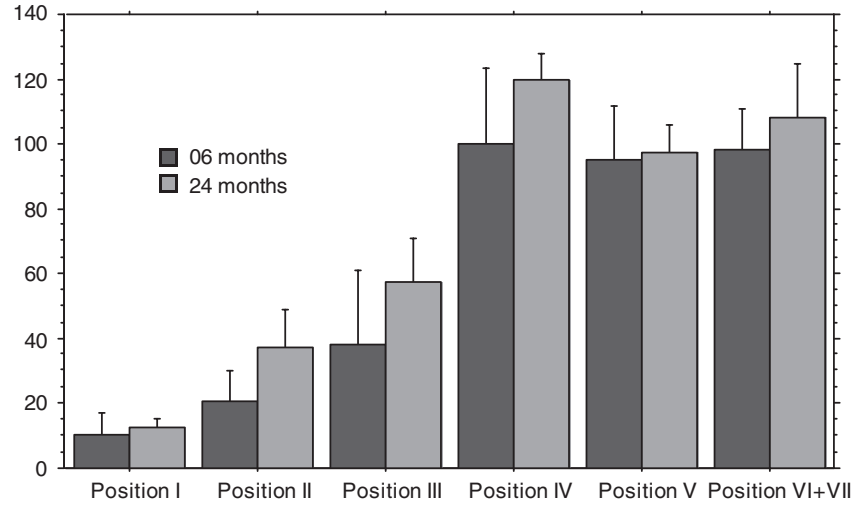

Fig. 10. Cementum thickness (μm) in the various positions after 6 and 24 months of healing. Mean values and SD (For explanation of positions I–VII see Fig. 2).

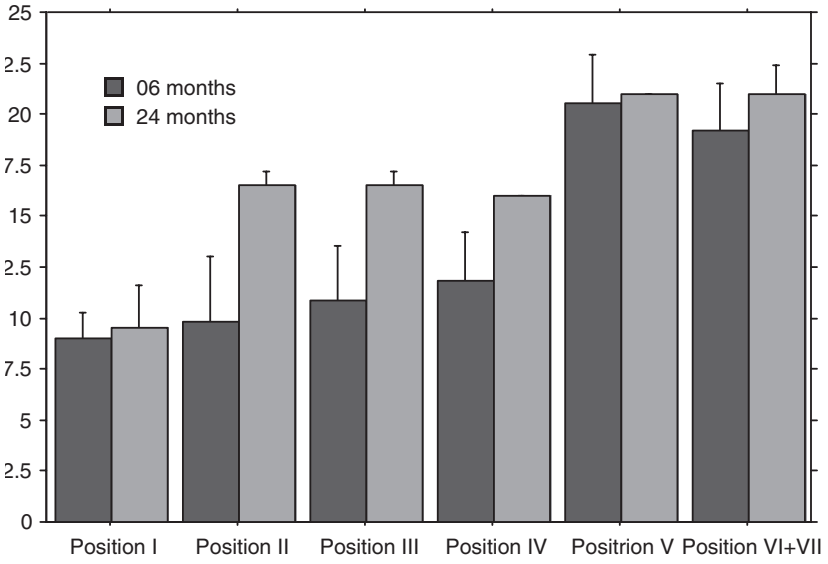

Fig. 11. Number of extrinsic fibres per 100 μm in the various positions after 6 and 24 months of healing. Mean values and SD. (For explanation of positions I–VII see Fig. 2).

11.8 ± 2.3 in position IV. The extrinsic fibre density of the pristine cementum amounted to 20.5 ± 2.4 in position V and 19.2 ± 2.3 in positions VI and VII. After 2 years of healing the extrinsic fibre density in positions II–IV was about 50% higher than in the 6 months specimens and reached values close to that of the pristine cementum. The fibre density at 2 years in position I was almost similar to that at 6 months.

PDL width (Fig. 12).

The PDL formed after 6 months and 2 years of healing was wider than the pristine PDL. At 6 months, the PDL width amounted to 440 ± 66 in position II, 300 ± 114 in position III and 285 ± 59 in position IV. The corresponding values in positions V and VI+VII were 186 ± 44 and 184 ± 37, respectively. There was no difference between the 6 months and the 2 years specimens with regard to the PDL width in the various positions.

Composition of PDL (Fig. 13)

After 6 months of healing, the connective tissue in the regenerated PDL consisted of 61% collagen, 23% fibroblasts, 13% vessels and 4% residual tissue. The corresponding values obtained after 2 years were 62% collagen, 23 fibroblasts, 14% vessels and 2% residual tissue and revealed, that the composition of the connective tissue in the regenerated PDL was similar to that in the pristine PDL.

Discussion

This experimental study described the structure of the periodontal tissues formed following GTR therapy of surgically created but chronically inflamed IBD in the monkey.

It was demonstrated that after 6 months of healing, NA with NC, PDL and bone had formed within the defect. While the resolution of the IBD appeared to be almost complete, the NC was thinner than the pristine cementum and exhibited an extrinsic fibre density that was about 50% that of the pristine periodontium. After 2 years of healing the cementum in the subcrestal compartment was wider and the extrinsic fibre density higher than after 6 months. Thus, the structure of the regenerated periodontium after 2 years of healing resembled that of the pristine cementum.

In the present study, NC was identified within almost the entire new attachment and seemed to be firmly anchored to the circum-pulpal dentin. Although similar observations regarding cementum-to-dentin anchorage have been reported previously (Schüpbach et al. 1993, Araujo et al. 1996, Graziani et al. 2005), most studies on periodontal wound healing found a split between the NC and the underlying dentin (e.g. Listgarten 1972, Gottlow et al. 1984, Gottlow et al. 1990, Sculean et al. 1999, Sculean et al. 2000, Cochran et al. 2003, Kostopoulos & Karring 2004). This split was suggested to be an artefact resulting from the histological preparation (Listgarten 1972) but may also be explained by the presence of a residual smear layer on the dentin surface that may prevent proper anchorage of the NC to the circum-pulpal dentin (Bosshardt et al. 2005). Lindskog and Blomlof (1992, 1994) and Blomlof and Lindskog (1994) evaluated the quality of periodontal healing using different kinds of periodontal wound healing models in the monkey and concluded that formation of NC on exposed dentin was not preceded by surface resorption and that the new ''reparative cementum'' was not attached to the dentin surface. In the present study, the exposed dentin surface had an irregular outline, which

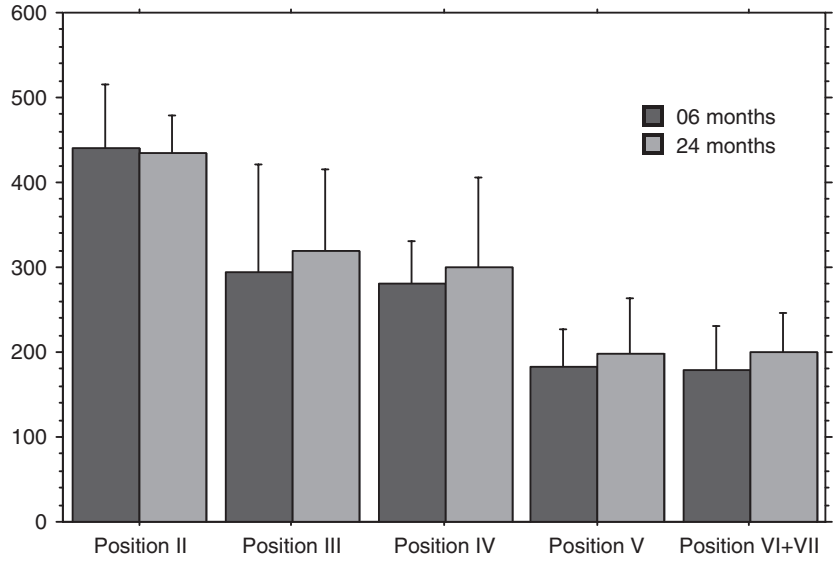

Fig. 12. Periodontal ligament width (μm) in the various positions after 6 and 24 months of healing. Mean values and SD. (For explanation of positions I–VII see Fig. 2).

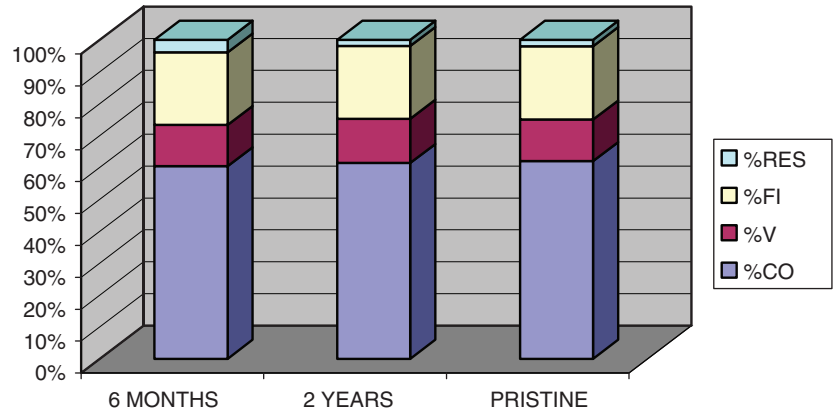

Fig. 13. Percentage composition of regenerated and pristine periodontal ligament regarding collagen (CO) vessels (V), fibroblasts (FI) and residual tissue (RES).

was interpreted as being the result of the root surface instrumentation during surgery and an early resorption of the dentin surface (Schüpbach et al. 1993, Araujo et al. 1997).

In the pristine periodontium, the cementum consists of a narrow zone of AEFC and a thicker zone of CMFC (Schroeder 1986, Bosshardt & Selvig 1997). Consequently, in order for the GTR therapy to be truly regenerative, deposition of AEFC upon the instrumented dentine surface should be considered a crucial part of the healing process (Hammarstrom et al. 1997). In the present study, the NC consisted of two distinct layers; a thin layer of AEFC formed on the exposed circum-pulpal dentin surface and, in the sub-crestal compartment, a thicker layer of CMFC laid down on top of the AEFC. Thus, after 6 months of healing the structure of the NC formed after GTR therapy resembled that of the pristine cementum. This finding is in contrasts with other experimental studies on periodontal regeneration (Gottlow et al. 1984, Lindskog & Blomlof 1992, 1994, Araujo et al 1996, Sculean et al. 1999, Sculean et al. 2000, Kostopoulos & Karring 2004) in which NC was consistently defined as cellular cementum. The present results are, however, in agreement with the findings presented by Schüpbach et al. (1993). They studied the periodontal healing 14 weeks after GTR therapy in furcation defects in the dog and found that AEFC was formed directly on circum-pulpal dentine.

In the present study, the thickness of the sub-crestal cementum increased in apical direction as measured from the bone crest (position II) to the bottom of the defect (position IV). In addition, the thickness of the cementum in the sub-crestal compartment increased between 6 months and 2 years. Thus, after 2 years of healing the regenerated cementum at the bottom of the defect was as thick as the pristine cementum. A gradual thickening of the cementum in the apical direction was also reported in other animal experimental studies (e.g. Nyman et al. 1982a,b, Gottlow et al. 1984, Gottlow et al. 1990). Berglundh et al. (1991) compared cementum in young and old dogs and found that the cementum in young dogs was acellular, narrow and of equal thickness along the root. In old dogs the cementum in the sub-crestal compartments was defined as cellular, 5–10 times thicker as compared with the cementum in young dogs and widened in apical direction. As seen in the present study and in the studies by Berglundh et al. (1991) and Graziani et al. (2005), the cementum in the supra-crestal compartment was thin and acellular.

In the present study, the numerical extrinsic fibre density in the sub-crestal compartment increased over time. At 6 months of healing the sub-crestal fibre density was about 50% and after 2 years 85% that of the pristine cementum. The reason for increased extrinsic fibre density over time found in the present study and in the study by Graziani et al. (2005) and for the gradual thickening of the sub-crestal CMFC seen in the present and other studies (Berglundh et al. 1991, Schüpbach et al. 1993, Graziani et al. 2005) is presently not understood. It may be suggested that concomitant with the re-modelling of the newly formed bone, cementum formation continues by the appositional growth of a CMFC which in turn will result in increasing numbers of collagen fibres from the re-modelling PDL. This process may continue in order to meet functional demands in the area until a balance between functional load on the tooth and the capacity of the new PDL to withstand the load is achieved (Graziani et al. 2005).

In the present study, the dimension and composition of the new PDL were established after 6 months of healing and no further changes seemed to occur up to 2 years. When comparing regenerated and pristine PDL the proportion of fibroblasts, vessels and collagen in regenerated PDL at 6 months and 2 years was similar to that of pristine PDL. However, the new PDL was wider than the pristine PDL. In addition the new PDL widened in coronal direction

from around 280 μm in position IV to about 450 μm in position II.

In conclusion, GTR therapy of IBD does result in true regeneration of the periodontium with the formation of both AEFC and CMFC. In qualitative, terms, the periodontal healing that takes place following GTR therapy of IBD will result in a restitutio ad integrum, i.e. healing by regeneration. In quantitative terms there seems to be some restriction of the qualitative process as complete regeneration with formation of new attachment of the entire defect never occurred in the present study.

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Clinical Relevance

Background: GTR is a treatment modality by which it is possible to promote regeneration of periodontal tissues lost through periodontitis. In the clinic, the outcome of GTR therapy of IBD is evaluated by clinical attachment level (CAL) and radiographic measurements. It is not conclusive, however, whether the healing following GTR represents a ''restitutio ad integrum'' or a healing by repair.

Principle findings: GTR therapy of IBD will result in the formation of a true new attachment including AEFC and CMFC, PDL and bone.

Clinical Implication: Findings in the present study provides information on the biological background to CAL gain. For the clinician it is important to realize that the quality of gained attachment and bone fill may improve over time.