Journal of Clinical
Periodontology

Periodontal wound healing following GTR therapy of dehiscence-type defects in the monkey: short-, medium- and long-term healing

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Abstract

Objective: To describe periodontal wound healing in dehiscence-type defects following guided tissue re-generation (GTR) therapy.

Methods: Ten adult *Macaca fascicularis* monkeys were used. Buccal dehiscence-type defects were created at the maxillary second pre-molars and second molars. After 3 months, GTR surgery was performed. The animals were euthanized at 6 weeks, 6 months and 2 years after surgery. Block biopsies were harvested, and prepared for histological analysis.

Results: A new attachment apparatus was structured already after 6 weeks of healing. A 10–20 μm thin layer of acellular extrinsic fibre cementum (AEFC) had formed along the instrumented root surface. At 6 months, the thickness of the supracrestal cementum was comparable with that at 6 weeks, while the thickness of the subcrestal cementum had increased to 40–60 μm. In this zone, the cementum consisted of an inner layer of AEFC attached to the circum–pulpal dentin and an outer layer of cellular mixed fibre cementum (CMFC). The numerical extrinsic fibre density was twice that at 6 weeks. At 2 years, the periodontal tissues resembled the pristine periodontium.

Conclusion: Periodontal healing following GTR therapy of recession-type defects will result in a restitutio ad integrum, i.e. healing by re-generation. A continuous maturation process occurs over at least 2 years.

One of the goals in periodontal therapy is to restore periodontal tissues lost through periodontal disease. Re-generation is defined as the process by which architecture and function are completely renewed (The American Academy of Periodontology, *Glossary of periodontal terms* 1992). By this definition, periodontal regeneration would include the formation of a new connective tissue attachment including new cementum (NC) and supporting bone.

Several methods have been applied to achieve periodontal re-generation, such as the use of autogenous bone grafts, root surface conditioning, growth factors and occlusive membranes (for a review, see Gottlow et al. 1986) In recent years, the use of enamel matrix proteins was introduced (Hammarstrom 1997, Hammarstrom et al. 1997, Heijl 1997, Heijl et al. 1997).

The re-generated periodontium is less described than pristine periodontium (Bosshardt & Selvig 1997, MacNeil & Somerman 1999). NC has generally been characterized as a cellular cemen-

tum that increases in width in the apical direction. The NC may further exhibit the presence or absence of an inserting fibre, and is usually poorly attached to the dentin surface (e.g. Gottlow et al. 1984, Araujo et al. 1996, Hammarstrom et al. 1997, Cochran et al. 2003, Donos et al. 2003, Kostopoulos & Karring 2004). It may therefore be questioned whether guided tissue re-generation (GTR) therapy results in a *restitutio ad integrum*, i.e. healing by re-generation or by repair (Egelberg 1987). In a series

of experimental studies in dogs, re-generated periodontium was characterized and compared with pristine periodontium in mandibular furcation degree III defects (Araujo et al. 1996–1998). It was suggested that the features of the NC were ''reparative'' rather than regenerative.

Limited information is available on the morphogenesis of the regenerated periodontal tissues. Araujo et al. (1997, 1998) described the dynamics of periodontal tissue formation in degree III furcation defects in dogs after GTR therapy from 2 weeks up to 5 months. Three distinct phases of cementum formation were distinguished. In the first phase (2 weeks of healing), adjacent collagen fibres aligned in a direction perpendicular to the root surface. In the second phase (4 weeks), a matrix ground substance in which collagen fibres were embedded was deposited on the root surface. In the third phase (8–20 weeks), almost the entire root surface was covered by NC containing intrinsic collagen fibres. Alveolar bone developed during the different phases of healing. Thus, a fibrous connective tissue differentiated into woven bone and eventually into lamellar bone and bone marrow. Simultaneous with the bone maturation process, the periodontal ligament (PDL) was developed and, hence, fibres connecting the bone to the cementum were formed (Araujo et al. 1997).

Thus, it may not be possible from available data to judge whether GTR procedures will result in a restitutio ad integrum of the periodontal tissues or in a periodontium that is functionally but not morphologically, equivalent to the pristine periodontium. The healing period used in most studies may have been too short to evaluate whether true regeneration occurs. The aim of the present study was to describe the periodontal tissues formation in chronically inflamed dehiscence-type defects from 6 weeks up to 24 months after GTR therapy,

Materials & Methods

Ten 5–7-year-old monkeys (*M. fascicularis*) were used. The study protocol was approved by the local Ethics Committee for Animal Research. Surgical procedures were performed under general anaesthesia (Ketalar® 10 mg/kg bodyweight; Pfizer Inc., New York, NY,

USA) and local anaesthesia (Xylocain®-Adrenalin 5 mg/ml; Astra, Sweden).

The outline of the experiment is depicted in Fig. 1.

Creation of chronically inflamed dehiscence-type defects

The first pre-molars, first and third molars in the maxilla were extracted at minimum 2 months prior to initiation of the experiment (Gottlow et al. 1986). Two months after tooth extraction, plaque-exposed and chronically inflamed dehiscence-type defects were created at the maxillary second pre-molars and second molars as described by Gottlow et al. (1994). In brief, buccal full-thickness flaps were raised extending from the canine to the second molar on each side of the jaw. The buccal bone support within the mesio–buccal and disto–buccal line angles and to a level of about 5 mm apical to the cemento–enamel junction (CEJ) of each root of the experimental teeth was removed using a bone chisel. A cotton floss ligature was placed over each defect and the flaps were re-positioned and sutured. Sutures were removed after 1 week. The ligatures were removed 4 weeks post-surgery, and the experimental sites were exposed to plaque formation for an additional 1-month period. Two months after the defect surgery, the teeth were cleaned using rubber cups and pumice, and a 1-month plaque control programme including polishing and topical irrigation of 0.2% chlorhexidine digluconate solution twice weekly, was instituted.

Re-generative surgery

One month after the start of the plaque control regimen, reconstructive surgery was performed. Mucoperiostal flaps were raised buccally and palatally around the experimental teeth and granulation tissue was removed. Root cementum was carefully removed to the level of the reduced bone crest (BC) using perio-diamond burs, and hand instruments. Root planing was performed with an ultrasonic instrument (Titan-S, Lone Star Dental Corp, Adington, TX, USA). GTR barriers were attached around the neck of the teeth to cover the defect and 2–3 mm of the surrounding bone. On one side of the jaw, the defects were covered with a bio-resorbable PLA barrier (Guidor® Matrix Barrier, Guidor AB, Huddinge,

Sweden). In the contra-lateral segment, the roots were covered with a non-resorbable ePTFE barrier (Gore-tex® Periodontal Material, W. L. Gore & Associates, Flagstaff, AZ, USA).

The flaps were coronally displaced to cover the barriers and sutured (Gore-tex®, USA). Antibiotics (Medicyklin® vet. 5 mg/kg bodyweight through intra-muscular injection; Norbrook Lab Ltd., Newry, Co. Down, UK) was provided once daily for 3 days. Post-surgical care including topical application of a 0.2% chlorhexidine digluconate solution, and gentle supragingival polishing was provided twice weekly for 6 weeks.

The ePTFE barriers were surgically removed 4 weeks after implantation. An incision was made and a buccal flap was raised. The membrane was dissected free and carefully removed. The flap was resutured, and the sutures were removed 1 week later.

Two monkeys were euthanized with an overdose of a Pentothal–sodium solution at 6 weeks post-surgery. The remaining animals were subjected to a plaque control programme consisting of topical application of a 0.2% chlorhexidine digluconate solution and gentle toothbrushing twice weekly until euthanization was performed at 6 months (six animals) and 2 years (two animals) post-surgery.

Biopsy, histological processing and analysis

The jaws were removed, and block biopsies containing the experimental teeth and the periodontal tissues were dissected and placed in 10% buffered formalin. The specimens were decalcified in trifluoracetic acid, dehydrated and embedded in paraffin. Bucco–lingual sections of the two roots in each biopsy were produced with the microtome set at 5 μm. The sections were stained with haematoxylin–eosin and Mallory's connective tissue stain.

Histological analysis was carried out using a Leitz DM RBE® microscope (Leica, Wetzlar, Germany) set for light microscopy, also using polarized light and interference contrast. Histometric and morphometric measurements were performed in a Leica DMRBE microscope (Leica) equipped with an image system Q-500MC® (Leica).

From each root, 3 s, about 50 μm apart, and representing the central portion of the dehiscence, were selected for the analysis.
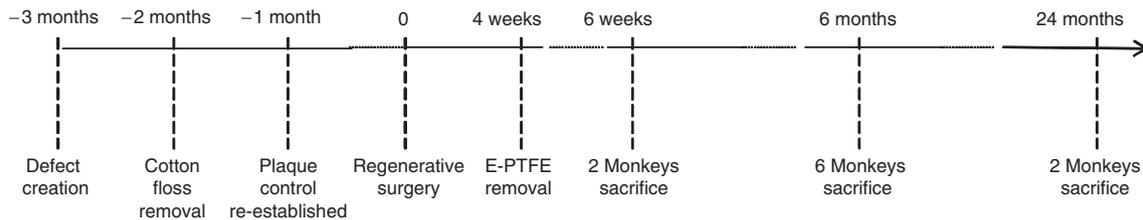
Fig. 1. Study outline.

The following linear measurements were made at × 25 magnification:

- the height of the defect (mm) i.e. the distance from the CEJ to the apical extension of the root instrumentation (aRI);
- the amount of new attachment (NA) i.e. the distance from the apical termination of the dento–gingival epithelium (apical extension of junctional epithelium (aJE)) to the aRI (mm and percentage of defect height);
- the amount of NC i.e. clearly identified NC with inserting collagen fibres as measured in the coronal direction from the aRI (mm and percentage of defect height and percentage of NA); and
- the amount of new bone (NB) measured from the BC to the aRI (mm and percentage of defect height).

Analysis of cementum and the PDL fibres was carried out at different positions of dehiscence and pristine tissue areas (Fig. 2) as follows:

Position I, supracrestal area of the dehiscence site;
Position II, at the level of the crest of the newly formed bone;
Position III, subcrestally, at the mid-level of the newly formed bone;
Position IV, at the aRI;
Position V, pristine tissue 200 μm apical to the aRI;
Position VI, pristine tissue at the palatal aspect of the root at the level of position IV; and
Position VII, pristine tissue at the palatal aspect of the root at the level of position II.

The following assessments were made:

- the thickness (μm) of the newly formed cementum at positions I–IV (× 400);

- the thickness (μm) of the pristine cementum at positions V–VII (× 400);
- the width (μm) of the newly formed PDL at positions II–IV (× 200);
- the width (μm) of the pristine PDL positions V–VII (× 200);
- the number of extrinsic fibres within a 100 μm zone of the newly formed cementum at positions I–IV (× 400); and
- the number of extrinsic fibres within a 100 μm zone of the pristine cementum at positions V–VII (× 400).

A mean value was calculated from the assessments conducted in positions VI and VII.

Morphometric analysis

Morphometric assessments representing the composition of the connective tissue within the PDL were performed in positions II–VII. The proportions of the PDL tissue representing collagen (Co) fibres vascular (V) structures, fibroblasts (C) and residual (R) tissue 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–IV (re-generated PDL) and in positions V–VII (pristine PDL), respectively.

Statistics

Mean values for each animal were determined for each of the variables. Group mean values and standard deviations were calculated for the different healing periods (6 weeks, 6 months, 2 years).
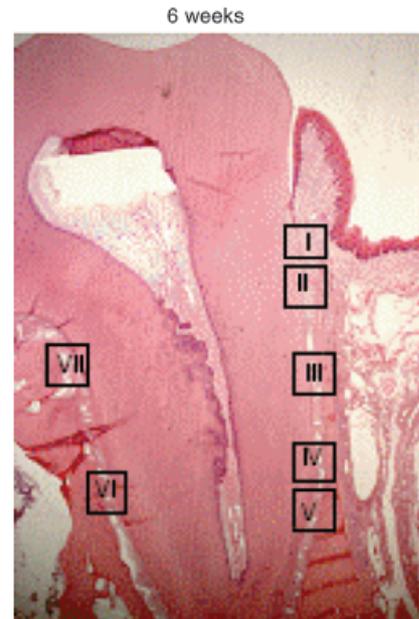

Fig. 2. Bucco–lingual section of an experimental tooth showing the various positions (I–VII) in which histometric and morphometric measurements were performed.

Results
Clinical observations

Soft tissues healed with only minor signs of inflammation or other adverse tissue reactions following re-generative surgery. Gingival recession was virtually absent or very minor at all teeth. Barrier exposure occurred in few sites during the first 2 weeks of healing and was limited to the coronal 1–2 mm of the barrier. When resorbable membranes were exposed, no action was taken and the exposed material disappeared spontaneously after 4–6 weeks. Exposed non-resorbable ePTFE membranes were left in situ until membrane removal after 4 weeks.

Histological observations

The results of the histometric assessments are presented in Table 1, and are depicted in Figs 3–5.

Table 1. Linear measurements of defect height

	Defect (aRI-CEJ) (mm)	New bone		New attachment		New cementum		
		mm	% of defect	mm	% of defect	mm	% of defect	% of NA
6 weeks	4.54 ± 0.21	1.27 ± 0.61	28 ± 15	3.79 ± 0.38	83 ± 5	2.37 ± 0.39	52 ± 7	63 ± 4
6 months	4.60 ± 0.60	2.53 ± 0.73	55 ± 10	3.72 ± 0.55	80 ± 4	2.65 ± 0.73	60 ± 11	74 ± 12
2 years	4.51 ± 0.02	2.60 ± 0.05	58 ± 2	3.57 ± 0.02	80 ± 0.3	2.66 ± 0.08	60 ± 2	74 ± 3

NA, new attachment; CEJ, cemanto-enamel junction; aRI, apical extension of the root instrumentation.
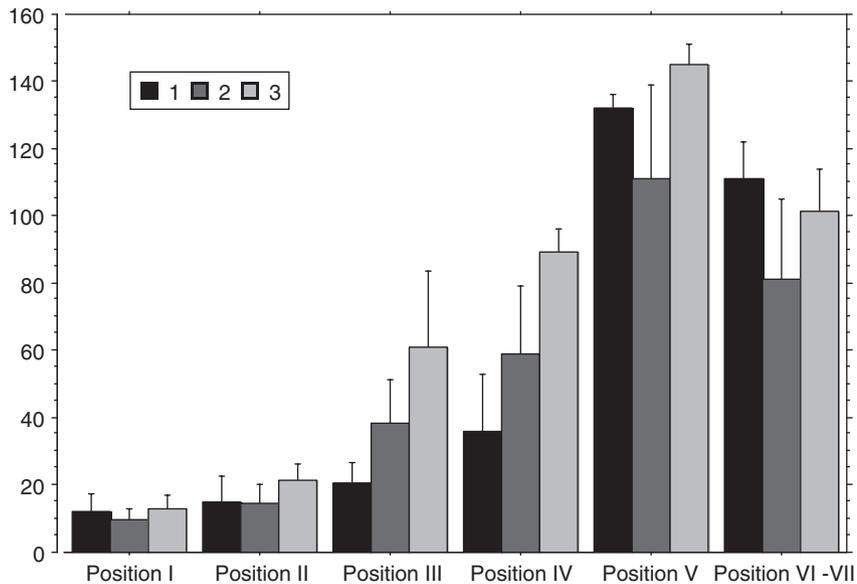

Fig. 3. Cementum thickness (μm) in various positions after 6 weeks (1), 6 months (2) and 2 years (3) of healing. Positions V–VII represent pristine tissue. Mean values and SD.
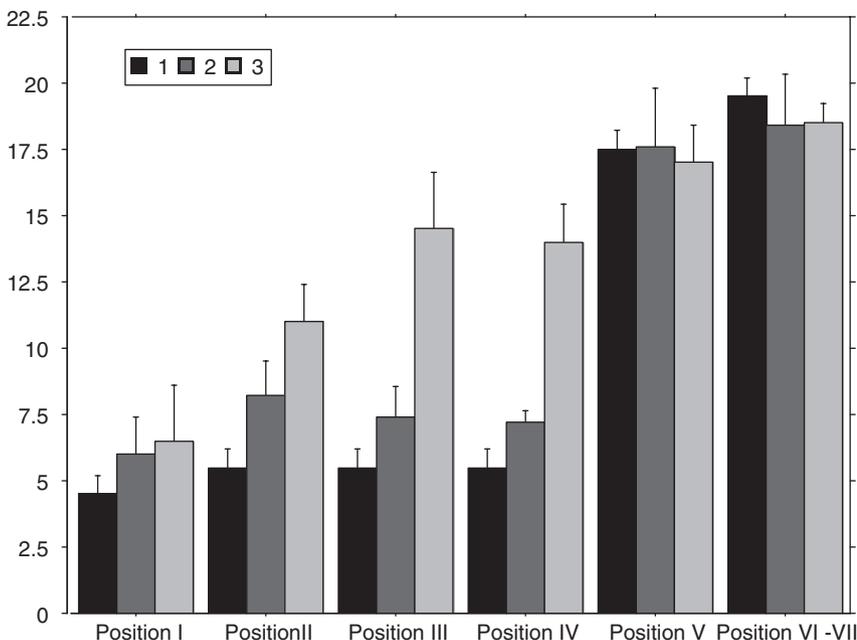

Fig. 4. Number of extrinsic fibres in various positions after 6 weeks (1), 6 months (2) and 2 years (3) of healing. Positions V–VII represent pristine tissue. Mean values and SD.

Pristine tissues

The pristine cementum (positions V–VII) varied between 90 and 130 μm in thickness (Fig. 3). A representative area of the pristine periodontium is presented in Fig. 6. The inner layer of the cementum, which was continuous with the periphery dentin, was characterized as a thin acellular extrinsic fibre cementum (AEFC). The major part of the pristine cementum had the appearance of cellular mixed fibre cementum (CMFC) consisting of layers of extrinsic and intrinsic fibre cementum. The extrinsic fibre density in the CMFC amounted to about 18(± 2) fibres per 100 μm (Fig. 4).

The width of the pristine PDL amounted to about 200 ± 30 μm (Fig. 5). The PDL was characterized by a high content of densely packed collagen fibres between the bone and the cementum running in a course perpendicular to the root surface. Epithelial remnants of Malassez were occasionally seen.

Regenerated tissues

Six weeks healing period (Figs 3–5 and 7–9). The defect height, i.e. the distance from the CEJ to the aRI amounted to 4.5 ± 0.2 mm (Table 1). The new periodontal tissues were organized but immature (Fig. 7). In most specimens, the aJE was slightly apical to the CEJ. The NA including collagen fibres anchored to the root surface had a vertical dimension (aJE to the aRI) of 3.8 ± 0.4 mm or 83% of the defect height. A thin cementum-like structure covered the instrumented root surface (Figs 8 and 9). The interface between this NC and the underlying dentin had a ruffled appearance. NC, however, was only identified at 63% of the distance between the aRI and the aJE i.e. of the NA. Thus, between the aJE and the coronal extension of the NC, there was an area, where collagen fibres seemed to attach directly to the dentin surface in an irregular, criss-cross-like fashion (Fig. 8 position I and Fig. 11).
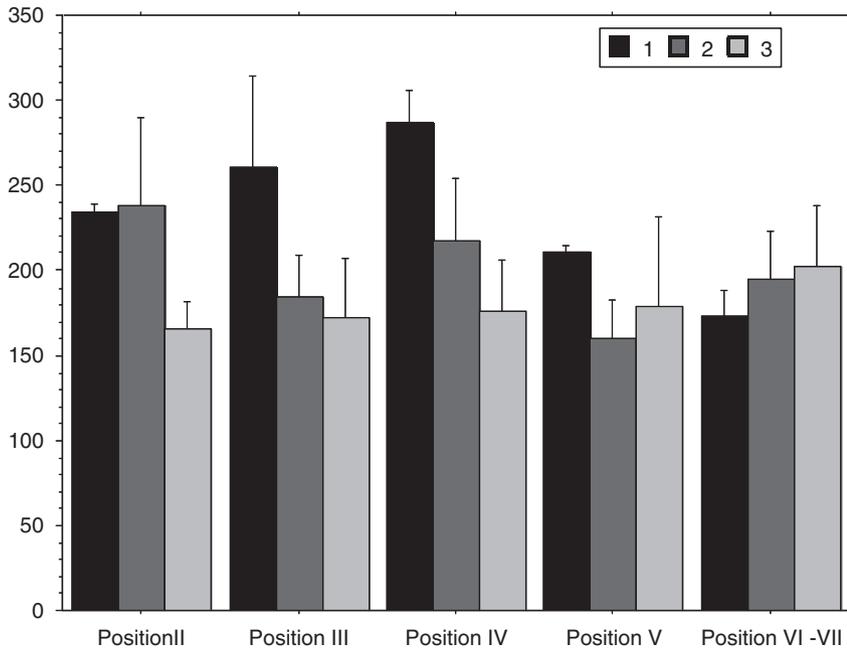
Fig. 5. Periodontal ligamentwidth (μm) in various positions after 6 weeks (1), 6 months (2) and 2 years (3) of healing. Positions V–VII represent pristine tissue. Mean values and SD.
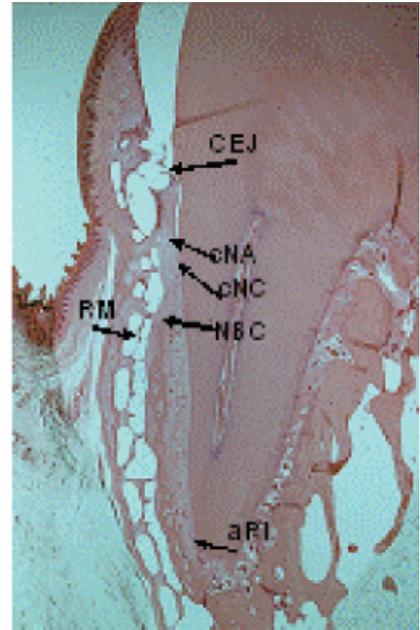

Fig. 7. Bucco–lingual section of a 6-week specimen showing landmarks for linear measurements. CEJ, cemento–enamel junction; cNA, coronal extension of new attachment that is identical to the apical extension of the junctional epithelium; cNC, coronal extension of new cementum; NBC, new bone crest; aRI, apical extension of root instrumentation; RM, Guidor resorbable membrane. Original magnification $\times$ 25.
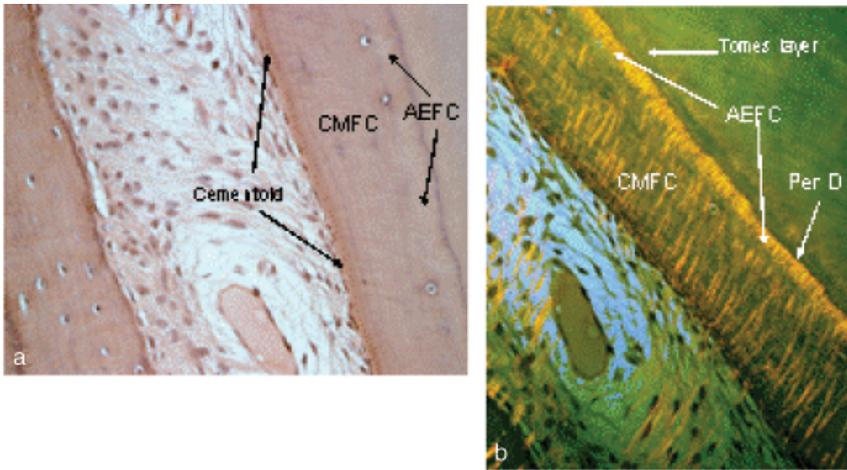

Fig. 6. Pristine cementum. (a) Normal light, (b) polarized light. CMFC, cellular mixed fibre cementum; AEFC, acellular extrinsic fibre cementum; Per.D, periphery dentin. Original magnification $\times$ 400.

The NC was acellular, covered with a mono-layer of cells. The thickness of the NC varied between $12 \pm 5\,\mu$m in position I and $36 \pm 17\,\mu$m in position IV (Figs 3 and 8). The structure of this NC was mainly extrinsic fibre cementum (Fig. 9) and fibres of very small caliber traversed the cementum and extended to the dentin surface. The numerical extrinsic fibre density (in the following referred to as extrinsic fibre density) was uniform along the entire extension of the NC and amounted to around $5/100\,\mu$m (Fig. 4) which was less than 1/3 of the fibre density in the pristine cementum.

Bone fill had occurred to about 30% of the defect height (Table 1). Coronal to the newly formed bone, there was a well-organized dense connective tissue rich in collagen. The newly formed PDL was wider than the pristine PDL (Fig. 5) and fairly loosely arranged (Fig. 9).

Six months healing period (Figs 10–13). The defect height at 6 months amounted to 4.6 ± 0.6 mm (Table 1). The healed tissue exhibited NA, including NC, new PDL and new supporting bone (Fig. 10). NA with collagen fibres

arranged mainly perpendicular to the root surface had a vertical dimension (aJE to aRI) of 3.72 ± 0.55 mm i.e. $80 \pm 4\%$ of the defect height (Table 1 and Fig. 10). Seventy-four percent of this NA exhibited identified NC (Table 1). Between the aJE and the most coronal extension of detectable NC, collagen fibres attached directly to the dentin surface angulated to, but not in parallel alignment with the root surface (Fig. 11). Furthermore, the NC always extended on top of the pristine cementum to a level about $200\,\mu$m apical to the aRI.

In the supracrestal compartment and at the crest level (positions I and II), the cementum was mainly of acellular extrinsic fibre character and had a thickness similar to that at 6 weeks (Figs 3 and 11). The extrinsic fibre density at 6 months of healing was just slightly higher than at 6 weeks healing (Fig. 4).

At positions III–IV, the re-generated cementum was considerably thicker than in the 6-week sample (Figs 3 and 12), the thickness increasing in apical
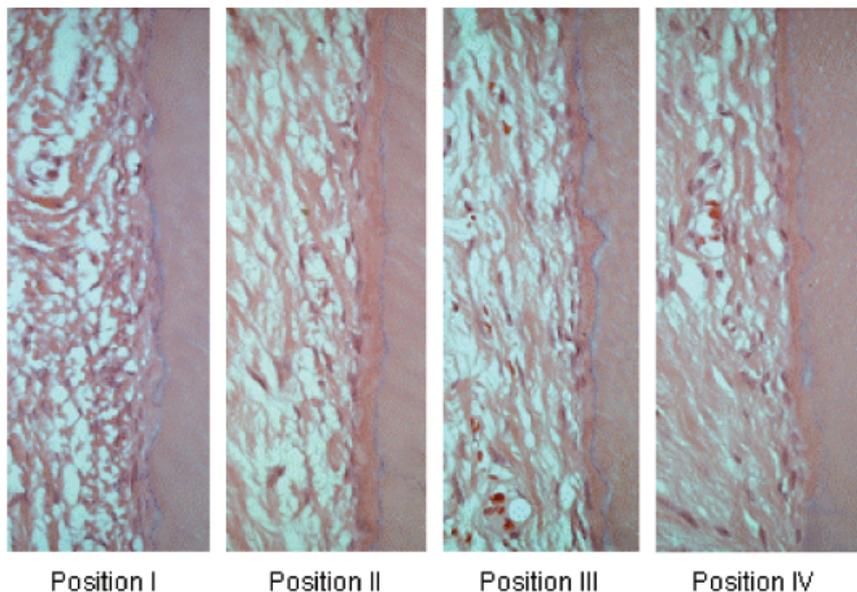
Fig. 8. Six weeks healing period. New cementum in positions I–IV, i.e. from the supracrestal area to the apical extension of root instrumentation. The connective tissue is loosely arranged. The cementum, which is of almost equal thickness along the defect, seems to be firmly anchored to the circumpulpal dentin, the surface of which seems to have undergone a transient demineralization. In the most coronal part of position I, fibres seem to attach directly to the dentin surface. Original magnification × 200.
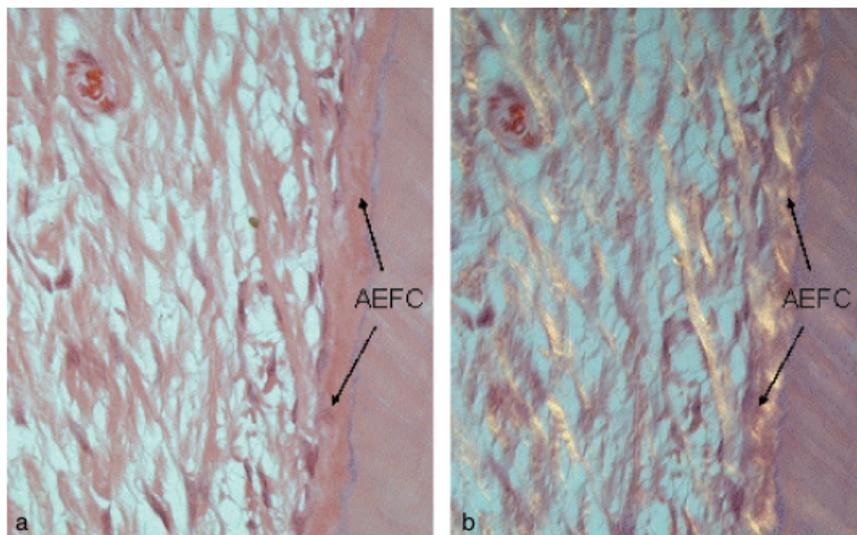

Fig. 9. Higher magnification of position II in Fig. 8. Normal (a) and polarized (b) light. The new cementum is acellular with extrinsic fibres. Note the blue resorption line.
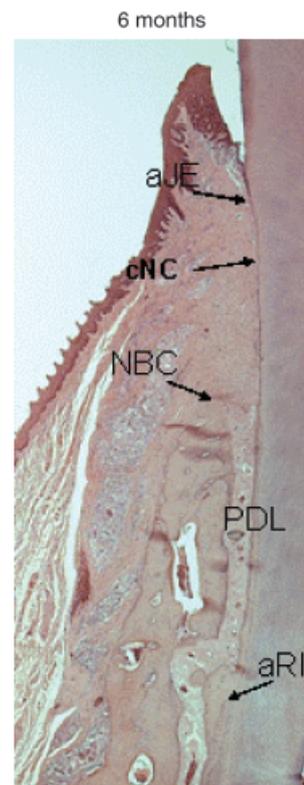

Fig. 10. Six months healing period. AJE, apical extension of the junctional epithelium, which is identical to coronal extension of new attachment; cNC, coronal extension of new cementum; NBC, new bone crest; a R1, apical extension of root instrumentation. Original magnification × 25.

The width of the newly formed PDL at 6 months was smaller than at 6 weeks and almost of the same width as the pristine PDL (Fig. 5). The collagen fibre bundles were of larger caliber and higher density as compared with 6 weeks and appeared in directions both perpendicular to the root, and in a criss-cross fashion. The newly formed bone amounted to 2.5 ± 0.7 mm or 55% of the defect height (Table 1 and Fig. 10). This NB had the appearance of lamellar bone.

Two years healing period. The defect height of the 2-year specimen averaged 4.5 ± 0.02 mm (Table 1). The newly formed attachment apparatus had an appearance similar to that at 6 months in terms of extension and tissue composition. As in the 6-month specimen, the NA extended to about 80% of the defect height (aRI to aJE), with NC visible along 74% (Table 1). While the supracrestal cementum (position I) was as thin as at the 6-week healing period, the thickness of the NC in positions

direction from $15 \pm 7\,\mu$m in position II to $59 \pm 20\,\mu$m in position IV. In positions II–IV, two distinct layers of cementum were identified (Fig. 13). Anchored to the dentin and continuous with the supracrestal cementum, there was a thin layer of AEFC. On top of the AEFC, there was a thick layer of cellular cementum containing both extrinsic and intrinsic fibres, i.e. a CMFC. On the cementum surface, there was a cemen- toid and a layer of cells, most likely cementoblast. While the thickness of the inner layer of the cementum was more or less uniform along its entire extension, the thickness of the CMFC layer increased in the apical direction. The extrinsic fibre density in positions II–IV at 6 months was about 50% higher than at 6 weeks but was still less than half that of the pristine cementum (Fig. 4).
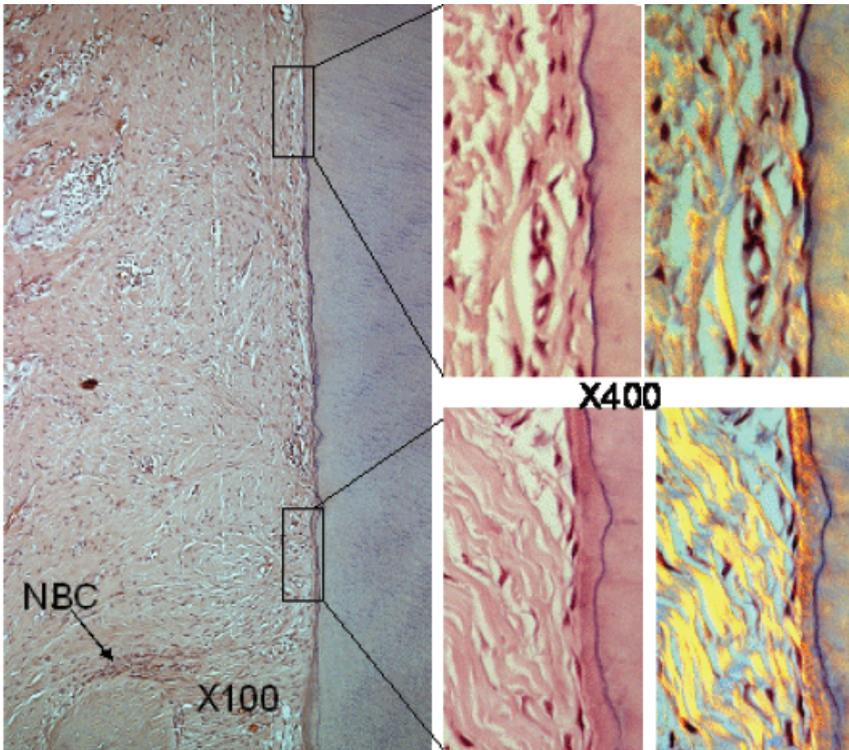
Fig. 11. Six months healing period. Medium (original magnification × 100)- and high-power magnification (original magnification × 400) of the supracrestal area. In the most coronal compartment, the new attachment consisted of collagen fibres anchored to the circum–pulpal dentin without visible new cementum. More apically in the supracrestal compartment, a thin layer of acellular extrinsic fibre cementum had formed on the circum–pulpal dentin.

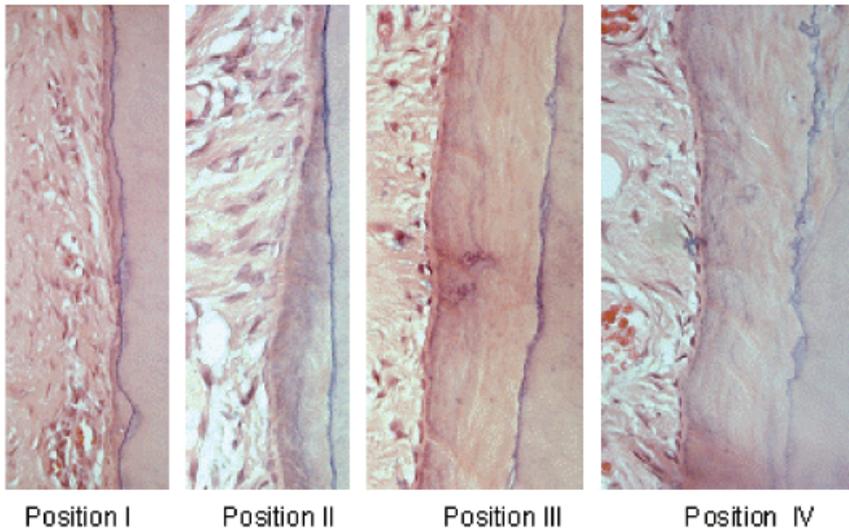Position I   Position II   Position III   Position IV

Fig. 12. Six months of healing. The new cementum increased in thickness in the apical direction. The most significant thickening of the cementum is seen at the bone crest level, position II. Original magnification × 400.

II–IV was about 50% thicker than in the 6-month sample (Fig. 3).

The density of extrinsic fibres in position I was slightly higher than at 6 months. In positions II–IV, the extrinsic fibre density was twice that at the 6-month healing period amounting to 15 ± 2 per 100 μm, which corresponded to about 80% of the fibre density of pristine cementum (Fig. 4).

The re-generated PDL was of the same width and density as the pristine PDL. The newly formed bone amounted to 2.6 ± 0.05 mm corresponding to 58% of the defect height. This NB had the appearance of lamellar bone.

Morphometric measurements

The results of the morphometric analyses are depicted in Figs 14 and 15.

Six weeks healing period. In the re-generated PDL (positions II–IV), collagen constituted 47.6%, vessels 21.2%, cells 12.1% and residual tissue 19.2% of the tissue composition. The pristine PDL (positions V-VII) consisted of 60.9% collagen, 14.3% vascular structures, 13.1% fibroblasts and 11.7% residual tissue. Evidently, at this time point, there was less collagen and more residual tissue in re-generated than in pristine PDL.

Six months and 2-year healing periods. The composition of the connective tissue of the PDL re-generated areas at 6 months was similar to that in pristine compartments and included 58.9% of collagen, 14.5% vascular structures, 15.7% cells and 10.9% residual tissue. The values obtained from the 2-year specimens were also similar in regenerated and pristine areas. From 6 months onwards, there was evidently no difference in tissue composition between regenerated and pristine PDL.

Discussion

In this experiment, the formation of periodontal tissues following GTR therapy of chronically inflamed dehiscence-type defects was described. One of the unique features of the study was the experimental outline, which made it possible to evaluate the healing from 6 weeks up to 2 years. To our knowledge, there are no other studies on periodontal wound healing with healing periods exceeding 6 months.

It was demonstrated that after 6 weeks, a NA had formed with a thin AEFC, a loosely arranged PDL and bone.

At 6 months of healing, the supracrestal cementum was as thin and the numerical extrinsic fibre density almost as low as at 6 weeks of healing. The subcrestal cementum was thicker and consisted of an inner layer of AEFC
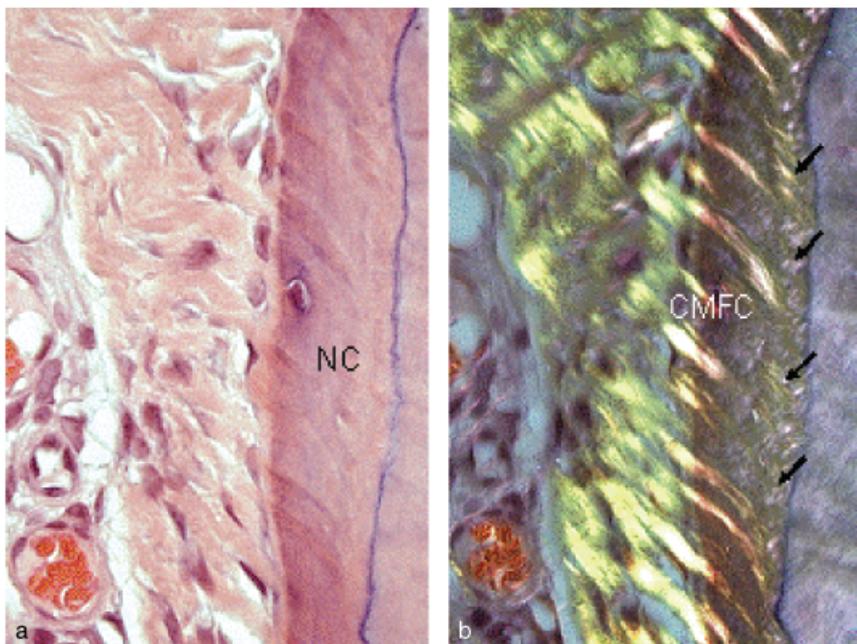
Fig. 13. Six months healing. Position III. (a) Normal light and (b) polarized light. The new cementum consists of an inner narrow layer of acellular extrinsic fibre cementum (arrows) on top of which is a thicker layer of cellular mixed fibre cementum. Original magnification × 400.
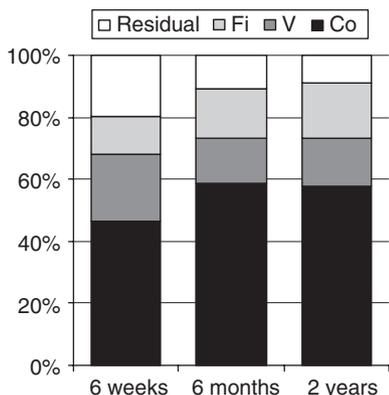

Fig. 14. Tissue composition in regenerated periodontium after 6 weeks, 6 months and 2 years of healing. Means of positions II–IV.
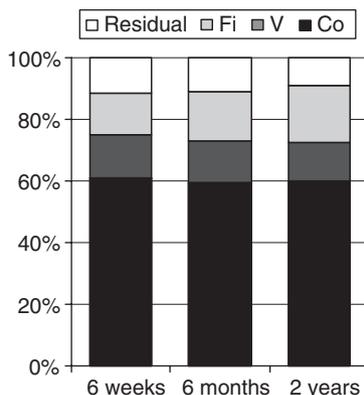

Fig. 15. Tissue composition in pristine periodontium at 6 weeks, 6 months and 2 years of healing. Means of positions V–VII.

and an outer layer of CMFC, and exhibited increased extrinsic fibre density.

The re-generated periodontium at 2 years of healing resembled that of pristine tissues.

The current results revealed that a NA apparatus was structured already after 6 weeks of healing. At this time point, a thin NC had formed along the entire defect and seemed to be firmly anchored to the underlying circumpulpal dentin. This observation is not in agreement with findings reported in previous experimental studies on periodontal wound healing (Listgarten 1972, Got-

tlow et al. 1984, Sculean et al. 1999, 2000, Cochran et al. 2003, Kostopoulos & Karring 2004). In the studies referred to, it was consistently reported that a split occurred between the newly formed cementum and the underlying dentin surface. It was suggested that this split represented an artefact from the demineralization process during the histological preparation (Listgarten 1972) or a residual smear layer on the dentin surface that prevented proper anchorage of the NC to the circumpulpal dentin (Bosshardt et al. 2005). During the re-generative surgeries in the present

study, great care was given to the final cleaning of the root surface using a sonic scaler. It may be suggested that meticulous cleaning is an effective means to remove the smear layer. The ruffled appearance of the exposed dentin surface was interpreted as being the result of the root surface instrumentation and an early transient demineralization of the dentin surface (Schupbach et al. 1993, Araujo et al. 1997), allowing for an anchorage of the NC fibres to the dentin collagen matrix as demonstrated by Schupbach et al. (1993).

In the 6-week specimens of the present study, the previously exposed root was covered with a thin AEFC including collagen fibres that extended from the circumpulpal dentin surface into a loosely arranged connective tissue. In addition, the numerical extrinsic fibre density was low along the entire extension of the NC (Fig. 4). This observation is in agreement with findings reported in an experimental study by Hammarstrom et al. (1997). They produced dehiscence-type defects in monkeys and performed treatment including the application of enamel matrix proteins. After 8 weeks of healing, a NA had formed, which included a thin layer of AEFC of about 60–80% of the defect height. Our findings also conquer with those reported by Araujo et al. (1997). They studied periodontal healing following GTR in through and through furcation defects in dogs. At 4 weeks of healing, collagen fibres projected from the dentin surface into the surrounding connective tissue (Araujo et al. 1997). This finding by Araujo et al. (1997), which was referred to as phase II of cementum formation, resembled the AEFC structure observed in the 6-week healing sample in the present study.

At 6 months of healing in the current experiment, two distinctive types of cementum could be identified. Attached to the circumpulpal dentin was a thin layer of acellular cementum with similar characteristics as the thin AEFC observed in the 6-week specimens. Thus, in the supracrestal compartment, the thickness of the NC and the extrinsic fibre density was similar in the 6-month and the 6-week healing periods. In the subcrestal zones, however, i.e. in positions opposite newly formed bone, a layer of CMFC had formed on top of the AEFC. In this area, at 6 months, the build-up of the NC resembled that of the pristine cementum. Similar findings were also reported by Araujo et al.

(1997). They found that after 5 months of healing, the early formed AEFC was covered with cellular extrinsic/intrinsic fibre cementum. The finding in the present study is also in agreement with Schupbach et al. (1993). They evaluated NA in through and through furcation defects in dogs treated according to the principles of GTR. They reported that AEFC had formed directly on the circumpulpal dentin without the re-establishment of a peripheral dentin and that a CMFC evidently developed on top of the AEFC during a later stage of the healing.

Whereas the new supracrestal cementum remained thin up to 2 years in the present study, the subcrestal cementum not only became thicker over time but also grew thicker in the apical direction. After 2 years of healing, the thickness of the NC in position IV was 60–90% of that of the pristine cementum. The thickening of the cementum in the apical areas of the root was also reported in other animal experimental studies (e.g. Nyman et al. 1982, 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 width along the root. In old dogs, the cementum was cellular, 10 times thicker and widened in the apical direction. In addition, the PDL was not narrower in the old than in the young animals, which was explained as follows: while the cementum grows wider, there is a concomitant compensatory resorption on the bone surface on the opposite side of the PDL. Thus, both the increase in thickness and the progressive thickening of the cementum in the apical direction observed in the present study may be natural features of maturation and remodelling.

In the present study, also the extrinsic fibre density in the subcrestal compartment increased over time. Following 6 weeks of healing, the fibre density amounted to 30% of that of the pristine cementum. At 6 months of healing, the subcrestal fibre density was about 50% and after 2 years, 85% that of the pristine cementum. At the same healing time, the dimension and composition of the new PDL were established to levels comparable with the pristine PDL. Between 6 weeks and 2 years, the supracrestal cementum thickness and the fibre density remained unchanged. The reason for this gradual thickening of the subcrestal cellular cementum and

the increased extrinsic fibre density over time is presently not understood. It may be suggested that concomitant with the development of NB, cementum formation continues by the appositional growth of a CMFC that will result in embedding of collagen fibres from the developing PDL. This process will continue in order to meet functional demands in the area until a balance between functional load on the tooth and the capability of the new PDL to withstand the load is achieved. The remodelling of the periodontium may also be the result of a natural maturation process aiming at re-establishing the biological dimensions. In fact, at 2 years, the regenerated periodontium resembled that of the pristine tissues (Schüpbach et al. 1993, Bosshardt & Selvig 1997).

Taken together, our findings suggest that periodontal healing following GTR therapy occurs in two sequences. Following the initial healing phase, which includes the formation of a blood clot and a transient root resorption/demineralization, an AEFC is deposited on the root surface and a connective tissue is formed. This event represents a wound healing that will establish continuity between various tissues in the area. The next phase of healing is a remodelling process governed by function. The morphology of re-generated cementum, which is the key tissue in periodontal regeneration, will become similar to pristine cementum as maturation proceeds over time, i.e. a thin AEFC covered with a CMFC. Simultaneously, a new PDL is formed, the composition of which is similar to the pristine PDL. Thus, from a *qualitative* perspective, the periodontal healing that takes place following GTR therapy of recession-type defects will result in a restitutio ad integrum, i.e. healing by regeneration. From a *quantitative* perspective, however, there seems to be a restriction of the *qualitative* process in the coronal direction.

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Clinical relevance

In the clinic, the outcome of GTR therapy of dehiscence-type defects is primarily evaluated by clinical attachment level (CAL) measurements. The findings in the present study will thus provide the biological background of CAL gain but also of quantitative limitations, although minor. For the clinician, it is important to realize that the quality of the gained attachment will improve over time.