Clin Oral Invest (2012) 16:239–249
DOI 10.1007/s00784-010-0478-7

ORIGINAL ARTICLE

Wound healing following regenerative procedures in furcation degree III defects: histomorphometric outcomes

Nikolaos D. Gkranias · Filippo Graziani · Anton Sculean · Nikolaos Donos

Received: 4 May 2010 / Accepted: 28 September 2010 / Published online: 22 October 2010

Abstract Degree III furcation involvements were surgically created at four first molars in each of three monkeys. Following 6 weeks of healing, full-thickness flaps were elevated. Following 24% EDTA gel conditioning, the defects were treated with one of the following: (1) enamel matrix proteins (EMD), (2) guided tissue regeneration (GTR) or (3) a combination EMD and GTR. The control defects did not receive any treatment. After 5 months of healing, the animals were sacrificed. Three 8 μm thick histological central sections, 100 μm apart, were used for histomorphometric analysis in six zones of each tooth either within the furcation area or on the pristine external surface of the root. In all specimens, new cementum with inserting collagen fibres was formed. Following GTR or GTR+EMD, cementum was formed up to and including the furcation fornix indicating complete regeneration on the defect periphery. Periodontal ligament fibres were less in all four modalities compared to pristine tissues. In the teeth treated with GTR and GTR+EMD a higher volume of bone and periodontal ligament tissues was observed compared to EMD. After 5 months of healing, regenerated tissues presented quantitative differences from the pristine tissues. In the two modalities where GTR alone or combined with EMD was used, the regenerated tissues differed in quantity from the EMD-treated sites.

Keywords Wound healing · Regenerative procedures · Furcation degree III defects

N. D. Gkranias · F. Graziani · N. Donos (✉)
Department of Periodontology, UCL Eastman Dental Institute for Oral Health Care Sciences University of London,
256 Gray's Inn Road,
London WC1X 8LD, UK
e-mail: n.donos@eastman.ucl.ac.uk

F. Graziani
Department of Surgery, Section of Oral Surgery,
University of Pisa,
Pisa, Italy

A. Sculean
Department of Periodontology, School of Dental Medicine,
University of Berne,
Berne, Switzerland

Introduction

Clinical use of guided tissue regeneration (GTR) or Emdogain® (EMD) results in regeneration of the lost periodontal tissues in a predictable manner [1–4]. Histological observations in animal studies as well as human biopsies have shown that following GTR the regenerated cementum is in continuum with the existing one at the apical part of the defect, while very often an "artefact" is present between new cementum and underlying dentine [5]. Previous qualitative histological studies in degree III furcation defects have shown that periodontal ligament fibres in the regenerated periodontium differ to those of the pristine periodontium at 5 months of healing [5, 6]. However, it has been shown that as the healing process continues regenerated periodontal tissues mature and at 2 years of healing they present similar characteristics to those of the pristine tissues [7, 8].

Initial histological publications in animals [9] and human biopsies [10] showed that EMD could promote new attachment formation [9, 10] characterised by mixed cellular/acellular cementum [11–15]. However, more recent studies based on human biopsies suggested that the cementum observed at 2–6 weeks after the EMD application shared characteristics of the cellular intrinsic fibre

cementum and bone [16, 17]. Largely, there are only few studies in literature that compare the quality and quantity of the regenerated periodontal tissues between EMD and GTR.

Therefore, the aim of the present pilot study was to evaluate histomorphometrically the regenerated tissues following the use of GTR or EMD in degree III furcation defects.

Material and methods

Three adult male monkeys (*Macaca fascicularis*) were included in the study. Before operatives procedures, the animals have been sedated with intramuscular injection of Kethalar® (10 mg/kg body weight, Park–Davies Co. Inc., Warner Lambert Nordic A/B, Solna, Sweden), combined with local infiltration anaesthesia (Xylocain® 2%, Astra, Copenhagen, Denmark). The surgical protocol was approved by the ethical committee for experimental animal use of the Royal Dental College, University of Aarhus, Denmark as well as from the Regional Ethics Committee for experimental animal research.

Production of experimental defects

The mandibular second premolar and third molar were extracted 2 months prior to the initiation of the experiment. Following intra-sulcular incisions, full-thickness flaps were elevated on the buccal and lingual aspect of the mandibular molars. Degree III furcation defects were created surgically at the first and the second mandibular molars with the use of bone chisels and slowly rotating diamond burs. A "horizontal" pattern of bone loss was created by removing the alveolar bone of the buccal and lingual aspect of the mesial and distal root of each molar. However, the height of the alveolar bone on the mesial and distal interproximal aspect of each experimental tooth was maintained.

The dimensions of furcation defects were approximately 4 mm wide and 3 mm high. In order to enhance plaque accumulation and prevent spontaneous healing, the defect was filled with impression material (Impregnum F®, Espe, Seefeld, Germany).

Subsequently, the flaps were repositioned and stabilised with 4–0 silk sutures. Ten days following surgery, the sutures were removed. No oral hygiene measures were performed for 4 weeks, thus allowing plaque accumulation to take place. At that point, the impression was removed from the defects. During the following 2 weeks, a plaque control regimen was established, which included tooth brushing and local application of chlorexidine digluconate (0.2%) twice per week.

Reconstructive surgery

Six weeks post-operatively, intrasulcular incisions were performed and full-thickness buccal and lingual mucoperiosteal flaps were elevated and the furcation defects and adjacent roots and bone were exposed. No vertical releasing incisions were performed. All granulation tissue was removed and the exposed root surface was carefully scaled and root planned. With the help of a small round bur, reference notches (N), serving as landmarks for the measurements to be made in histological sections, were prepared at the level of the reduced bone crest in the buccal and interradicular surfaces of the two roots of each molar. Thus, any periodontal ligament tissue, which later may develop coronally to the notch on the root surface, will be clearly distinguished as "de novo" formed in the histological sections.

Subsequently, the root surfaces were conditioned with 24% ethylenediaminetetraacetic acid (EDTA) containing gel (Biora AB, Malmö, Sweden) for 2 min according to the manufacturer's instructions. The EDTA remnants were removed with copious sterile saline rinsing.

In each monkey, (1) the first molar in one side of the jaw was treated with a coronally displaced flap (control, three defects in total); (2) the second molar on the same side had a bioresorbable membrane (Resolut® Regenerative Material, W. L. Gore Associates, Flagstaff, AZ, USA) trimmed and adapted on the buccal and lingual aspect of the furcation before flap closure (three defects in total); (3) the first contralateral molar was treated with EMD (Emdogain®, Biora AB, Malmö, Sweden) before flap closure (three defects in total) while the (4) second molar in the same side was treated with a combination of Emdogain® and bioresorbable membranes (Resolut® Regenerative Material, W.L. Gore Associates, Flagstaff, AZ, USA; three defects in total). The membranes were fixed with bioresorbable sutures (Dexon® II, Davis & Geck, Inc, Manati, PR, USA) around the tooth neck.

The Emdogain® gel was supplied by the manufacturer in two bottles. One bottle contained sterile, lyophilised form of the enamel matrix proteins and the other one contained the vehicle solution. The bottles were stored in the refrigerator before use and the gel was mixed just before each surgical procedure. Following thorough removing of all EDTA from the root surfaces by rinsing, the Emdogain® gel was applied on the root surfaces and into the defect. The furcation was completely filled with the gel. At the sites were the Emdogain® was combined with membranes, the membranes were adapted first loosely on the furcation defects and after the Emdogain® application they were tightly sutured around the experimental teeth.

In all experimental teeth, a periosteal incision was performed at the base of the flap and subsequently, the flaps were coronally displaced in such a way that they

completely covered the membranes and the furcation areas. All flaps were secure in position with horizontal and vertical mattress sutures (Goretex ®, Flagstaff, AZ, USA).

The animals received a single dose of post-operative intravenous antibiotics (0.5 ml/kg Clamoxycillin®, Pfizer Italiana SPA, Borgo San Michele (Latina) Italy). For the first 6 weeks, plaque control with tooth brushing and local application of chlorexidine was performed twice per week and thereafter once a week.

Following 5 months of healing, the animals were euthanised, the oral tissues were fixed, the jaws and the surrounding soft tissues were dissected and decalcified with EDTA, dehydrated and embedded in paraffin. Serial sections in mesio-distal direction and parallel to the long axis of the tooth were cut with the microtome set at 8 μm. The sections were stained with Haematoxylin and Eosin, Van Gieson's connective tissue staining or the aldehyde-fuchsin-Halmi staining method.

Histomorphometric measurements

Three sections, 100 μm apart, representative of the central area of the furcation were selected from each tooth for the measurements.

Analyses were made at six zones (Fig. 1):

Zone 1: apical to the notch on the right root of the specimen (1a) and on the left root of the specimen in the furcation area (1b);

Zone 2: at the level of the notch on the right root of the specimen (2a) and on the left root of the specimen in the furcation area (2b);

Zone 3: at the level of the most coronal newly formed bone between the notch and the fornix of the furcation on the right root of the specimen (3a) and on the left root of the specimen (3b);

Zone 4: at the level of the fornix of the furcation

Zone 5: (Pristine area) at the interdental surface of the tooth at the level of the furcation fornix on the right root of the specimen (5a) and on the left root of the specimen (5b). If that was over the bone crest, the measurements were taken at the level of the bone crest;

Zone 6: (Pristine area) at the interdental surface of the tooth at the level of the notch, on the right root of the specimen (6a) and on the left root of the specimen (6b).

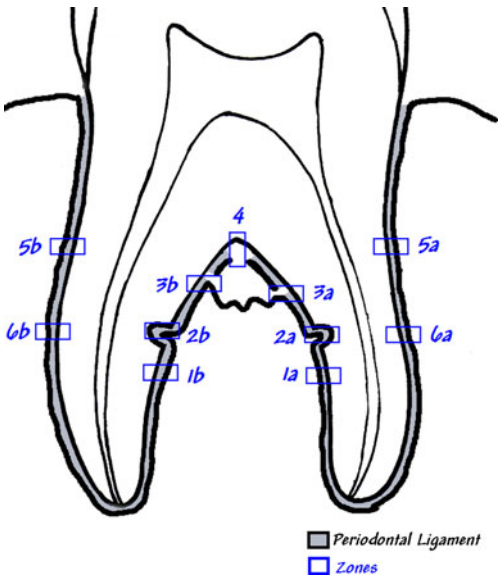

Fig. 1 Zones of histomorphometric measurements

Histological analysis was carried out using a light microscope set for light microscopy and polarised light microscopy. Histometric and morphometric measurements were performed on digitally captured images from the microscope using an image analysis system (Image-Pro Plus 4.5; Media Cybernetics, Silver Spring, MD, USA).

Histometric measurements

Histometric measurements were made:

(a) Under light microscopy at ×100 magnification for:

- width of cementum expressed in micrometre. Where new cementum was formed on top of the old one, the border between the new and old cementum was not always apparent and as such the width of both cementum was measured together.
- width of periodontal ligament expressed in μm

(b) Under polarised microscopy at ×400 magnification for:

- number of collagen fibres (per 100 μm of linear root length) inserting cementum

Morphometric analysis

Morphometric measurements were also performed in the above-mentioned zones.

The measurements were made at:

- ×400 magnification to assess the proportion of periodontal ligament representing (a) vascular structures, (b) collagen fibres, (c) fibroblasts, and (d) residual tissue. The proportions were determined using a point-counting procedure [18] superimposing a lattice comprising of 100 points over the tissue at the magnification (×400).
- ×50 times magnification for evaluating the proportions of the healed tissues above the notch level that

were lamellar bone, bone marrow spaces, periodontal ligament and connective tissue and granulation tissue and epithelial tissue. The calculation was done by outlining the surfaces with a mouse cursor.

Statistical analysis

The examiner performing the histological measurements (NG) was initially trained by a researcher with previous experience (FG) in histological measurements and familiarity to the software used. After a training period, 10% of the measurements where performed twice (7 days apart) and intra-examiner reproducibility was checked by means of the Wilcoxon test. No statistical significant differences were found between the two lots of measurements (Fig. 2).

Mean values at tooth level and group level (treatment modality) as well as standard deviations were determined for each of the variables. In addition, the results of the pristine zones 5 and 6 have been calculated as an average (control) and are presented as a mean as well as independently in the tables. Statistical analysis was restricted to descriptive statistics (Fig. 3).

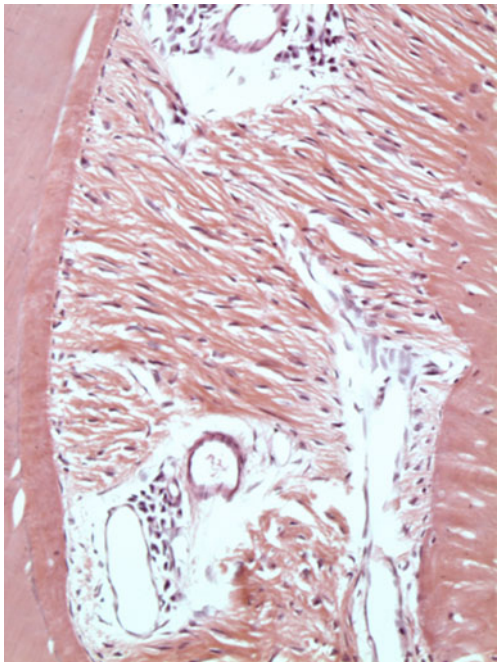

Fig. 3 EDTA regenerated tissues under light microscopy (original magnification ×100)

Results

Clinical observations

During the second postoperative week, exposure of the membrane occurred in two sites treated with GTR alone. In the third postoperative week, a defect treated with a combination of GTR and EMD presented exposure of the membrane. The exposed parts of the membranes disintegrated within a few days without any adverse effects (Fig. 4).

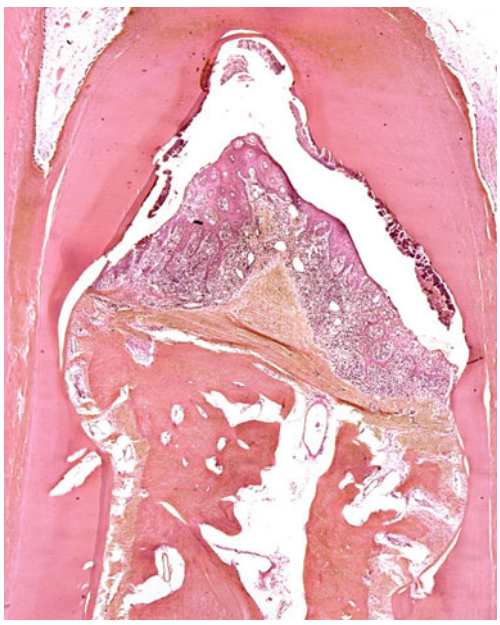

Fig. 2 Furcation treated with EDTA. Regeneration was observed only at the level of notch, with connective tissue and granulation tissue plus epithelium covering the rest of the space (original magnification ×25)

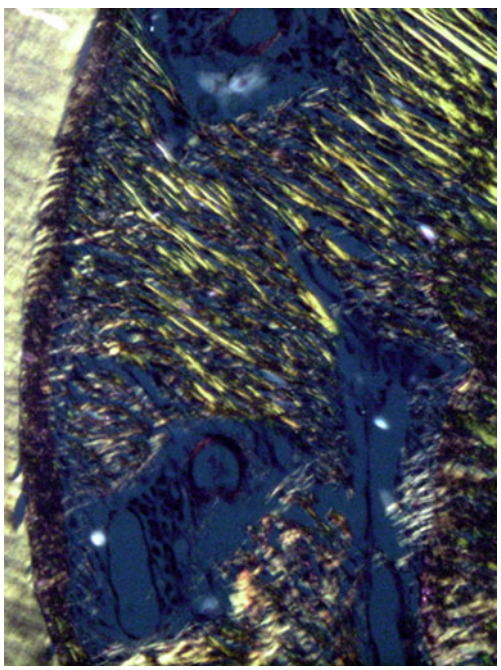

Fig. 4 EDTA regenerated tissues under polarised light microscopy (original magnification ×100)

Table 1 Width of periodontal ligament space in micrometres

Periodontal ligament width in micrometres												
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 5		Zone 6	
	μm	SD	μm	SD	μm	SD	μm	SD	μm	SD	μm	SD
GTR+EMD	216.1	43.5	267.7	67.1	237.1	46.8	196.3	66.1	287.9	58.6	204.0	37.9
									Mean control			
GTR	236.9	47.5	214.1	39.5	244.5	10.6	161.5	17.7	μm		SD	
									245.9		64.5	
EMD	236.1	50.4	236.2	34.0	241.0	77.6						
EDTA	218.8	34.9	225.7	31.0	196.3	58.6						

One defect treated with EMD presented recession of the flap to the level of the fornix of the furcation. In the remaining defects, no adverse events were observed.

Histometric results

Periodontal ligament width The width of the periodontal ligament space was measured in six zones per root and averaged per tooth, as described in the material and methods. The results per zone and treatment modality are summarised in Table 1 (Fig. 5).

In *pristine areas*, the width of periodontal ligament was found to be narrower apically (zone 6; 204±37.9 μm) and wider coronally (287.9±58.6 μm; zone 5).

In all four treatment modalities, the periodontal ligament was overall narrower than that of the corresponding pristine areas (zones 5 and 6). It ranged between 225.7±31 (zone 2) and 196.3±58.6 (zone 3) for EDTA, 236.1±50.4 (zone 1), and 241±77.6 (zone 3) for EMD, 244.5±10.6 (zone 3) and 161.5±17.7 (zone 4) for GTR and 267.7±67.1 (zone 2), and 196.3±66.1 μm (zone 4) for GTR+EMD. Only in GTR and GTR+EMD defects were periodontal ligament tissues observed in the furcation fornix (Fig. 6).

Cementum width The width of the cementum was measured in six zones per root and averaged per tooth, as described in the "Material and methods" section. Histologically, the newly created cementum was always, irrespectively of the treatment modality, composed of layers of acellular extrinsic fibre cementum and layers of cellular extrinsic and intrinsic fibre cementum. Therefore, the regenerated cementum observed was mostly of the mixed stratified type. Exceptions were only areas near the notch at the teeth treated with EMD or GTR+EMD (zone 1) where an area of solely acellular extrinsic fibre

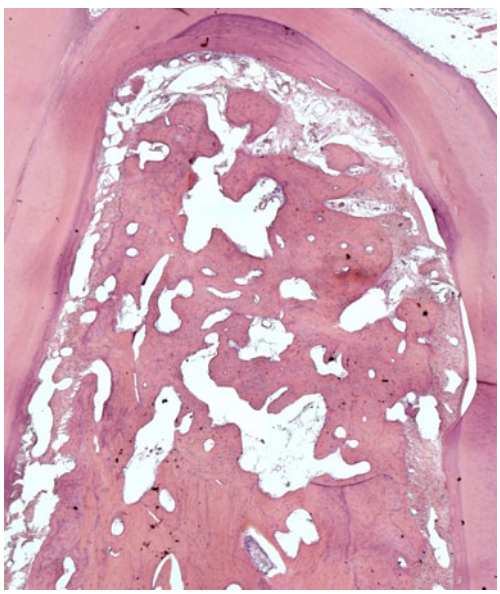

Fig. 5 Furcation treated with GTR+EMD. Regeneration was observed up to the fornix (original magnification ×25)

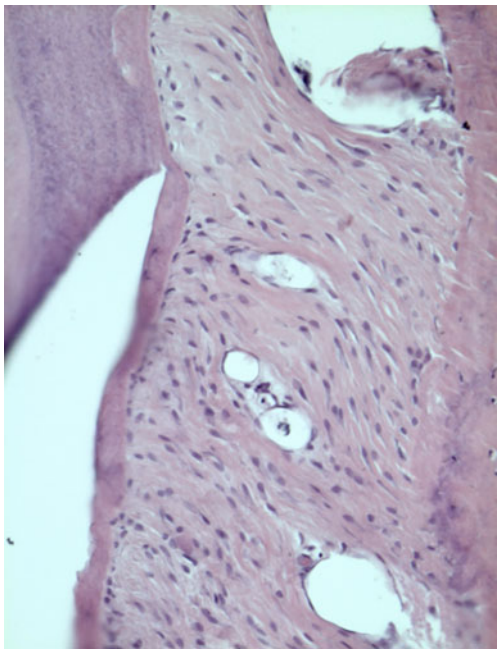

Fig. 6 GTR+EMD regenerated tissues under light microscopy (original magnification ×100)

Table 2 Width of cementum in micrometres

Cementum width in micrometres												
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 6		Zone 6	
	μm	SD	μm	SD	μm	SD	μm	SD	μm	SD	μm	SD
GTR+EMD	92.5	14.5	51.0	29.0	24.9	11.4	22.8	19.8	42.9	14.3	51.8	18.3
									Mean control			
GTR	74.3	40.3	49.0	26.6	28.6	30.8	34.3	38.5	μm		SD	
									47.4		16.7	
EMD	82.4	34.3	31.0	13.9	30.5	18.4						
EDTA	75.0	9.1	49.9	28.2	34.3	40.7						

cementum was observed. In the same defects, further coronally, the acellular cementum was replaced by the same mixed stratified type of cementum observed to the other two treatment modalities. In almost all histological samples, irrespectively of the type of the regenerated cementum, the cementum appeared detached from the underlying circumpulpal dentine. The only areas where the cementum was still in contact with the underlying surface were areas where underlying old cementum was present. In the control pristine areas (zones 5 and 6), the cementum observed, was purely acellular extrinsic fibre cementum. The results per zone and treatment modality are summarised in Table 2 (Fig. 7).

In pristine areas, the cementum was found wider apically (zone 6) 51.8±18.3 μm and narrower coronally at 42.9±14.3 μm (zone 5).

In all four treatment modalities, the cementum was overall wider than that of the corresponding pristine areas (zones 5 and 6). It ranged between 75±9.1 (zone 1) and 34.3±40.7 (zone 3) for EDTA, 82.4±34.3 (zone 1) to 30.5±18.4 (zone 3) for EMD, 74.3±40.3 (zone 1) and 28.6±30.8 (zone 3) for GTR and 92.5±14.5 (zone 1) and 22.8±19.8 μm (zone 4) for GTR+EMD. Only in GTR and GTR+EMD defects regenerated cementum occurred (Fig. 8).

Number of sharpey fibres per 100 μm The number of fibres inserting the cementum per 100 μm of root surface were measured in six zones per root and averaged per tooth, as described in the "Material and methods" section. In all pristine areas and regenerated tissues, Sharpey's fibres were always oriented in a perpendicular way to the cementum surface. The results per zone and treatment modality are summarised in Table 3 (Fig. 9).

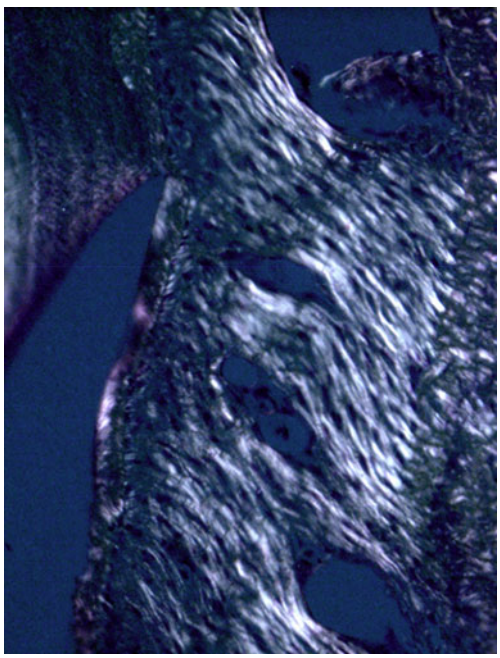

Fig. 7 GTR+EMD regenerated tissues under polarised light microscopy (original magnification ×100)

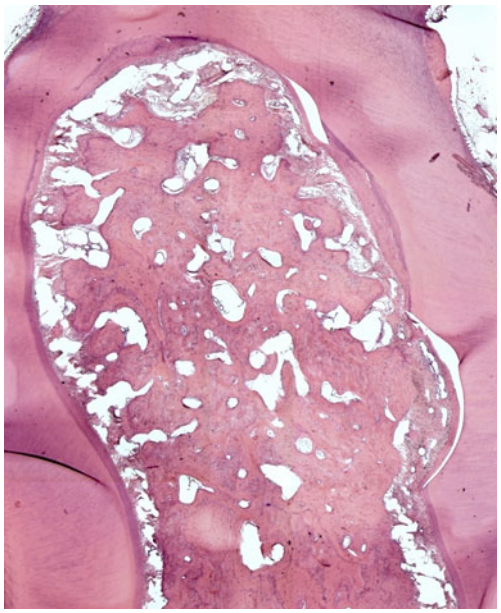

Fig. 8 Furcation treated with GTR. Regeneration was observed up to the fornix in one specimen, new cementum with inserting collagen fibres perpendicularly oriented to the root surfaces was covering the entire circumference of the defect and newly formed trabecular bone filled the defect almost completely (original magnification ×25)

Table 3 Number of collagen fibres inserting cementum per 100 μm

Number of collagen fibres inserting cementum per 100 μm												
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 5		Zone 6	
	n	SD	n	SD	n	SD	n	SD	n	SD	n	SD
GTR+EMD	15.4	1.1	15.6	1.1	10.6	6.0	9.9	8.5	20.1	4.0	18.3	4.4
									Mean control			
GTR	15.3	2.9	16.2	1.8	7.2	9.0	4.1	7.2	*n*		SD	
									19.2		4.3	
EMD	13.7	4.6	12.0	5.5	7.0	9.6						
EDTA	14.1	2.9	13.0	3.7	6.9	7.3						

In pristine areas, the Sharpey's fibres per 100 μm of cementum were found to be more numerous apically and less coronally ranging from 18.3±4.4 (zone 6) to 20.1±4 (zone 5).

In all four treatment modalities, the Sharpey's fibres per 100 μm of cementum were overall less than those of the corresponding pristine areas ranging from 14.1±2.9 (zone 1) to a 6.9±7.3 (zone 3) for EDTA, 13.7±4.6 (zone 1) to 7±9.6 (zone 3) for EMD, 16.2±1.8 (zone 2), and 4.1±7.2 (zone 4) for GTR and 15.6±1.1 (zone 2), and 9.9±8.5 (zone 4) for GTR+EMD. Only in GTR and GTR+EMD-treated defects were Sharpey's fibres observed (Fig. 10).

Morphometric results

Zone morphometry The tissues were analysed by zone and treatment modalities and were compared with those obtained by the measurements in pristine areas (by zone or average). There were not marked differences between the pristine areas and the zone 1 (Table 4) but at more coronal zones (zones 2–4), differences were observed. The percentage of connective tissue fibres was gradually reduced for all treatment modalities except the EDTA (64% in zone 3) while the proportion of the vascular structures was increased (especially in GTR-treated sites) again with the exception of the EDTA-treated sites. Overall, the tissues with the most deviation from the pristine tissue morphometry are the tissues of zone 4 (furcation fornix). These tissues were only formed in the GTR or GTR+EMD-treated furcations (Table 4; Fig. 11).

Furcation tissues morphometry An analysis of the percentage of the newly formed tissues (surface area) covering the furcation defect above the level of the notches was performed.

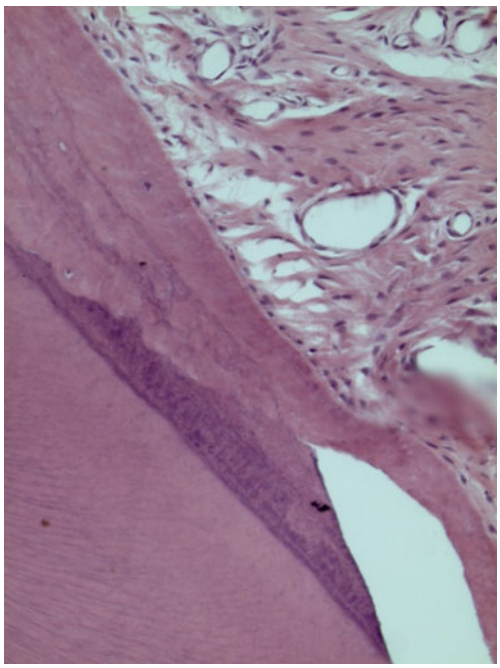

Fig. 9 GTR regenerated tissues under light microscopy (original magnification ×100)

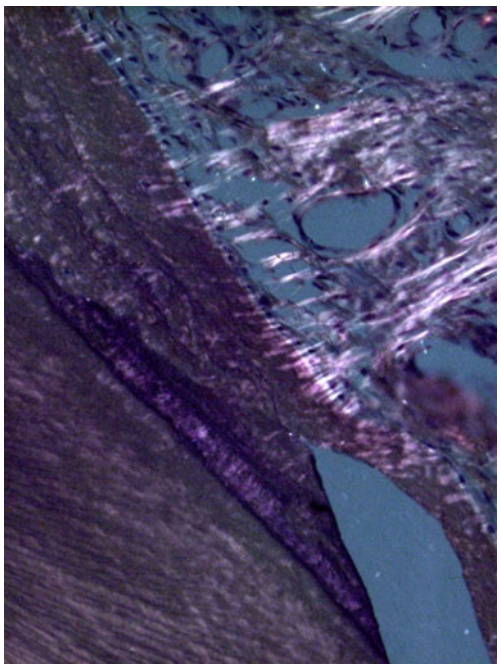

Fig. 10 GTR regenerated tissues under polarised light microscopy (original magnification ×100)

Table 4 Table of morphometric characteristics by zone and treatment and control measurements from pristine area

Morphometric analysis of PDL tissue per zone per treatment and control									
	Treatment	Connective tissue fibres		Vascular structures		Fibroblasts		Residual tissue	
		%	SD%	%	SD%	%	SD%	%	SD%
Zone 1	EMD+GTR	63	7	13	4	12	3	12	4
	GTR	60	2	16	4	10	3	13	2
	EMD	63	3	14	5	10	3	13	4
	EDTA	62	4	15	4	10	1	13	5
Zone 2	EMD+GTR	62	4	17	5	11	3	10	2
	GTR	63	5	13	5	10	2	14	3
	EMD	58	5	15	4	14	4	13	5
	EDTA	57	3	18	8	12	2	13	4
Zone 3	EMD+GTR	60	4	14	2	13	4	13	3
	GTR	52	5	21	6	10	6	15	7
	EMD	54	3	17	3	11	3	17	3
	EDTA	64	5	10	2	13	1	14	2
Zone 4	EMD+GTR	58	5	14	6	13	6	15	7
	GTR	44	6	25	13	15	13	16	16
	EMD	–	–	–	–	–	–	–	–
	EDTA	–	–	–	–	–	–	–	–
Control	Zone 5	65	3	11	3	13	3	11	3
	Zone 6	65	6	13	5	11	2	11	2
	Total	65	4	12	4	12	3	11	2

PDL periodontal ligament

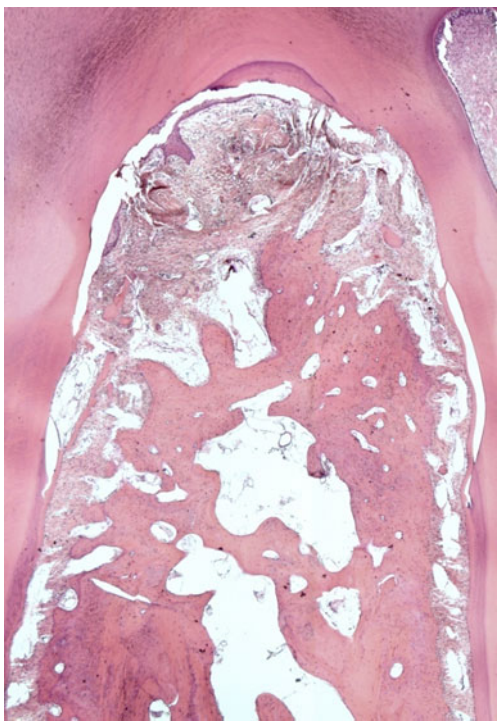

Fig. 11 Furcation treated with EMD. One of the furcations treated with EMD was completely regenerated, while two presented an epithelialised inflamed connective tissue downgrowth at the upper part of the defect (original magnification ×25)

In the sites treated with GTR+EMD the majority of the tissues consisted of connective tissue (44.81%) closely followed by lamellar bone (44.23%), bone marrow (9.63%) and some granulation tissue and epithelial downgrowth (1.33%). Epithelial down growth and granulation was relatively low also in EMD-treated sites (10.68%). In the same sites, lamellar bone was 39.06%, connective tissue 44.81% and bone marrow spaces 9.63%. On the contrary, granulation tissue and epithelial tissue was higher in both the GTR- and EDTA-treated furcation with percentages of 17.85% and 23.08%, respectively. The proportions of bone marrow (7.71% and 8.58%, respectively) or connective tissue (36.96% and 35.42%) were not markedly different in those two treatments but the proportion of lamellar bone was markedly lower in EDTA (32.92%)-treated sites compared to GTR sites (37.76%; Table 5).

Discussion

The present study evaluated the healing of degree III furcation defects following four treatment modalities, namely EDTA root conditioning, GTR, EMD, or combination of GTR and EMD. It was observed that following healing of 5 months different histological results were

Table 5 Table of morphometric characteristics of furcation tissues by treatment

Proportions of tissues covering healed furcation area per treatment modality

	Lamellar bone		Bone marrow		Granulation tissue and epithelium		Connective tissue (including PDL)	
	%	SD	%	SD	%	SD	%	SD
GTR+EMD	44.23	5.45	9.63	4.86	1.33	1.23	44.81	8.06
GTR	37.76	14.29	7.71	4.17	17.85	12.56	36.69	5.40
EMD	39.06	7.18	9.29	3.56	10.68	7.98	40.98	13.10
EDTA	32.92	3.97	8.58	1.92	23.08	9.12	35.42	7.16

PDL periodontal ligament

obtained for each treatment. Sites where the furcation defects presented regeneration up to the fornix were observed only in defects treated with GTR and a combination of GTR and EMD (Fig. 12).

In defects treated either with EDTA (and coronally advanced flaps) or with GTR, the newly formed cementum was predominantly cellular with extrinsic fibres or mixed stratified (acellular and cellular extrinsic fibre). When the defects were treated with EMD or combination of GTR and EMD, the cementum was characterised apically as acellular extrinsic fibre and coronally as mixed stratified which is in agreement with Araújo et al. [11] and could partially suggested to be due to the active presence of the enamel matrix proteins for less than 2 weeks on the root surface [19]. The granulation tissue corresponding higher in the furcation area (zone 3) might require more than 2 weeks to be populated by periodontal ligament fibroblast-like cells and therefore it might not benefit from the effects of the enamel matrix proteins (Fig. 13).

The regenerated cementum was thinner than the pristine one when compared at the same level for all four treatments (zone 2 compared to zone 6, and zone 4 compared to zone 5). In addition, in all treatment sites, as well as in pristine sites, the cementum had a tendency to become thinner towards the coronal direction. This is in accordance with Graziani et al. [7] and Laurell et al. [8] that also had similar observations in dehiscence-type and infrabony defects in monkeys following application of GTR. However, it is different to Araújo et al. [5] that showed no major differences in the width of healed cementum, between the different zones, following GTR in degree III furcation defects in dogs. This could be attributed to the differences

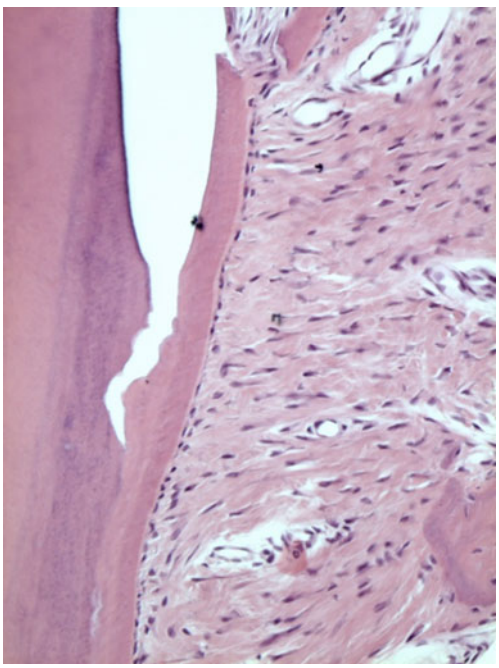

Fig. 12 EMD regenerated tissues under light microscopy (original magnification ×100)

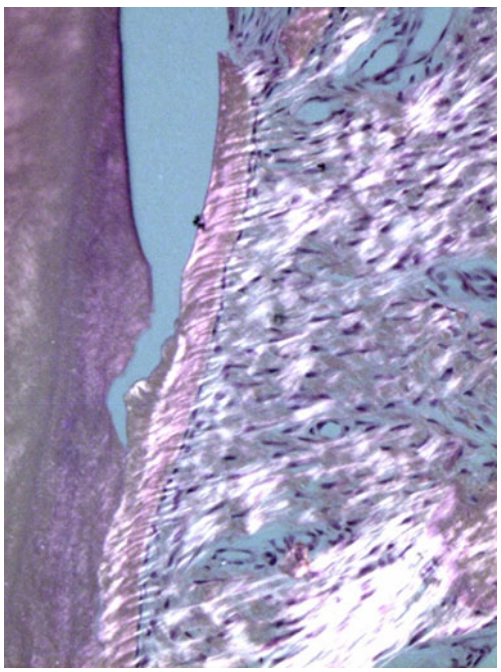

Fig. 13 EMD regenerated tissues under polarised light microscopy (original magnification ×100)

in animal species between the studies, as well as by the incidence of membrane exposure in the current study that may have affected the quantity of regenerated tissues and the healing process per se.

In all histological samples and irrespectively of the treatment modality, splits between the regenerated cementum and the dentine surface were consistently observed. In the past, this has been attributed as either an artefact due to the demineralisation process [20] or due to the presence of smear layer on the dentine [21]. In this study, the artefact was not observed when the new cementum was laid on top of old cementum, irrespectively if this occurred on the bottom or roof of the furcation. A plausible explanation may lay in the presence or absence of the cemento-dentine interface zone (Thomes granular layer) that is absent when new cementum regenerates on the dentine of root surfaces [5].

The periodontal ligament width did not present marked differences between the different treatment modalities. Overall, it was narrower in the treated sites (161–267 μm) than in the pristine sites (204–288 μm). In the pristine sites, the periodontal ligament was wider coronally while in treatment sites it was narrower coronally. It has been suggested that the width of the periodontal ligament depends partly on the functional loading applied to the area [22]. Therefore, differences in the functional forces applied within the furcation compared to the external root surface might explain the observed difference in the present investigation.

Collagen fibres inserting the new cementum were observed in all treatment modalities except in the EDTA and EMD-treated defects where the defect remained open during healing. Regenerated tissues were found up to the furcation fornix only in defects treated with GTR or a combination of GTR and EMD. In all cases of GTR and GTR+EMD the new collagen fibres were inserting the cementum surface in perpendicular direction. However, the number of fibres per 100 μm, in all treated sires was less than the pristine sites. This is similar to results obtained in 6 months in other animal models testing GTR in dehiscence [7] or infrabony [8] defects. In those studies observation periods of up to 2 years were required in order for the number of the inserted collagen fibres to reach an amount closer to the one of the pristine areas. In our model in the pristine areas the number of fibres was slightly higher in zone 5 (alveolar bone margin) compared to zone 6 (more apically). This difference may be attributed to the total number of fibres at the level of the alveolar bone margin is the sum of the number of Sharpey's fibres in addition to the number of gingival fibres (since at this histological level it is difficult to differentiate between the origins of each collagen fibre).

The findings of this study indicate that periodontal regeneration of furcation grade III defects might be possible up to a certain extent at the central part of the defect but the outcome remains unpredictable in terms of complete defect closure. The only treatments that might have the ability to regenerate the periodontal tissues up to the furcation fornix were the ones that used barrier membranes alone (GTR) or in combination with EMD (GTR+EMD). In all cases, the new cementum was thinner than the pristine one and in the majority; it was mixed stratified with external Sharpey's fibres. The number of periodontal ligament fibres was less than in the pristine tissues.

The tissues that are produced from the three regenerative modalities used in the current study (GTR, GTR+EMD, EMD) can lead to "true" periodontal tissues regeneration to a different extent. Those regenerated tissues are still under maturation 5 months after the regenerative operation.

On a clinical level, the outcomes of a regenerative procedure are evaluated based on clinical attachment level gain and in the case of furcation defects in horizontal and vertical probing changes, that are crudely expressed in change of defect degree. Especially for the degree III furcation defects, only a change to degree I or 0 would provide significant clinical benefit, in terms of risk of progression of the disease [23]. Therefore, although mandibular grade III furcation defects seem to be, according to the results from this study, amenable to regeneration with the use of barrier membranes (alone or in combination with Emdogain) the clinical outcomes remain unpredictable [24], with most common risks partial or no filling of the furcation defect.

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Esposito M, Grusovin MG, Coulthard P, Worthington HV (2005) Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. Cochrane Database Syst Rev (4)
2. Needleman I, Worthington H, Giedrys-Leeper E, Tucker R (2006) Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev 19(2)
3. Jepsen S, Eberhard J, Herrera D, Needleman I (2002) A systematic review of guided tissue regeneration for periodontal furcation defects. What is the effect of guided tissue regeneration compared with surgical debridement in the treatment of furcation defects? J Clin Periodontol 29(Suppl 3):103–116
4. Jepsen S, Heinz B, Jepsen K, Arjomand M, Hoffmann T, Richter S, Reich E, Sculean A, Gonzales JR, Bodeker RH, Meyle J (2004) A randomized clinical trial comparing enamel matrix derivative and membrane treatment of buccal Class II furcation involvement in mandibular molars. Part I: study design and results for primary outcomes. J Periodontol 75:1150–1160
5. Araújo M, Berglundh T, Lindhe J (1996) The periodontal tissues in healed degree III furcation defects. An experimental study in dogs. J Clin Periodontol 23:532–541
6. Araújo MG, Berglundh T, Lindhe J (1997) On the dynamics of periodontal tissue formation in degree III furcation defects. An experimental study in dogs. J Clin Periodontol 24:738–746

7. Graziani F, Laurell L, Tonetti M, Gottlow J, Berglundh T (2005) Periodontal wound healing following GTR therapy of dehiscence-type defects in the monkey: short-, medium- and long-term healing. J Clin Periodontol 32:905–914
8. Laurell L, Bose M, Graziani F, Tonetti M, Berglundh T (2006) The structure of periodontal tissues formed following guided tissue regeneration therapy of intra-bony defects in the monkey. J Clin Periodontol 33:596–603
9. Hammarström L, Heijl L, Gestrelius S (1997) Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. J Clin Periodontol 24:669–677
10. Heijl L (1997) Periodontal regeneration with enamel matrix derivative in one human experimental defect. A case report. J Clin Periodontol 24:693–696
11. Araújo MG, Lindhe J (1998) GTR treatment of degree III furcation defects following application of enamel matrix proteins. An experimental study in dogs. J Clin Periodontol 25:524–530
12. Sculean A, Donos N, Windisch P, Brecx M, Gera I, Reich E, Karring T (1999) Healing of human intrabony defects following treatment with enamel matrix proteins or guided tissue regeneration. J Periodontal Res 34:310–322
13. Sculean A, Donos N, Brecx M, Reich E, Karring T (2000) Treatment of intrabony defects with guided tissue regeneration and enamel–matrix proteins. An experimental study in monkeys. J Clin Periodontol 27:466–472
14. Sculean A, Donos N, Brecx M, Karring T, Reich E (2000) Healing of fenestration-type defects following treatment with guided tissue regeneration or enamel matrix proteins. An experimental study in monkeys. Clin Oral Investig 4:50–56
15. Donos N, Sculean A, Glavind L, Reich E, Karring T (2003) Wound healing of degree III furcation involvements following guided tissue regeneration and/or Emdogain. A histologic study. J Clin Periodontol 30:1061–1068
16. Bosshardt DD, Sculean A, Windisch P, Pjetursson BE, Lang NP (2005) Effects of enamel matrix proteins on tissue formation along the roots of human teeth. J Periodontal Res 40:158–167
17. Bosshardt DD, Sculean A, Donos N, Lang NP (2006) Pattern of mineralization after regenerative periodontal therapy with enamel matrix proteins. Eur J Oral Sci 114(Suppl 1):225–231
18. Schroeder HE, Münzel-Pedrazzoli S (1973) Correlated morphometric and biochemical analysis of gingival tissue. Morphometric model, tissue sampling and test of stereologic procedures. J Microsc 99(3):301–329
19. Gestrelius S, Andersson C, Johansson AC, Persson E, Brodin A, Rydhag L, Hammarstrom L (1997) Formulation of enamel matrix derivative for surface coating. Kinetics and cell colonization. J Clin Periodontol 24:678–684
20. Listgarten MA (1972) Electron microscopic study of the junction between surgically denuded root surfaces and regenerated periodontal tissues. J Periodontal Res 7:68–90
21. Bosshardt DD, Nanci A (2003) Immunocytochemical characterization of ectopic enamel deposits and cementicles in human teeth. Eur J Oral Sci 111:51–59
22. ElDeeb ME, Andreasen JO (1991) Histometric study of the effect of occlusal alteration on periodontal tissue healing after surgical injury. Endod Dent Traumatol 7:158–163
23. McGuire MK, Nunn ME (1996) Prognosis versus actual outcome. III. The effectiveness of clinical parameters in accurately predicting tooth survival. J Periodontol 67:666–674
24. Donos N, Glavind L, Karring T, Sculean A (2004) Clinical evaluation of an enamel matrix derivative and a bioresorbable membrane in the treatment of degree III mandibular furcation involvement: a series of nine patients. Int J Periodontics Restor Dent 24:362–369