Clin Oral Invest (2005) 9: 58–64
DOI 10.1007/s00784-004-0288-x

ORIGINAL ARTICLE

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Formation of human cementum following different modalities of regenerative therapy

Received: 27 July 2004 / Accepted: 16 September 2004 / Published online: 6 January 2005

Abstract The aim of the present study was to compare newly formed cementum following different types of regenerative therapy in humans. Eighteen patients, each displaying one advanced intrabony defect around teeth scheduled for extraction, were included in this study. The defects were treated with either guided tissue regeneration (GTR), enamel matrix protein derivative (EMD), EMD plus bioactive glass, bovine-derived xenograft (BDX), BDX plus GTR, or BDX plus EMD. After healing, the teeth were removed together with their surrounding soft and hard tissues. Cellular content, presence of artifactual splits between the new cementum and the old one or the dentin surface, and thickness of the new cementum were evaluated. Irrespective of treatment, the new cementum was of a reparative, cellular, extrinsic and intrinsic fiber type. There were no differences in cementum thickness among treatments. These findings indicate that in humans, (a) the new cementum formed after different types of regenerative therapy was, irrespective of the treatment, of a reparative, cellular, extrinsic and intrinsic fiber type, and (b) the regenerative modality does not seem to influence the type of newly formed cementum.

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Keywords Bone grafts · Enamel matrix derivative · Guided tissue regeneration · Human cementum · Periodontal regeneration

Introduction

The goal of regenerative periodontal therapy is to reform a tooth's supporting tissues which have been lost following dental trauma or periodontal disease [15]. Histologically, regenerative periodontal therapy should result in the formation of new cementum, new periodontal ligament, and new alveolar bone [15]. In humans, periodontal regeneration has been shown to occur following the use of intra- or extraoral autografts, demineralized freeze-dried bone allografts, guided tissue regeneration (GTR), bovine-derived xenografts (BDX), enamel matrix protein derivative (EMD), growth factors, and various combinations of these techniques [3–7, 9–11, 13, 14, 20–22, 24–28, 31–40].

In spite of the key role that formation of new cementum plays in periodontal regeneration, very little is known about its repair and regeneration in humans [1, 29]. So far, the data regarding new cementum following various regenerative modalities in humans are sparse and controversial [11, 13, 19, 20, 21, 32–34]. Findings from previous studies have indicated that, in humans, the cementum formed after treatment with various types of bone grafts or GTR is mainly of a cellular type, and artifacts (i.e., splits between the new cementum and the old one or the dentin surface) are often present [3, 4, 9, 11, 19, 32, 34]. An enamel matrix protein derivative (EMD) has also been introduced as a new modality for predictably achieving periodontal regeneration [12].

The rationale for using EMD was based on observations from studies on tooth development which have indicated that enamel matrix proteins (EMP), synthesized and secreted by cells of the Hertwig's epithelial root sheath, induce the differentiation of dental follicle cells into cementoblasts, which in turn may specifically be responsible for the formation of acellular extrinsic fiber cementum (AEFC), the type which mainly participates in tooth an-

chorage [12]. In those studies, the newly formed cementum appeared to be similar to AEFC, and artifacts were either not or only very sparsely observed [12, 13]. This lack of artifacts was interpreted as additional evidence of the superior quality of EMD-induced cementum to that formed after other regenerative techniques such as GTR [12]. On the other hand, human histological studies evaluating the healing of intrabony defects following treatment with GTR or EMD have indicated that the cementum formed after both treatments was of a predominantly cellular type, with frequent artifacts [32, 33].

Taken together, the data from human biopsies regarding new cementum after different regenerative modalities are still very controversial. Moreover, to the best of our knowledge, none of the available studies has attempted to evaluate this question systematically. Thus, there are virtually no data from human material attempting to quantify parameters such as cementum thickness, cellular content, and the presence of artifacts between the new cementum and the old one or the dentin surface following different regenerative modalities for intrabony defects. Therefore, the aim of the present study was to shed more light on this subject.

Material and methods

Study structure and patients

Eighteen patients suffering from advanced marginal periodontitis and displaying one advanced intrabony defect each were included in the study. All 18 teeth were scheduled for extraction due to advanced periodontitis and/or further prosthetic considerations. All patients volunteered for the study and received verbal and written information about its purpose, possible risks, and the possibility to withdraw at any time. In every case, written informed consent was obtained prior to the start of the study.

The study protocol was approved by the ethics committee of Semmelweis University of Medicine in Budapest, Hungary. Two to 3 months before surgery, all patients received oral hygiene instructions and full-mouth supra- and subgingival scaling in order to reduce soft-tissue inflammation to a minimum. Furthermore, to reduce mobility when needed, the teeth were included in temporary bridge reconstructions or splinted with orthodontic wires. Prior to and 6 months after the surgical procedures, plaque index, gingival index [18], pocket depth, gingival recession, and clinical attachment level were recorded (Table 1).

Surgical procedures and postoperative care

All surgical procedures were performed under local anesthesia. Following intracrevicular incisions, mucoperiosteal flaps were raised at both the vestibular and lingual aspects of the teeth. After removing all granulation tissue from the bone defects, the root surfaces were scaled and planed by means of hand and ultrasonic instruments. Notches were prepared in the root surfaces using a small round bur (2 mm in diameter) to indicate the most apical level of the calculus or the bottom of the defect in cases where no calculus was present. Thus, any periodontal ligament tissue which might be present on the root surface coronally from the notch was considered de novo formed connective tissue.

During surgery and after complete removal of granulation tissue from the defects, the following measurements were made: distance from cementoenamel junction to the bottom of the defect (CEJ-BD) and distance from CEJ to the most coronal extension of the alveolar bone crest (CEJ-BC). The intrabony component (INTRA) of the defects was defined as (CEJ-BD)–(CEJ-BC). After thorough rinsing of the wound with sterile saline, three defects each were assigned to the following treatment groups:

1. GTR
2. EMD
3. EMD plus bioactive glass (BG)
4. BDX (bovine-derived xenograft) plus GTR
5. BDX
6. EMD+BDX

In the GTR group, a bioabsorbable membrane (Resolut) (Gore, Flagstaff, Ariz., USA) of appropriate configuration was selected, trimmed, and fitted to the defect in such a manner that the entire defect and 2–3 mm of the surrounding alveolar bone were covered. The membrane was fixed to the affected tooth or neighboring teeth with bioabsorbable sutures (Dexon II) (Davis and Geck, Manati, P.R.).

In the defects receiving treatment with EMD, EMD+BG, or EMD+BDX, the root surfaces were conditioned for 2 min with a 24% ethylenediamine tetra-acetate (EDTA) gel (PrefGel) (Biora, Malmö, Sweden) according to the instructions given by the manufacturer. The EDTA residues were removed by copious rinsing with sterile saline. The EMD gel (Emdogain) (Biora) was then applied to the root surfaces and the defects with a sterile syringe. In defects treated with EMD+BG (Perioglas) (U.S. Biomaterials, Alachua, Fla., USA) or EMD+BDX (Bio-Oss) (Geistlich, Wolhusen, Switzerland), the remaining EMD

Table 1 Clinical characteristics of the treated defectsprior to and during surgery (means±SD). *PI* plaque index,*GI* gingival index, *INTRA* intrabony component, *PD* pocket depth, *CAL* clinical attachment level

Treatment	PI	GI	INTRA (mm)	Baseline PD (mm)	Baseline CAL (mm)
GTR	0.8±0.6	1.4±0.5	5.3±1.2	12.0±1.6	14.3±1.2
EMD	0.9±0.7	1.5±0.8	4.3±0.5	11.7±2.1	12.3±2.5
EMD+BG	1.0±0.5	1.3±0.6	5.0±0.8	10.3±1.2	12.0±0.8
BDX+GTR	0.9±0.7	1.6±0.7	5.0±0.8	9.7±1.2	12.0±0.8
BDX	0.8±0.8	1.6±0.5	5.0±0.8	11.3±1.2	12.0±0.8
EMD+BDX	0.7±0.6	1.5±0.7	6.3±0.5	12.0±1.6	13.0±1.4

was mixed with the respective graft material (either BDX or BG). Care was taken not to overfill the defects. At the defects treated with GTR, BDX, or BDX+GTR, no root surface conditioning was performed.

In the BDX group, the defects were filled with the graft material only, whereas in the BDX+GTR group, a bioresorbable collagen membrane of porcine origin (BioGide Perio) (Geistlich) was additionally placed over the defect so as to cover 2–3 mm of the surrounding alveolar bone and ensure stability of the graft material. No sutures or pins were used for membrane fixation or stabilization.

Finally, in all groups, the mucoperiosteal flaps were repositioned coronally and fixed with vertical or horizontal mattress sutures. Postoperative care consisted of administration of antibiotics for 1 week (1 g/day of amoxicillin) and rinsing with 10 ml of a 0.2% chlorhexidine solution twice a day for 6 weeks. The sutures were removed 14 days following surgery. Recall appointments associated with professional tooth cleaning were performed once per week for the first 4 weeks and once per month for the remaining period. No subgingival instrumentation in the operated areas was performed during the entire experimental period of 6 months.

Biopsy removal and histological preparation

Following local anesthesia, paramarginal incisions were performed and full-thickness mucoperiosteal flaps were raised. The teeth were then removed together with their surrounding soft and hard tissues. After postsurgical healing, all patients received complete prosthodontic treatment.

Immediately upon removal, the biopsies were fixed in 10% buffered formalin, decalcified in EDTA, dehydrated, and fixed in paraffin. Mesiodistal serial sections were cut parallel to the long axes of the teeth with the microtome set at 8 μm. The sections were stained alternatively with hematoxylin and eosin, van Giesson's connective tissue stain, Ladevig's connective tissue stain, or the oxytalan-aldehyde-fuchsin-Halmi method [30]. Histological evaluation was performed by one blinded investigator. Only the sections representing central parts of the defects were selected for this purpose. In an attempt to provide quantitative data, the following measurements and semiquantitative analysis were performed: (1) thickness of new cementum in μm, (2) presence of artifacts, and (3) number of cells in the new cementum.

Results

The surgical procedure, postoperative care, clinical results, and linear histologic measurements (i.e., height of new cementum and new bone) for most of the defects included in this study have been reported previously [32, 34–36]. However, three defects were treated exclusively for this paper (one in the EMD+BDX group and two in the BDX group). Attempts were also made to include defects with comparable clinical and histometric characteristics (Table 1). For this reason, only three defects per group were included. The clinical characteristics of the treated defects prior to and during surgery are presented in Table 1. All treated defects were combined one-to-two-walled defects.

Briefly, the healing following all six different types of regenerative treatment resulted to a varying extent in formation of cementum, periodontal ligament, and bone. Neither ankylosis nor root resorption was observed. The new cementum displayed a predominantly cellular character and comparable thickness in all six treatment groups (Figs. 1, 2, 3, 4, 5, 6) (Table 2). Collagen fibers were observed to run parallel but also to insert into the newly formed cementum, irrespective of the treatment. Artifacts were observed in all biopsies.

Discussion

The results of the present comparative study show that, in humans, the cementum formed after different types of regenerative modalities was, irrespective of the treatment provided, of a reparative, cellular, extrinsic and intrinsic fiber type. The newly formed cementum was of comparable thickness in all six treatment groups, varying from 150.0±57.2 μm to 200.0±29.4 μm. These findings are in contradiction to those from previous investigations which indicated that new cementum formed after EMD treatment was of a predominantly acellular extrinsic fiber type and with no artifacts [13, 20].

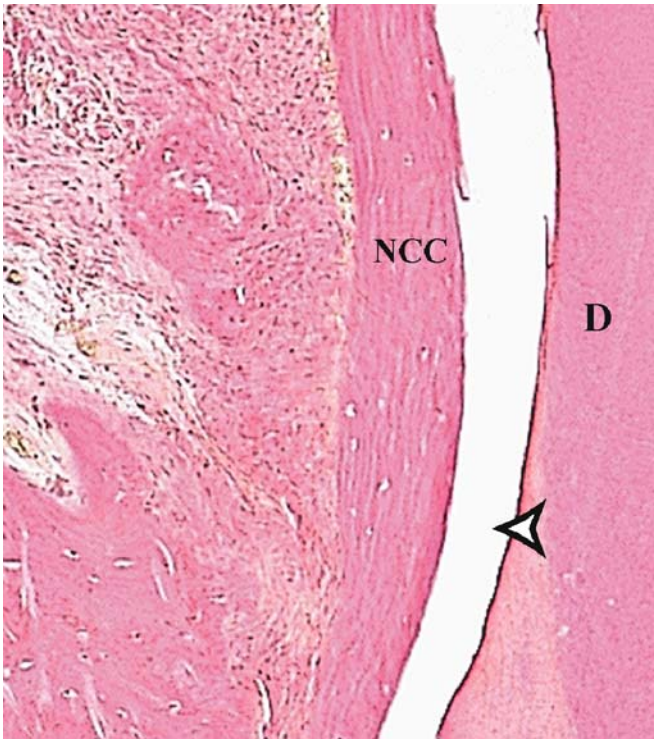

Fig. 1 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with GTR. *Arrowhead* indicates the presence of an artifact. *D* dentin. Original magnification ×350

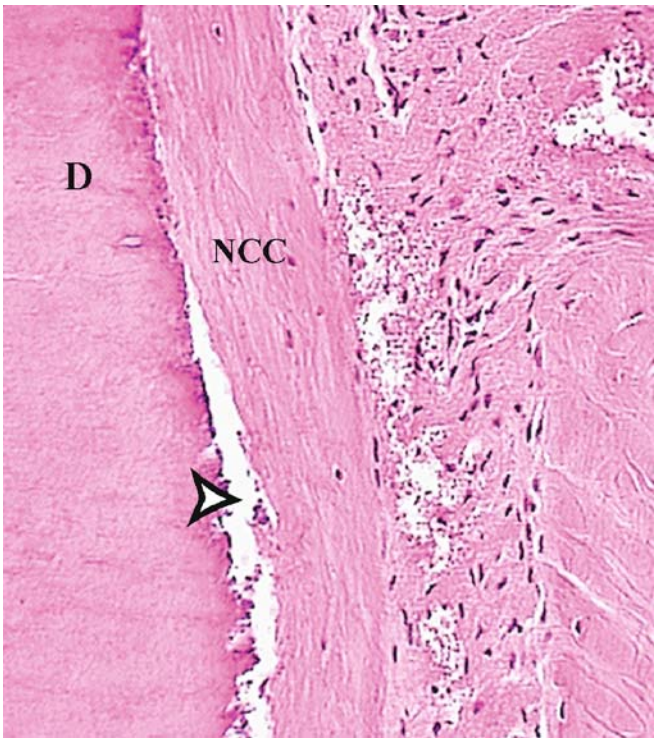

Fig. 2 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with EMD. *Arrowhead* indicates the presence of an artifact. *D* dentin. Original magnification ×350

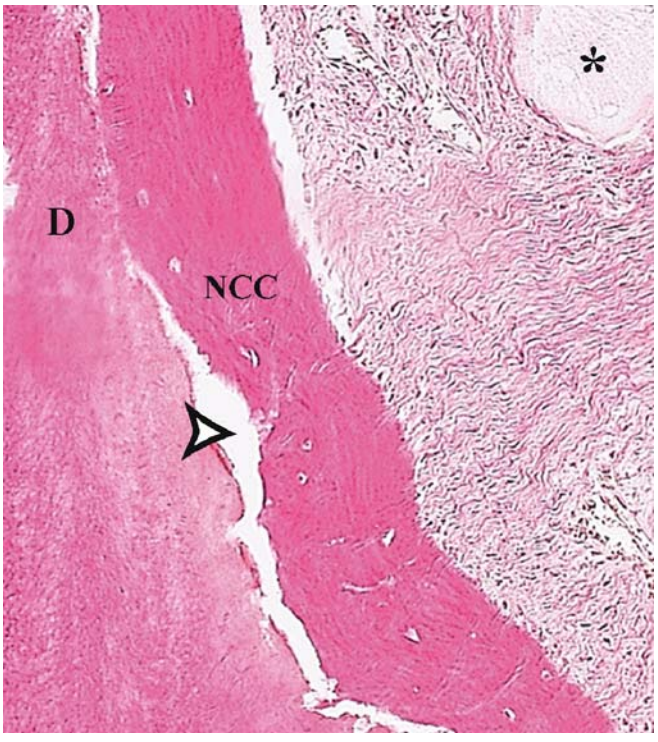

Fig. 4 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with BDX+GTR. *Arrowhead* indicates the presence of an artifact. *D* dentin, * BDX particle. Original magnification ×350

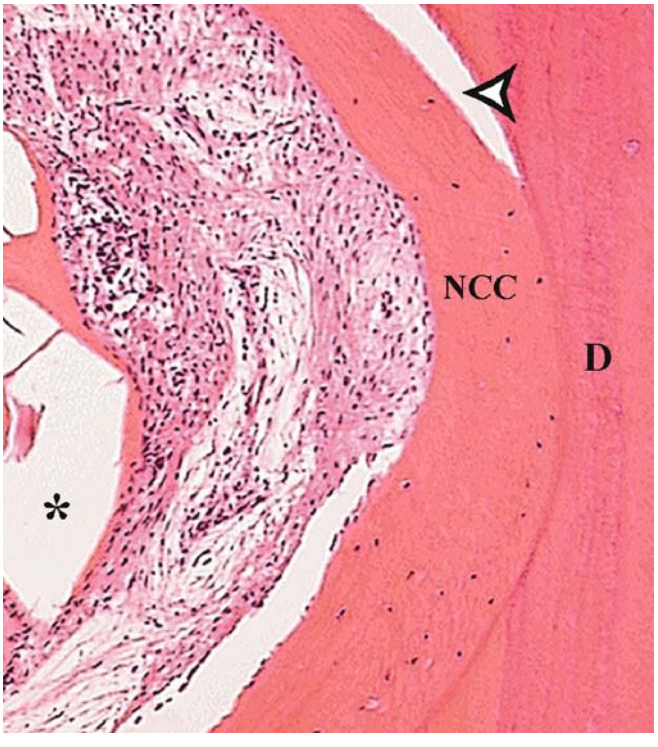

Fig. 3 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with EMD+BG. *Arrowhead* indicates the presence of an artifact. *D* dentin, * BG particle. Original magnification ×350

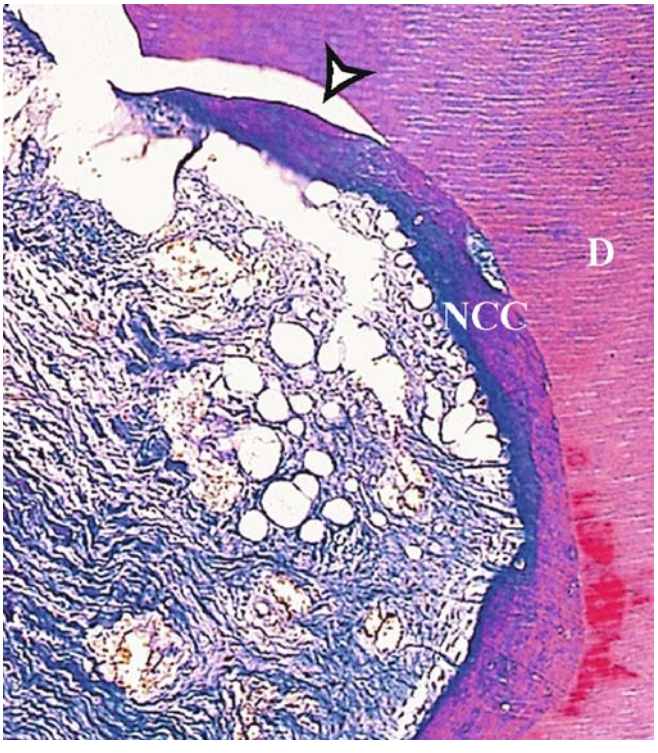

Fig. 5 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with BDX. *Arrowhead* indicates the presence of an artifact. *D* dentin. Original magnification ×350

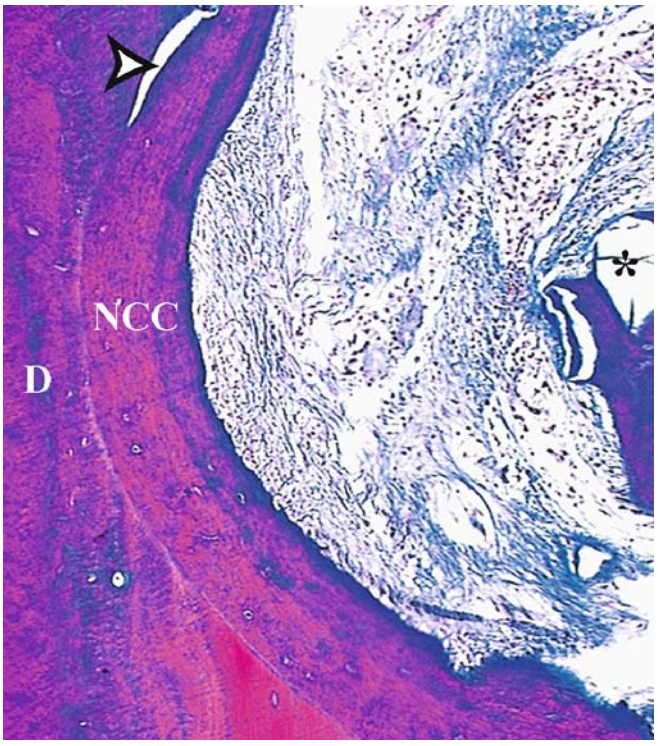

Fig. 6 Photomicrograph of newly formed cellular cementum (*NCC*) following treatment with EMD+BDX. *Arrowhead* indicates the presence of an artifact. *D* dentin, * BDX particle. Original magnification ×350

Table 2 Analysis of newly formed cementum following regenerative periodontal therapy

Treatment	Thickness of cementum in μm (mean±SD)	Artifacts	*N* single cells in cementum
GTR	193.3±69.4	Present	>10
EMD	166.7±23.6	Present	>10
EMD+BG	150.0±57.2	Present	>10
BDX+GTR	160.0±43.2	Present	>10
BDX	173.3±17.0	Present	>10
EMD+BDX	200.0±29.4	Present	>10

Based on the above observations, it was suggested that treatment with EMD may predictably enhance the formation of an acellular type of cementum closely resembling AEFC [12]. However, if this assumption were true, treatment with EMD should promote the formation of acellular cementum, while the use of any other regenerative techniques should yield a cellular (i.e., reparative) type. The present results, however, failed to reveal any difference between the different regenerative modalities in terms of cellular content and presence of artifacts. On the other hand, these findings are in agreement with those from an electron microscopic study evaluating the nature and attachment of cementum formed after GTR treatment in humans [19] revealing that the new cementum bore a close resemblance to cementum formed during spontaneous repair of root resorption.

The present results are also in line with very recent findings from a study investigating the association between Hertwig's epithelial rooth sheath cells, enamel matrix proteins (EMP), and cementogenesis in porcine teeth. That study failed to demonstrate a causal link between EMP and the formation of AEFC [2]. In this context, it should also be pointed out that results from previous studies on cementogenesis in humans indicate that the formation of AEFC is an extremely slow process.

Zander and Hürzeler [41] measured the cementum thickness in 233 teeth from patients between 11 and 76 years old and concluded that, over decades, the appositional rate of AEFC is on average 1.7–3.9 μm/year. Comparable findings were reported by Dastmalchi et al. [8], who calculated that in erupted human premolars and molars, AEFC increases in thickness by an average of 2.9 μm/year, while Bosshardt and Schroeder [1] showed that, in human premolars, it grew in thickness by 2.0–2.5 μm/year.

In contrast to the slow formation of AEFC, cellular cementum forms very rapidly. It was suggested that the reason for cell (cementocyte) incorporation into the reformed cementum may be dependent upon the speed of cementum formation [1]. This view seems to be corroborated by the present results, in which a rather high amount of new cementum was formed in a relatively short period of time (6 months).

Together with the data from the literature, the present findings indicate that, in humans, AEFC does not seem to form predictably after any of the regenerative modalities used. Furthermore, the fact that no differences in cementum thickness and cellular content were observed between the six different regenerative therapies may indicate that, once the process of periodontal wound healing is initiated, the resulting cementum is, irrespective of treatment modality, always of a reparative, cellular, extrinsic and intrinsic fiber type.

The fact that splits occurred in all treatment groups may indicate that, in humans, the type of regenerative therapy itself does not seem to influence (i.e., reduce) significantly the occurrence of such artifacts. It should be kept in mind that the significance of such histological artifacts for the clinical outcome of treatment is still controversially discussed in the literature.

While some authors consider the presence of splits between the new cementum and the old one or dentin to result mainly from the decalcification process during histological preparation and do not necessarily reflect poor quality of the regenerated tissues [17], others have interpreted similar findings as representing a weakness which might negatively affect the supporting apparatus of the tooth [23]. It is, however, unknown to what extent the type of new cementum and the presence of artifacts may affect the clinical outcome of the therapy.

In this context, a recent monkey experiment compared the susceptibility of GTR-regenerated periodontal attachment after ligature-induced periodontitis with that of pristine periodontium [16]. The histologic analysis indicated that the root surfaces treated with GTR were covered

by newly formed cementum of the reparative, cellular, extrinsic and intrinsic fiber type, while the cementum on pristine roots was mainly AEFC. However, the results failed to show that teeth with a periodontal attachment apparatus formed by GTR are more susceptible to periodontitis than those with pristine periodontium.

It is also important to emphasize that, to the best of our knowledge, no other studies have attempted systematically to evaluate and compare newly formed cementum following different regenerative therapies, and thus direct comparisons with other studies are not possible.

Conclusions

The present findings indicate that in humans (1) the new cementum formed after six different types of regenerative therapy was, irrespective of treatment, of a reparative, cellular, extrinsic and intrinsic fiber type and (2) the regenerative modality does not seem to influence the type of newly formed cementum.

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