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Histological characteristics of newly formed cementum in surgically created one-wall intrabony defects in a canine model

Jung-Chul Park, Yoo-Jung Um, Ui-Won Jung, Chang-Sung Kim, Seong-Ho Choi, Chong-Kwan Kim*

Department of Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, Seoul, Korea

Purpose: Periodontal regenerative therapies for defects created by severe periodontitis are mainly focused on bone regeneration. Although cementum regeneration needs to be better understood, it is believed to play an important role in periodontal regeneration. The first step toward a full understanding of cementum regeneration is to compare repaired cementum to pristine cementum. This study, which used histological techniques, was designed to focus on cementum regeneration and to compare pristine cementum to repaired cementum after surgical procedures with 8 and 24 week healing periods in a canine model.

Methods: Buccal and lingual mucoperiosteal flaps of 10 beagle dogs were surgically reflected to create critical-sized defects. Intrabony one-wall defects, of which dimension is 4 mm width and 5 mm depth, were made at the distal aspect of mandibular second premolars and the mesial aspect of mandibular fourth premolars in the right and left jaw quadrants. Animals were sacrificed after 8 and 24 weeks post-surgery for histological specimen preparation and histometric analysis.

Results: The repaired cementum was composed mostly of acellular cementum and cellular mixed fiber cementum and was thicker in the apical area than in the coronal area. The acellular cementum of the supracrestal area appeared to be amorphous. The newly formed cellular cementum was partially detached from the underlying circumpulpal dentin, which implied a weak attachment between new cementum and dentin, and this split was observed to a lesser extent in the 24 week group than in the 8 week group. The vertical height of the repaired cementum was greater in the 24 week group than in the 8 week group.

Conclusions: Within the limitations of this study, we can conclude that repaired cementum after root planing was mainly acellular cementum and cementum tissue that matured to a shape similar to pristine cementum as the healing progressed from 8 to 24 weeks.

Keywords: Animal models, Dental cementum, Periodontal guided tissue regeneration.

INTRODUCTION

Periodontitis is a common infectious disease of periodontal tissue. During the 1950s and the 1960s, resective surgical therapy with or without osseous recontouring was regarded as the standard treatment based on the belief that retention of shallow pocket depth was important. Now however, attention has shifted from resective surgeries to regenerative and reconstructive therapies.

The final goal of regenerative periodontal therapy is to restore the structure and function of the periodontium destroyed or lost due to periodontitis, and this includes the for-

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***Correspondence:** Chong-Kwan Kim

Department of Periodontology, Yonsei University College of Dentistry, Shinchon-dong, Seodaemun-gu, Seoul 120-752, Korea

E-mail: ckkim@yuhs.ac, Tel: +82-2-2228-3185, Fax: +82-2-392-0398

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mation of a new connective tissue attachment, new cementum and supporting bone [1]. When considering periodontal regeneration, it is important to note that at least four criteria must be met in order for regeneration to occur. First, a functional epithelial seal must be reestablished at the most coronal portion of the tissues. Second, new connective tissue fibers must be inserted into the previously exposed root surface to reproduce both the periodontal ligament and the dentogingival fiber complex. Third, new acellular extrinsic fiber cementum (AEFC) must be reformed on the previously exposed root surface. Lastly, alveolar bone height must be restored to within 2 mm of the cementoenamel junction [2].

A critical step toward achieving periodontal regeneration is the new attachment of connective tissue fibers to the previously contaminated root surface. In this process, root cementum plays an important role, because it invests and securely attaches the periodontal ligament fibers to the root surface. Although cementum tissue was first described in 1835 [3], it has remained a poorly defined tissue. To the best of our current knowledge, cementum is a histologically unique tissue [4,5]. It does not have the lamellar organization found in bone, is avascular, is not innervated, does not contain bone marrow, and does not undergo physiological remodeling [6]. There are basically two varieties of cementum, which are distinguished on the basis of the presence or absence of cells within it and the origin of the collagen fibers of the matrix. The first type of cementum is AEFC which is found on the cervical half to two thirds of the root. The other form of cementum is cellular intrinsic fiber cementum (CIFC). This type is distributed along the apical third to half of the root and functions as a repair tissue that fills resorptive defects and root fractures. CIFC constitutes the intrinsic component of cellular mixed fiber cementum (CMFC), which possesses a stratification that is derived from consecutively deposited, alternating layers of AEFC and CIFC.

To the best of our current knowledge, the predictability and quality of cementum regeneration in everyday clinical situations appear to be low [2,7]. Ideally, the regenerated cementum should closely resemble the AEFC, because it contributes mostly to the attachment function [5,8]. However, in most periodontal regeneration studies, the quality of the attachment function is questionable, because the newly formed cementum is cellular, the numerical density of inserting fibers is low, and the interfacial tissue bonding appears to be weak [9-13]. It has further been suggested that new "reparative cementum" is of osteoblast origin and, hence, resembles bone tissue [11,14].

There have been several experimental studies designed to characterize and compare regenerated tissue with pristine periodontal tissues [15-17]. These results showed that the new cementum was in fact different from the pristine cementum and it was suggested that the healing after guided tissue regeneration had the characteristics of 'reparative' rather than 'regenerative' healing. On the other hand, there have been other studies showing that the healing was regenerative [18]. Also, Graziani et al. [19] reported that healing from 6 weeks to 2 years resulted in pristine tissue-like periodontal tissues. In these results, after 2 years of healing, the new cementum consisted of a narrow band of AEFC bound to the circumpulpal dentin and was covered by relatively thicker CMFC.

There has been little study, however, of the histological characteristics after a natural healing period of 8 and 24 weeks without any regenerative therapy. Although a 24 week healing period would not result in the same maturation of periodontium as in a 2 year healing period, the difference between naturally repaired periodontium and intentionally regenerated periodontium would indicate the process of regeneration. The aim of this study, which used histological techniques, was to focus on cementum regeneration and to compare pristine cementum and repaired cementum after surgical procedures with 8 and 24 week healing periods in canine models.

MATERIALS AND METHODS

Animal model

Ten male Beagle dogs, with an approximate mean age of 15 months and weight of 15 kg, obtained from experimental animal breeder (Samtako Co., Osan, Korea), were used. The animals were divided into 2 experimental groups with different healing periods—one of 8 weeks and one of 24 weeks. Upon their receipt, a health examination of the animals was performed. The animals were examined for any abnormalities in posture or movement. Only healthy animals were included in the study. The animals were acclimatized for 1 week prior to experimentation.

Animal selection and management, surgery protocol, and periodontal defect preparation followed a study protocol approved by the Institutional Animal Care and Use Committee, Yonsei University, Seoul, Korea. The animals were housed in stainless steel cages with a floor area of 7,056 cm^2 and a height of 60 cm labeled with cards identifying study number, species/strain, sex, cage number, and animal ID. The cages were housed in an air conditioned room with 10 to 20 air changes/hours. The temperature was 22±3°C and the relative humidity 50 to 60%. Temperature and humidity was monitored daily. A light/dark cycle of 12 hours light/12 hours darkness was applied.

The animals had *ad libitum* access to water and a pelleted laboratory diet with the exception of one week immediately postextraction and postsurgery when they were fed a diet of canned soft dog food.

Surgical protocol

Food was withheld the night preceding surgery. The surgical procedure was performed under general anesthesia induced by intravenous injection of 0.04 mg/kg atropine (Kwangmyung Pharm., Seoul, Korea) and intramuscular injection of a combination of xylazine (Rompun™, Bayer Korea Co., Seoul, Korea) and ketamin (Ketara™, Yuhan Co., Seoul, Korea) followed by inhalation anesthesia (Gerolan™, Choongwae Pharm., Seoul, Korea). Routine dental infiltration anesthesia was used at the surgical sites.

The mandibular first premolars (P1) and third premolars (P3) were extracted prior to the experimental surgery and the extraction sites were allowed to heal for 2 months. The remaining dentition received oral prophylaxis in conjunction with the extractions.

The experimental surgery included elevation of buccal and lingual mucoperiosteal flaps to surgically create critical-size, "box-type" (4 mm width and 5 mm depth), one-wall, intrabony defects at the distal aspect of mandibular second premolars (P2) and the mesial aspect of mandibular fourth premolars (P4) in the right and left jaw quadrants [20]. Following root planing, a reference notch was made with a round bur on the root surface at the base of the defect (Fig. 1). In five animals from the 8 weeks observation group, defects were made unilaterally and contralateral sites received treatments reported elsewhere. In another five animals from the 24 weeks observation group, defects were made unilaterally and sham surgeries were done only on the mesial side of P4. The distal side of P2 and the contralateral sides received treatments reported elsewhere. Pristine tissue was evaluated from the mesial side of P2.

The mucogingival flaps were advanced, adapted, and sutured using a resorbable suture material (Vicryl® 5.0, Ethicon Inc., Somerville, USA). Therefore, no regeneration techniques of any kind were applied and only sham surgeries were performed. Radiographs of the defect sites were taken immediately postsurgery and at the day of euthanasia.

Postsurgery care

Postsurgery care included intramuscular administration of Cefazoline Sodium with 20 mg/kg dosage (Yuhan Co., Seoul, Korea) and daily topical application of a 0.2% chlorhexidine solution (Hexamedin®, Bukwang Pharm., Seoul, Korea) for infection control. Observations of the experimental sites to monitor for gingival health, suture line closure, edema, and evidence of tissue necrosis or infection were made daily until suture removal and at least twice weekly thereafter.

Euthanasia

The animals were euthanized at week 8 and 24 postsurgery using an 90 to 120 mg/kg IV injection of pentobarbital (Yuhan Co., Seoul, Korea). Block sections including defect sites and surrounding alveolar bone and mucosal tissues were collected. The block specimens were rinsed in sterile saline and were immersed in 10% neutral buffered formalin at a volume 10 times that of the block section for 10 days.

Histological preparation

After rinsing in sterile water, the sections were decalcified in 5% formic acid for 14 days, trimmed, dehydrated in a graded ethanol series, and embedded in paraffin. Step-serial sections, 5-μm thick, were cut in a mesial-distal vertical plane, at approximately 80-μm intervals. The sections were stained using hematoxylin-eosin stains. The four most central sections of each defect site selected based on the width of the root canal were used for the histological and histometric analysis.

Histological analysis

One experienced masked examiner performed the histopathologic evaluation of the tissue specimens using incandescent and polarized light microscopy (Olympus BH2™ multi-view microscope, Olympus Optical Co., Tokyo, Japan) and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, USA). Four central sections stained with hematoxylin/eosin were evaluated. The follow-

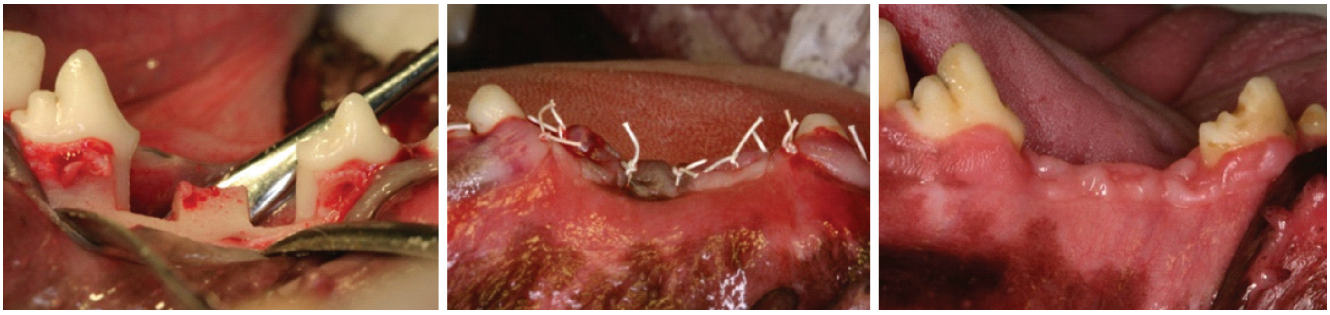

Figure 1. Surgically created, critical-size, one-wall, intrabony, periodontal defect at the distal aspect of mandibular second premolars and the mesial aspect of mandibular fourth premolars. Mucoperiosteal flaps are adapted and sutured for primary intention healing. The right panel shows healing at week 8 postsurgery.

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ing parameters were analyzed:

- Defect height: distance from the cementoenamel junction (CEJ) to the base of the reference notch.
- Junctional epithelium/recession: distance from the CEJ to the apical extension of the junctional epithelium.
- Connective tissue adhesion: distance from the apical extension of the junctional epithelium to the coronal extension of cementum formation.
- Cementum regeneration: distance from the base of the reference notch to coronal extension of the newly formed cementum on the root surface.

Statistical analysis

Summary statistics (mean±SD) based on animal means for the experimental treatments were calculated using the four central sections from each defect, defects being averaged for each site. Animal means were used to test for differences between experimental conditions using one-way analysis of variance and a post hoc test. The level of significance was set at 5%.

RESULTS

Clinical observation

Surgical procedures were uneventful and without complication. Despite the extent and size of the surgically involved areas, wound closure was successfully maintained throughout the experiment for all defects. During this study, no signs of infection or clinical complication were found.

Histological observations

There was no histologically observable pathological tissue reaction at the time of necropsy. However, some cases seemed to cause an initial inflammation of the bone surrounding the intrabony 1-wall defects that were subsequently repaired along with the bone healing of the defects.

Defect height, new cementum, epithelium attachment and connective tissue attachment were measured and the results are shown in Table 1. The periodontal healing is illustrated as a percentage of defect height after 8 and 24 weeks postsurgery in Fig. 2.

In the pristine group, the cementum layer consisted of a narrow zone of AEFC lateral to the peripheral dentin in the upper supracrestal area and a wider zone of CMFC containing both extrinsic and intrinsic collagen fibers in the subcrestal area. Densely packed collagen fibers were inserted perpendicularly into the AEFC in the supracrestal area. The cementum was consistently thicker in its apical portion than in more coronal portions (Fig. 3).

In the 8 week group, a new attachment including new cementum, periodontal ligament (PDL) and bone had formed within the defect compartments. New bone and cementum formations were observed extending coronally to the notch. The measurements are shown in Table 1. Most of the supracrestal cementum was observed as acellular cementum and its shape was amorphous. Newly repaired cementum of the supracrestal and notch areas was CMFC. In some specimens, the coronal extension of the new cementum did not reach the junctional epithelium. In the gap between the coronal extension of the new cementum and the junctional epithelium, collagen fibers were directly attached to the dentin surface. The new cementum was in direct contact with the circumpulpal dentin, the ruffled surface of which indicated transient root resorption. There were a few specimens that showed separation between the new cementum and the underlying den-

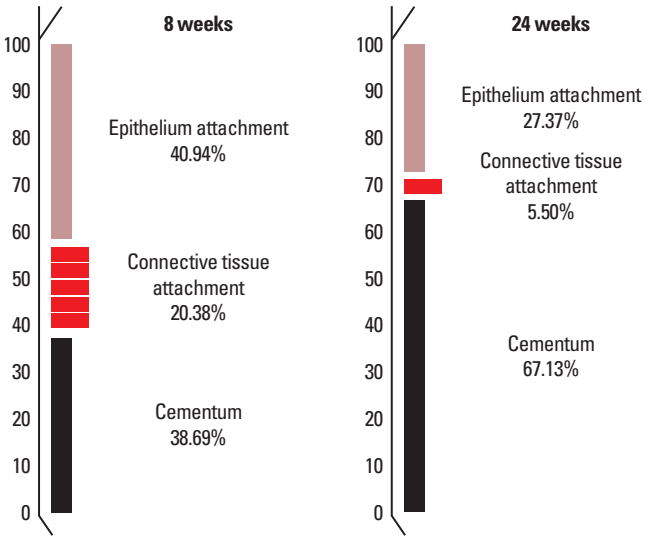

Figure 2. Periodontal healing illustrated as a percentage of defect height after 8 weeks and 24 weeks postsurgery.

Table 1. Defect height (CEJ to apical extension of root instrumentation), thickness of new cementum, epithelium attachment and connective tissue attachment in mm (mean±SD).

	Defect height	Cementum thickness	Epithelium attachment	CT attachment
8 weeks (n=5)	4.83 ± 0.62	1.84 ± 0.50	1.98 ± 0.51	0.99 ± 0.01
24 weeks (n=5)	4.80 ± 0.45	3.28 ± 1.65	1.27 ± 1.15	0.25 ± 0.28

CEJ: cementoenamel junction, CT: connective tissue.

tin. Also, newly formed cementum was consistently thicker in its apical portion than in its more coronal portion (Fig. 4).

In the 24 week group, observations regarding the magnitude of cementum formation were similar to those observed of the 8 week group. New bone and cementum formation were observed extending from the coronal portion to the notch. The newly formed cementum appeared irregular in thickness, generally formed a thin strip along the root surface, and appeared as acellular cementum (Fig. 5).

In the supracrestal compartment, the new cementum was very thin and had the appearance of acellular cementum (Fig. 6). In the areas apical to the bone crest, the new cementum consisted of two layers. A thin layer of acellular cementum which was continuous with the supracrestal AEFC, was covered by a thicker layer of CMFC that contained extrinsic and intrinsic collagen fibers (Fig. 7). Extrinsic fiber bundles extended from the circumpulpal dentin through the new cementum and continued into the new PDL.

Although no significant root resorption was observed along the root surface, small resorption pits were evident. These limited resorption pits were mainly detected on the root surface coronally to the newly formed cementum.

DISCUSSION

It was demonstrated that after 8 and 24 weeks, new connective tissue attachment with new cementum, PDL and bone had formed within the defect. In this study, the focus was on the cementum regeneration. Many studies indicate the important role of cementum in the formation and regeneration of periodontal tissues [8,21,22], because new attachment requires new cementum formation to replace diseased root surfaces contaminated with bacteria.

Cementum regeneration requires cementoblasts. The origin of cementoblasts and the molecular factors regulating their recruitment and differentiation are not fully understood. However, it has been suggested that new cementum is formed by cells which migrate from an existing layer of

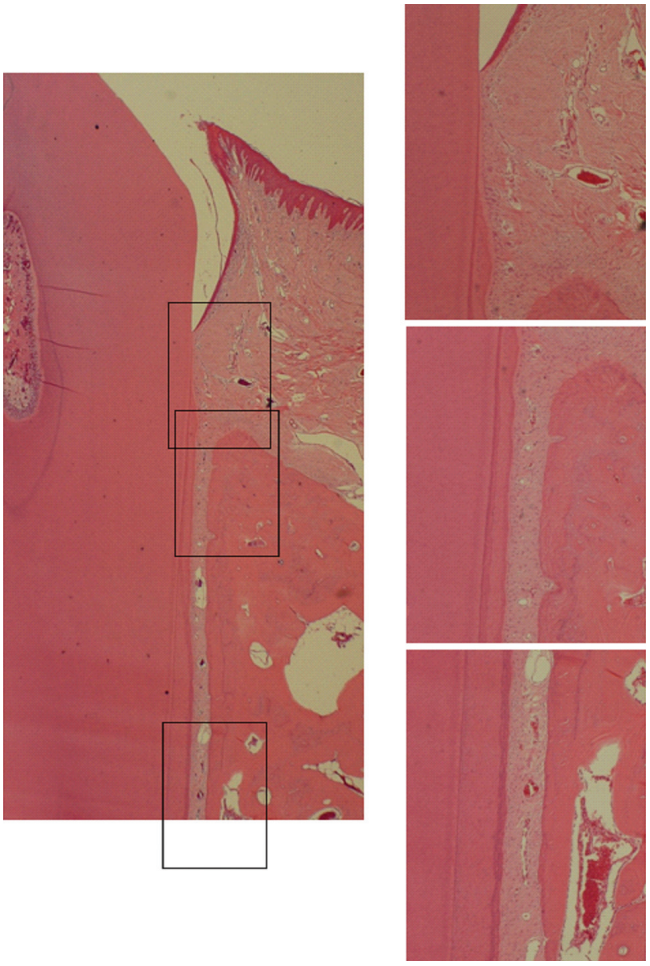

Figure 3. Mesio-distal section of a pristine specimen (H&E stain, left ×20 and right ×200).

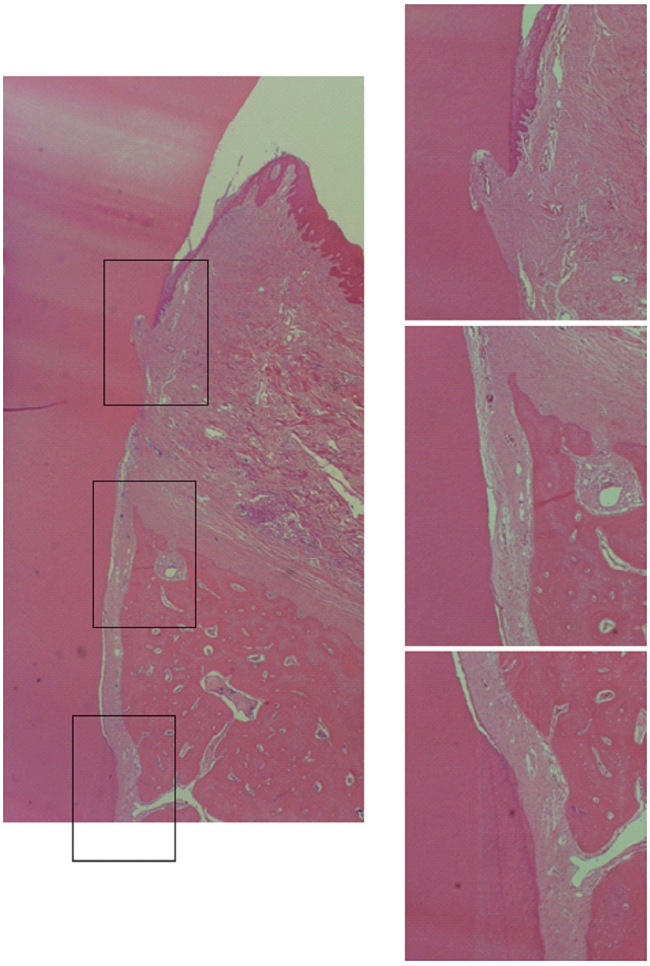

Figure 4. Mesio-distal section of an 8 week specimen (H&E stain, left ×20 and right ×200).

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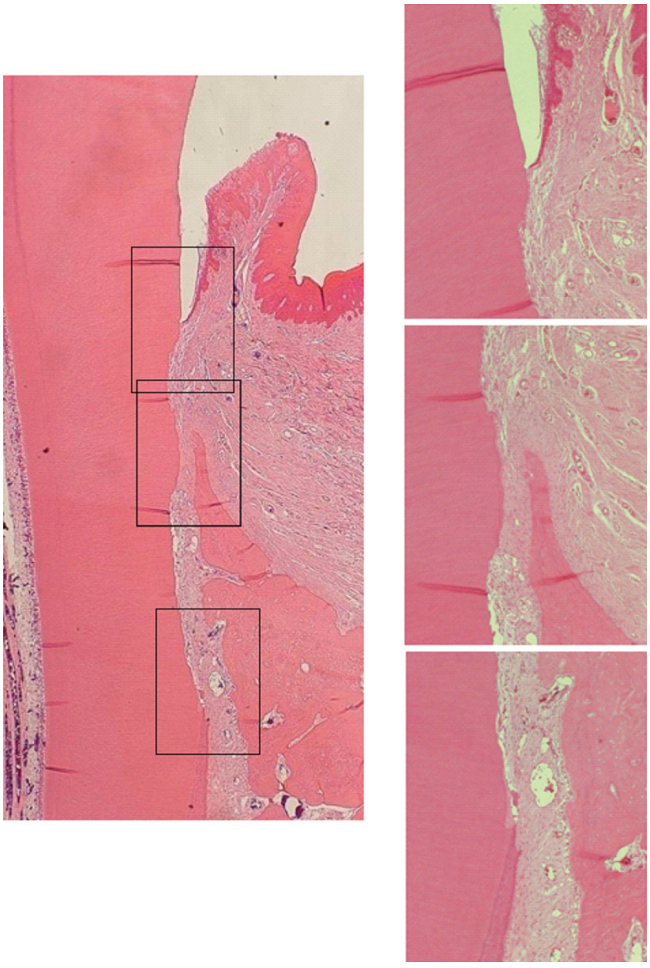

Figure 5. Mesio-distal section of a 24 week specimen (H&E stain, left ×20 and right ×200).

cementum or periodontal ligament and this is believed to result in dimensional differences in apico-coronal direction. Also, despite many years of research and the important role that cementum is thought to play in the reparative process following periodontal disease, very little is known about the differences or similarities between pristine and repaired cementum. Therefore, evaluation of periodontal regeneration is usually focused on the amount of bone regeneration, and the importance of cementum regeneration is usually ignored.

As shown in this article, pristine periodontium consists of a narrow zone of AEFC and a thicker zone of CMFC [5]. Therefore, in order to achieve true regeneration, deposition of AEFC upon the instrumented dentine surface should be considered a crucial part of the healing process [23]. In this study, the new cementum was found to consist of two distinct layers; a thin layer of acellular cementum formed on the exposed circumpulpal dentin surface and, in the subcrestal compartment, a thicker layer of CMFC formed on top of and covering the AEFC. Within the limitations of this study, natural healing of 8 and 24 week periods allowed the re-creation of cementum tissue similar to pristine tissue. However, the supracrestal area showed minor changes and the subcrestal areas exhibited changes showing more similarity to pristine tissue. These findings are in contrast with other experimental studies on periodontal regeneration [10,14,24] in which new cementum was consistently defined as cellular cementum. However, the present results are in agreement with the findings presented by Schupbach et al. [18].

In this study, new cementum seemed to be firmly anchored to the underlying circumpulpal dentin except in several spec-

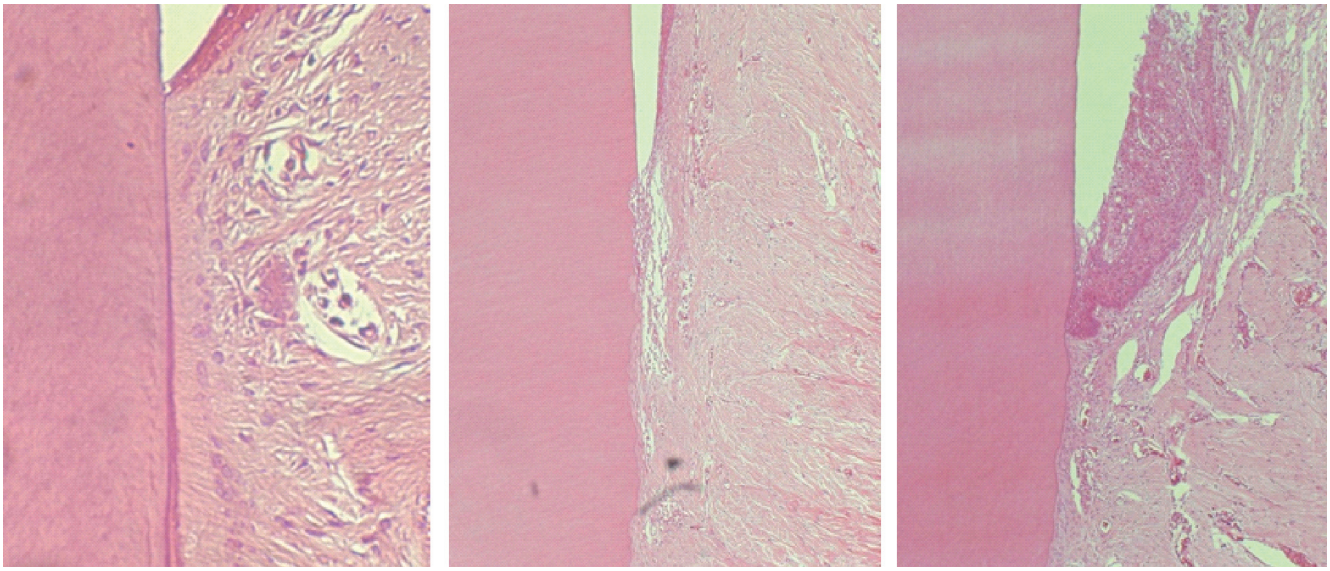

Figure 6. Acellular cementum coverage between junctional epithelium and alveolar crest. Pristine, 8 weeks and 24 weeks (H&E stain, ×400).

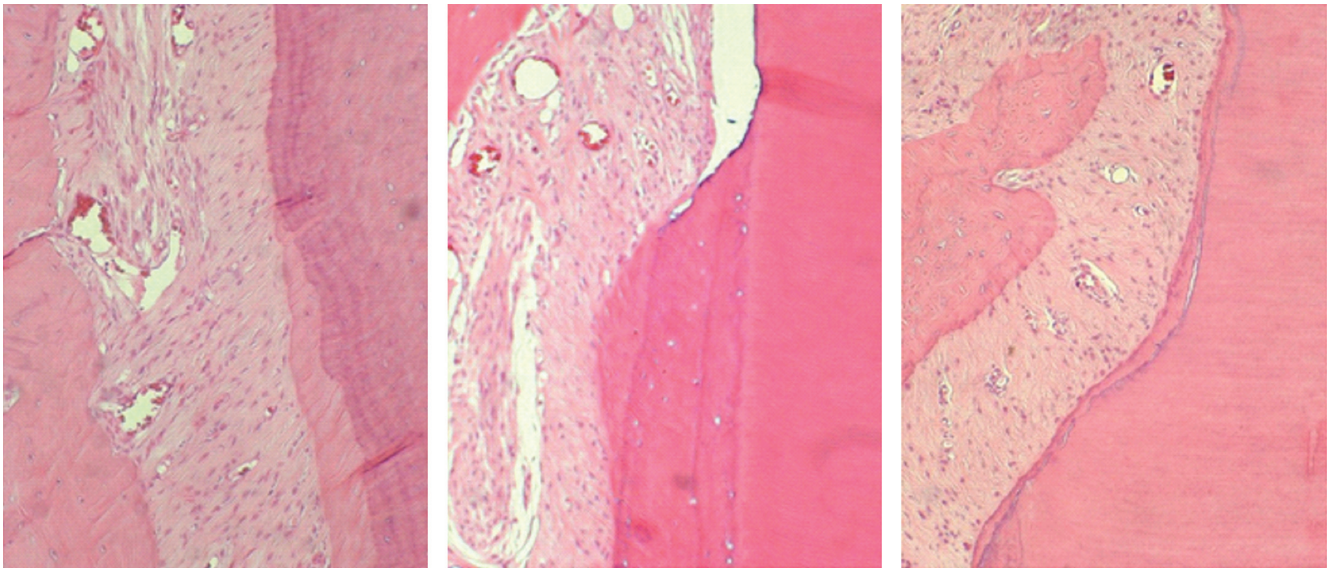

Figure 7. Cellular cementum coverage over notch area (H&E stain, ×1,000). Corresponding area for notch formation is shown in pristine group. Pristine, 8 weeks and 24 weeks.

imens in the 8 week group. Most studies on periodontal wound healing found a similar split between the new cementum and underlying dentin [10,12,24-26]. It has been suggested that this separation is an artifact resulting from histological preparation [25]. However, the current understanding is that the split is caused by a residual smear layer on the dentin surface after surgical instrumentation which prevents proper anchorage of the new cementum to the circumpulpal dentin [27].

Root resorption was observed in some root surfaces in both the 8 and 24 week groups and it appears to be a common sequel to connective tissue repair after periodontal surgery [28]. Undermining resorption in the cervical regions is not uncommon, especially when the connective tissue flaps are adapted directly onto a denuded instrumented root surface. It has been suggested that root resorption and the subsequent exposure of dentinal collagen fibers always precedes new cementum formation [29]. It has also been proposed that the formation of a cementum matrix might prevent root resorption [8].

In conclusion, repaired cementum is composed of acellular cementum and CMFC. Natural healing did not result in complete regeneration in either the 8 week or the 24 week period. In quantitative terms, there seems to be some limitation to natural healing in surgically created intrabony defects. Repaired cementum was thicker in the apical area than in the coronal area and acellular cementum of the supracrestal area appeared amorphous. The vertical height of the repaired cementum was greater in the 24 week group than in the 8 week group. Newly formed cellular cementum was partially detached from the underlying circumpulpal dentin which implies weak attachment between new cementum and dentin and this split was less common in the 24 week group than the 8 week group. Longer-term studies are necessary to fully evaluate the regeneration of cementum.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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