Presence of oxytalan fibers in human regenerated periodontal ligament


Abstract. The aim of the present study was to investigate whether oxytalan fibers are formed in the regenerated human periodontal ligament. 6 patients, each of them exhibiting an advanced intrabony defect, were treated with a biodegradable membrane according to the GTR-principle. Following a healing period of 6 months, the teeth were extracted together with their surrounding soft and hard tissues and subsequently fixed in 10% buffered formalin. Following decalcification in EDTA, the specimens were embedded in paraffin and 8-μm histological sections were cut in the mesio-distal direction, parallel to the long axes of the teeth. The sections were stained with haematoxylin and eosin, or with the oxone-aldehyde-fuchsin-Halmi staining method and examined in the light microscope. A regenerated periodontal ligament containing newly-formed oxytalan fibers was observed in all specimens. Many of them inserted into the newly formed cementum on the root surface. It is concluded that oxytalan fibers are formed de novo in human regenerated periodontal ligament tissue.


Periodontal regeneration is defined as the healing characterized by the formation of a new connective tissue attachment (i.e., new cementum with inserting collagen fibers) and new alveolar bone (Caton & Greenstein 1993). One of the most predictable treatment modalities by which this type of healing can be achieved is termed guided tissue regeneration (GTR). It implies the placement of a barrier membrane covering the root surfaces and the periodontal defects thus, allowing periodontal ligament cells to selectively repopulate the previously periodontitis affected root surface (Nyman et al. 1982, Gottlow et al. 1986, Karring et al. 1993). Histological and clinical studies have demonstrated that both non-biodegradable and bioabsorbable membranes can be used to predictably achieve this goal (Cafose et al. 1994, 1997, Tindhe et al. 1995, Cortellini et al. 1996, Furuzel et al. 1997).

Recently, in a monkey experiment, oxytalan fibers have been observed in the newly formed periodontal ligament following regenerative periodontal treatment (Sculean et al. 1997). These regenerated oxytalan fibers displayed a similar array and morphological appearance as those in the normal periodontal ligament. They often inserted into the newly formed cementum thus, suggesting a strong relationship between these two tissues.

Although the reported results have clearly demonstrated the presence of oxytalan fibers in the monkey regenerated periodontal ligament it is still not...
known whether they are also present in the human regenerating periodontium.

Therefore, the aim of the present study was to investigate whether oxytalan fibers are present in the human regenerating periodontal ligament.

**Material and Methods**

6 patients, each of them displaying an advanced intrabony periodontal defect at a tooth which was already scheduled for extraction for periodontal or prosthesis reasons, were included in this study. All patients volunteered for the study and received verbal and written information about its purpose and possible risks and about the possibility to withdraw at any time. In every case a written informed consent was obtained prior to the start of the study. The study protocol was approved by the ethical committee of the Semmelweis University of Medicine, Budapest, Hungary. Three months before surgery, all patients received oral hygiene instructions and supra- and subgingival scaling in order to reduce the soft tissue inflammation to a minimum.

**Surgical procedure and postoperative care**

The treated intrabony defects were located at teeth 12, 22, 34, 35, 46, and 47. All surgical procedures were performed under local anesthesia. Following intraoperative incisions full-thickness mucoperiosteal flaps were raised at both the vestibular and the lingual aspects of the teeth. After the removal of 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 to indicate the most apical level of the calculus or the bottom of the defect when no calculus was present. Thus, any periodontal ligament tissue which might be present on the root surface in histological sections should be considered de novo formed connective tissue attachment. Following rinsing of the wound with sterile saline a bioabsorbable sutures (Dexon® II, Davis & Geck, Inc., Manati, PR). Flap closure was achieved with horizontal or with vertical mattress sutures using a non-bioabsorbable material (Gore-Tex®, Flagstaff, Arizona, USA). The postoperative care consisted of administration of antibiotics for one week (Amoxicillin 3×375 mg/day and Metronidazole 3×250 mg/day) (Van Winkelhock et al. 1989) 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 every second week during the entire experimental period.

**Biopsy removal and histological preparation**

Following local anesthesia paramarginal incisions were placed and full-thickness mucoperiosteal flaps were raised. The teeth were then removed together with their surrounding soft and hard membranes were fixed to the affected tooth, or to the neighbouring teeth with bioabsorbable sutures (Dexon® II, Davis & Geck, Inc., Manati, PR). Flap closure was achieved with horizontal or with vertical mattress sutures using a non-bioabsorbable material (Gore-Tex®, Flagstaff, Arizona, USA). The postoperative care consisted of administration of antibiotics for one week (Amoxicillin 3×375 mg/day and Metronidazole 3×250 mg/day) (Van Winkelhock et al. 1989) 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 every second week during the entire experimental period.

**Fig. 1. Photomicrograph of a healed intrabony defect after GTR treatment.** A new periodontal ligament (NPL), new cementum (NC) and new alveolar bone (NB) can be observed coronal to the notch (N) in the root surface. Oxytalan fibers (OX) can be distinguished both apically and coronally from the notch. A=apex, D=dentin, V=blood vessel. Oxone-aldehyde-fuchsin-Halmy stain, original magnification ×100.

**Fig. 2. Higher magnification of the area apical to the notch (N) where an intact periodontal ligament (PL) is still present.** Oxytalan fibers (OX) can be observed running in a mainly apico-occlusal direction and some of them insert into the root cementum (C). Oxone-aldehyde-fuchsin-Halmy stain, original magnification ×400.

**Fig. 3. Higher magnification of the area coronal to the notch shown in Fig. 1 (arrows).** Regenerated oxytalan fibers (OX) are seen in the newly formed periodontal ligament (NPL) and some of them insert into the newly formed cementum (NC). V=blood vessel. Oxone-aldehyde-fuchsin-Halmy stain, original magnification ×400.
tissues. After post-surgical healing all patients received complete prosthodontic treatment.

Immediately upon removal, the biopsies were fixed in 10% buffered formalin, decaified in EDTA, dehydrated and fixed in paraffin. Recto-rectal distal serial sections were cut parallel to the long axis of the teeth with the microtome set at 8 μm. The sections were stained with hematoxylin and eosin and every 10th section was stained with the oxalate-aldehydehyd-fuchsain-Halni staining method as described by Fullmer et al. (1974). As controls, sites with an intact periodontal ligament were used.

Results
Postoperative healing was uneventful in all cases and no adverse reactions against the membrane material were observed. Membrane exposure did not occur. At the central sites, the oxalate fibers displayed a mainly spic-o-cuscal orientation with a localization closer to the cementum than to the alveolar bone (Figs. 1, 2). Some of them were seen inserting into the cementum. At the test sites, periodontal regeneration (i.e., new cementum with inserting collagen fibers and new alveolar bone) had occurred coronally to the notch in all six biopsies (Figs. 1, 3). The newly formed attachment extended in the coronal direction to a varying extent. Regenerated oxalate fibers were observed in all cases where a newly formed periodontal ligament was present. The regenerated oxalate fibers were always localized closer to the newly formed cementum than to the alveolar bone and had a similar morphological appearance to those observed at sites with an intact periodontal ligament (Figs. 2, 3). Many of them were inserting into the regenerated cementum on the previously periodontitis affected root surface (Fig. 3).

Discussion
The present investigation has demonstrated for the first time the presence of oxalate fibers in human regenerated periodontal ligament. Newly formed oxalate fibers, morphologically similar to those from intact sites, were present in all sites where a new cementum and a new periodontal ligament had formed. The presence of oxalate fibers in regenerated periodontal ligament was demonstrated previously in monkey (Scuían et al. 1997). In that study, oxalate fibers were identified in a new periodontal ligament formed following regenerative surgical treatment of intra-bony defects with plates-derived growth-factors alone or in combination with GTR. The oxalate fibers in the regenerated periodontal ligament had a light - and electron microscopic appearance similar to that in the normal periodontal ligament. The histological examination of the present material has also shown that the regenerated oxalate fibers displayed a similar morphological appearance to those previously described by others in the normal animal and human periodontal ligament (Fullmer 1958, 1959, 1960, 1961, 1962, Fullmer et al. 1974, Ronne 1963, Jonas & Riede 1969, Edwards 1968, Carmsichet 1968, Hurst 1971, Sims 1975, 1977, 1979, Itoh et al. 1979, Sampson & Sims 1979, Edmonds et al. 1979, Sculean et al. 1997).

In the present investigation, oxalate fibers were observed in the newly formed periodontal ligament formed coronal to the notch in the root surface in all treated cases. They were often inserting into the newly formed cementum on the root surface while none of the fibers were inserting into the alveolar bone. These observations are in agreement with the findings from previous studies and suggest a close relationship between cementum, periodontal ligament and oxalate fibers (Fullmer 1959, 1961, Fullmer et al. 1974, Goggius 1966, Sims 1973, 1977, 1997, Sculean et al. 1997).

However, it is important to realize that in sections oxidized with peracetic acid or ozone not only oxalate fibers, but also most mucopolysaccharides and epithelial mucins, keratin, zymogen granules in the pancreas and granules in α cells of the pancreatic and pancreatic islets stain with aldehyde fuchsain (Fullmer 1974). Thus oxalate fibers are selectively but not specifically stained with this method. Furthermore, the violet aldehyde fuchsain stained material is a consequence of a differentiation step in 70% alcohol. The shorter the differentiation time the more contaminants will be stained. A prolonged differentiation, however, results in less intensely stained oxalate fibers. The function of oxalate fibers has not yet been clarified but the observation that these fibers are formed in the regenerated periodontal ligament in both monkey and man suggests that they play an important role in the physiology of this connective tissue. Furthermore, the observations in the present study and in the previous study in monkey where oxalate fibers regenerated both after the treatment with growth factors and GTR (Scuían et al. 1997) indicates that the regeneration of oxalate fibers is related to the neoformation of a periodontal ligament rather than to the treatment-type by which the regeneration was accomplished.

Zusammenfassung

Résumé
Présence de fibres oxalates dans le demonto- dente humain régénéré Le but de la présente étude était d'examiner s'il se forme des fibres oxalates dans le demonto- dente humain régénéré. Chez 6 patients présentant chacun une lésion infra-osseuse, des biopsies de parodont ont été prises avec une membrane biocompatible suivant la méth- ode de la Résection Tissulaire Guidée (GTR). Après une période de cicatrisation de 6 mois, les tissus ont été extraites en même temps que les tissus normaux et l'exam- ination, puis fixées dans une solution tampon- née de formol à 10%. Après décalcification dans l'EDTA, les spécimens ont été inclus dans la paraffine, et des coupes histologiques de 8 μm ont été taillées en direction néo- donnée, parallèlement aux axes longitudinaux des décès. Les coupes ont été colorées à l'ét- matine-fuchsaine et examinées au microscope optique. Un dem- ments régénérés contiennent des fibres oxalate- nes formées ont été observées dans tous les
specimens. A grand nombre de ces fibres s’insertent dans le yeux nouvellement foré sur la surface radiculaire. Nous en concluons que les fibres ostéales se forment de nouveau dans le desmodonte human segment.

References


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