Various therapeutic procedures have been designed to promote new attachment and regeneration of periodontal tissues lost in periodontal disease.

New attachment may be defined as the formation of a new cementum with inserting extrinsic collagen fibres occurring on a root surface deprived of its periodontal ligament, whether or not this has occurred because of periodontal disease or by mechanical means [1].

Periodontal regeneration may be defined as “regeneration of the tooth’s supporting tissues, including alveolar bone, periodontal ligament and cementum” [2]. The term regeneration, however, implies restoration of the original morphology and function of a lost tissue. Thus, periodontal regeneration is better defined as new attachment occurring in combination with the formation of new bone and periodontal ligament which fully restore the morphology and function of the original tissues. Likewise, the term periodontal repair may be defined as the formation of a periodontal tissue which differs from the original tissue in terms of morphology and/or function.

The evidence that exists regarding the potential of various procedures used in periodontal therapy to promote new attachment and periodontal regeneration was recently reviewed [2–6]. It was generally agreed that periodontal regeneration in humans is possible following surgical procedures that include the use of bone grafts or barrier membranes (guided tissue regeneration; GTR).

The findings from the clinical studies reviewed in the publications cited above which evaluated the outcome of GTR were corroborated by histological evidence from animal experiments using different models [7–17]. Thus, the findings indicate that (i) new attachment or bone regrowth may occur on or adjacent to an instrumented root surface and may extend up to 3 mm in the apical-coronal direction.

V furcation defect healing after GTR with and without Emdogain® application

Maurício G. Araújo

Kliniska och histologiska studier har visat att styrd vävnadsregeneration (GTR) främjar bildandet av nytt tandfäste och benåterväxt. Trots att de kliniska effekterna av GTR-behandling är väl kända, är informationen om de kvalitativa egenskaperna hos de nybildade vävnaderna begränsad.

I denna översikt presenteras den strukturella uppbyggnaden av ny parodontal vävnad i experimentellt framställda grad III furkationsdefekter hos hundar, behandlade med GTR och GTR i kombination med emaljproteiner (Emdogain®).

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direct direction [11, 17] and (ii) the new cementum is of varying thickness, is tapered coronally and has matured to become of cellular type.

In the clinical and experimental studies referred to above, attention was paid to the amount of new cementum, new periodontal ligament, and new bone that had formed following various regenerative procedures. In other words, the findings were reported in terms of mm gain – in the apical-marginal direction – in cementum and bone. Despite the amount of data demonstrating a gain in mm of new periodontal tissue, very little is known about the reformation and quality of such tissues.

The aim of this review was, therefore, to present a series of studies whose aim was to evaluate the formation and quality of the new tissues formed after GTR. In addition, data regarding the quality of these new periodontal tissues formed after GTR combined with application of enamel matrix proteins (Emdogain®) are also presented.

The healing dynamics

Araújo et al. [18] performed an investigation with the aim of describing, in a chronological sequence, the dynamics of periodontal tissue formation in degree III furcation defects after GTR. The study was performed in eight foxhound dogs. Degree III furcation defects were produced and reconstructive surgery was performed. Resolut® Regenerative Material barriers (W.L. Gore & Associates, Flagstaff, AZ, USA) were applied at the experimental sites on both sides of the mandible. The dogs were divided into four groups. Each group contained two dogs and, hence, four experimental teeth. The dogs in the four groups were scheduled for sacrifice 2, 4, 8, and 20 weeks after GTR. Biopsies were obtained and prepared for histological analyses.

At 2 weeks of healing, the furcation was occupied by granulation tissue in the coronal portion and by connective tissue (CNT) in the remaining area. The CNT, which was rich in cells and fibres was related to the root surface in two different ways. In some areas, the collagen fibres in the CNT lateral to the root ran parallel to the root surface. In other areas, however, this CNT contained strands of fine collagen-like fibres extending in a perpendicular direction from the dentine surface (phase I in cementum formation). At 4 weeks of healing, the tissues in the furcation comprised a small area of granulation tissue, frequently at the most coronal portion of the defect; a large area of connective tissue; and a limited area of newly formed bone. The CNT in direct contact with the root presented the previously described features, but in some areas, the fibres extending from the root were embedded in a matrix substance (phase II in cementum formation). At 8 weeks, the furcation was occupied by a large area of woven bone and to a lesser extent by connective tissue, bone marrow, lamellar-like bone (including primary and secondary osteons), and periodontal ligament. The root surface was covered by new cementum in different phases of formation. In some areas the new cementum contained intrinsic fibres and cells (phase III in cementum formation). At 20 weeks of healing, the furcation defect was occupied by lamellar bone, bone marrow, periodontal ligament, and some residual connective tissue. The lamellar bone along with a layer of bundle bone was found mainly at the periphery of the bone tissue, while in the centre a large bone marrow space was present. Lamellar bone, however, frequently failed to form in the fornix region of the furcation. The entire root surface was covered by new cementum containing cells and extrinsic and intrinsic fibres.

These results indicate that tissue formation and differentiation in a furcation defect after GTR follow an orderly sequence of events. Thus, the granulation tissue that occupies a large area of the furcation defect at 2 weeks of healing is gradually replaced by connective tissue. In later stages of healing and depending on its location, this connective tissue is replaced by either cementum, periodontal ligament, or bone tissue. The observed pattern of healing is in agreement with findings from experiments of others describing healing after GTR in degree III furcation defects [19, 20].

In the present model, new cementum started to form as early as 2 weeks after reconstructive therapy. This observation supports findings from new attachment studies in monkeys [21, 22] which documented that new cementum had started to form 2 weeks after root instrumentation. The build-up of new cementum was found to occur in three phases, (i) the organisation of collagen fibres adjacent and perpendicular to the root surface, (ii) the deposition of a matrix substance plus the assembly of collagen fibres, and (iii) the addition of cells and fibres organised parallel to the root surface. This pattern of cementum formation is consistent with data previously reported [23–25]. In addition, it also matches the sequence of events characterising the formation of cementum that takes place in resorption bays in humans [26].

The healed tissue

From the definitions of regeneration and repair
reported previously in the present review, it is clear that one can only determine the nature of the periodontal tissues obtained after GTR if such tissues are compared with tissues from pristine sites. Thus, Araújo et al. [27] performed a study to address this question. The study included ten dogs. In six of the dogs (group A), degree III furcation defects were produced and GTR was performed. The four remaining dogs (group B) represented healthy untreated furcations and were used as controls. Five months after reconstructive surgery, all ten animals (groups A + B) were killed and biopsies were obtained and prepared for histological analyses.

The authors observed that the periodontal tissues in the healed furcation defects differed markedly in many respects from the corresponding tissues in the pristine furcation (Fig. 1). The pristine furcations were occupied to about 70% by bone tissue (64% mineralised bone and 7% bone marrow) and 30% by periodontal ligament tissue. The healed furcation was also occupied to about 70% by bone tissue, but the relative proportions of mineralised bone and bone marrow were significantly different, 28% (vs. 64%) and 41% (vs. 7%) respectively. The remaining area of the healed furcation was occupied to 16% by periodontal ligament and to 14% by residual connective tissue.

The root cementum in the pristine furcations was of either an acellular, extrinsic fibre type or a cellular, mixed, stratified fibre type and was in direct continuity with a distinct layer of hyaline peripheral dentine. It has been suggested that this hyaline layer of peripheral dentine is composed of a connective tissue matrix and epithelial products and may play an important role in establishing the initial attachment of the acellular, extrinsic fibre cementum to the tooth surface [28]. In the healed furcation, however, the newly formed cementum that covered the entire previously instrumented root surface was continuous with the circumpulpal dentine. A layer of hyaline peripheral dentine was not observed.

That the new cementum which formed after GTR was found to be of a cellular, extrinsic/intrinsic fibre type agrees with observations made in several studies on new attachment. Such experiments demonstrated the establishment of a new cellular cementum on root surfaces that had been instrumented in re-constructive procedures which also included, for example, acid conditioning [23, 29, 30], GTR [8, 9, 12–14, 16, 17, 24], application of acid/fibronectin [31], bone morphogenetic proteins [32–34], platelet-derived and insulin-like growth [35], and placement of demineralised freeze-dried bone allografts with...
and without osteogenin [36–38]. Furthermore, several studies also confirmed that the newly formed reparative cellular cementum contains extrinsic and intrinsic collagen fibres [24–26]. Based on these findings, it is suggested that a new cementum of a cellular, extrinsic/intrinsic fibre type will consistently form after reconstructive periodontal therapy.

In addition, the results from the analyses performed in the different zones of the newly formed tissues indicated that in the apical portion of the furcation, the periodontal tissues were better organised than the tissues in the more coronal portions. In fact, in the most coronal portion of the furcation, lamellar bone frequently failed to form. When this occurred, (i) bone marrow tissue substituted for the lamellar bone, (ii) the periodontal ligament failed to become established and was replaced by loose connective tissue, and (iii) only a few collagen fibres were observed to insert in the reparative cementum.

The effect of enamel matrix proteins

From the data reported before, it is evident that the new cementum contains relatively few extrinsic fibres, which may impair its function of support. The formation of a new cementum which contains a larger number of extrinsic fibres (i.e. an acellular, extrinsic fibre cementum) represents, therefore, an advantageous goal. Data from experimental studies suggest that enamel matrix proteins (EMD) may, during root development, be involved in the formation of acellular, extrinsic fibre cementum [39–44]. In vitro cell culture experiments indicate that EMD (enamel matrix derivatives) stimulates the proliferation of periodontal ligament cells and protein synthesis [45]. Experimental studies in monkeys demonstrate that the application of EMD to the root surface of surgically-created defects promotes the formation of acellular cementum [46, 47]. Furthermore, the clinical use of a commercially-available porcine EMD gel (Emdogain®) in bone defects improves the clinical attachment levels and reduces pocket depth [48].

The effect on periodontal tissue healing of GTR followed by application of Emdogain® to the root surface was investigated by Araújo and Lindhe [49]. The experiment was performed in five foxhound dogs. Degree III furcation defects were produced and GTR was performed. In the experimental site on one side of the mandible (test group), phosphoric acid gel was applied to the exposed root surfaces for 15 seconds. The acid was removed by flushing the root surfaces with sterile saline. Subsequently, a gel of enamel matrix derivative (Emdogain®, Biora AB, Malmö, Sweden) was applied to all the instrumented parts of the root before a Resolut® barrier was placed. The contralateral premolar (control group) received the same treatment, but acid etching was not performed and EMD gel was not applied before barrier installation. Four months after reconstructive therapy the dogs were sacrificed and biopsies were obtained and prepared for histological analysis.

The histological analyses demonstrated the furcation defects of both the test and control groups to be occupied with similar amounts of mineralised bone, bone marrow, periodontal ligament tissue and residual connective tissue. In the apical half of the furcation in the test group, however, the new cementum was found to be acellular, thin and to contain extrinsic and intrinsic fibres (Fig. 2). In addition, it was reported that this new acellular cementum contained more inserting collagen fibres (extrinsic fibres) than did the new cementum in the remaining half of the test group or the entire instrumented surface of the root in the control group. This observation agrees with findings from studies in the monkey by Hammarström [46] and Hammarström et al. [47]. The mechanism behind the ability of Emdogain® to promote the formation of an acellular cementum is not properly understood but may be related to the potential of

**Figure 2.** High power magnification of the apical portion of the furcation treated with GTR and Emdogain® application. D = dentine, NC = new acellular cementum, P = periodontal ligament. H&E staining; original magnification x200.
the compound to prevent the apposition of intrinsic fibres and cells which in the present model characterises phase III in cementum formation.

**Summary**

Clinical and histological studies have demonstrated that guided tissue regeneration (GTR) therapy promotes new attachment formation and bone regrowth. Despite the great amount of publications on the clinical outcome of GTR, limited information exists regarding the quality and the course of tissue formation after GTR. This review surveys studies which describe the features of the newly formed periodontal tissues obtained following GTR in degree III furcation defects in dogs. These newly formed tissues of healed furcations were also compared with tissues from pristine furcations. In addition, the effect of the application of enamel matrix proteins (Emdogain®) combined with GTR is reviewed.

**References**

PERIODONTAL HEALING


Address
Maurício G. Araújo, Departamento de Odontologia, Universidade Estadual de Maringá, Av. Colombo, 5790 - Campus Universitário CEP 87020-900, Maringá - Paraná - BRAZIL