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®).

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

Author

Maurício G. Araújo, DDS, Odont Dr, Department of Periodontology, Institute of Odontology, Göteborg University, Göteborg, Sweden.

Key words

Furcations; periodontal healing; guided tissue regeneration; enamel matrix proteins

Accepted for publication September 1998

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

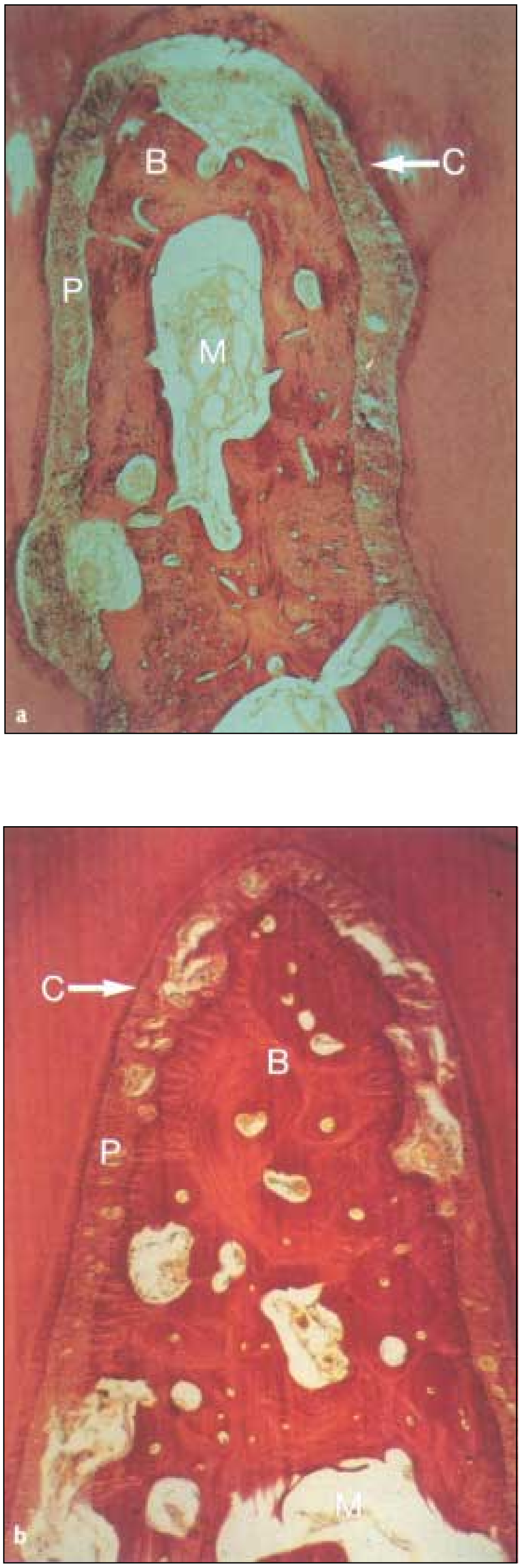

Figure 1. Mesial-distal section of the mid-portion of **(a)** the healed and **(b)** the pristine furcations. B = mineralised bone, C = cementum, M = bone marrow, P = periodontal ligament. Van Gieson staining; original magnification x50.

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].

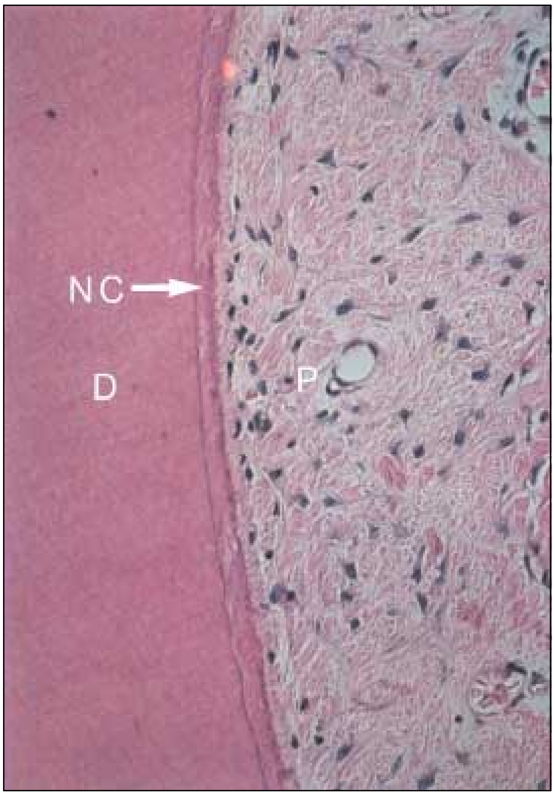

Figure 2. High power magnification of the apical portion of the furcation treated with GTR and Emdogain® application. D = dentine, NC = new acellular cementum, P = periodontal ligament. H&E staining; original magnification x200.

The 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 Hammarstöm [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 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

1. Karring T, Lindhe J, Cortellini P. Regenerative periodontal therapy. In: Clinical Periodontology and Implant Dentistry. Lindhe J, Karring T, Lang N, editors. Copenhagen: Munksgaard, 1997.
2. Garret S. Periodontal regeneration around natural teeth. Annals of Periodontology 1996; 1: 621–66.
3. Callens A. Growth factors in periodontal regeneration – a review. In: Proceedings of the 2nd European Workshop on Periodontology. Chemicals in Periodontics. Lang N P, Karring T, Lindhe J, editors. Berlin: Quintessenz Verlag, 1997: 284–302.
4. Klinge B. Root surface conditioning. In: Proceedings of the 2nd European Workshop on Periodontology. Chemicals in Periodontics. Lang N P, Karring T, Lindhe J,editors. Berlin: Quintessenz Verlag, 1997: 276–83.
5. Lioubavina N, Karring T. Systemic and local antibiotics in periodontal regeneration with barrier membranes. In: Proceedings of the 2nd European Workshop on Periodontology. Chemicals in Periodontics. Lang N P, Karring T, Lindhe J, editors. Berlin: Quintessenz Verlag, 1997: 303–12.
6. Ouhayoun J-P. Bone grafts and biomaterials used as bone graft substitutes. In: Proceedings of the 2nd European Workshop on Periodontology. Chemicals in Periodontics. Lang N P, Karring T, Lindhe J, editors.

Berlin: Quintessenz Verlag, 1997: 313–58.
7. Nyman S, Gottlow J, Karring T, Lindhe J. The regenerative potential of the periodontal ligament. An experimental study in the monkey. J Clin Periodontol 1982; 9: 257–67.
8. Aukhil I, Simpson DM, Schaberg TV. An experimental study of new attachment procedure in beagle dogs. J Periodont Res 1983; 18: 643–54.
9. Aukhil I, Pettersson E, Suggs C. Guided tissue regeneration. An experimental procedure in beagle dogs. J Periodontol 1986; 57: 727–34.
10. Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. J Clin Periodontol 1984; 11: 494–503.
11. Gottlow J, Karring T, Nyman S. Guided tissue regeneration following treatment of recession-type defects in the monkey. J Periodontol 1990; 61: 680–5.
12. Magnusson I, Nyman S, Karring T, Egelberg J. Connective tissue attachment formation following exclusion of gingival connective tissue and epithelium during healing. J Periodont Res 1985; 20: 201–8.
13. Caffesse RG, Smith BA, Castelli WA, Nasjleti CE. New attachment achieved by guided tissue regeneration in beagle dogs. J Periodontol 1988; 59: 589–94.
14. Caffesse RG, Dominguez LE, Nasjleti CE, Castelli WA, Morrison EC, Smith BA. Furcation defects in dogs treated by guided tissue regeneration (GTR). J Periodontol 1990; 61: 45–50.
15. Claffey N, Motsinger S, Ambruster J, Egelberg J. Placement of a porous membrane underneath the mucoperiosteal flap and its effect on periodontal wound healing in dogs. J Clin Periodontol 1989; 16: 12–6.
16. Caton J, Wagener C, Polson A, Nyman S, Frantz B, Bouwsma O, Bliende T. Guided tissue regeneration in interproximal defects in the monkey. Int J Periodont Restor Dent 1992; 12: 267–77.
17. Sigurdsson TJ, Hardwick R, Bogle C, Wikesjö UME. Periodontal repair in dogs: space provision by reinforced e-PTFE membranes enhances bone and cementum regeneration in large supraalveolar defects. J Periodontol 1994; 65: 350–6.
18. Araújo MG, Berglundh T, Lindhe J. On the dynamics of periodontal tissue formation degree III furcation defects. An experimental study in dogs. J Clin Periodontol 1997; 24: 738–46.
19. Herr Y, Matsuura M, Lin W-L, Genco RJ, Cho M-I. The origin of fibroblasts and their role in the early stages of horizontal furcation defect healing in the beagle dog. J Periodontol 1995; 66: 716–30.
20. Matsuura M, Herr Y, Han K-Y, Lin W-L, Genco RJ, Cho M-I. Immunohistochemical expression of extracellular matrix components of normal and healing periodontal tissues in the beagle dog. J Periodontol 1995; 66: 579–93.
21. Caton JG, DeFuria EL, Polson AM, Nyman S. Periodontal regeneration via selective cell repopulation. J Periodontol 1987; 8: 546–52.
22. Ogura N, Mera T, Sato F, Ishikawa I. Longitudinal observation of cementum regeneration through multiple fluorescent labeling. J Periodontol 1991; 62: 284–91.
23. Selvig KA, Ririe CM, Nilvéus R, Egelberg J. Fine structure of new connective tissue attachment following acid treatment of experimental furcation pockets in dogs. J Periodont Res 1981; 16: 123–9.
24. Schüpbach P, Gaberthüel T, Lutz F, Guggenheim B. Periodontal repair or regeneration structures of different types of new attachment. J Periodont Res 1993; 28: 181–93.
25. Nalbadian J, Frank RM. Electron microscopic study of the cementum and periodontal connective tissue attachment in the cat. J Periodont Res 1980; 15: 71–89.
26. Bosshardt DD, Schroeder HE. How repair cementum becomes attached to the resorbed roots of human permanent teeth. Acta Anat 1994; 150: 253–66.
27. Araújo M, Berglundh T, Lindhe J. The periodontal tissues in healed degree III furcation defects. An experimental study in dogs. J Clin Periodontol 1996; 23: 532–41.
28. Ten Cate AR. The role of epithelium in the development, structure and function of the tissues of tooth support. Oral Diseases 1996; 2: 55–62.
29. Crigger M, Bogle G, Nilvéus R, Egelberg J, Selvig KA. The effect of topical acid application on the healing of experimental furcation defects in dogs. J Periodont Res 1978; 13: 538–49.
30. Dyer BL, Caffesse RG, Nasjleti CE, Morrison E. Guided tissue regeneration with dentine biomodification. J Periodontol 1993; 64: 1052–60.
31. Caffesse RG, Nasjleti CE, Anderson GB, Lopatin DE, Smith BA, Morrison EC. Periodontal healing following guided tissue regeneration with citric acid and fibronectin application. J Periodontol 1991; 62: 21–9.
32. Kinoshita A, Oda S, Takahashi K, Yokota S, Ishikawa I. Periodontal regeneration by application of recombinant human bone morphogenetic protein-2 to horizontal circumferential defects created by experimental periodontitis in beagle dogs. J Periodontol 1997; 68: 103–9.
33. Ripamonti U, Heliotis M, van den Heever B, Reddi AH. Bone morphogenetic proteins induce periodontal regeneration in the baboon (Papio ursinus). J Periodont Res 1994; 29: 439–45.
34. Sigurdsson TJ, Lee MB, Kubota K, Turek TT, Wozney J M, Wikesjö UME. Periodontal repair in dogs recombinant human bone morphogenetic protein-2 significantly enhances periodontal regeneration. J Periodontol 1995; 66: 131–8.
35. Rutherford RB, Niekrash CE, Kennedy JE, Charret MF. Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys. J Periodont Res 1992; 27: 285–90.
36. Bowers G, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part I. J Periodontol 1989a; 12: 664–74.
37. Bowers G, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part II. J Periodontol 1989b; 12: 675–82.
38. Bowers G, Felton F, Middleton C, Glynn D, Sharp S, Mellonig J, Corio R, Emerson J, Park S, Suzuki J, Ma S, Romberg E, Reddi A H. Histological comparison of regeneration in human intrabony defects when osteogenin is combined with demineralized freeze-dried bone allograft and with purified bovine collagen. J Periodontol 1991; 62: 690–702.
39. Slavkin H C, Bringas P Jr, Bessem C, Santos V, Nakamura M, Hsu M-Y, Snead ML, Zeichner-David M, Fincham AG. Hertwig's epithelial root sheath differentiation and initial cementum and bone formation during long-term organ culture of mouse mandibular first molars using serumless, chemically-defined medium. J Periodont Res 1988; 23: 23–40.
40. Slavkin HC, Bessem C, Fincham AG, Bringas P Jr, Santos V, Snead ML, Zeichner-David M. Human and mouse cementum proteins immunologically related to enamel proteins. Biochem Biophys Acta 1989; 991: 12–8.

41. Lindskog S. Formation of intermediate cementum. I: early mineralization of aprismatic enamel and intermediate cementum in monkey. J Craniofac Genet Dev Biol 1982a; 2: 147–60.
42. Lindskog S. Formation of intermediate cementum. II: a scanning electron microscopic study of the epithelial root sheath of Hertwig in monkey. J Craniofac Genet Dev Biol 1982b; 2: 161–9.
43. Lindskog S, Hammarström L. Formation of intermediate cementum III: ^{3}H-tryptophan and ^{3}H-proline uptake into the epithelial root sheath of Hertwig in vitro. J Craniofac Genet Dev Biol 1982; 2: 171–7.
44. MacNeil RL, Thomas HF. Development of the murine periodontium. II. Role of the epithelium root sheath in formation of the periodontal attachment. J Periodontol 1993; 64: 285–91.
45. Gestrelius S, Andersson C, Lidström D, Hammarström L, Somerman M. In vitro studies on periodontal ligament cells and enamel matrix derivate. J Clin Periodontol 1997; 24: 685–92.
46. Hammarström L. Enamel matrix, cementum development and regeneration. J Clin Periodontol 1997; 24: 658–68.
47. Hammarström L, Heijl L, Gestrelius S. Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. J Clin Periodontol 1997; 24: 669–77.
48. Heijl L, Heden G, Svärdström G, Östgren A. Enamel matrix derivate (EMDOGAIN®) in the treatment of intrabony periodontal defects. J Clin Periodontol 1997; 24: 705–14.
49. Araújo MG, Lindhe J. GTR treatment of degree III furcation defects following application of enamel matrix proteins. An experimental study in dogs. J Clin Periodontol 1998; 25: 524–30.

Lecture at the Scandinavian Society of Periodontology Annual Meeting at Kolmården, Sweden, 8–10 May 1998.

Address

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