A biological approach to periodontal regeneration

Lars Hammarström

Under senare år har ett flertal metoder introducerats för att åstadkomma regeneration av förlorade parodontala vävnader. Exempel på sådana metoder är användning av olika operationsmetoder, demineralisering av rotytan, bentransplantat, membranteknik och tillförsel av tillväxtfaktorer. I denna korta presentation beskrivs bakgrunden till den metod som imiterar den normala utvecklingen av parodontiet och utnyttjar en fraktion av emaljproteinerna för att åstadkomma parodontal regeneration. Sambandet mellan emalj- och cementbildning illustreras bl a av det faktum att ett stort antal gräsätande djur har cement ovanpå emaljen. Det betonas att alla tre parodontiets vävnader hör till tanden och att det alveolära benets tillväxt styrs av celler nära rotytan. Regeneration av rotytan och dess celler med hjälp av emaljproteiner leder således till regeneration av såväl cement som rothinna och alveolärt ben.

First of all I will express my deep gratitude to the Scandinavian Society of Periodontology for awarding me *The 1998 Jens Waerhaug Lecture in Periodontology*. I feel very honoured and I admire the courage of the prize committee to select a person like me who is not a specialist in the field of periodontology. In my research I have been studying the development of the dental tissues including the periodontal ligament and alveolar bone. Research on the biology of mineralized tissues is now progressing very rapidly, and it will provide a better understanding of periodontal diseases and of the mechanisms involved in periodontal regeneration.

In recent years a number of special treatment procedures have been introduced to promote regeneration of lost periodontal tissues. These include different modalities of flap procedures often combined with implantation of bone grafts of various types of bone substitutes [1–3], demineralization of the root surface [4–7], guided tissue regeneration [8, 9] and combinations of these modalities. More recently growth factors and attachment proteins have been tried experimentally [10–13]. These studies have shown that although it is possible to modify the healing response in various ways, true periodontal regeneration, i.e. restoration of original structure and function of the periodontal tissues, has been an elusive goal. The most common divergence from the original morphology concerns the type of cementum and its cohesion to the dentine surface. The hard tissue formed at the root surface is often cellular, and it easily separates from the dentineeee [10, 14–17].

Studies on cementum formation and periodontal regeneration are hampered by the scarcity of suitable experimental models. Small laboratory animals such as mice and rats have molar teeth with limited growth and roots like human teeth, but the small size of these animals' molars make experimental studies very difficult. In addition,

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Kew words

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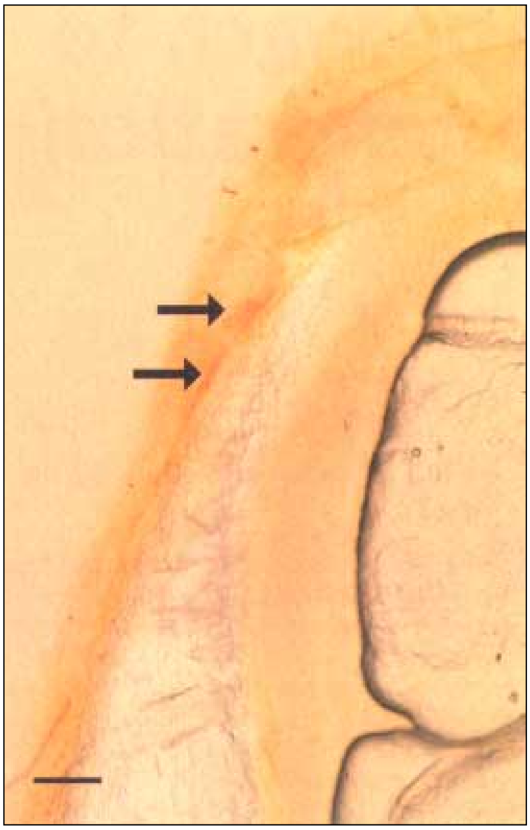

Figure 1. A frozen section of the apical end of a developing human premolar extracted for orthodontic reasons and incubated for immunohistochemical demonstration of the amelogenin fraction of enamel matrix. The staining (arrows) at the peripheral surface of the apical end of the root shows that amelogenin is present in the area where cementum formation is initiated. Bar = 100 µm.

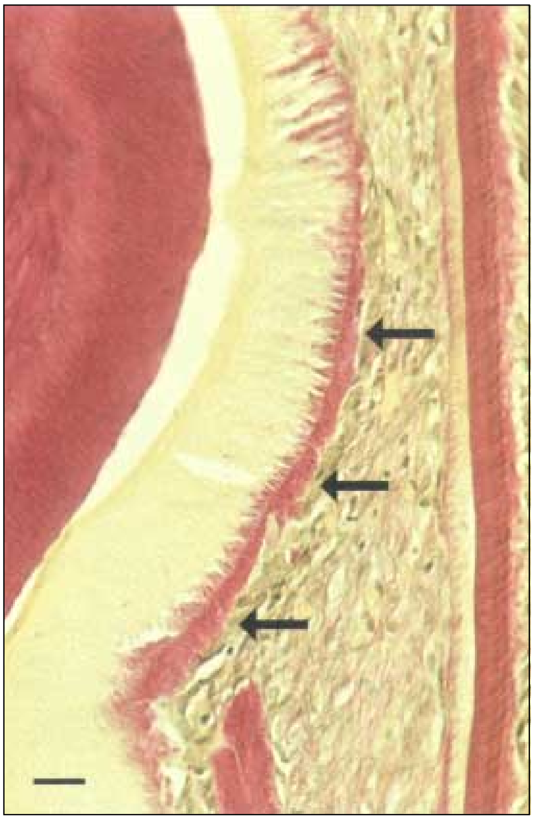

Figure 2. The enamel matrix of a maxillary developing molar of a 5-day-old rat exposed to the mesenchymal cells of the dental follicle for 10 days. A thin layer of a new hard tissue (arrows) has formed on top of the exposed enamel matrix. Bar = 50 µm.

the formation of the roots of, at least, rat molars seems to differ from that of human teeth. Other animals commonly used in the laboratory such as rabbits and guinea pigs have molars as well as incisors that grow continuously, which excludes them from studies on periodontal regeneration. Dogs have frequently been used for studies on periodontal regeneration, but it seems as if results in dogs are not directly transferable to the human situation. In dogs, the regeneration of the bone around the teeth seems to be less dependent on a normal periodontium than in monkeys and humans. So far the teeth of monkeys seem to be the best model for experimental studies on periodontal regeneration [18, 19]. However, a number of ethical, economical and other reasons limit the possibilities for experimental testing in monkeys. In order to progress in our understanding of different factors that influence the progression of periodontitis as well as periodontal regeneration, new experimental models have to be developed.

A new, alternative approach to obtain periodontal regeneration is to try to mimic the events that took place during the development of the periodontal tissues. It should then be remembered that the development of the periodontal ligament and the alveolar bone is associated with the development of the teeth [20–27]. Experimental studies indicate that the maintenance of the periodontal ligament and the alveolar bone is also regulated by cells close to the root surface [28]. Thus, if the ambition is to regenerate the periodontal ligament and the alveolar bone that have been lost due to periodontitis, it should aim at re-establishing a new cementum and neighbouring cells. If this is accomplished, the periodontal ligament and alveolar bone are regenerated as a result of the cells at a healthy root surface.

Root formation is initiated by the downgrowth of Hertwig´s epithelial root sheath. The epithelial root sheath constitutes an apical extension of the enamel organ, and ever since Slavkin and Boyde [29] suggested that cementum is an epithelial secretory product, numerous studies have been carried out to investigate this secretory activity [30–35]. Most of these studies have shown that the root sheath cells form and secrete enamel matrix proteins during root formation, but there seem to be some differences between species.

At early stages the developing enamel consists of about equal amounts of proteins, minerals, and water with minor fractions of carbohydrates and lipids. Amelogenins are a family of proteins that is by far the most abundant of these proteins representing more than 90% of the protein content of the enamel. Studies of amelogenin from various species have shown that is remarkably well conserved, which means that there are very small differences in the composition between species [36, 37].

Amelogenin has been demonstrated in epithelial cells at the apical end of infected roots of rat molars [38]. It has also been found at the surface of the developing apical end of the roots of human premolars and within the peripheral root dentine [39, 40] (Fig.1). In the peripheral root dentine of human teeth, the space occupied by amelogenin deposits remain as Tomes' granular layer in the fully formed tooth. At the root surface, acellular cementum is formed in the area where the amelogenin has been found. The deposition of amelogenin in the peripheral dentine makes this part of the tooth a very special tissue. In the clinical treatment of periodontitis, no attention has been paid to this fact. Root planing aims at obtaining a smooth root surface with no concern for the biological function of the tissues that are removed. In my opinion, more attention should be paid to the possible role of the peripheral (mantle) dentine for a successful periodontal regeneration.

Experimental studies on developing rat molars have shown that cementum is formed when mesenchymal cells of the dental follicle are exposed to denuded enamel matrix [40, 41]. A few days after the removal of the enamel epithelium and exposure of the enamel matrix, a thin layer of a collagenous hard tissue can be observed (Fig. 2). It is interesting to note that coronal cementogenesis in a number of herbivorous species seems to be initiated by exposure of the mesenchymal cells of the dental follicle to the developing enamel. The coronal cementum formation starts as islets in fenestrations in the enamel epithelium. In some species it then continues to develop into a complete coverage of the enamel, while it remains as islets or pearls in others [42–46]. At the developing ends of the roots of human teeth, the epithelial root sheath also fenestrates in the area where cementum formation starts [47]. With increasing age the windows in the epithelium increase in size and the epithelial network becomes less dense [48].

The effect of enamel matrix proteins on periodontal regeneration has been tested in a buccal dehiscence model in monkeys. Those experiments showed that it was possible to regenerate acellular cementum as well as the periodontal ligament and alveolar bone. The model was also used for quantitative studies on the effect of different fractions of the enamel matrix on the periodontal regeneration. It was found that the amelogenin fraction was efficient

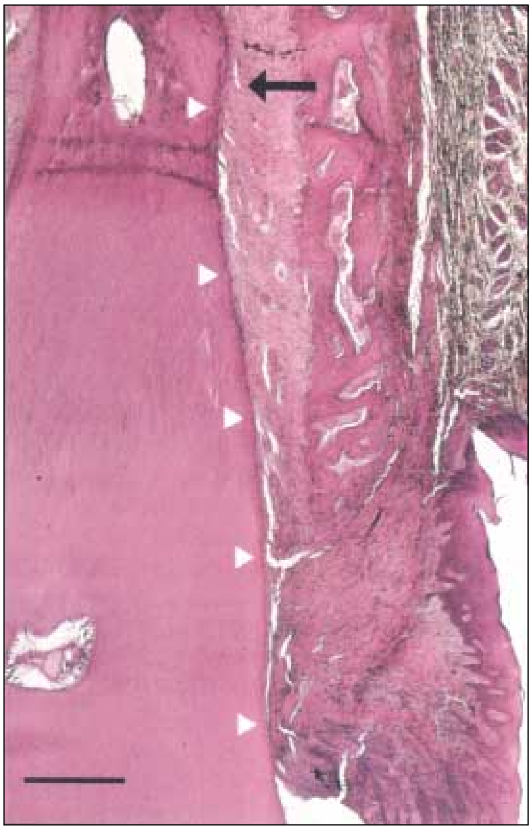

Figure 3. A buccal dehiscence in a maxillary premolar of a monkey 8 weeks after the application of the amelogenin fraction of the enamel matrix on the exposed dentine surface. The black arrow indicates the apical end of the mechanically removed cementum, periodontal ligament, and alveolar bone. Note the regeneration of cementum (white arrow heads), periodontal ligament, and alveolar bone. Bar = 100 µm.

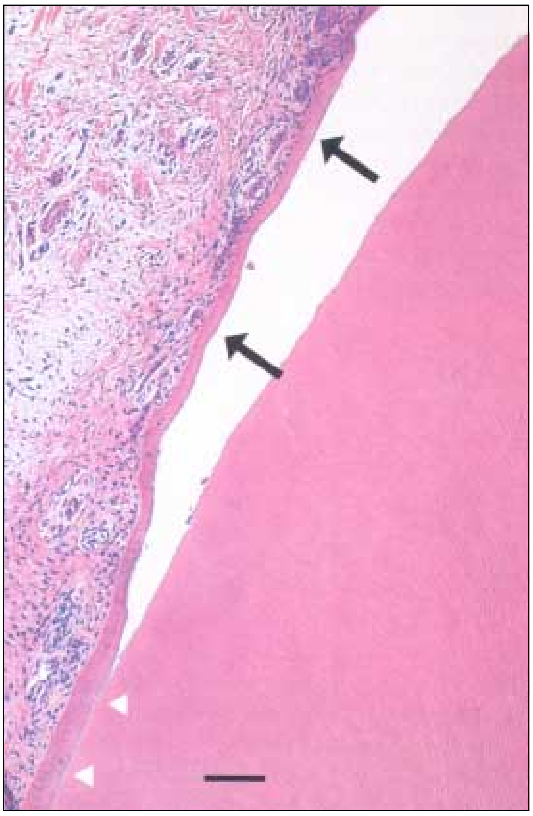

Figure 4. The cervical area of a sheep incisor showing the cementum covering the surface of the enamel (black arrows) as well as the root surface (white arrow heads). Note that there is no observable difference between the coronal and radicular cementum. Note also that the cemento-dentinal junction is an apical extension of the cervical edge of the enamel. Bar = 50 µm.

while matrix components with a higher molecular weight did not promote periodontal regeneration [49] (Fig. 3). A product based on the amelogenin fraction, called EMDOGAIN® (BIORA AB, Malmö, Sweden) is now being marketed for the promotion of periodontal regeneration.

The observation that cementum formation seems to be associated with the enamel proteins should not come as a surprise, since a number of herbivorous animals have cementum on top of the enamel of the crown. In this position the coronal cementum constitutes a part of the occlusal surface and takes part in the grinding of the food. Human teeth also have regions with coronal cementum. Based on structural studies, most of the coronal cementum of human teeth has been defined as acellular, afibrillar cementum [50]. However, coronal cementum of some herbivorous animals has a structural appearance that is similar to that of the radicular acellular extrinsic fibre cementum [44, 45, 51] (Fig. 4).

Comparative studies of the developing teeth in various species are very informative for the understanding of the relation between the formation of all the dental tissues including enamel and cementum. It might be said that nature has made all the good experiments. The scientific challenge is to identify them and to compare and interprete them. After almost four billion years of evolution, nature has learned what works. Recently, biomimicry was introduced as a name for a new science that studies nature´s models and then imitates them [52]. The word *biomimicry* comes from the greek words *bios* (life) and *mimesis* (imitation). It introduces a new way of viewing and valuing nature and tries to find what we can learn from it The use of enamel matrix proteins to promote regeneration of the periodontal tissues is, in my opinion, a good example of biomimicry.

Summary

During the last decades a number of methods have been introduced to promote the regeneration of periodontal tissues. These include different flap procedures, demineralization of the root surface, bone grafts, guided tissue regeneration, and administration of growth factors. This short presentation describes the background of the method that imitates the normal development of the periodontium and which involves the application of a fraction of the enamel proteins to the root surface to promote periodontal regeneration. The link between enamel and cementum formation is, e. g., illustrated by the fact that a great number of herbivorous animals have cementum on top of the enamel. It is emphasized that the three tissues of the periodontium are dental tissues and that growth and maintenance of the alveolar bone are regulated by cells at the root surface. Regeneration of the root surface by means of enamel proteins will thus result in regeneration of the cementum as well as of the periodontal ligament and alveolar bone.

References

1. Yuktanadana I. Bone graft in the treatment of infrabony periodontal pockets in dogs. A histological investigation. J Periodontol 1959; 30: 17–26.
2. Moscow BS, Karsh F, Stein SD. Histological assessment of autogenous bone graft: a case report and critical evaluation. J Periodontol 1979; 50: 291–300.
3. Yukna RA. Synthetic bone grafts in periodontics. Periodontology 2000 1993; 1: 92–9.
4. Register AA. Bone and cementum induction by dentine, demineralized in situ. J Periodontol 1973; 44: 49–54.
5. Register AA, Burdick FA. Accelerated reattachment with cementogenesis to dentine, demineralized in situ. I. Optimum range. J Periodontol 1975; 46: 646–55.
6. Register AA, Burdick FA. Accelerated reattachment with cementogenesis to dentine, demineralized in situ. II. Defect repair. J Periodontol 1976; 47: 497–505.
7. Lowenguth RA, Bleiden TM. Periodontal regeneration: root surface demineralization. Periodontology 2000, 1993; 1: 54–68.
8. Karring T, Nyman S, Gottlow J, Laurell L. Development of the biological concept of guided tissue regeneration – animal and human studies. Periodontology 2000 1993; 1: 26–45.
9. Gottlow J, Nyman S. Barrier membranes in the treatment of periodontal defects. Curr Opin Periodontol 1996; 3: 140–8.
10. Caffesse RG, Nasjleti C E, 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.
11. Rutherford RB, Niekrash CE, Kennedy JE, Charette MF. Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys. J Periodont Res 1992; 27: 285–90.
12. Caffesse RG, Quinones CR. Polypeptide growth factors and attachment proteins in periodontal wound healing and regeneration. Periodontology 2000 1993; 1: 69–79.
13. Howell HT, Martuscelli G, Oringer RJ. Polypeptide growth factors for periodontal regeneration. Curr Opin Periodontol 1996; 3: 149–56.
14. Listgarten MA. Electron microscopic study of the junction between surgically denuded root surfaces and regenerated periodontal tissues. J Periodont Res 1972; 7: 68–90.
15. Listgarten MA, Rosenberg MM. Histologic study of repair following new attachment procedures in human periodontal lesions. J Periodontol 1979; 50: 333–45.
16. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. J Clin Periodontol 1982; 9: 290–6.
17. 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.
18. Caton JG, Zander HA. Primate model for testing periodontal treatment procedures: I. Histologic investi-

gation of localized periodontal pockets produced by orthodontic elastics. J Periodontol 1975; 46: 71–4.
19. Caton JG, Kowalski CJ. Primate model for testing periodontal treatment procedures: II. Production of contralaterally similar lesions. J Periodontol 1976; 47: 506–10.
20. Hoffman RL. Formation of periodontal tissues around subcutaneously transplanted hamster molars. J Dent Res 1960; 36: 781–98.
21. Hoffman RL. Bone formation and resorption around developing teeth transplanted into the femur. Am J Anat 1966; 118: 91–102.
22. Hoffman RL. Tissue alterations in intra-muscularly transplanted developing molars. Arch Oral Biol 1967; 12: 713–20.
23. Ten Cate AR, Mills C, Solomon G. The development of the periodontium. A transplantation and autoradiographic study. Anat Rec 1971; 170: 365–80.
24. Ten Cate AR, Mills C. The development of the periodontium: The origin of alveolar bone. Anat Rec 1972; 173: 69–78.
25. Freeman E, Ten Cate AR, Dickinson J. Development of a gomphosis by tooth germ implants in the parietal bone of the mouse. Arch Oral Biol 1975; 20: 139–40.
26. Ten Cate AR. Formation of supporting bone in association with periodontal ligament organization in the mouse. Arch Oral Biol 1975; 20: 137–8.
27. Al-Talabani NG, Smith CJ. Continued development of 5-day-old tooth-germs transplanted to syngenic hamster (Mesocreitus auratus) cheek pouch. Arch Oral Biol 1978; 23: 1069–76.
28. Andreasen JO. Interrelation between alveolar bone and periodontal ligament repair after replantation of mature permanent incisors in monkeys. J Periodont Res 1981; 16: 228–35.
29. Slavkin HC, Boyde A. Cementum: An epithelial secretory product? J Dent Res 1975; 53: 157.
30. Lindskog S. Formation of intermediate cementum I: Early mineralization of aprismatic enamel and intermediate cementum. J Craniofac Genet Dev Biol 1982; 2: 147–60.
31. Lindskog S. Formation of intermediate cementum II: A scanning electron microscopic study of the epithelial root sheath of Hertwig. J Craniofac Genet Dev Biol 1982; 2: 161–9.
32. Lindskog S, Hammarström L. Formation of intermediate cementum III: ^{3}H-proline and ^{3}H-tryptophan uptake into the epithelial root sheath of Hertwig in vitro. J Craniofac Genet Dev Biol 1982; 2: 171–7.
33. Slavkin HC, Bringas Jr P, Bessem C, Santos V, Nakamura M, Hsu M, Snead ML, Zeichner-David M, Fincham A. 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: 28–40.
34. Slavkin HC, Bessem C, Fincham PB, Santos VJr, Snead ML, Zeichner-David M. Human and mouse cementum proteins immunologically related to enamel proteins. Biochemica et Biophysica Acta 1989; 991: 12–8.
35. Luo W, Slavkin HC, Snead ML. Cells from Hertwig´s root sheath do not transcribe amelogenin. J Periodont Res 1991; 26: 42–7.
36. Brookes SJ, Robinson C, Kirkham J, Bonass WA. Biochemistry and molecular biology of amelogenin proteins of developing enamel. Arch Oral Biol 1995; 40: 1–14.
37. Simmer JP, Snead ML. Molecular biology of the amelogenin gene. In: Dental enamel. Formation to destruction. Robinson C, Kirkham J, Shore R, editors. Boca Raton, New York: CRC Press, 1995: 59–84.
38. Hamamoto Y, Nakajima T, Ozawa H, Uchida T. Production of amelogenin by enamel epithelium of Hertwig´s root sheath. Oral Surg Oral Med Oral Pathol 1996; 81: 703–9.
39. Hammarström L. The role of enamel matrix in the development of cementum and periodontal tissues. In: 1997 Dental enamel. Wiley, Chichester (Ciba Foundation Symposium 205) 1997a: 246–60.
40. Hammarström L. Enamel matrix, cementum development and regeneration. J Clin Periodontol 1997b; 24: 658–68.
41. Heritier M. Experimental induction of cementogenesis on the enamel of transplanted mouse tooth germs. Arch Oral Biol 1982; 27: 87–97.
42. Hunt AM. A description of the molar teeth and investing tissues of normal guinea pigs. J Dent Res 1959; 38: 216–31.
43. Listgarten MA. A light and electron microscopic study of coronal cementogenesis. Arch Oral Biol 1968; 13: 93–114.
44. Listgarten MA, Kamin A. The development of a cementum layer over the enamel surface of rabbit molars – a light and electron microscopic study. Arch Oral Biol 1969; 14: 961–85.
45. Ainamo J. Morphogenetic and functional characteristics of coronal cementum in bovine molars. Scand J Dent Res 1970; 78: 378–86.
46. Listgarten MA, Shapiro IM. Fine structure and composition of coronal cementum in guinea-pig molars. Arch Oral Biol 1974; 19: 679–96.
47. Hammarström L, Alatli I, Fong CD. Origins of cementum. Oral Diseases 1996; 2: 63–9.
48. Simpson HE. The degeneration of the rests of Malassez with age as observed by apoxestic technique. J Periodontol 1965; 36: 288–91.
49. 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.
50. Schroeder HE. The periodontium. In: Handbook of Microscopic Anatomy. Oksche A, Wollrath L, editors. Berlin: Springer Verlag, 1986.
51. Weinreb MM, Sharav Y. Tooth development in sheep. Am J Vet Res 1964; 25: 891–908.
52. Benyus JM. Biomimicry – innovations inspired by nature. New York: W Morrow & Comp Inc., 1997.

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