

Biomaterials for the Reconstructive Treatment of Periodontal Intrabony Defects.

Part II. Guided Tissue Regeneration, Biological Agents and Combination Therapies

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The aim of this paper is to present a critical overview, with emphasis on the evidence from human histology, on guided tissue regeneration, different types of biological agents and various combinations used in regenerative periodontal therapy of intraosseous defects. The available evidence indicates that surgical periodontal therapy employing the use of guided tissue regeneration, enamel matrix proteins or growth factors may result in periodontal regeneration. However, it is yet unknown to what extent a combination of guided tissue regeneration, enamel matrix proteins, growth factors and various types of bone grafts or bone substitutes may provide additional histological and clinical benefit.

Key words: periodontal regeneration, human histology, controlled clinical studies, guided tissue regeneration, enamel matrix proteins, growth factors, review

Guided Tissue Regeneration (GTR)

The biological principle of GTR is based on experimental studies demonstrating that the progenitor cells capable of forming new cementum with inserting collagen fibers (i.e. new connective tissue attachment) have their source in the periodontal ligament. By placing a mechanical barrier to cover the periodontal defects, the downgrowth of the epithelial cells can be stopped and the stability of the blood clot ensured. Consequently, the wound area and the previously 'plaque-infected' root surfaces can be repopulated by cells originating from the intact periodontal ligament and alveolar bone (Nyman et al, 1982; Gottlow et al, 1984, 1986; Karring et al, 1993).

The first clinically tested mechanical barrier was the Millipore[®], filter followed by the nonresorbable e-PTFE membranes (Nyman et al, 1982; Gottlow et al, 1984, 1986; Karring et al, 1993). The first human case treated with GTR was reported by Nyman et al, (1982). A deep intrabony defect localized at a lower incisor scheduled for extraction

due to very advanced destruction of the attachment apparatus was treated with a Millipore[®], filter according to the biological principle of GTR. Following removal of granulation tissue an 11 mm deep periodontal defect was diagnosed. A membrane was adapted in order to fully cover the intrabony defect and 2–3 mm of the surrounding alveolar bone. Histological evaluation after 3 months of healing revealed that new cementum with inserting collagen fibers had formed on the previously plaque-exposed root surface.

In a later investigation Gottlow et al (1986) treated 12 intrabony defects with GTR. Histological documentation was also presented in five out of the 12 cases. The results demonstrated the formation of considerable but varying amounts of new cementum, new periodontal ligament and new bone. The observed variability in the results was attributed to factors such as the amount of remaining periodontal ligament, defect morphology, surgical technique and bacterial contamination of the membrane. These observations were later confirmed in controlled animal and human studies

(Pontoriero et al, 1992; Cortellini et al, 1993, 1996; Selvig et al, 1993). It was shown that treatment with GTR may predictably enhance formation of new connective tissue attachment and of new alveolar bone in intrabony and mandibular degree II furcation defects.

Nonresorbable ePTFE membranes require a second surgical procedure to remove the membrane which subsequently may lead to a damage of the newly formed tissues. Furthermore, a common complication associated with the use of nonresorbable ePTFE membranes is membrane exposure followed by subsequent bacterial colonization and even infection. In order to minimize this complication various barrier materials were developed such as collagen, polylactic acid and synthetic biodegradable barriers.

Results from controlled clinical trials have shown that comparable results in terms of probing depth reduction, gain of clinical attachment and radiological hard tissue remodelling can be obtained by using non bioresorbable or bioresorbable barriers (Caffesse et al, 1997; Laurell et al, 1994; Christgau et al, 1995; Eickholz et al, 1998). The materials are degraded by hydrolysis and eliminated from the organism through the Krebs cycle as carbon dioxide and water. Results of animal studies using bioresorbable materials have shown the histological formation of new cementum, new connective tissue attachment and new supporting bone (Gottlow et al, 1994; Caffesse et al, 1994; Hürzeler et al, 1997). The histological results from animals were later confirmed by findings from human case reports (Sculean et al, 1999a, 1999b) (Figs. 1–3).

In a meta-analysis comparing the clinical results following treatment of intrabony defects no statistically significant differences between non resorbable and bioresorbable membranes were found (Karring et al, 2003). The mean gain in clinical attachment following treatment of a total of 351 intrabony defects with non resorbable membranes was 3.7 mm. The mean gain in clinical attachment following treatment of a total of 592 intrabony defects with bioresorbable membranes was 3.6 mm. A further analysis of the results showed that a clinical attachment gain of 2–3 mm was observed in 29.2% of the defects, gains of 4–5 mm in 35.4% of the defects, and gains of 6 mm or more in 24.9% of the defects. A gain of less than 2 mm was observed in 10.5% of the

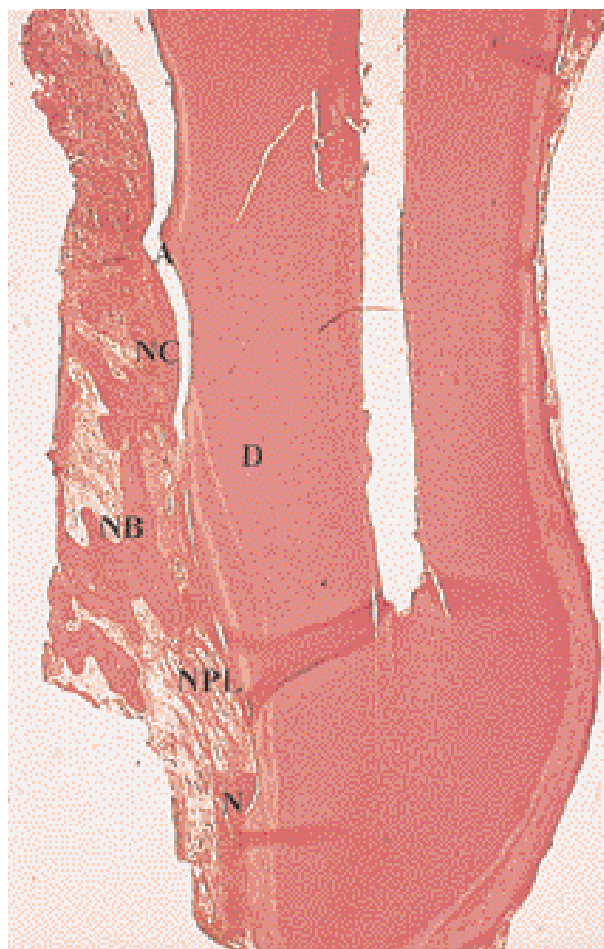


Fig. 1 Healing of a human intrabony defect following treatment with a bioresorbable membrane. The healing occurred through formation of new cementum (NC), new periodontal ligament (NPL) and new bone (NB). D: dentin, N: notch, A: artifact. (Hematoxylin und Eosin stain, original magnification: x 25).

treated defects whereas no change occurred in two cases. The reported results indicate that GTR procedures may predictably result in greater clinical improvements when compared to flap surgery alone. This conclusion was furthermore supported by the analysis of a total of 11 controlled randomized clinical studies in which GTR was compared to conventional flap surgery. In 9 out of the 11 investigations GTR resulted in statistically greater gains in clinical attachment level than flap surgery. Moreover, the weighted mean of the results reported in the 11 studies have shown a gain

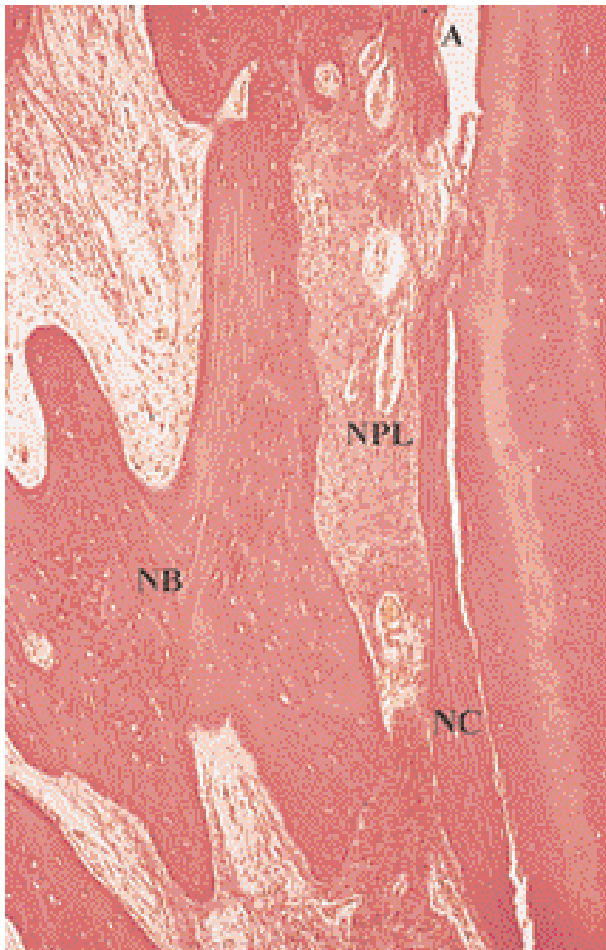


Fig. 2 Higher magnification of the middle part of the defect shown in Fig. 1. The newly formed cementum (NC), the new periodontal ligament (NPL) and the new bone (NB) are clearly visible. A: artifact (Hematoxylin and Eosin stain, original magnification: x 150).

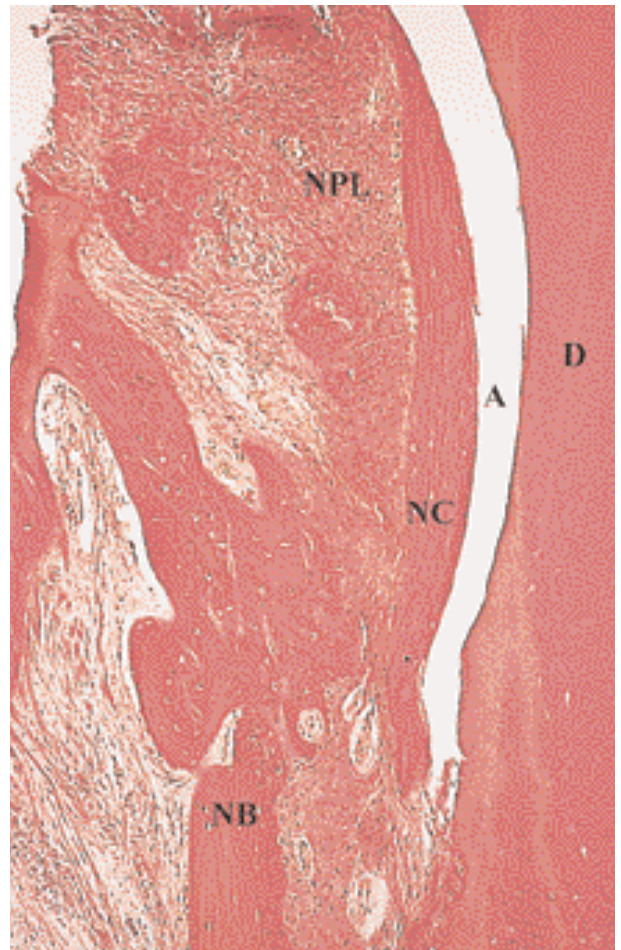


Fig. 3 Higher magnification of the coronal part of the defect shown in Fig. 1. Formation of new cementum (NC), new periodontal ligament (NPL) and new bone (NB) is evident. D: dentin, A: artifact (Hematoxylin und Eosin stain, original magnification: x 150).

in clinical attachment level of 3.3 ± 1.8 mm following treatment with GTR and of 2.1 ± 1.5 mm following flap surgery (Karring et al, 2003). Similarly, a recent systematic review demonstrated that GTR was more effective than open flap debridement in improving clinical attachment levels (Needlemann et al, 2002).

Root Surface Conditioning

It was suggested already thirty years ago that removal of bacterial deposits and of endotoxins from the root surface as well as demineralization of the root surface to expose the collagen of the root dentin may facilitate the deposition of cementum by inducing mesenchymal cells from the adjacent tissue to differentiate into cementoblasts. The exposure of collagen fibers of the dentin matrix may enhance the adhesion of the blood clot to the root surface which subsequently might facilitate fi-

broblast migration (Stahl et al, 1971). Several authors demonstrated the formation in humans of a new connective tissue attachment following flap surgery and root surface demineralization with citric acid (Frank et al, 1983; Stahl and Froum, 1991; Cole et al, 1980). Cole et al (1980) provided histological evidence that in human intrabony defects flap surgery followed by root surface demineralization with citric acid may result in formation of new cementum, new periodontal ligament and new alveolar bone.

Several studies in animal models were able to demonstrate periodontal regeneration following citric acid or tetracycline root surface demineralization; however many specimens demonstrated ankylosis and root resorption (Bogle et al, 1981; Magnusson et al, 1985). Recently, root surface demineralization with 24% EDTA resulted in an exposure of collagen fibers from the dentin matrix without any necrotizing effects on the neighboring soft and hard tissues (Blomlöf et al, 1996a, 1996b). Controlled clinical studies have failed to demonstrate any additional benefit of surgical or non surgical periodontal therapy and root surface demineralization with citric acid or EDTA compared to surgical or non surgical periodontal therapy alone (Moore et al, 1987; Blomlöf et al, 2000a, 2000b).

Growth Factors

Growth factors are a class of polypeptide hormones which stimulate a great variety of cellular events such as proliferation, chemotaxis, differentiation and production of extracellular matrix proteins (Terranova and Wikesjö, 1987). Proliferation and migration of periodontal ligament cells and synthesis of extracellular matrix as well as differentiation of cementoblasts and osteoblasts are prerequisites for obtaining periodontal regeneration. Therefore, it was suggested that the topical application of growth factors may enhance periodontal regeneration. Histological studies examined the effect of treating naturally occurring periodontal defects in dogs by means of flap surgery and the combination of platelet-derived growth factors (PDGF) and insulin-like growth factors (Lynch et al, 1989, 1991). Healing in the test sites was characterized by formation of new cementum, new

periodontal ligament and new bone whereas the control sites (treated with flap surgery only) presented with a long junctional epithelium and no periodontal regeneration. Similar results were reported by other authors following treatment of experimentally induced periodontitis in monkeys with the combination of PDGF and IGF (Rutherford et al, 1992; Giannobile et al, 1994, 1996). In a clinical study the periodontal intrabony and degree II furcation defects were treated with PDGF and IGF. At re-entry after 9 months, significant increase in bone fill was only observed in the furcation defects treated with the growth factor combination (Howell et al, 1997). Therefore, further data from controlled clinical studies are needed before any definitive conclusions can be drawn on the applicability of this type of regenerative treatment in humans.

Bone Morphogenetic Proteins (BMPs) are osteoinductive factors that possess the potential to stimulate mesenchymal cells to differentiate into bone-forming cells (Wozney et al, 1998). Several histological studies in animals have demonstrated periodontal regeneration following treatment of furcation-type defects with BMPs (Sigurdsson et al, 1995; Ripamonti et al, 1994; Jepsen and Terheyden, 2002).

However, human histological studies and controlled clinical studies are needed in order to evaluate the possible role of BMPs in periodontal regeneration.

Enamel Matrix Proteins

Findings from basic research have pointed to the key role of enamel matrix proteins in the development of root cementum, periodontal ligament and alveolar bone (Hammarström, 1997). The biological concept is based on the assumption that the proteins contained in the enamel matrix (especially the amelogenins) play a decisive role in cementogenesis and may mimic the events that took place during the development of the periodontal tissues (Hammarström, 1997). On the basis of these findings the enamel matrix derivative (EMD) from the tooth buds of unerupted teeth from young pigs was isolated, purified and lyophilized (Hammarström et al, 1997). Since enamel matrix proteins are extremely hydrophobic, they had to be brought into soluble form by means of a propy-

Table 1 Evidence for (partial) periodontal regeneration in intraosseous defects from human histology and for superior clinical outcomes compared to open flap debridement from RCTs, meta-analyses and/or systematic reviews

	GTR	Matrix proteins/Growth factors	
	Barrier Membranes	EMD	GF
Human Histology	Yes	Yes	Yes*
RCT (compared to OFD)	Yes	Yes	n/a
Systematic Review (compared to OFD)	Yes	Yes	n/a

HA= hydroxyapatite, EMD = enamel matrix derivative, GF = growth factors, *rhPDGF in combination with DFDBA, n/a = not available

Table 2 Mean differences between test and control groups (open flap debridement) in changes of PPD, CAL and extent of defect fill as assessed by means of meta-analyses

	GTR	Matrix proteins
	Barrier Membranes*	EMD**
Mean difference in PPD change (mm)	0.80	1.60
Mean difference in CAL change (mm)	1.11	1.33
Mean difference in defect fill (mm)	1.39	n/a
Mean difference in defect fill (%)	n/a	n/a

*Needleman I, Tucker R, Giedrys-Leeper E, Worthington H. A systematic review of guided tissue regeneration for periodontal infrabony defects. *J Periodont Res* 2002; 37: 380–388.
 **Trombelli L, Heitz-Mayfield L, Needleman I, Moles D, Scabbia A. A systematic review of graft materials and biological agents for periodontal intraosseous defects. *J Clin Periodontol* 2002; 29(Suppl. 3): 117–135.

lene glycol alginate (PGA) carrier before their use in regenerative periodontal therapy (Gestrelus et al, 1997a). Results from in vitro studies have shown that EMD may not only enhance cementogenesis, but may also inhibit epithelial proliferation (Gestrelus et al, 1997b; Kawase et al, 2000). It could also be demonstrated that EMD promotes the release of autocrine growth factors from periodontal ligament fibroblasts (Lyngstadaas et al, 2001). Thus, one possible explanation for the positive effect of EMD upon periodontal wound healing may be the release of autocrine growth factors from the wound area (Lyngstadaas et al, 2001). In a human immunohistochemical study it was shown that EMD can be detected on the root surfaces for a period of up to 4 weeks following therapy (Sculean et al, 2002a). Recently, certain antibacterial effects and disturbances of bacterial adherence were observed following application of EMD (Van der Pauw et al, 2000;

Sculean et al, 2001a; Spahr et al, 2001; Arweiler et al, 2002). In an ex vivo study involving 24 patients with chronic periodontitis a plaque sample was taken after 4 days of plaque accumulation and divided into 5 equal parts (Sculean et al, 2001a). Each part was mixed with 5 µl of the following solutions: 1) NaCl; 2) EMD dissolved in water; 3) EMD dissolved in PGA vehicle; 4) PGA vehicle; and 5) Chlorhexidine digluconate (CHX). Subsequently, the vitality of the plaque flora was evaluated under a vital fluorescent microscope. EMD dissolved in the PGA vehicle had a very strong antibacterial effect, however it was concluded that this antibacterial effect is mainly due to the effect of the PGA carrier. In a further investigation it was shown that EMD inhibits the growth of the periodontal pathogenic bacteria *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. No living colonies of these pathogenic bacteria could be observed 24 hours

following the application of EMD (Spahr et al, 2001; Newman et al, 2003). Moreover, EMD demonstrated no negative effect on gram-positive bacteria.

Histological studies from animals also provided evidence of periodontal regeneration following treatment of acute and chronic periodontal defects with EMD (Hammarström et al, 1997; Sculean et al, 2000a, 2000b). In a controlled histological study, recession defects were created by surgically removing the entire buccal bone plate and the root cementum (Hammarström et al, 1997). The test defects were treated with EMD, while a coronally repositioned flap was made in the control defects. Eight weeks after surgery the animals were sacrificed and the jaw segments histologically evaluated. In all test defects a new periodontium (i.e. acellular cementum with inserting collagen fibers and new alveolar bone) had developed. In the control defects, the healing was characterized by a long junctional epithelium with very limited cementum and new bone formation. Where new cementum had formed in the control defects, it was mostly more cellular and only partly attached at the root surface. Interestingly, in the test defects no root resorption occurred, while in the control defects root resorption was a very frequently-found phenomenon. It is important to mention that during the entire study period no oral hygiene measures were carried out. In two further studies in monkeys, recession-type and intrabony defects were created surgically (Sculean et al, 2000a, 2000b). The defects were treated with one of the following therapies: a) GTR; b) EMD; c) EMD + GTR; or d) flap surgery alone (control). The histological investigation showed that in the control defects healing was characterized by a long junctional epithelium and a limited periodontal regeneration while treatment with GTR, EMD and EMD + GTR predictably resulted in periodontal regeneration.

Results of the first human histological biopsy were published by Heijl (1997). In this study a recession defect on a lower incisor was surgically created and treated with EMD. After a healing period of 4 months, the tooth including the surrounding soft and hard tissues was extracted and histologically evaluated. A new layer of acellular root cementum covered 73% of the original defect depth. These findings were confirmed in subsequent reports by other authors, not only in recession-type but also in intrabony defects (Sculean et

al, 1999b, 2000c; Mellonig, 1999; Rasperini et al, 2000; Yukna and Mellonig, 2000) (Figs. 4 and 5). However, nonsurgical periodontal therapy (i.e. scaling and root planing with hand or ultrasonic instruments) and subsequent application of EMD did not promote periodontal regeneration in human intrabony defects (Sculean et al, 2003b). Data from controlled clinical studies demonstrated that treatment of intrabony defects with EMD results in a significant reduction of the probing depths and gain of clinical attachment (Heijl et al, 1997; Pontoriero et al, 1999; Silvestri et al, 2000; Okuda et al, 2000; Froum et al, 2001; Sculean et al, 1999c, 2001b; Tonetti et al, 2002; Trombelli et al, 2002; Zuchelli et al, 2002). A randomized, placebo-controlled multicenter study examined the effectiveness of EMD in a split-mouth study in 33 patients (Heijl et al, 1997). The results after 36 months showed a mean CAL gain of 2.2 mm in the test group and 1.7 mm in the control group (open flap debridement). The radiologically determined bone gain amounted to 2.6 mm in the test group, with a 66% fill of the bone defects. However, the control teeth did not show any bone gain. In another controlled clinical study Froum et al (2001) compared the treatment of deep intrabony defects by open flap surgery with and without EMD. Twenty-three patients (with at least 2 intrabony defects each) with a total of 53 defects were treated with open flap surgery + EMD; and 31 defects were treated with open flap surgery alone. After a healing phase of 12 months the defects were re-entered and the defect fill was measured. Treatment with open flap surgery + EMD resulted in a 3 times larger defect fill than flap surgery alone (74% defect fill after flap surgery + EMD vs 23% defect fill after flap surgery alone). In a prospective, controlled clinical study a total of 40 patients were treated by surgical therapy with either EMD or GTR (with a non-bioabsorbable or with 2 bioabsorbable barriers) and compared to a group of patients treated with open flap surgery (control) (Pontoriero et al, 1999). All 4 regenerative procedures were equally effective regarding probing depth (PD) reduction and CAL gain and the results were significantly better than the control treatment.

A recent prospective, randomized, multicenter clinical study reported the treatment of intrabony defects employing the papilla preservation technique with and without application of EMD (Tonetti et al,

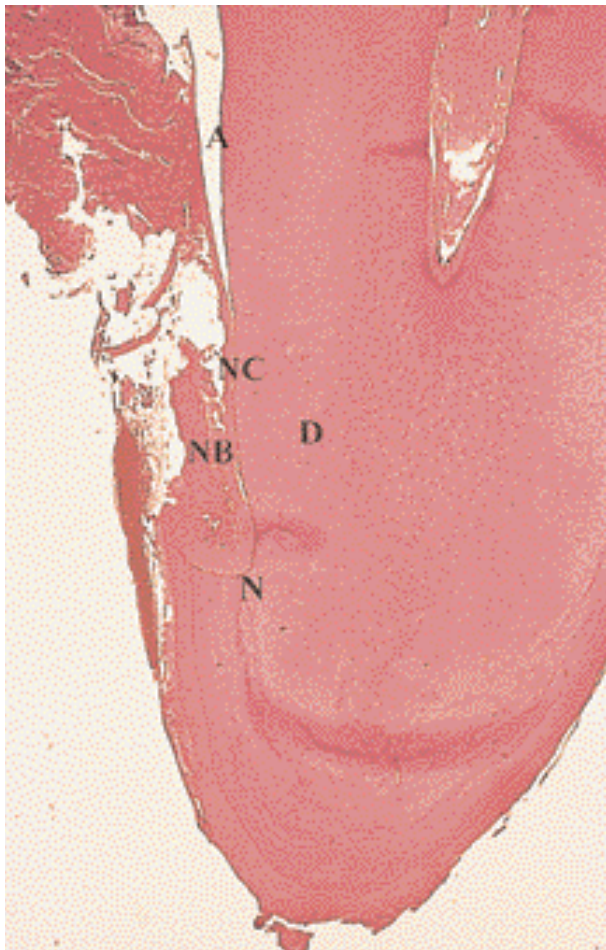


Fig. 4 Healing of a human intrabony defect following treatment with an enamel matrix protein derivative. The healing occurred in formation of new cementum (NC), new periodontal ligament (NPL) and new bone (NB). D: dentin, N: notch, A: artifact. (Hematoxilin und Eosin stain, original magnification: x 25).

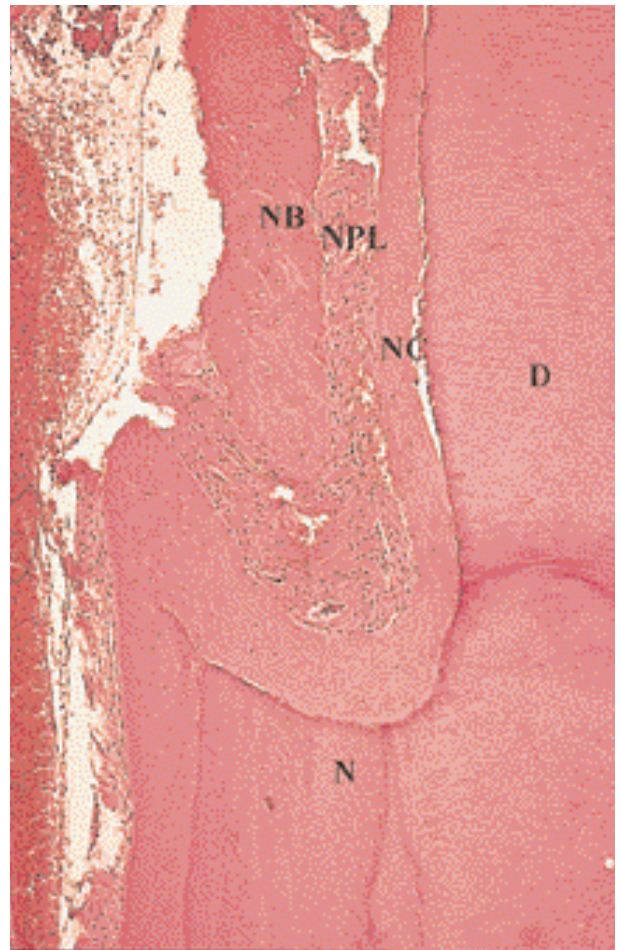


Fig. 5 Higher magnification of the defect shown in Fig. 4. The newly formed cementum, periodontal ligament (NPL) and new bone (NB) are clearly visible. N: notch, D: dentin (Hematoxilin und Eosin stain, original magnification: x 150).

2002). A total of 83 test and 83 control defects were treated. After 1 year there was a significantly greater CAL gain in the test group than in the control group. Comparative studies reported comparable results after treatment of intrabony defects with EMD or GTR, whereby the type of the GTR barrier (non bioabsorbable or bioabsorbable) did not play a role (Sculean et al, 1999b, 1999c, 2001b; Pontoriero et al, 1999; Silvestri et al, 2000; Zuchelli et al, 2002). In a prospective, controlled, clinical study the treatment of intrabony defects was evaluated following treatment with EMD, GTR, a combination of EMD + GTR, and

open flap surgery (Sculean et al, 2001b). The results have shown that all 3 regenerative procedures resulted in a significantly greater improvement of the clinical parameters compared to conventional flap surgery; the combination of EMD + GTR led to no additional improvement. Very recent data also indicated that the clinical results after treatment of intrabony defects with EMD can be maintained over a longer time period (4 and/or 5 years) (Sculean et al, 2001c, 2003c, 2004b).

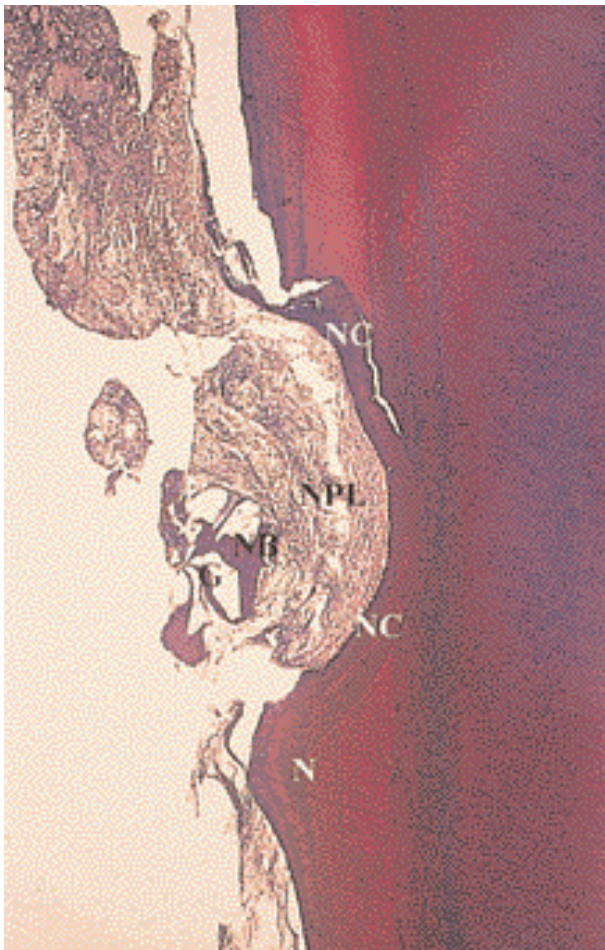


Fig. 6 Healing of a human intrabony defect following treatment with a combination of enamel matrix protein derivative and bioactive glass. The healing resulted in formation of new cementum (NC) and new periodontal ligament (NPL). The graft particles are surrounded by new bone (NB). N: notch. (Ladevig's connective tissue stain, original magnification: x 25).

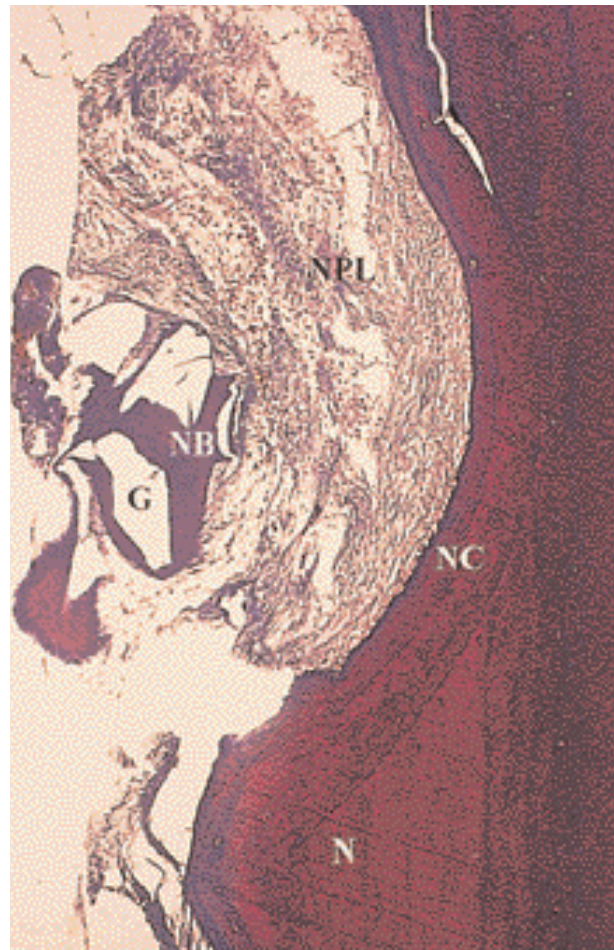


Fig. 7 Higher magnification of the defect shown in Fig. 6. The newly formed cementum (NC) and periodontal ligament (NPL) are evident. The graft particles (G) are surrounded by bone. N: notch. (Ladevig's connective tissue stain, original magnification: x 150).

Combination Therapies

Experimental and clinical studies have indicated that the extent of the regeneration is determined by the available space under the mucoperiosteal flap (Wikesjö and Selvig, 1999; Gottlow et al, 1986; Karring et al, 1993; Selvig et al, 1993). A collapse of the mucoperiosteal flap may limit the area available for the regenerative process and may thus affect the result. In order to avoid these disadvantages, combination therapies of bone substitutes and a cell binding peptide, bone

grafts/substitutes and GTR or PDGF, EMD and GTR, EMD and bone substitutes were tested (Camelo et al, 1998, 2003; Nevins et al, 2003a, 2003b; Sculean et al, 2000a, 2000b, 2001b, 2002b, 2002c, 2003a, 2003d, 2004a, 2004b, 2004c; Yukna et al, 2003). Observations from animal-histological and human-histological studies demonstrated periodontal regeneration after treatment of intrabony defects with some of these combinations (Camelo et al, 1998, 2003; Nevins et al, 2001, 2003a, 2003b; Sculean et al, 2000a, 2000b, 2003a,



Fig. 8 Healing of a human intrabony defect following treatment with a combination of enamel matrix protein derivative and bioactive glass. The healing resulted in formation of new cementum (NC) and new periodontal ligament (NPL). The graft particles are surrounded by new bone (NB). (Ladevig's connective tissue stain, original magnification: x 25).



Fig. 9 Treatment of a human intrabony defect following treatment with a bovine derived xenograft and a collagen membrane. The healing is characterized by formation of new cementum (NC), new periodontal ligament (NPL) and new bone (NB). The graft material (G) is surrounded by bone (NB). D: dentin, A: artifact. (Hematoxylin and Eosin stain, original magnification: x 25).

2004a, 2004c; Yukna et al, 2003) (Figs. 6–10). However, data from clinical studies are controversial and until now no definitive conclusions can be drawn on the possible clinical benefit of a combination therapy in relation to the single therapies (Schallhorn and McClain, 1988; Chen et al, 1995; Mellado et al, 1995; Gouldin et al, 1996; Kim et al, 1996; Kilic et al, 1997; Lekovic et al, 2000; Trejo et al, 2000; Yukna et al, 2000; Scheyer et al, 2002; Velasquez-Plata et al, 2002; Sculean et al, 2002b, 2002c; Stavropoulos et al, 2003).

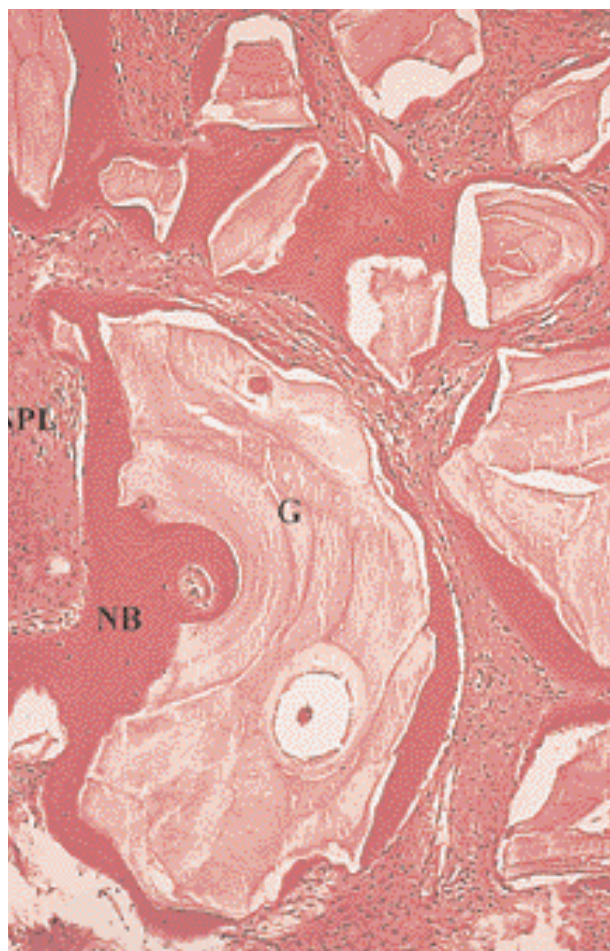


Fig. 10 High power magnification of the defect depicted in Fig. 9. The graft material (G) is surrounded by new bone (NB). PL: new periodontal ligament. (Hematoxylin und Eosin stain, original magnification: x 250).

CONCLUSIONS

In conclusion, the available evidence from human histological studies indicates that surgical periodontal therapy employing the use of GTR, enamel matrix proteins, growth factors, or a combination of these materials may result in periodontal regeneration. However, it is yet unknown to what extent a combination of GTR, enamel matrix proteins, growth factors and various types of bone grafts or bone substitutes may provide additional histological and clinical benefit.

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