Biomaterials for the Reconstructive Treatment of Periodontal Intrabony Defects.
Part I. Bone Grafts and Bone Substitutes

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The aim of the paper is to present a critical overview, with emphasis on the evidence from human histology, on the different types of bone grafts and bone substitutes used in regenerative periodontal therapy of intraosseous defects. The available evidence indicates that surgical periodontal therapy employing the use of autogenous bone, demineralized freeze dried bone allograft, bovine bone derived xenografts, may result in periodontal regeneration. At present there is no evidence from human histological studies demonstrating predictable periodontal regeneration following the use of alloplastic (synthetic) materials.

Key words: periodontal regeneration, human histology, controlled clinical studies, bone grafts, bone substitutes, review

Regeneration is defined as a reproduction or reconstruction of a lost or injured part in a way that both form and function of lost tissues are completely restored (Wikesjö and Selvig, 1999; Caton and Greenstein, 1993; Machtei, 1997; World Workshop in Periodontology, 1996). Regenerative periodontal therapy aims to predictably restore the tooth's supporting apparatus which has been lost following periodontitis or dental trauma. Histologically, these treatment procedures should lead to the formation of new root cementum with inserting collagen fibers, new periodontal ligament and new alveolar bone (Wikesjö and Selvig, 1999; Caton and Greenstein, 1993; Machtei, 1997; World Workshop in Periodontology, 1996). Non-surgical and conventional surgical periodontal therapy may usually result in successful clinical outcomes such as probing depth reduction and gain of clinical attachment. However, histologically these types of treatment are characterized by a long junctional epithelium along the treated root surfaces and no formation of cementum and periodontal ligament (Caton and Greenstein, 1993). Although in some cases a conventional periodontal therapy may result in bone regrowth, histological studies clearly demonstrated that an epithelial lining was interposed between the root surface and the newly formed bone (Caton and Greenstein, 1993). Consequently, histology represents the only way to definitively demonstrate periodontal regeneration (Caton and Greenstein, 1993; World Workshop in Periodontology, 1996). Within the last decades many treatment modalities have been performed in order to predictably obtain periodontal regeneration. Generally, these modalities consisted of various types of surgical techniques involving conditioning of the root surfaces, implantation of various types of bone grafts, guided tissue regeneration (GTR), enamel-matrix-proteins, and growth factors. There is consensus that in order for a procedure to be considered as an approach which facilitates periodontal regeneration the following criteria must be fulfilled (World Workshop in Periodontology, 1996):

1. Controlled histological animal studies demonstrating formation of new cementum, new periodontal ligament and new alveolar bone.
2. Controlled clinical studies demonstrating gains of clinical attachment level and alveolar bone.

3. Human biopsies which demonstrate formation of new cementum, new periodontal ligament and new alveolar bone onto a previously 'plaque infected root surface'.

An overview is presented in the following about the available materials employed in regenerative periodontal therapy of intraosseous defects with special emphasis on the available evidence from human histology.

Bone Grafts / Bone Replacement Materials

The use of bone grafting or replacement materials is based on the assumption that these materials may facilitate formation of alveolar bone, periodontal ligament and root cementum through one of the following mechanisms:

1. Contain bone forming cells (osteoneogenesis).
2. Serve as scaffold for bone formation (osteoconduction).
3. Contain bone-inducing substances (osteoinduction).

The various types of bone grafting and replacement materials can be categorized as follows:

1. Autogenous grafts: grafts transferred from one position to another within the same individual. These grafts can be harvested either from extraoral (for example iliac crest) or intraoral (for example maxillary tuberosities or mandibular retromolar area) donor sites.
2. Allogeneic grafts (allografts): grafts transferred between genetically dissimilar members of the same species.
3. Xenogeneic grafts (xenografts): grafts taken from a donor of another species.
4. Alloplastic materials: synthetic or anorganic materials that can be used as substitutes for bone grafts.

Autogenous Grafts

Autogenous grafts may contain some viable cells which influence bone healing through osteogenesis and / or osteoconduction. They are gradually resorbed and replaced by new viable bone.

Intraoral Autogenous Grafts

These grafts can be harvested either from edentulous areas of the jaw, maxillary tuberosities, mandibular retromolar area, or the chin region. The histological evaluation of human biopsies has demonstrated formation of root cementum, periodontal ligament and alveolar bone following treatment of intrabony periodontal defects with intraoral autogenous grafts (Ross and Cohen, 1968; Nabers et al, 1972; Hiatt et al, 1978; Stahl et al, 1983). Other studies have shown the formation of a long junctional epithelium along the debrided root surface (Froum et al, 1983; Hawley and Miller, 1975; Listgarten and Rosenberg, 1979). The results from controlled clinical studies are controversial. While some studies have indicated higher gains of clinical attachment and defect fill following treatment of intrabony defects with flap surgery and additional placement of intraoral bone grafts than following flap surgery alone, other studies have failed to show any differences between the two procedures (Moskow et al, 1979; Rivault et al, 1971; Froum et al, 1976; Carraro et al, 1976; Ellegaard and Löe, 1971). Taken together the available data from the literature indicate that the use of intraoral autogenous bone grafts may result in periodontal regeneration.

Extraoral Autogenous Grafts

Extraoral autogenous hip marrow grafts have been used for the treatment of intrabony and furcation defects (Renvert et al, 1985; Schallhorn, 1968; Patur, 1974; Froum et al, 1975; Elegaard et al, 1974; Dragoo and Sullivan, 1973a, 1973b). Histological studies from monkeys and humans have shown formation of new cementum, periodontal ligament and alveolar bone following flap surgery and defect fill with iliac crest marrow grafts in intrabony defects (Renvert et al, 1985; Schallhorn, 1968; Patur, 1974; Froum et al, 1975; Elegaard et al, 1974; Dragoo and Sullivan, 1973a, 1973b). Dragoo and Sullivan (1973a, 1973b) have demonstrated periodontal regeneration at 8 months following treatment with iliac marrow grafts, not only in the intrabony component of the defects, but also about 2 mm in the supracrestal sites. However, clinical evidence of root resorption was noted in 7 out of the 250 grafted sites. Ellegaard et al (1974) have evaluated the healing of intrabony lesions in monkeys following placement of iliac crest marrow grafts. The histological evaluation indicated that periodontal regeneration occurred more frequently at grafted than at ungrafted sites, but their use was

frequently associated with ankylosis and root resorption.

Although the use of iliac crest marrow grafts has been shown to enhance periodontal regeneration, these grafts are no longer used in regenerative periodontal therapy due to the high rate of root resorption and patient morbidity associated with the donor site.

Allogeneic grafts

Allogeneic grafts (allografts) have been introduced in order to stimulate bone formation without the additional morbidity associated with the donor site. Although minute, the use of allografts still carries the risk of antigenicity and disease transmission. In regenerative periodontal therapy the most widely used types of allografts are the mineralized freeze dried bone allografts (FDBA) and decalcified freeze-dried bone allografts (DFDBA).

FDBA is a mineralized bone graft, which through the manufacturing process has lost cell viability and thus, promotes bone regeneration through osteoconduction (Goldberg and Stevenson, 1987). Results from clinical studies have shown that treatment of intrabony defects with a combination of FDBA and autogenous bone graft may lead to superior results compared to treatment with FDBA alone (Mellonig, 1991). On the other hand, comparative studies have indicated that treatment with FDBA may render comparable results as treatment with DFDBA or hydroxyapatite (Rummelhart et al, 1989; Barnett et al, 1989). However, in the only controlled clinical study comparing treatment of intrabony defects with flap surgery with and without FDBA, no differences for clinical attachment level gain and bone fill were found between the treatments (Altiere et al, 1979). Furthermore, the evaluation of human biopsies indicated that treatment of intrabony defects with FDBA resulted in the formation of a long junctional epithelium and no periodontal regeneration (Dragoo and Kaldahl, 1983).

Results from animal studies have demonstrated the osteogenic potential of a demineralized bone allograft (DFDBA) due to the release of bone morphogenetic proteins (BMPs) that have the potential to induce bone formation (Urist and Strates, 1970; Mellonig et al, 1981). Controlled clinical studies have shown that treatment of intrabony defects with flap surgery and DFDBA resulted in significantly higher CAL gains and defect fill than treatment with flap surgery alone (Pearson et al, 1981; Meadows et al, 1993; Flemmig et al, 1998). In a randomized controlled clinical trial, Flemmig et al (1998) assessed the long-term maintenance of alveolar bone gain after implantation of autolyzed, antigen-extracted, allogenic (AAA) bone. In each of 14 patients, AAA bone was implanted into the intraosseous defect of 1 tooth (test) whereas a second tooth with an intraosseous defect was treated by modified Widman flap surgery alone (control). All patients were offered supportive periodontal therapy at 3 to 6-month intervals following treatment. Clinical measurements were taken prior to surgery, 6 months, and 3 years following surgery. Of the 14 patients enrolled, 11 patients completed the 6-month and 8 patients the 3-year examination. In test teeth, bone gain was significantly greater compared to control teeth at 6 months (2.2±0.5 mm and 1.2±0.5 mm, respectively) and 3 years (2.3±0.7 mm and 1.1±0.8 mm, respectively) ($P<0.05$). Also, more probing attachment was gained in test compared to control teeth at 3 years (2.0±0.7 mm and 0.8±0.5 mm, respectively; $P<0.05$). The data indicated that alveolar bone gain after implantation of AAA bone may be maintained over a minimum of 3 years in patients receiving periodontal supportive therapy.

Results from a controlled clinical study comparing treatment with DFDBA to FDBA have failed to demonstrate differences in terms of CAL gain and defect fill between the treatments (Rummelhart et al, 1989). Histologic evidence of periodontal regeneration following treatment of human intrabony defects with DFDBA was provided by Bowers et al (1989a, 1989b). However, these results were not confirmed in histological animal studies (Sonis et al, 1985; Caplanis et al, 1998). These controversial data on the regenerative and osteogenic potential of DFDBA could probably be explained by the differences in the osteoinductive potential (from very high to minute) between the various commercially available grafts (Becker et al, 1994, 1995; Shigeyema et al, 1995; Schwartz et al, 1996; Garraway et al, 1998).

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

	Bone grafts/ Bone replacement materials						
	Grafts			Alloplastic materials			
	auto	allo	xeno	Bioactive glass	HA	β-TCP	Polymers
Human Histology	yes/no	yes	yes/no	no	no	no	no
RCT (compared to OFD)	yes	yes	yes/n/a	yes/no	yes	n/a	no
Systematic Review (compared to OFD)	n/a	yes	yes/n/a	yes	yes	n/a	no

HA = hydroxyapatite, 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

	Bone grafts/ Bone replacement materials						
	Grafts*			Alloplastic materials*			
	auto	allo	Xeno**	Bioactive glass	HA	β-TCP	Polymers
Mean difference in PPD change (mm)	n/a	0.41	0.04	0.60	0.98	n/a	n/a
Mean difference in CAL chance (mm)	n/a	0.36	0.90	1.04	1.40	n/a	n/a
Mean difference in defect fill (mm)	n/a	0.00–2.54	1.10–3.50	0.4–1.75	0.55–2.80	n/a	n/a
Mean difference in defect fill (%)	n/a	11.1–53.8	35.2–52.9	–8.5–27.2	22.4–35.9	n/a	n/a

* 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.

** Coralline calcium carbonate

n/a = not available.

Xenogeneic Grafts

Xenogeneic grafts (xenografts) from bovine material were introduced several years ago in regenerative periodontal therapy. Results from human histologic material have demonstrated formation of root cementum, periodontal ligament and alveolar bone following treatment of deep intrabony defects with a bovine derived xenograft (BDX) (Camelo et al, 1998; Sculean et al, 2003). Camelo et al (1998) treated four deep intrabony defects with a bovine derived xenograft. Two defects were additionally covered with a bioresorbable collagen membrane. The histological analysis at 6 and 8 months respectively indicated periodontal regeneration in all four biopsies.

Comparable observations were made also by other authors following treatment of intrabony defects with BDX (Sculean et al, 2003). At six months following the treatment of a deep intrabony defect localized at the distal aspect of a lower molar the histological analysis revealed formation of new cellular cementum with inserting collagen fibers onto the previously 'plaque infected' root surface.

The downgrowth of epithelium stopped at the most coronal aspect of the newly formed cementum whereas most BDX particles were surrounded by a bone-like tissue. Very recent results have also provided histological evidence that a newly developed BDX combined with collagen (BDX Coll) may also promote periodontal regeneration in human intrabony defects (Nevins et al, 2003). Two deep intrabony defects, localized at single rooted teeth were treated with flap surgery and defects were filled with BDX Coll. The clinical evaluation at 9 months after regenerative surgery demonstrated CAL gains of 5 mm and of 9 mm, respectively. Healing occurred in both cases through formation of new cementum, new periodontal ligament and new alveolar bone. Controlled clinical studies indicated that treatment of intrabony defects with BDX may lead to comparable results than treatment with DFDBA (Richardson et al, 1999). However, until now there are no published data from controlled clinical studies comparing the healing of intrabony or furcation defects by means of flap surgery with or without BDX.

The use of coralline calcium carbonate as a bone substitute was introduced some decades ago. Depending on the pre-treatment modality, the natural coral is transformed into non-resorbable porous hydroxyapatite or into a resorbable calcium carbonate. Controlled clinical studies have demonstrated higher PD reduction, CAL gain and defect fill at grafted sites compared to the non-grafted ones (Kenney et al, 1985; Yukna, 1994; Mora and Ouhayoun, 1995; Yukna and Yukna, 1998). Comparative studies indicated that in intrabony defects similar results may be obtained following grafting with FDBA, DFDBA or porous hydroxyapatite on natural coral basis (Barnett et al, 1989; Bowen et al, 1989). Moreover, results from a controlled clinical study have found results in favor of this material when compared to DFDBA (Oreamuno et al, 1990). Histological studies from animals and humans have, however, failed to show evidence for periodontal regeneration following grafting with natural coral. The healing was predominantly characterized by formation of a long junctional epithelium and connective tissue encapsulation of the graft particles (West and Brustein, 1985; Ettel et al, 1989; Carranza et al, 1987; Stahl and Froum, 1987).

Alloplastic Materials

Alloplastic materials are synthetic, inorganic, biocompatible and/or bioactive bone substitutes which are believed to promote healing of bone defects through osteoconduction. The following alloplastic materials have been employed in regenerative periodontal therapy: hydroxyapatite (HA), beta tricalcium phosphate (β-TCP), polymers and bioactive glasses.

Hydroxyapatite (HA)

HA is available in either non-resorbable or resorbable form. Histological studies from animals and humans indicated only limited and unpredictable periodontal regeneration following treatment of intrabony defects with HA (Barney et al, 1986; Minabe et al, 1988; Wilson and Low, 1992; Froum et al, 1982; Moskow and Lubarr, 1983; Ganeles et al, 1986; Sapkos, 1986). The healing was mostly characterized through formation of a long junctional epithelium, whereas most HA particles were encapsulated in connective tissue. Formation of new bone occurred only occasionally, and only in the proximity of the bony walls. Controlled clinical studies have shown higher PD reductions, CAL gains and defect fill at grafted than at non grafted sites (Meffert et al, 1985; Yukna et al, 1985, 1986; Galgut et al, 1992). The results of a recent systematic review, however, demonstrated a high heterogenity between the studies (Trombelli et al, 2002).

Beta-tricalcium phosphate (β-TCP)

Implantation of beta-tricalcium phosphate (β-TCP) in intrabony defects resulted in significant CAL gains and defect fill (Strub et al, 1979; Snyder et al, 1984; Baldock et al, 1985). Histological studies in animals and humans indicated that in periodontal defects, the material is either rapidly resorbed or encapsulated in connective tissue (Barney et al, 1986; Levin et al, 1974; Bowers et al, 1986; Froum and Stahl, 1987; Stahl and Froum, 1986; Saffar et al, 1990). Formation of new cementum and new periodontal ligament did not occur predictably, whereas formation of new bone was found only occasionally.

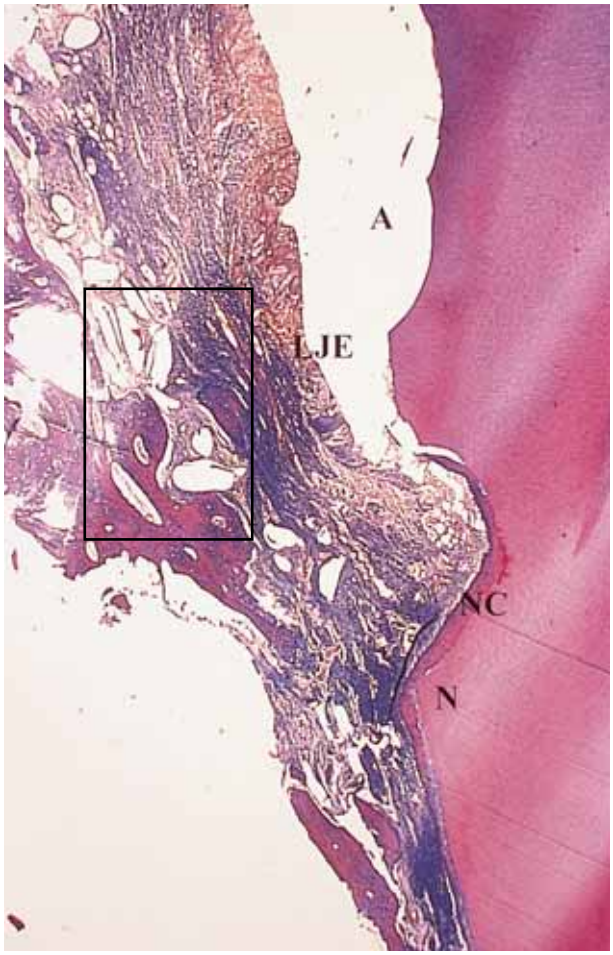

Fig. 1 Healing of a human intrabony defect following treatment with bioactive glass (G). The healing occurred in formation of a long junctional epithelium (LJE) until the coronal part of the notch (N). Formation of cementum (NC) can only be observed in the area of the notch. The graft particles (G) are encapsulated in connective tissue. A: artifact. (Ladevig's connective tissue stain, original magnification: x25.)

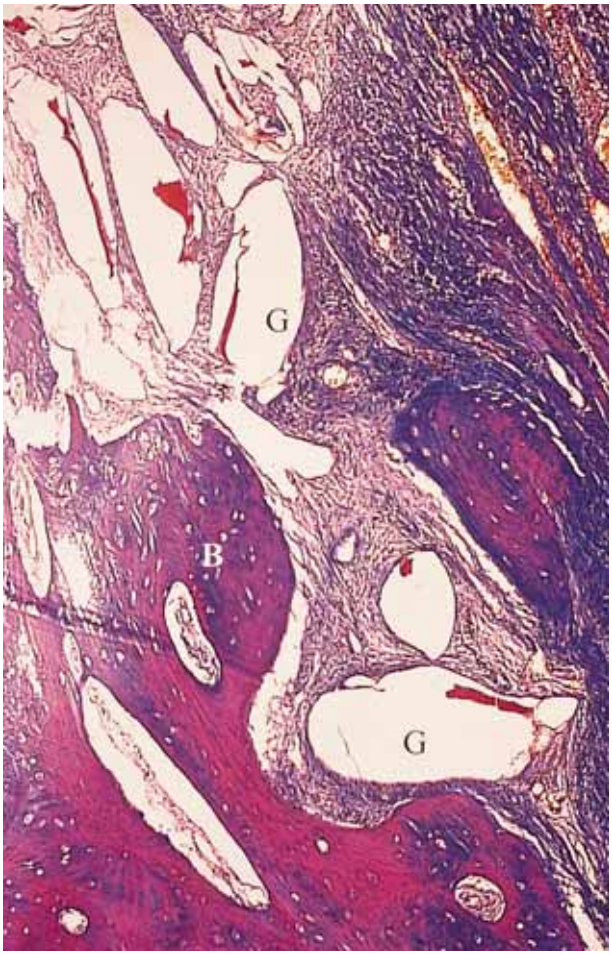

Fig. 2 High power magnification of the area shown in Fig. 1. The bioglass particles (G) are encapsulated in connective tissue. (Ladevig's connective tissue stain, original magnification: x250.)

Polymers

Two types of polymers have been used as bone substitutes in the treatment of intrabony defects:

a) non-resorbable calcium hydroxide coated co-polymer of poly-methyl-methacrylate (PMMA) and poly-hydroxylethyl-methacrylate (PHEMA), which is often referred to as HTR-polymer (hard tissue replacement graft);
b) resorbable polylactic acid (PLA).

Histological studies have failed to demonstrate periodontal regeneration following implantation of HTR polymer in periodontal defects (Plotzke et al, 1993; Stahl et al, 1990; Froum, 1996). In all biopsies the HTR particles were shown to be encapsulated in connective tissue without any formation of cementum and periodontal ligament. Bone formation was found only occasionally. In controlled clinical studies no significant differences in PD reduction and CAL gain were found between HTR grafted and non grafted sites (Yukna, 1990; Shahmiri et al, 1992). Moreover, results from a controlled clinical study have found less PD reduction and CAL gain at PLA grafted sites than at sites treated with flap surgery alone (Meadows et al, 1993).

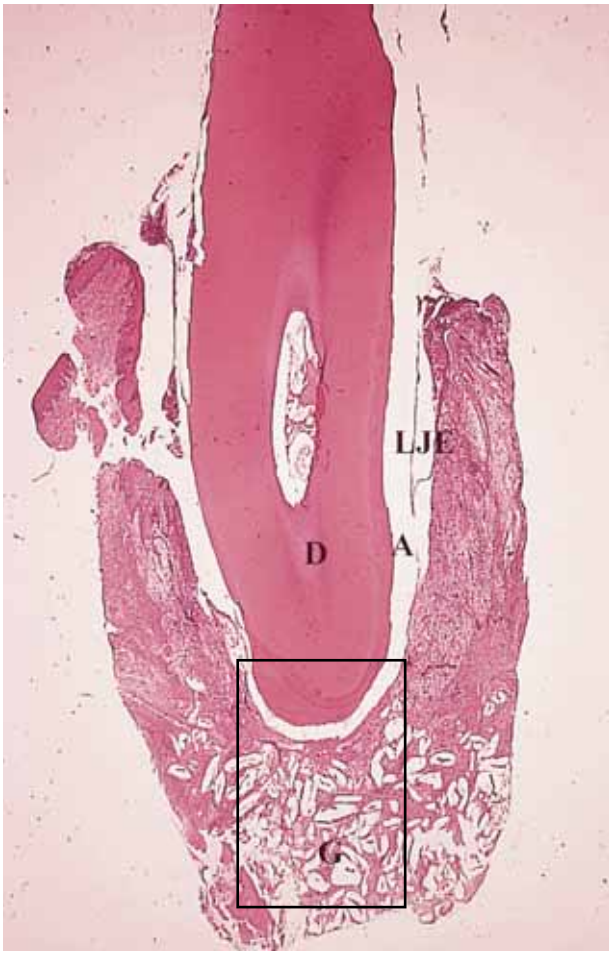

Fig. 3 Healing of a human intrabony defect following treatment with bioactive glass (G). A long epithelium (LJE) has developed along the debrided root surface and there is no evidence for periodontal regeneration. The graft particle (G) are encapsulated in connective tissue. D: dentin, A: artifact. (Hematoxilin and Eosin stain, original magnification: x25.)

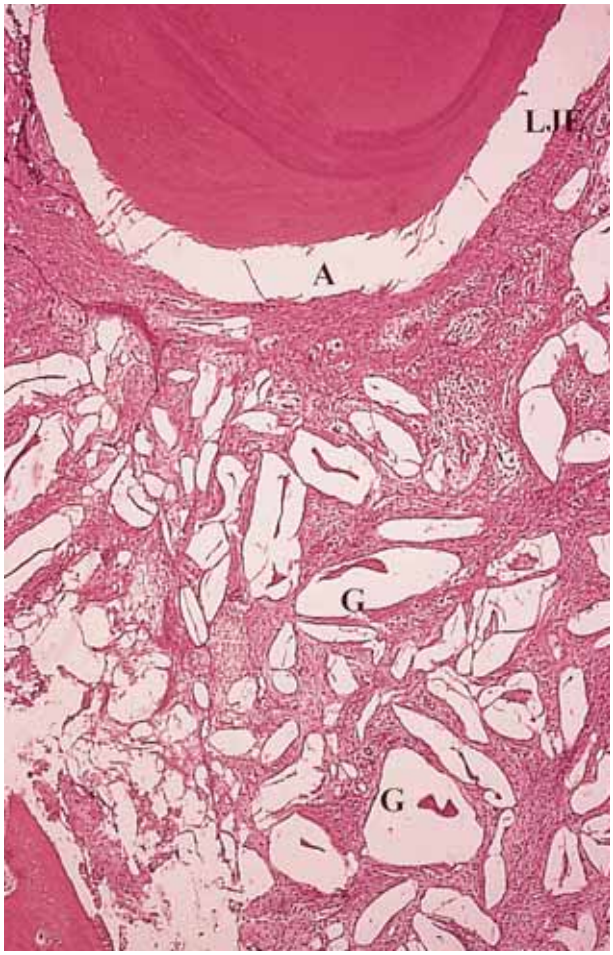

Fig. 4 High power magnification of the defect depicted in Fig. 1. The bioglass particles (G) are encapsulated in connective tissue. (Hematoxilin and Eosin stain, original magnification: x250.) A: artifact, LJE: long junctional epithelium.

Bioactive Glasses

Bioactive glasses are composed of SiO_2, Na_2O und P_2O_5 and are resorbable or non-resorbable depending on the relative proportion of the components. When bioactive glasses are exposed to tissue fluids, a double layer of silica gel and calcium phosphate is formed on their surface. It is believed that this layer promotes absorption and concentration of proteins used by osteoblasts to form extracellular bone matrix which in turn, may promote bone formation. Histological studies from animals have shown that bioactive glasses have good osteoconductive properties, inhibit epithelial downgrowth and enhance the formation of root cementum, periodontal ligament and bone (Karatzas et al, 1999). Recent observations from a human histological case report have reported partial regeneration of root cementum, periodontal ligament and bone following treatment of an intrabony defect with bioactive glass (Rühling and Plagmann, 2001).

However, results from two other human histological studies evaluating a total of eight biopsies have failed to demonstrate predictable periodontal regeneration following grafting with bioactive

glass (Nevins et al, 2000; Sculean et al, 2004). Following grafting of intrabony defects with bioactive glass Nevins et al (2000) obtained substantial PD reductions and CAL gain, but histologically the healing was characterized by a long junctional epithelium and connective tissue encapsulation of the graft particles. A minute amount of new cementum and periodontal ligament was found in only one out of the five evaluated biopsies. Similar findings were also made in a very recent human histological study (Sculean et al, 2004). Following grafting of three deep intrabony defects with bioactive glass, the histological evaluation revealed some signs of periodontal regeneration in only one defect (Figs. 1–4). The material displayed good osteoconductive capacity, but did not seem to predictably inhibit epithelial downgrowth. In a controlled clinical study comparing treatment of intrabony defects by means of flap surgery with and without a bioactive glass, treatment with bioactive glass resulted in significantly higher PD reductions and CAL gains than flap surgery alone (Froum et al, 1998). However, in two other controlled clinical studies grafting with bioactive glass failed to result in superior results compared to flap surgery alone (Zamet et al, 1997; Ong et al, 1998). On the other hand, a comparative clinical study reported similar results following grafting of intrabony defects with bioactive glass or DFDBA (Lovelance et al, 1998).

CONCLUSIONS

The available evidence from human histological studies indicates that surgical periodontal therapy employing the use of autogenous bone, demineralized freeze dried bone allograft and bovine derived xenografts may result in periodontal regeneration. At present there is no evidence from human histological studies demonstrating predictable periodontal regeneration following the use of alloplastic (synthetic) materials.

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