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Bone substitutes and growth factors as an alternative/ complement to autogenous bone for grafting in implant dentistry

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Autogenous bone, with its osteogenic, osteoinductive and osteoconductive properties, has long been considered the ideal grafting material in bone reconstructive surgery (26, 85). However, drawbacks with autogenous bone include morbidity, availability and unpredictable graft resorption (85, 93, 94, 128, 167, 174).

Recent advances in biotechnology have provided the implant surgeon with access to a great variety of bone grafting materials and the possibility of easier implant treatment for the patient as well as for the surgeon. However, the perfect grafting material has yet to be identified. Current research focuses on proteins and carriers for delivering growth factors to the surgical site; however, drawbacks of high production costs and unpredictable results exist. The clinical usefulness of a great variety of materials for bone augmentation in implant dentistry has been seriously questioned (56). The use of osteconductive osteobiologics in implant dentistry remains an experimental procedure until more knowledge becomes available regarding the clinical and biologic aspects of these materials.

Osteoinduction denotes a process of accelerated bone formation that provides an abbreviated healing period. Using solely an osteoconductive grafting material may prolong the healing period with 2–6 months, which may be of clinical significance. Uncontrolled case reports, which suggest a graft healing period of 3–4 months for osteoconductive deproteinized bovine bone or biphasic materials, may mislead the inexperienced dentist. Furthermore, clinical recommendations seem premature when based upon a few animal studies rather than upon comprehensive long-term investigations in humans.

This review discusses clinical studies of bone substitutes, growth factors and bone graft procedures employed with the purpose of augmenting peri-implant sites.

Experimental and clinical studies of autogenous bone grafts and growth factors

The graft

Autogenous grafting may include cortical, cancellous or cortico-cancellous bone, which can appear in one piece, *en bloc*, or in a particulated form. The grafted bone can, on the one hand, be regarded as mainly a partially necrotic tissue that in an unknown time-frame goes through stages of resorption, later to act as a scaffold for new bone formation. On the other hand, swift and gentle handling of the bone graft may permit cell survival and revitalization of the graft *in situ*. Because the survival of osteocytes depends on the presence of a vascular supply within a distance of 0.1 mm (43), cortical bone grafts lacking vascular and cellular pools on endosteal and periosteal surfaces may not be able to sustain cellular viability. Cancellous bone grafts may have a greater likelihood of supporting cell survival because of the possibility of diffusion of nutrients and revascularization from the recipient bed.

Healing of autogenous bone grafts entails both osteoconduction, where new bone is gradually formed around the resorbing graft, and osteoinduction, where released proteins are capable of stimulating osteoblasts or pre-osteoblasts to form new bone. In many aspects, the healing of bone grafts is similar to the healing of fractures. Gordh & Alberius (70) discussed factors of importance for the successful incorporation of autogenous bone grafts, including the embryonic origin of the graft, the rate and extent of revascularization, structural and biomechanical features, rigid fixation of the graft to the recipient site, graft orientation and the availability of local growth factors.

The survivability *in vivo* of cancellous and cortical bone grafts was studied by Albrektsson et al. in a rabbit tibia model with an implanted titanium chamber (3, 4). In this model, surgical trauma to a graft compromised cell viability and caused a delay in the revascularization and remodelling of the traumatized graft (15 days) compared with a carefully handled graft (7 days). Cancellous bone grafts also exhibited a faster rate of revascularization (maximum 0.2–0.4 mm / day) than cortical bone grafts (0.15–0.30 mm / day). The faster revascularization of cancellous bone grafts than of cortical bone grafts has been confirmed in several studies (34, 110, 144). Perhaps variation in the micro-architecture of grafted bone (relative cortical and cancellous composition) can explain study differences in graft volumetric stability and revascularization during healing (25, 133, 134). When a bone block in the rabbit chamber model was cut out, rotated and replaced, the development of new vessels was evident after 5–8 days and remodelling was apparent after 3 weeks of grafting. However, when the rabbit model revealed the presence of smaller vessels (>30 μm in diameter) and functional end-to-end anastomoses, remodelling of a graft was observed as early as 1 week after grafting (2).

The importance of the embryologic origin of bone is another important consideration in bone grafting. Experimental observations in different animal models and in human clinical studies have made it clear that membranous bone grafts (i.e. cranial bone), as a result of less resorption over time, are preferable to endochondral bone grafts (i.e. iliac crest bone) (50, 81, 204, 214, 215).

In the rabbit model, pure cortical membranous and cortical endochondral bone grafts and pure cancellous endochondral bone grafts, were placed as onlays onto the outside of the rabbit cranium (133). At the end of the 16-week study period, the cancellous bone grafts were almost totally resorbed, whereas the cortical bone grafts had lost only 50% of their original volume. Cortical bone of either membranous or endochondral origin showed a similar rate of resorption. The study also suggested that resorption of a graft placed under the periosteum takes place mostly in the height (and less along the perimeter) of the graft (133). The study also revealed a slow change in character of a dense cortical bone graft into a more cortico-cancellous type of bone when placed on a surface of the cranio-facial skeleton. Microcomputed analysis of the grafts during healing confirmed a decrease in mineralized bone content and an increase in internal surface area as a result of the appearance of more trabeculated bone, progressively resembling the structure of the recipient bone (134).

Soft tissue pressure from the periosteum and from the flap covering the graft can increase osteoclastic activity, as suggested by experiments that varied the pressure from the periosteum by means of pre-operative tissue expansion around the recipient bed (68, 69). Rigid fixation of a block bone graft is also important for healing, because there is probably a limit for the motion that is accepted by invading progenitor cells differentiating into soft-tissue-forming cells (fibroblasts) or bone-producing cells (osteoblasts). Studies have shown that a bone graft survives better when rigidly fixated to the recipient site (138, 139).

Gordh & Alberius (70) concluded that a unicortical cortico-cancellous bone graft is best placed with the cancellous part against the recipient site and the cortical part acting as a barrier and 'space-keeper' against the pressure from the flap. A dual-sided cortical bone graft may increase resorption of the recipient site. Also, exposure of the bone marrow of the recipient site by cortical perforation can facilitate revascularization. However, as a block bone graft may be difficult to adapt to the recipient site (i.e. in the maxilla), block bone is sometimes particulated in a mill to facilitate placement into bony grooves and pits (117, 182, 185, 186). Grafting with particulate bone can also reduce the risk of soft tissue ingrowth between the recipient bed and the graft.

Particulated bone grafts are used in sinus-inlay situations (21, 23) and in mandibular (31, 114, 119, 180) and maxillary (22, 185, 187) reconstructions. Bone harvested for particulation for use in maxillofacial reconstruction is obtained from intra-oral sites, such as the posterior lateral part of the mandible, or from extra-oral sites, such as the iliac crest. The bone chips created may vary in density depending upon the ratio of available cortical or cancellous bone, but are, after milling, transformed into a homogenous

paste-like mixture, which will be embedded in blood at the recipient site.

Other arguments for particulating bone include the possibility of more rapid vascular ingrowth and of obtaining a more homogenous and dense graft compared with a cortico-cancellous bone onlay graft from the iliac crest. However, the volumetric stability of the particulated graft has been questioned (42), especially if placed outside the skeletal envelope (22). How particle size, quality and viability, and how various methods of collecting the graft, may affect osteoconductive capability, have been addressed, although not to the same extent as the mode of periodontal surgery and the usefulness of xenografts (15, 162, 212). An orthopedic study found that bone particles collected by reamers retained vital osteoblasts (86), and more vital cells were found in unmilled and cancellous bone than in milled or cortical bone (85). A primate study found that the bone particles should exceed 125 μm in size in order to prevent removal by macrophages (162). Also, the use of sharp instruments for harvesting bone chips seems to benefit graft vitality (35, 54, 55).

Growth factors in bone and in tissue healing

Growth factors are present at low concentrations in bone matrix and plasma, but execute important biologic functions. Growth factors bind to transmembrane receptor molecules on mammalian cells and induce cytoplasmic cascade reactions, which give rise to transcription of mRNA and intracellular and extracellular protein release (192).

Levander (108) observed, in 1938, ectopic bone formation around periosteal-placed and surface-placed free bone grafts in nonskeletal sites. Urist (195) later showed that protein extract from demineralized bone matrix was able to induce bone formation and named, in 1971, the responsible factors bone morphogenetic proteins (197). The tissue-forming potential of bone morphogenetic proteins is closely related to the delivery matrix of the agent and is not species specific (157, 158). Bone morphogenetic proteins are not only capable of inducing bone and cartilage but are also important regulators of morphogenesis during development (11, 207). Bone morphogenetic proteins form a subgroup of the transforming growth factor-β superfamily, which is a large group of proteins that affect cell growth, migration and differentiation, and play a regulatory role in tissue homeostasis and repair in adult organisms (96, 101). The transforming growth factor-β superfamily includes bone morphogenetic proteins, osteogenic proteins, cartilage-derived morphogenetic proteins and growth differentiation factors and bone morphogenetic protein-like molecules (53). At least 30 bone morphogenetic proteins have been identified. Bone morphogenetic protein-2 to bone morphogenetic protein-8 show high osteogenic potential (170).

Bone morphogenetic proteins can induce a local immediate action, bind to extracellular antagonists at the site of secretion, or interact with extracellular matrix proteins and subsequently target cells. *In vitro*, mesenchymal stem cells, from which osteoblasts differentiate, exhibit a great number of bone morphogenetic protein receptors (154). Mesenchymal stem cells also synthesize the bone morphogenetic protein antagonists noggin, gremlin, follistatin and sclerostin, which are capable of blocking osteogenesis as mesenchymal stem cells differentiate into osteoblasts. Bone morphogenetic protein-blocking factors are important also in normal bone turnover and regulation. Bone morphogenetic protein-9 may be highly osteogenic because it is unable to bind to these regulatory molecules (i.e. noggin). Osteoblasts secrete bone morphogenetic proteins as well as their antagonists by a delicate regulatory mechanism during bone formation and remodeling (1).

Transforming growth factor-β has five isoforms, which have various biologic effects (101). Transforming growth factor-β is found at the highest concentration in platelets (9) but is quantitatively most abundant in bone, being present at a concentration of approximately 200 μg/kg of tissue (161). Transforming growth factor-β is produced by osteoblasts, stimulates the expression of bone matrix proteins (208) and suppresses the degrading activity of matrix metalloproteinases and other enzymes (131). Transforming growth factor-β also induces the differentiation or proliferation of osteoblastic cells while inhibiting the formation of osteoclast precursors and, in greater concentrations, may exert an inhibitory effect on mature osteoclasts (20). Smads are the signalling pathways from the membrane of the effector cell to the nucleus for the transforming growth factor-β superfamily (45). Smad-proteins are found in several animal species, which has enabled scientists to use more simple models to understand the transcriptional events of cells affected after cytokine stimulation (171). In contrast to bone morphogenetic proteins, transforming growth factor-β does not induce ectopic bone formation (109).

Transforming growth factor-β release (transforming growth factor-β1, -β2 and -β3), as well as the release of bone morphogenetic proteins 1–8 and growth differentiation factors 1, 5, 8 and 10, are abundant during the healing of fractures (36). Signalling molecules of importance during fracture healing can be categorized into three groups: (i) the pro-inflammatory cytokines (interleukin-1, interleukin-6 and tumor necrosis factor-α), (ii) the transforming growth factor-β superfamily (bone morphogenetic proteins and transforming growth factor-β) and other growth factors (platelet-derived growth factor, fibroblast growth factor and insulin-like growth factors I and II) and (iii) the angiogenic factors [vascular endothelial growth factor, angiopoietins 1 and 2 and matrix metalloproteinases (that degrade bone and cartilage and enable vessel invasion)] (46).

The cytokines interleukin-1, interleukin-6 and tumor necrosis factor-α occur early in the repair cascade. These cytokines are secreted by macrophages and mesenchymal cells present in the periosteum and respond to injury with a peak in expression during the first 24 hours, but are also active in the cartilaginous and remodelling phase of a fracture. These cytokines exert chemotactic activity on inflammatory cells, enhance cellular matrix synthesis and stimulate angiogenesis (46, 104).

Platelet-derived growth factor is a potent mitogen for mesenchymal cells from, for example, the periosteal layer. Platelet-derived growth factor is synthesized by platelets, monocytes, macrophages, endothelial cells and osteoblasts (5). The platelet-derived growth factor is composed of two polypeptide chains (A and B) and these chains form either a heterodimer or a homodimer. Of the three platelet-derived growth factors (platelet-derived growth factor AB, AA or BB), platelet-derived growth factor BB is biologically most potent. In the early stages of fracture healing, platelet-derived growth factor is a powerful chemotactic agent for inflammatory cells and a stimulus for osteoblasts and macrophages (109).

Fibroblast growth factor is produced by monocytes, macrophages, mesenchymal cells, chondrocytes and osteoblasts. Fibroblast growth factor is important in chondrogenesis and bone resorption. The target cells are mesenchymal and epithelial cells as well as chondrocytes and osteoblasts. Two isoforms exist: α-fibroblast growth factor and β-fibroblast growth factor. The α-fibroblast growth factor plays a role in chondrocyte proliferation, and the β-fibroblast growth factor (the more potent isoform) is important for the maturation of chondrocytes and bone resorption during fracture healing (46).

The role of insulin-like growth factors in bone formation has been disputed (12, 61). Sources of insulin-like growth factor are bone matrix, endothelial cells, osteoblasts and chondrocytes (109, 173). Of the two isoforms of insulin-like growth factor, insulin-like growth factor-I is the more potent and involved in bone matrix formation. Insulin-like growth factor-II acts in the later stages of endochondral bone formation. The insulin-like growth factor-binding proteins modulate the action of insulin-like growth factor in a cell-specific manner (163).

In the late phases of fracture healing (i.e. endochondral ossification), and in bone remodeling, cartilage and bone are degraded by matrix metalloproteinases. This allows angiogenic factors to regulate vessel ingrowth by either the vascular-endothelial growth factor-dependent pathway or the angiopoietin-dependent pathway (67). Vascular endothelial growth factor is found in four isoforms (A, B, C and D) and the protein is produced by several cells, including macrophages, smooth muscle cells and osteoblasts. Hypoxia has been found *in vitro* to stimulate vascular endothelial growth factor production by smooth muscle cells and osteoblasts (24, 176). Vascular endothelial growth factor induces the migration and proliferation of endothelial cells by the use of transmembrane adhesion proteins ('intergrins'), which transmit signals from the extracellular surroundings to the cellular genes (106). Vascular endothelial growth factor also induces relaxation in the cell-to-cell contact of endothelial cells, resulting in hyperpermeability of blood vessels. In addition, the stimulated endothelial cells produce matrix-degrading enzymes, which facilitate cell migration (106). Vascular endothelial growth factor was recently shown to be an important factor for enhancing and directing stem cell motility (189).

Platelet-rich plasma in human reconstructive surgery

Platelets

Platelets are un-nucleated fragments of bone marrow megakaryocytes, have a diameter of 1.5–3.0 μm and are the second most abundant particulate body in blood (43, 213). In a resting state, platelets circulate in blood for 9–10 days. Their role is central to hemostasis, wound healing and inflammation. Several activators of platelets are known and some are

produced by platelets themselves. Platelet activators include collagen, thrombin, thromboxane A_2, adenosin phosphate, P-selectin and molecules that ligand to protease-activated receptors, of which three of four identified are expressed on platelets (40, 213). Inhibitors of platelet activation work through the blockade of different receptors on the platelet surface. Inhibitors include several of the coagulation factors, adenosine diphosphate-receptor inhibitors and aspirin (88).

The granules of platelets, released upon activation, are of three major types: dense core granules, α-granules and lysosomes. The dense core granules contain nucleotides, cations and amines, such as serotonin and histamine. The α-granules contain: (i) adhesion molecules (P-selectin, platelet endothelial cell adhesion molecule-1, glycoprotein IIb/IIIa, von Willebrand factor, thrombospondin-1, vitronectin and fibronectin), (ii) mitogenic factors (platelet-derived growth factor, vascular endothelial growth factor and transforming growth factor-β), (iii) coagulation factors (fibrinogen, plasminogen, protein S, kininogens and factors V, VII, XI, and XIII) and (iv) protease inhibitors (C1 inhibitor, plasminogen activator inhibitor-1 and tissue factor pathway inhibitor). Lysosomes contain glycosidases, proteases and cationic proteins (213).

Literature reviews of platelet-rich plasma

The rationale for adding 'extra' platelets in tissue wound healing has been reviewed by Anitua et al. (7) and Soffer et al. (172). Further reviews on the subject are available; some enthusiastic as to the clinical outcome (7, 8, 115, 155, 190), and others more critical (21, 62, 71, 143, 172, 201). The concept of adding platelet-rich plasma to bone grafts was introduced to the maxillofacial and dental community primarily by Tayapongsak et al. (180), Whitman et al. (205) and Marx et al. (116).

The usefulness of platelet-rich plasma has also been reviewed in foot and ankle surgery (66, 72), knee surgery (60), spine fusion surgery (111), thoracic surgery (97) and general surgery (58). Other medical fields that have used autologous platelets are plastic surgery (17, 113), healing of skin and diabetic ulcers (10, 52, 142), eye surgery (135) and sports medicine with tendon and ligament repair (124, 125).

The literature uses different terms for platelet-rich plasma, such as platelet-rich plasma-gel, platelet gel, platelet-rich plasma-clot, plasma-rich in growth factors and modified concept of platelet-rich fibrin (18, 37, 38, 47–49).

From fibrin glue to platelet-rich plasma

Hematologic and immunologic research have examined platelet-rich plasma and platelet-poor plasma for about 50 years (100). The clinical use of platelet-rich plasma evolved from the idea of a fibrin glue (149, 164), which is used during surgical intervention to control bleeding and adhere and seal tissues together. Recently, fibrin was suggested to act as a protector of the blood clot by preventing a leukocyte-mediated premature degradation. Fibrin glue can be prepared from platelet-rich plasma or by mixing concentrated fibrinogen solutions with thrombin, by fibrinogen precipitation (separation by centrifugation of blood elements and concentration of fibrinogen), by cryoprecipitation (fibrinogen is concentrated from plasma by freezing and thaw cycles), or by chemically induced precipitation (ammonium sulphate, ethanol or polyethylene glycol). A wide range of applications have been described for fibrin glue, similar to areas where platelet-rich plasma has been used, including fracture repair, bone grafting, tendon repair, nerve sealing and spine surgery (164).

Preparation of platelet-rich plasma

By using the common centrifugation technique for separating whole blood in hematology, platelet-rich plasma can be prepared in the operating room during surgery. Depending on the amount of platelet-rich plasma needed, blood is drawn from a large or a small peripheral vein of the patient. The whole blood is treated with citrate–phosphate–dextrose to prevent coagulation. Differential centrifugation is achieved using a first 'hard spin' that separates the platelet-poor plasma from the red blood cells and platelet-rich plasma. The second 'soft spin' then separates red blood cells from the platelet-rich plasma. The blood components with the highest specific gravity are located on the bottom of the plasma-filled tube. The platelets are found in a small 'pellet' that, before use, must be dispersed evenly in the plasma. Calcium chloride and thrombin are subsequently added for recalcification and initiation of clot formation.

Human studies on platelet-rich plasma

Platelet-rich plasma has been studied extensively in human and animal models. However, as many clinical reports on platelet-rich plasma are merely case studies, which lack controls, they will not be reviewed here. Published data on platelet-rich plasma-assisted

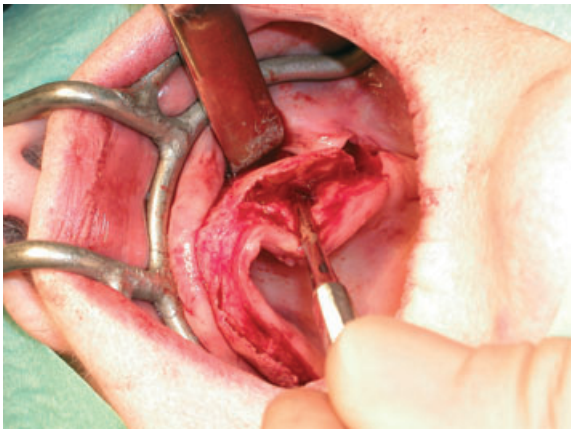

Fig. 1. Extensive resorption of a maxillary alveolar process, requiring bone augmentation prior to implant placement.

grafting show a great variation in clinical outcome, regardless of whether or not the study authors are in support of the platelet-rich plasma concept. The placement of a combined graft of platelet-rich plasma and particulated bone for alveolar ridge augmentation before implant installation is illustrated in Figs 1–5 for the maxilla and in Fig. 6a–d for the mandible.

The clinical use of platelet-rich plasma (sinus grafting as a model) was recently reviewed by Boyapati et al. (21). Platelet-rich plasma was proposed to improve the handling of particulate grafts, to facilitate graft placement and stability, to improve the rate and quality of vascular ingrowth, to increase bone regeneration, to enhance soft tissue healing, and to exert mitogenic effects on critical cells (21). Platelet-rich plasma may also constitute an inexpensive and readily available source of growth factors and a natural biologic sealant that is free of disease-transmission risks.

Grageda (71) proposed a research protocol, based upon histomorphometric specimen analysis, to quantify platelet yield in whole blood, platelet-rich plasma, and growth factors. The need for studies to correlate histomorphometric results with growth factor levels has been emphasized (21).

Periodontal defects and platelet-rich plasma

Periodontal defects have been resolved using membrane-mediated guided tissue regeneration. The usefulness of bovine porous bone mineral and platelet-rich plasma, in conjunction with guided tissue regeneration, has also been evaluated (29, 30, 80, 107). However, the effect of platelet-rich plasma is difficult to determine, as all studies show a good healing response of periodontal defects in both test and control groups. Ouyang et al. (130) found an

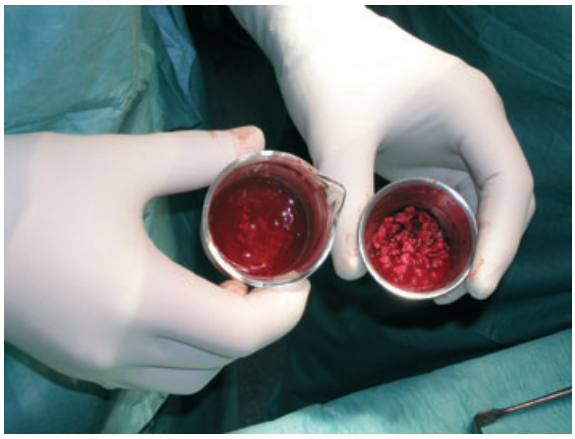

Fig. 2. Platelet-rich plasma in gel form (left) and particulated bone (right) were mixed together before use as an onlay graft in the reconstruction of alveolar bone. A platelet-rich plasma-particulated bone graft is allowed to heal for 4 months before implant placement.

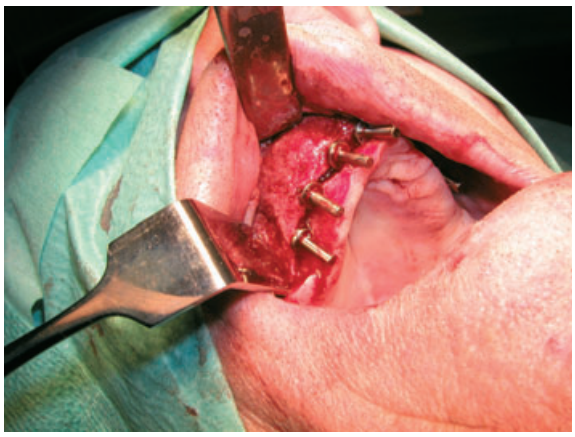

Fig. 3. The grafted maxilla at the time of implant installation.

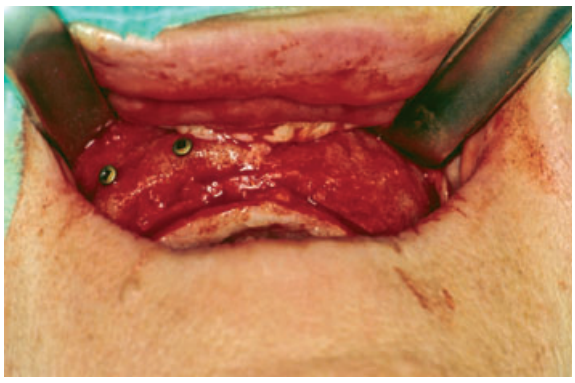

Fig. 4. Block bone grafting with fixation screws (left) and particulated bone (right) after 6 months of healing. This illustration shows two common modes of bone grafting, but also points to the common problem of extensive resorption of the grafted bone (Thor et al. [185]).

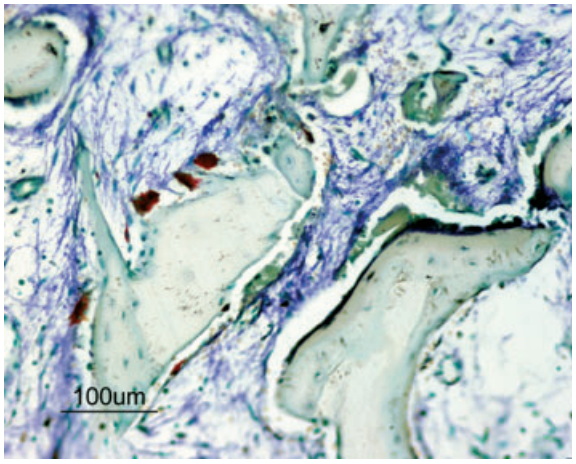

Fig. 5. A nondecalcified histologic specimen of alveolar bone treated with platelet-rich plasma. Alkaline phosphatase positivity (blue staining) was observed on bone surfaces (indicating osteoid and osteoblastic activity) and within the bone (indicating osteocyte activity). Acid phosphatase positivity (red staining) was indicative of osteoclasts. An abundance of alkaline phosphatase activity was detected within the soft tissue cavities.

additive effect of platelet-rich plasma with bovine porous bone mineral in intrabony periodontal defects in a small group of patients. Okuda et al. (129) compared hydroxyapatite and platelet-rich plasma with hydroxyapatite and saline and found, after 1 year, more bone regeneration in patients receiving platelet-rich plasma. In the treatment of gingival recession defects with a coronally advanced flap, no effect of platelet-rich plasma was detected (89).

Simon et al. (165) studied patients undergoing mandibular third molar extraction, with seven patients randomly receiving platelet-rich plasma inserted into the extraction socket and seven patients serving as controls. Clinical parameters, such as mouth opening, use of analgesics, and swelling, were evaluated, despite these features being of unknown relevance with respect to platelet-rich plasma efficacy, but histomorphometric analysis was not performed. Radiographic analysis showed that the use of platelet-rich plasma after a few weeks yielded evidence of better bone formation, an observation that, together with other favourable signs of soft tissue healing, led the authors to recommend the use of platelet-rich plasma after molar extraction. Sammartino et al. (156), in a somewhat better designed study of 18 patients, extracted bilateral mesioangular-positioned third molars that, pre-operatively, exhibited a probing depth of ≥7.5 mm and a probing attachment level of ≥6 mm. The authors used platelet-rich plasma in half of the extraction sites in a split-mouth design study. Sited treated with platelet-rich plasma showed a significant ($P < 0.05$) reduction in probing depth and probing attachment level compared with control sites. However, re-entry procedures with bone

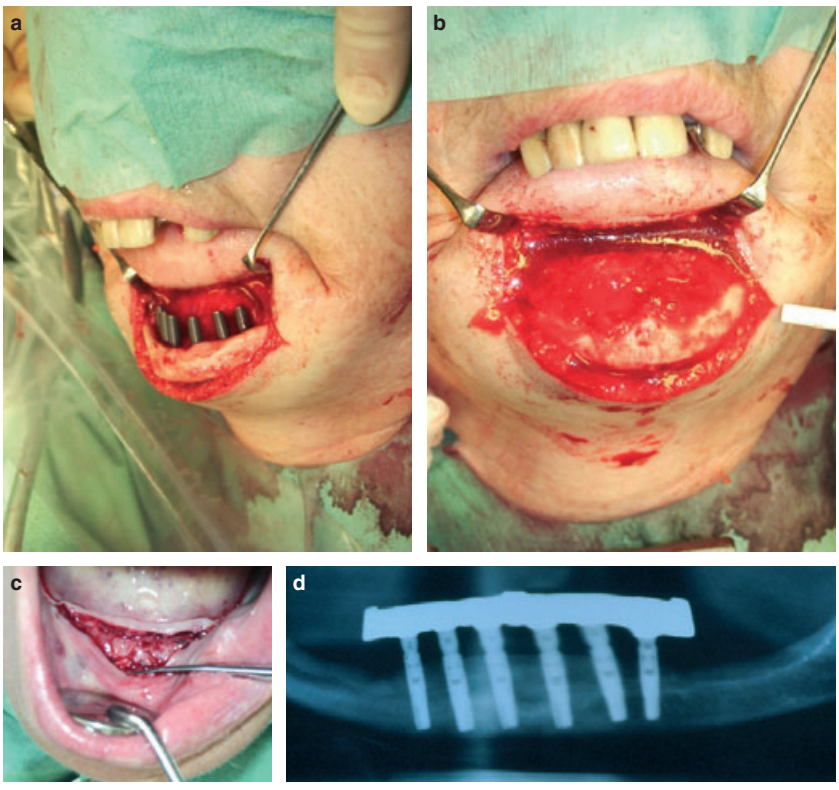

Fig. 6. (A–D) Extra-oral installation of implants in a severely resorbed mandible, performed according to the technique of Marx et al. (119). A mixture of platelet-rich plasma and particulated bone was packed around 15-mm-long implants (Astra Tech, Mölndal, Sweden). Abutment surgery was performed after 10 weeks of healing. The radiograph shows a stable clinical situation at 2 years of follow-up.

biopsies were only performed in sites treated with platelet-rich plasma, and no data were reported on the level of bone formation other than 'considerable observable bone regeneration'.

Platelet-rich plasma with implants

In a small study population, Anitua (6) reported positive effects on bone integration and adjunctive soft tissue healing after introducing platelet-rich plasma into surgical sites before implant installation. Monov et al. (126), in a split-mouth study, inserted platelet-rich plasma into surgical sites in the posterior part of the mandible before implant placement, and the same clinical procedure was followed in contralateral jaw sites, but without the addition of platelet-rich plasma. The implants were analyzed using resonance frequency analysis from installation to 44 days. No difference in healing was observed between the test and control implant sites. Additionally, the implant stability quotient value between days 0 and 4 was reduced significantly in both test and control groups.

Sinus augmentation and platelet-rich plasma

The posterior edentulous maxilla is a difficult region for implant placement because of a limited amount of remaining bone. Initial reports on decreased healing time for platelet-rich plasma grafts (6, 116) and high morbidity of autogenous bone grafts (118, 183) originally made some dentists reluctant to use platelet-rich plasma for sinus augmentation. However, studies on platelet-rich plasma in sinus augmentation are mostly case report series without controls (73, 112, 120, 136, 137, 150).

Thor et al. (184), in a split-mouth study, found, after 3 months, significantly more new bone in biopsies from sinuses grafted with autogenous particulated bone and platelet-rich plasma than in controls where no platelet-rich plasma was used. However, allografts used in conjunction with platelet-rich plasma can demonstrate a relatively large variation in the level of bone formation (73, 98, 112, 126, 136, 137, 150, 175, 200). Raghoebar et al. (146) suggested that the use of allografts together with platelet-rich plasma in sinus augmentation procedures is unlikely to produce growth of new bone, as allografts lack cellular elements upon which platelet-rich plasma-associated growth factors can act. Some researchers have used autogenous bone to ensure cellular supply (126).

Wiltfang et al. (206) studied the ability of platelet-rich plasma to enhance bone formation and resorption of β-tricalcium phosphate (Cerasorb®, ceramic granules of 1,000–2,000 μm) with sinus augmentation. Seventeen sinuses received β-tricalcium phosphate and platelet-rich plasma, and 18 sinuses received β-tricalcium phosphate alone. Ten additional sinuses were grafted with β-tricalcium phosphate and platelet-rich plasma but were excluded from the study because of sinus membrane lacerations and a low platelet-rich plasma concentration (less than three times the average platelet concentration of normal plasma). The mean concentration of platelets in the platelet-rich plasma was 4.1 times higher than that of normal plasma. The mean bone formation in the test group was 38% (range 32–43%) and in the control group was 29% (range 25–37%). No difference in degradation of β-tricalcium phosphate was seen between the test and control groups. The authors concluded that platelet-rich plasma was not beneficial in sinus augmentations in the absence of osteoblasts or osteocytes. Raghoebar et al. (146) performed a randomized split-mouth study of platelet-rich plasma in combination with autogenous bone grafts from the iliac crest in five patients. At 3 months postgrafting, bone biopsies harvested with a trephine were evaluated using microradiographs and histomorphometry. The level of transforming growth factor-β did not change as a result of the platelet-rich plasma procedure, as evaluated in serum before and after the procedure, and platelet-rich plasma did not appear to enhance bone formation. Oyama et al. (132), in a study with relatively few patients, used autogenous bone and platelet-rich plasma (test) and autogenous bone and fibrin glue (control) to treat patients with cleft palate. A computed tomography scan at 5–6 months suggested that platelet-rich plasma increased the volume of bone. Ueda and co-workers (83, 194, 209) found beneficial effects of platelet-rich plasma in extracorporal expansion of mesenchymal stem cells. They also transplanted injectable bone (mesenchymal stem cells, platelet-rich plasma, and β-tricalcium phosphate) into human sinuses and alveolar clefts, and concluded that platelet-rich plasma can be beneficial in those types of treatment.

Platelet-rich plasma in large bony defects

Marx et al. (116) reported on 88 patients with mandibular continuity defects, who were treated with autogenous bone grafts, with or without the addition of platelet-rich plasma. A monoclonal

antibody study at baseline demonstrated a match between the sequestered platelets and the particulated iliac donor bone, the presence of transforming growth factor-β and platelet-derived growth factor in the platelet-rich plasma preparation, and the presence of transforming growth factor-β and platelet-derived growth factor receptors in the grafting bone. At 6 months postgrafting, implants were placed with at least one implant in each grafted area. A histomorphometric serologic analysis of the grafted bone confirmed the presence of transforming growth factor-β, but not of platelet-derived growth factor. The histomorphometric analysis also revealed more trabecular bone in the platelet-rich plasma group (74 ± 11%) than in the grafting group receiving no platelet-rich plasma (55 ± 8%) or in the native mandible (39 ± 6%). Furthermore, panoramic radiographs were used to evaluate, in a subjective manner, maturation of the grafts after 2, 4, and 6 months. A twofold faster bone maturation rate was found for the platelet-rich plasma-treated defects than for the controls.

Merkx et al. (122) reported on eight patients with malignant mandibular tumors, where the resection gab in six of the patients was successfully closed by means of autogenous bone grafts supplemented with platelet-rich plasma. One remaining patient died and another patient had an unsuccessful graft as only fibrous tissue was formed in the resection gab. In a consecutive report from the same research group (166), 11 ameloblastomas were removed by resection osteotomy. After 50 GY of radiation, six patients had the cortical part of the mandibular bone serving as a scaffold for combined grafts of particulated autogenous bone and platelet-rich plasma. The remaining five patients were reconstructed by titanium plates and bone grafts. This immediate reconstruction procedure rendered satisfactory results, as assessed by a quality-of-life study. Seven patients were free of complications, but four patients, all of whom were the first treated in the series, experienced problems postoperatively, including fracture of the scaffold and infections. The failures may partly have been caused by a learning-curve effect.

Robiony et al. (148), who combined platelet-rich plasma and autogenous bone, successfully distracted three patients for later implant placement. Kitoh et al. (102) used marrow-derived mesenchymal stem cells and platelet-rich plasma to enhance osteogenesis distraction of femur and tibia bones. However, a preliminary study of nonunion patients after orthopedic long-bone trauma found bone morphogenetic protein-7 to yield a significantly better outcome than platelet-rich plasma in a nongrafting procedure (28).

Experimental and clinical studies on bone graft substitutes: osteobiologics

Allografts

Allografts consist of bone tissue from a donor of the same species. They contain no viable cells (196). Allografts are probably incorporated into existing bone by a process similar to that of autogenous bone grafts, but proceed more slowly as a result of the absence of living cells. In animal studies, allografts have been found to possess bone-stimulating proteins and, consequently, osteoinductive properties (13, 147). Examples of allografts are fresh-frozen bone, freeze-dried bone and demineralized freeze-dried bone. Fresh-frozen bone grafts are rarely used in implant surgery (105) because of the risk of immunologic rejection and transfection; however, more than 150,000 fresh-frozen bone graftings are performed yearly by orthopedic surgeons in the U.S.A. Donors are tested twice for infectious diseases (human immunodeficiency virus infection and hepatitis) before using their bone in another individual. Immunologic risks are minimized by freezing of the bone.

Prospective studies of fresh-frozen bone grafts in implant dentistry are still lacking. However, in a histologic case report, in which fresh-frozen bone was used to widen the alveolar crest of an edentulous maxilla, fresh-frozen bone was found to integrate with a healing time similar to that of autogenous bone (87). In another study, four patients with severely resorbed maxillae underwent rehabilitation with onlays of allogeneic femoral head bone for later placement of titanium implants (105). At 20 months, 22 of 23 implants were osseointegrated. At 6 months postgrafting, biopsies revealed vital bone and blood samples absence of auto-antibodies (105).

Freeze-dried bone and demineralized freeze-dried bone allografts are reported to be less imunogenic than fresh-frozen bone allografts (63). These materials are frequently used in mixtures with osteoinductive autogenous bone or bone substitutes (19, 169). However, the clinical importance of the osteoinductive potential is questionable (27). Boeck-Neto et al. (19) found no histomorphometric difference between demineralized freeze-dried bone mixed with autogenous bone, and hydroxyapatite mixed with autogenous bone.

Xenografts

Xenografts consist of bone mineral from animals or bone-like minerals (calcium carbonate) derived from corals or algae (59, 92).

Deproteinized bovine bone is the most researched grafting material and is widely used in dentistry because of its similarity to human bone. Proteins in deproteinized bovine bone have been extracted to avoid immunologic rejection after implantation; however, as the deproteinizing procedure eliminates the osteoinductive capacity, deproteinized bovine bones act solely as an osteoconductive scaffold.

In a rat calvarial model, three grafting materials were compared: plaster of Paris, particulated dentine and deproteinized bovine bone (99). All three grafting materials generated new bone and all were found to be suitable for use as bone substitutes. However, deproteinized bovine bone showed the largest and most rapid bone formation. In a histomorphometric study in dogs, Berglundh et al. (16) found a reduction from 17 to 11% in the amount of deproteinized bovine bone particles in biopsies obtained during a 3–7-month time period. In a chimpanzee study, McAllister et al. (121) detected a decrease in the percentage of anorganic area, from 19 ± 14% to 6 ± 3% over a time period of 7.5–18 months. Other animal studies also found promising bone formation using deproteinized bovine bone (65, 74, 91, 103). However, whether deproteinized bovine bone is resorbable or nonresorbable is a topic of discussion. A study on defects in rabbit skulls found that deproteinized bovine bone particles had almost completely disappeared after 14 days of healing, as a result of the action of multinucleated cells (103). In a chimpanzee study, Hürzeler et al. (91) identified osteoclastic resorption of deproteinized bovine bone. In a dog study where a biologic glue (Tisseel®, Duo Quick; Immuno, Vienna, Austria) was added to the graft, no bone formation was found around the deproteinized bovine bone particles but a large number of the particles had disappeared as a result of giant cell resorption (32).

In a minipig model, augmentation of the maxillary sinus was performed using deproteinized bovine bone, with or without recombinant human osteogenic protein (= bone morphogenetic protein-7) (181). A significant increase in bone formation was found (38.6–80.0%) when recombinant human osteogenic protein was added to the graft. Other studies, which compared recombinant human osteogenic protein and platelet-rich plasma in bilateral sinus grafts using anorganic bone as a carrier, found platelet-rich plasma to be ineffective but recombinant human osteogenic protein to stimulate bone formation (151, 152).

In human studies of maxillary sinus augmentation, deproteinized bovine bone with or without a mixture with autogenous bone has histologically been associated with active bone formation (64, 75, 77, 159, 168, 179, 198, 199, 210). Froum et al. (64) used platelet-rich plasma together with anorganic bovine bone in the maxillary sinus, but found no statistical increase in the amount of vital bone produced as a result of adding platelet-rich plasma, confirming the findings in the experimental animal studies. Piattelli et al. (140) performed maxillary sinus augmentation using deproteinized bovine bone and autogenous bone in 20 patients and found, in biopsies harvested after 6–9 months of healing, an average of 30% newly formed bone, 30% deproteinized bovine bone, and 40% bone marrow. Increased mineralization was detected after 14 months, and osteoclastic resorption of the deproteinized bovine bone particles was found after 4 years. Valentini et al. (199) used deproteinized bovine bone in the floor of the maxillary sinus in 15 patients. Biopsies harvested after 6 months revealed 21% new bone, 39% deproteinized bovine bone, and 40% marrow, and after 12 months 28% new bone, 27% deproteinized bovine bone, and 45% marrow was found. The reason for the 12% decrease in the amount of deproteinized bovine bone particles during the second 6-month study interval is not known. Yildirim et al. (210) studied maxillary sinus augmentation using deproteinized bovine bone mixed with blood in 15 sinuses of 11 patients. After 4–9.5 months of healing, biopsies revealed 14.7% new bone, 55.6% soft tissue, and 29.7% deproteinized bovine bone. Twenty-nine per cent of the deproteinized bovine bone particles were in contact with newly formed bone (210). In another study, by Yildirim et al. (211), 13 maxillary sinuses in 12 patients were treated with a mixture of deproteinized bovine bone and autogenous bone along with a resorbable membrane on the lateral side of the graft (BioGide®; Geistleich, Pharma AG, Wolhusen, Switzerland). Biopsies harvested after 6–9 months revealed 19% new bone, 51% connective tissue and 30% deproteinized bovine bone. Thirty-three per cent of the deproteinized bovine bone particles were in contact with newly formed bone (211). Hallman et al. (75) used an 80:20% mixture of deproteinized bovine bone and autogenous bone in the floor of the sinus. Biopsies harvested after 6 months of healing showed 54% fibrous connective tissue, 21% lamellar bone, 14% deproteinized bovine bone particles and 10%

immature bone; 28% of the particles were in contact with newly formed bone. The corresponding figures from 3-year biopsies were 36% fibrous connective tissue, 51% lamellar bone, 12% deproteinized bovine bone particles and 1% immature bone; 54% of the particles were in contact with newly formed bone. The area of deproteinized bovine bone particles did not decrease during the 3-year follow-up period (75). In another study carried out by Hallman et al. (77), an 80:20% mixture of deproteinized bovine bone and autogenous bone was compared with deproteinized bovine bone only or with autogenous bone only. Micro-implants harvested with a surrounding bone core at 6–9 months after placement showed no statistical difference in implant osseointegration and bone formation for the three grafting materials used (77). This finding, and those of other studies (56, 76, 210), indicate that autogenous bone may not be needed as a grafting material in the floor of the maxillary sinus.

Several long-term follow-up studies have concluded that osteoconduction proceeds for several years, but no data are available to determine whether a mixture of autogenous bone and deproteinized bovine bone may shorten the healing time of the graft (75–77, 140, 191). However, a healing period of 8 months is recommended for deproteinized bovine bone when used as the only grafting material, compared with a healing period of 6 months for autogenous bone grafts (76, 77). Excellent results of implant survival for implants placed after sinus floor augmentation with DBB have also been presented and it is concluded that today this material is considered the standard of care for sinus augmentation procedures (56).

The resorption of deproteinized bovine bone by means of osteoclastic activity is a topic of controversy, especially when comparing results from experimental animal studies and clinical human studies. Some human studies have detected resorption of deproteinized bovine bone particles (140, 202). Wallace et al. (202) found no signs of deproteinized bovine bone particles after 20 months of healing; whether this finding reflects the biopsy technique used or was the result of true resorption is unclear. Piatelli et al. (140) observed osteoclastic activity around deproteinized bovine bone particles in 4-year specimens, although the deproteinized bovine bone particles seemed to remain intact. Schlegel & Donath (159) found no signs of resorption of deproteinized bovine bone particles after 6 years. Skovlund et al. (168) suggested that deproteinized bovine bone particles were slowly degraded (168). However, most studies failed to detect evidence of deproteinized bovine bone resorption (75, 77, 159, 179, 198, 199, 210, 211). The reported variation in resorption of deproteinized bovine bone may stem from differences in response from animal studies, surgical technique, biopsy technique, and histologic preparation technique (decalcified preparation or standard sectioning). The histologic decalcification process of the deproteinized bovine bone particles always causes shrinkage, which might be misinterpreted as resorption.

From a biologic point of view, resorption requires adhesion molecules (arginine–glycine–asparagine sequences) for the attachment of osteoclastic cells to plasma and extracellular matrix proteins, including fibronectin, fibrinogen, vitronectin, type I collagen, osteopontin and bone sialoprotein (141). As deproteinized bovine bone is free of proteins (14, 203), osteoclastic resorption can probably not occur. However, Schwartz et al. (160) found transforming growth factor-ß and bone morphogenetic protein-2 in deproteinized bovine bone particles, which might explain the lacunae found on some deproteinized bovine bone particles. Some degree of degradation by macrophagial phagocytosis may occur, but is probably too limited to explain the reported degradation of deproteinized bovine bone in some animal (16, 33, 78, 79, 91, 103, 121, 123) and human (140, 202) studies.

Studies examining biopsies harvested after 3 years (75) and 9 years (191) reported deproteinized bovine bone particles to be in close contact with giant cells, but without exhibiting signs of resorption (Fig. 7, Fig. 8a,b). However, Traini et al. (191) interpreted these giant cells as actually resorbing deproteinized bovine bone particles. Hallman et al. (75) interpreted

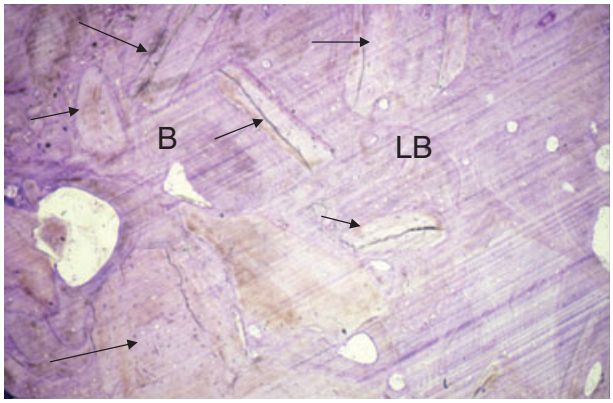

Fig. 7. Light micrograph of a nondecalcified specimen retrieved after 3 years, showing numerous deproteinized bovine bone (Bio-Oss®) particles incorporated in lamellar bone (LB) with no signs of resorption (arrows).

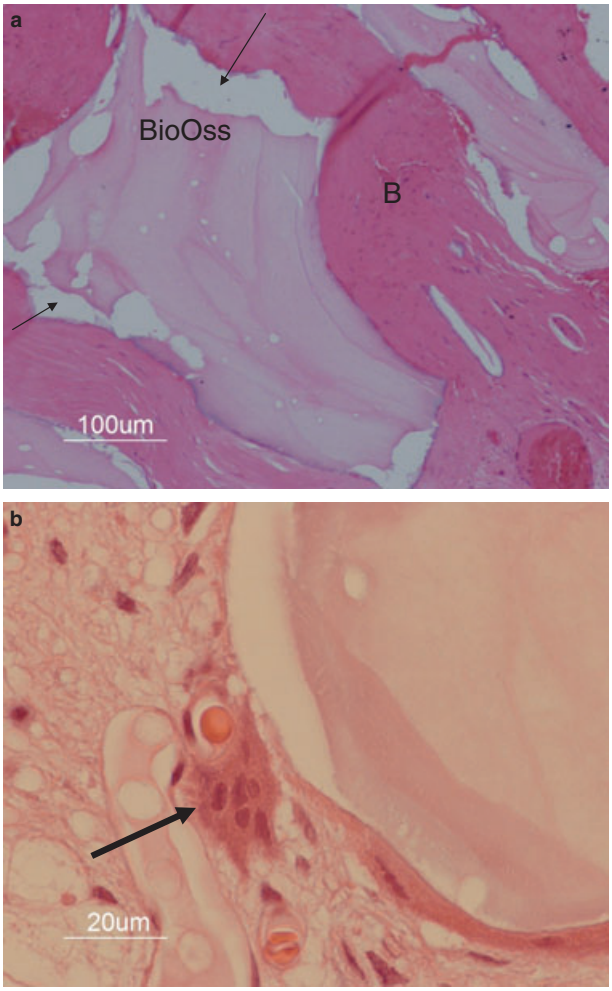

Fig. 8. (a) Light micrograph of a decalcified specimen harvested 7 years after a sinus floor augmentation with deproteinized bovine bone (Bio-Oss®) particles, showing shrinkage of the particles (arrows). Bone(B). (b) Light micrograph of a decalcified specimen harvested 7 years after a sinus floor augmentation, showing an osteoclast in close contact with a minor lacuna on a deproteinized bovine bone (Bio-Oss®) particle (arrow).

possible signs of resorption, such as lacunae on the surfaces of deproteinized bovine bone particles, as being lacunae present in the original donor material, or as evidence of osteoclastic resorption mediated by later surface adsorption of proteins containing arginine–glycine–asparagine sequences, such as fibronectin or fibrinogen (153). Adhesion proteins may originate either from the fibrin glue or from the patient's own blood. Nonetheless, the great reduction in deproteinized bovine bone particles seen in many animal studies is difficult to reconcile with available data from human studies. Even though the type of giant cells in the vicinity of deproteinized bovine bone particles may vary among species or individuals, osteoclastic cells are unlikely to resorb or degrade bovine bone that has been deproteinized. It seems safe to suggest that deproteinized bovine bone is basically a nonresorbable grafting material in humans.

In sinus augmentation, it may be of importance that deproteinized bovine bone is nonresorbable, whereas autogenous bone can experience more than 50% resorption (94). Cobb et al. (39) discussed the advantage of using a nonresorbable or a low-grade resorption bone substitute and concluded that a mixture of equal volumes of a nonresorbable and of autogenous bone was optimal for grafting. An increased amount of bone substitute enhances the risk of fibrous encapsulation. However, in some studies that used 100% deproteinized bovine bone as a grafting material, no encapsulation appeared to take place, with the results observed being similar to those obtained with a mixture of deproteinized bovine bone and autogenous bone (77, 84, 90, 91, 210, 211).

Lateral augmentation of edentulous alveolar ridge defects can also benefit from deproteinized bovine bone grafting (82, 84, 216). Hellem et al.(82), using a 50:50 (wt/wt) mixture of deproteinized bovine bone and autogenous bone, reported a 3-year implant survival rate of 96%. Zitzman & Scharer (216), harvested biopsies after 6–7 months, and found that deproteinized bovine bone particles (Bio-Oss®; Geistlich, Pharma AG, Wolhusen, Switzerland) had 37% contact with newly formed bone and that 31% of the biopsy area was occupied by the Bio-Oss particles.

Xenografts derived from marine carbonated algae, chemically converted into hydroxyapatite, have been used in implant dentistry for several years (59, 188). This material is probably also nonresorbable because it does not contain any proteins and is not dissolvable. Ewers (59) found a loss of 27 of 614 loaded implants in sinus sites grafted with the marine algae-based material, yielding an implant survival rate of 95.6%. Also, the volume of the xenograft material decreased by 14% after 6 months compared with 49.5% for autogenous bone (59).

Alloplastic bone substitutes

Alloplastic bone substitutes represent a large group of chemically diverse synthetic calcium-based biomaterials, including calcium phosphate, calcium sulphate, bioactive glasses, and polymers. These osteobiologics vary in structure, chemical composition, and mechanical and biological properties. Also, some osteobiologics are nonresorbable, whereas

others are chemically resorbable with a concomitant release of bioactive ions.

The pore size of osteobiologics seems to be a significant determinant of the ability to form bone. Pore sizes of >300 μm show enhanced formation of new capillaries and bone (95). Alloplastic material with pore sizes of <100 μm may not permit cell and capillary invasion and therefore may not induce bone formation. Most of the current commercial osteobiologics do not exhibit any pores. The manipulation of pore size is critical for creating an osteobiologic material that resembles natural bone.

Most alloplastic materials consist of hydroxyapatite, β-tricalcium phosphate, biphasic calcium phosphate, or some type of nonsintered calcium phosphate (reduced calcium content). Calcium phosphates are manufactured from tricalcium phosphate powder. Pure calcium phosphate is less stable than hydroxyapatite and thus can be dissociated more easily into potentially bone-stimulating ions (41). Tricalcium phosphate can be sintered into a uniform material, β-tricalcium phosphate (Cerasorb®; Curasan AG, Kleinostheim, Germany), which is one of the most frequently used alloplasts in implant dentistry (177). A drawback of β-tricalcium phosphate is a rapid resorption rate, which limits its use in bone augmentation procedures performed for esthetic purposes.

Biphasic alloplastic material is produced by sintering hydroxyapatite and tricalcium phosphate to a chemically united material. These grafting materials have pore sizes of >100 μm and have been shown to be effective in repairing skeletal defects (127). Synthetically produced alloplasts used in implant dentistry include Calcitec® Inc. (Austin, TX), Osteogen® (Impladent Ltd, Holliswood, NY), Tricos® (Baxter, Bern, Switzerland) and Bone Ceramic® (Straumann, Basel, Switzerland). However, sufficient documentation of the clinical utility of several of these alloplasts is still lacking.

Calcium phosphates can be bound to collagen carriers or mixed with fibrin. The concept is that collagen and fibrin form a network on which minerals can crystallize. Collagen can also bind to extracellular matrix proteins of importance in the mineralization process. Healos® (Orquest, Mountain View, CA) is a mixture of hydroxyapatite and bovine collagen, and Collagraft® (Zimmer Corp, Warsaw, IN) is composed of 65% hydroxyapatite and 35% tricalcium phosphate combined with bovine collagen. Tricos®, mentioned in the previous paragraph, is a mixture of hydroxyapatite, tricalcium phosphate, and fibrin.

Calcium sulphate has been used in craniofacial surgery for more than 100 years. Dreesman (51) was the first to use surgical plaster to fill skeletal defects, and De Leonardis & Pecora (44) have used the material for sinus floor augmentation in implant dentistry (44). Calcium sulphate resorbs quickly and is substituted by new bone. The rapid resorption rate can pose a potential problem because the volume of the graft may not be maintained for a sufficiently long period of time to yield reliable grafting results in the esthetic zone.

Bioglass consists of silica and has an optimal particle size of 300 μm for bone formation (178). Bioglass corrodes when placed in fluid followed by a migration of hydrogen ions to the surface of the material. Sodium and silica then precipitates and calcium ions are released, which can stimulate stem cells to produce bone-building cells (41). As Bioglass is slowly resorbed, it probably takes 12–16 months before the graft is replaced by newly formed bone, a factor that has to be considered when calculating the graft healing time (178, 193). Tadjoedin et al. (178) compared a mixture of autogenous bone and bioactive glass particles (300–355 μm in size) with autogenous bone alone. The biopsies showed more new bone in the autogenous bone group after 4–6 months and similar values for both groups after 16 months. Turunen et al. (193) compared a mixture of autogenous bone and bioactive glass granules (800–1,000 μm in size) with autogenous bone alone as a grafting material in the floor of the maxillary sinus. Biopsies harvested after 21–34 weeks and after 49–62 weeks revealed a similar bone-forming outcome in both study groups (193). The studies by Tadjoedin et al. (178) and Turunen et al. (193) suggest that bioglass can be used in a mixture with autogenous bone at the floor of the maxillary sinus, thus decreasing the amount of autogenous bone required.

Conclusion

Dentists have access to an increasing number of different biomaterials for use in bone augmentation procedures prior to implant placement. However, most of them are not well clinically documented (57).

It appears that a variety of biomaterials can provide excellent bone formation in the floor of the maxillary sinus. On the other hand, in some patients it is only necessary to tent the sinus membrane to achieve new bone for stability of

implants. Lateral bone augmentation of the alveolar ridge constitutes another important indication for biomaterial grafting. In general, prospective clinical studies are lacking for many of the bone augmentation materials and techniques currently available.

Resorption and complete remodelling into new bone is the ideal outcome of a grafting material. However, the resorption rate of a graft material can vary greatly. Some materials resorb quickly, which can severely compromise their usefulness, especially in the esthetic zone. Relatively rapid resorption of autogenous bone grafts has led to a search for more stable bone substitutes.

Currently, there is no clinical evidence for superiority of autogenous bone grafts in sinus augmentation procedures. The complex procedure of harvesting autogenous bone from the iliac crest is still necessary when a large amount is needed. However, studies during the past decade have suggested that nonresorbable grafting materials may be capable of predictably maintaining the graft volume. For minor grafting procedures, such as bone augmentation of the sinus floor or of alveolar defects, it seems feasible to use bone substitutes with or without adding autogenous bone harvested locally or from the mandibular ramus.

The platelet-rich plasma concept in reconstructive surgery has been evaluated in various experimental and clinical models, with conflicting results obtained. Some studies point to the need for the presence of appropriate target cells to interact with the growth factors in platelet-rich plasma. Moreover, in cell culture models, platelet-rich plasma seems to exert most activity in the early stages of cell proliferation. The effect of platelet-rich plasma in humans may be beneficial only in the early phases of the formation of bone and possibly soft tissue. Experimental and clinical investigations are needed to delineate the interaction among the various growth factors in platelet-rich plasma during tissue healing. Also, controversy still exists as to the clinical benefit of combining platelet-rich plasma with bone grafts, and to the utility of platelet-rich plasma in implant dentistry. Whether the extra cost and time spent on the platelet-rich plasma procedure is justified remains a topic for further study.

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