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Biomaterials Applicable for Alveolar Sockets Preservation: In Vivo and In Vitro Studies

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1. Introduction

Bone density and quantity are primary conditions for the insertion and stability of dental implants. In cases of a lack of adequate maxillary or mandibulary bone, e.g. in terms of front to back depth or thickness, bone augmentation will be necessary. Lack of bone or bone defects can be caused by inflammation, congenital malformation, trauma or oncological surgery. Several procedures and materials for augmenting bone height have been developed to overcome the problem of a reduced amount of bone. In dentistry, bone substitution materials were used for the following applications: (1) socket preservation; (2) periodontal defects; (3) third molar extraction sites to support 2nd molars; (4) ridge augmentation; (5) defects following cyst removal / apicoectomies; (6) sinus lifts; (7) distraction osteogenesis; and (8) implant dentistry. The treatment of bone-defects and socket preservation include autografting (from one location to another within the same individual), xenografting (from a donor of another species) and allografting (from a genetically dissimilar member of the same species) cancellous bone. After blood, bone is the most commonly transplanted tissue. Worldwide, an estimated 2.2 million grafting procedures are performed annually to repair bone defects in orthopaedics, neurosurgery, and dentistry (Giannoudis et al. 2005). The increasing number of grafting procedures and the disadvantages of current autograft and allograft treatments (e.g. limited graft quantity, risk of disease transmission) drive the quest for alternative methods to treat bone defects.

The use of synthetic bioactive bone substitute materials is of increasing importance in modern dentistry as alternatives to autogenous bone grafts. Various alloplastic bone substitution materials of different origin, chemical composition, and structural properties have been investigated in the last years. The materials commonly used in all approaches are ceramics, polymers or composites (Burg et al. 2000). These alloplastic materials are either absorbable or non-absorbable, as well as naturally derived or synthetically manufactured (Figure 1).

Various types of biomaterials (minerals and non-mineral based materials as well as natural and artificial polymers) with different characteristics have been used for studying ossification and bone formation. For example, calcium phosphate ceramics include a variety of ceramics such as hydroxyapatite, tricalcium phosphate, calcium phosphate cement, etc. These mentioned ceramics have excellent biocompatibility and bone bonding or bone regeneration properties. Recently non-biodegradable and degradable membranes have been

tested for their appliance in bone defects (Zhao et al. 2000). Cell-biomaterial interactions depend on surface characteristics, e.g. chemistry or topography. Surface characteristics determine the ionic exchange dynamics and the protein attachment as well as cell attachment, cell proliferation and cell differentiation.

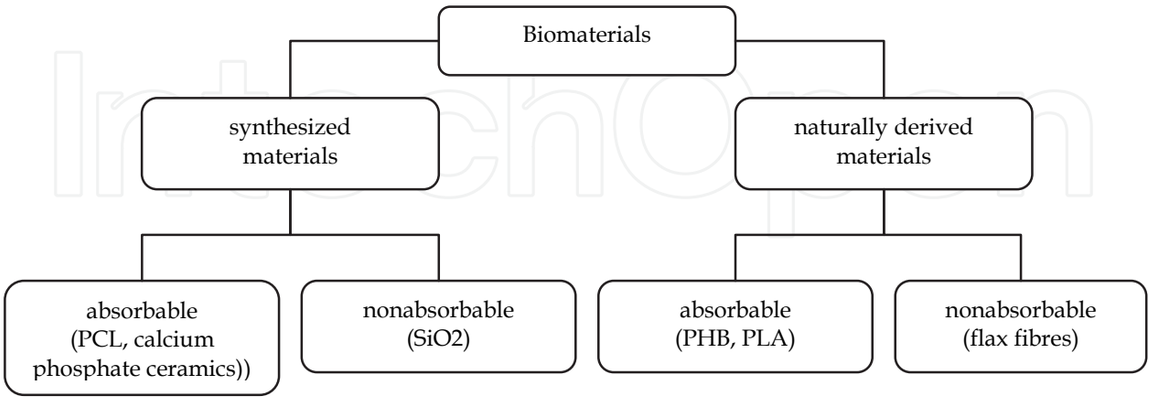

Fig. 1. The classification of used biomaterials for bone tissue engineering modified according to Burg (Burg et al. 2000)

Xenogenic grafting has been shown to be one of the most effective methods of creating bone in areas where there is none, whereas alloplastic graft material constitutes the second of the most popular forms of bone grafting material in dentistry.

2. Bone regeneration

Bone is a dynamic tissue that constantly undergoes remodelling. The signal that initiates bone remodelling has not been identified, but there is evidence that mechanical stress can alter local bone architecture. This can be sensed by osteocytes followed by secretion of paracrine factors such as insulin-like growth factor (IGF-1) in response to mechanical forces (Lean et al. 1996). Bone formations result from a complex cascade of events that involves proliferation of primitive mesenchymal cells (osteoinduction), differentiation into osteoblast precursor cells, maturation of osteoblasts, formation of matrix, and finally mineralization. Osteoblasts converge at the bottom of the resorption cavity and form osteoid which begins to mineralize after 13 days. The osteoblasts continue to form and mineralize osteoid until the cavity is filled (Hill 1998). Some of the osteoblasts differentiate into osteocytes and become embedded in the matrix.

Even if pre-existing osteoblasts may help to form new bone, it is generally agreed that such osteoblasts only contribute a minor portion of the new bone needed in a fracture-healing situation. The initial event must be the chemotactic attraction of osteoblasts or their precursors to sites of the resorption defect. This is likely to be mediated by the release of local, biochemical and biophysical messengers. The second event involved in the formation phase of the coupling phenomenon is proliferation of osteoblast precursors. This is likely to be mediated by osteoblast-derived growth factors and those growth factors released from bone during the resorption process. The third event of the formation phase is the differentiation of the osteoblast precursor into the mature cell. The differentiating osteoprogenitor cells express

several bone matrix macromolecules, namely alkaline phosphatase, type I collagen, bone sialoprotein, osteopontin, Cbfa1 and osteocalcin (Hill 1998).

It is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblast (Frost 1964). Bone remodelling occurs in small packets of cells called basic multicellular units (BMU), which turn bone over in multiple bone surfaces (Frost 1991). BMU consist of osteoblasts, other bone-forming cells such as osteocytes and bone-lining cells, bone-resorbing cells - osteoclasts, the precursor cells of both, and their associated cells like endothelial cells and nerve cells (Papachroni et al. 2009). Osteoblasts are key components of the bone multicellular unit and have a seminal role in bone remodelling, which is an essential function for the maintenance of the structural integrity and metabolic capacity of the skeleton. Osteoblasts originate from the non-hematopoietic part of bone marrow, which contains a group of fibroblast-like stem cells with osteogenic differentiation potential, known as the mesenchymal stem cells (MSCs) and also referred as skeletal stem cells (SSCs), bone marrow stromal cells (BMSCs) and multipotent mesenchymal stromal cells (MMSCs) (Abdallah and Kassem 2008; Heino and Hentunen 2008). MSCs are capable of multi-lineage differentiation into mesoderm-type cells such as osteoblasts, adipocytes and chondrocytes (Dezawa et al. 2004; Luk et al. 2005). Osteoblast growth and differentiation is determined by a complex array of growth factors and signalling pathways. The following three families of growth factors influence the main aspects of osteoblast activity and induce, mediate or modulate the effects of other bone growth regulators:

- the transforming growth factor-β (TGF-β) family
- the insulin-like growth factors (IGFs)
- bone morphogenetic proteins (BMPs) (Zhou et al. 1993; Mundy 1994; Bikle 2008).

Furthermore, other growth factors, such as the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) as well as platelet derived growth factor (PDGF) are involved in bone formation.

Many growth factors involved in the natural process of bone healing have been identified and tested as potential therapeutic candidates to enhance the regeneration process. *In vitro*, BMPs can differentiate mesenchymal stem cells into the osteoblastic phenotype (Cheng et al. 2003; Luu et al. 2007). Furthermore, the osteoinductive BMPs (e.g. BMP-2/-4/ and BMP-5 to -8) can initiate the complete cascade of bone formation when implanted ectopically (Wozney 2002). In general, TGF-β stimulates migration of skeletal stem cells and regulates cell proliferation, cell differentiation, and extra cellular matrix synthesis (Bostrom and Asnis 1998; Janssens et al. 2005). Stimulatory effects on bone healing and bone formation have been evaluated in various experimental settings (Janssens et al. 2005). FGFs are considered potent regulators of cell growth and wound healing and have mitogenic effects. The best studied member of FGFs is FGF-2. Although FGF-2 alone is not capable of inducing ectopic bone formation, it plays an important role in the regulation of normal bone healing (Marie 2003). In various animal studies exogenous recombinant FGF-2 enhances callus formation and stimulates bone healing (Kato et al. 1998; Nakamura et al. 1998; Radomsky et al. 1998). IGFs are the most abundant growth factors produced by bone cells and are stored at the highest concentration of all growth factors in the bone matrix. It was shown that IGF has an anti-apoptotic effect on pre-osteoblast cells and enhances bone matrix synthesis (Niu and Rosen 2005). In animal models and clinical trials of osteoporosis, systemic IGF infusion showed an increase in bone formation, bone volume, and / or bone turnover (Rosen 2004). VEGF is considered one of the key regulators of angiogenesis during bone formation

(Gerstenfeld et al. 2003). In various experimental models the stimulation of the bone regeneration process has been shown in response to VEGF administration (Street et al. 2002; Eckardt et al. 2005). In addition, PDGF regulates general tissue repair as well as enhances proliferation of various bone cell types and angiogenesis (Hollinger et al. 2008).

Mechanisms that promote skeletal tissue specificity are necessary, because none of these growth factors are specific for cells in the osteoblastic lineage and these involve interactions with other circulating hormones in addition to the action of specific intracellular mediators on bone-specific transcription factors. It is certain that bone remodelling is regulated by systemic hormones and by local factors (Canalis 1983), which affect cells of both the osteoclast and osteoblast lineages and exert their effects on the replication of undifferentiated cells, the recruitment of cells, and the differentiated function of cells (Hill 1998). The following hormones are involved in bone formation:

- Calcitonin (CT) – decreases the reabsorption of calcium from bones thereby lowering blood calcium levels. It can inhibit osteoclastic bone resorption and probably osteocytic osteolysis (Weiss et al. 1981; Wallach et al. 1993).
- Estrogens and androgens - slow the rate of bone remodelling and protect against bone loss. They help retain calcium in bones, thereby maintaining a strong bone matrix. Sex steroids not only influence the accrual of bone mass and bone mineral density but also bone growth (Manolagas et al. 2002; Bertelloni et al. 2010).
- Growth hormone (GH) - is one of the most important regulator substances for both bone growth and bone remodelling. It directly increases longitudinal bone growth by stimulating prechondrocites. GH is a polypeptide complex that regulates the processes of bone physiology, increases the rate of mitosis of chondrocytes and osteoblasts, and increases the rate of protein synthesis (collagen, cartilage matrix, and enzymes for cartilage and bone formation) (Ohlsson et al. 1998; Calvo-Guirado et al. 2011).
- Insulin – increases energy production from glucose. It has been established as an osteoblast regulator. In bone organ culture, insulin stimulates collagen synthesis at periphysiological hormone concentrations (Wettenhall et al. 1969; Cornish et al. 1996).
- Parathyroid hormone (PTH) - a major systemic regulator of bone metabolism, increases the reabsorption of calcium from bones to the blood, thereby raising blood calcium levels and increases the absorption of calcium by the small intestine and kidneys. The anabolic effect of intermittent PTH on bone has made it an effective treatment for osteoporosis in humans, where it was shown to increase bone mass and reduce fracture rate (Neer et al. 2001).
- Thyroxine – increases the rate of protein synthesis and increases energy production from all food types. It directly stimulates the metabolic activity of bone cells and profoundly affects bone turnover. Thyroxine influences the responsiveness of bone cells to 1,25-dihydroxycholecalciferol, parathyroid hormone, and calcitonin (High et al. 1981).

Besides growth factors and regulating hormones, expression of transcription factors is necessary and sufficient for mesenchymal cell differentiation. Runx2 is an essential bone-specific transcription factor. It was recently shown, that the complete Runx2 gene inactivation in transgenic mice leads to complete lack of intramembraneous and endochondral ossification owing to lack of mature osteoblasts (Komori et al. 1997). In addition, heterozygous Runx2 mice demonstrate specific skeletal abnormalities that are characteristic of the human heritable skeletal disorder cleidocranial dysplasia (Otto et al. 1997).

3. Pre-conditions of bone surrogates

Selection of graft materials is based on operator preference, type and size of the bone defect, resorbability of graft material as well as cost and patient acceptance. Furthermore, bone graft materials are generally evaluated based on their osteogenic, osteoinductive, or osteoconductive potential. Osteoinduction is a basic biological mechanism that occurs regularly, e.g. in fracture healing but also in implant incorporation (Albrektsson and Johansson 2001). Osteoconduction means that bone grows on a surface and is defined as the ability to stimulate the attachment, migration, and distribution of vascular and osteogenic cells within the graft material (Albrektsson and Johansson 2001). Several physical characteristics can affect the graft osteoconductivity, including porosity, pore size, and three-dimensional architecture. In addition, direct interactions between matrix proteins and their appropriate cell surface receptors play a major role in the host response to the graft material. An osteoconductive material guides repair in a location where normal healing will occur if left untreated (Kulkarni et al. 1971; Helm and Gazit 2005).

The ability of a graft material to independently produce bone is termed its direct osteogenic potential. The main critical considerations in bone tissue-engineering scaffold design are:

- Promotion of bone in-growth
- Average pore sizes approximately 200-400 µm
- Sterilization without loss of properties
- Absorbation with biocompatible components
- Good bony apposition
- Correct mechanical and physical properties for application
- Maximal bone growth through osteoinduction and/or osteoconduction
- Adaptation to irregular wound site (malleableness)
- Absorbation in predictable manner in concert with bone growth
- Availability to surgeon on short notice (Peter et al. 1998; Burg et al. 2000)

To have direct osteogenic activity, the graft must contain cellular components that directly induce bone formation. Polymers have been shown to be an excellent substrate for cellular or bioactive molecule delivery. They can differ in their molecular weight, polydispersity, crystallinity, and thermal transitions, allowing different absorption rates. Their relative hydrophobicity and percent crystallinity can affect cellular phenotype (Hollinger and Schmitz 1997). Various types of biomaterials (minerals and non-mineral based materials as well as natural and artificial polymers) with different characteristics have been used for studying ossification and bone formation. For example, calcium phosphate ceramics include a variety of ceramics such as hydroxyapatite, tricalcium phosphate, calcium phosphate cement, etc. Local tissue responses to polymers *in vivo* depend on the biocompatibility of the polymer as well as its degradative by-products (Hollinger and Battistone 1986). The mentioned ceramics have excellent biocompatibility and bone bonding or bone regeneration properties. They have been widely used in no or low load-bearing applications (Milosevski et al. 1999). Furthermore, natural polymers like collagen have been used for bone tissue engineering purposes (Hutmacher et al. 2001a; Lauer et al. 2001). Recently non-biodegradable and degradable membranes have been tested for their appliance in bone defects (Zhao et al. 2003). Scores of artificial polymers of diverse character are already in use for bone supply. One of them, poly(3)hydroxybutyrate (PHB) due to its form stability combined with little inflammatory response after implantation, may serve as a scaffold for tissue engineering (Gogolewski et al. 1993; Schmack et al. 2000).

4. Alloplastic materials in dentistry

Today, the above-mentioned clinical problems are solved by bone grafting. Autogeneic bone graft from the iliac crest is the main source of trabecular bone. It has a good osteoinductive capacity, but the sources are limited and the harvest procedure causes postoperative discomfort to the patient. Allogeneic bone is also widely used but provides mainly osteoconductive properties. Moreover, despite of extensive testing, there are still potential risks for transmitting diseases. Synthetic or alloplastic materials are alternative materials to avoid the drawbacks of autografts or allografts, because alloplastic materials show very little risk of morbidity and mortality. Alloplastic materials are divided into two main groups: (1) non-resorbable materials that acts as replacement bone and (2) resorbable frameworks or scaffolds for bone to grow into at its normal rate. A large percentage of alloplastic biomaterials are based on one raw material, such as hydroxyapatite (HA) and tri-calcium phosphate (TCP) or bioactive silicates (SiO2). Recent developments have focused on composites with different chemical phases, such as HA+TCP (Straumann® BoneCeramic, Straumann, Freiburg, Germany), SiO2+HA (Nanobone®,Artoss, Rostock, Germany) or SiO2+Ha+TCP (BonitMatrix®, DOT, Rostock, Germany).

4.1 Homogenous chemical composition

Until now HA-based biomaterials are the most abundant materials used in modern bone substitution. At the moment calcium phosphate cements play a secondary role in dentistry, although they often have an excellent biocompatibility (Noetzel and Kielbassa 2005). For HA scaffolds both pore size and porosity have effects on mineralisation and bone formation (Harris and Cooper 2004; Kruyt et al. 2004).

In vitro testing is used primarily as a first stage test for acute toxicity and cytocompatibility. A precondition for osteoconductivity and osteoinductivity are proliferating cells. Reichert and coworkers could show that neither Cerasorb® (TCP; Curason AG, Sonneberg, Germany), Geistlich Bio-Oss® (HA; Geistlich Pharma, Wolhusen, Germany) nor Perioglas® (SiO2; Novabone, Jacksonville, U.S.A.) has a negative influence on cellular proliferation, as compared to the control (Reichert et al. 2009). In fact, the tested bone substitution materials led to an increased AlamarBlue reduction over the observation period of 7d. Only PerioGlas® showed slight, but not significant decrease in AlamarBlue reduction, compared to controls (Reichert et al. 2009). The effect on the proliferation of osteoblast *in vitro* was investigated using phytogene HA (Algipore®), TCP (Bio-Base®) and bovine HA (Bio-Oss®). Kübler et al. have found that human osteoblast cells seeded with Bio-Oss® showed the lowest proliferation and differentiation rate after comparison with the other tested bone graft materials (Kubler et al. 2004). Furthermore, there were no obvious differences in cell migration and growth behaviour between BioOss® and Vitoss® scaffolds, but significantly higher osteocalcin expression in cells seeded on BioOss® scaffolds (Payer et al. 2010). In contrast, DNA content, LDH (lactate dehydrogenase) and alkaline phosphatase activity as well as expression of bone-related genes, such as alkaline phosphatase, osteonectin, osteopontin and bone sialoprotein II revealed proliferation and osteogenic differentiation of osteoblasts on Cerasorb®, but not on BioOss® during cultivation over 28 days (Bernhardt et al. 2010).

Bioactive glass ceramic was seeded with human primary osteoblasts and evaluated after 2, 6 and 12 days. Incubation with Bioglass 45S5 increased human osteoblast proliferation to 155% as shown by flow cytometric analysis (Xynos et al. 2000b). Analysis of osteoblast specific markers, such as alkaline phosphatase and osteocalcin indicate that Bioglass

advanced osteoblast augmentation. Furthermore, expression of IGF2 was 2,9fold increased compared to control cells (Xynos et al. 2000a).

However, *in vitro* characterization is not able to demonstrate the tissue response to materials. Animal models are essential for evaluating biocompatibility. Bone substitution materials, such as hydroxyapatite and β-tricalcium phosphate were shown to be osteoinductive (Okumura et al. 1997; Habibovic et al. 2008; Sun et al. 2008). The *in vivo* bone-regenerative capacity of calcium silicate was investigated in rabbits and results were compared with TCP. Micro-CT and histomorphometric analysis showed resorption and newly formed bone with both materials, but resorption as well as bone formation of calcium silicate was higher than that of TCP (Xu et al. 2008). Wistar rats were used to histologically evaluate the healing of surgically created defects on the tibia after implantation of bioactive glass. Bioactive glass promoted comparable bone formation independently of the size of glass granules (Macedo et al. 2005). Also new bone formation was found after implantation of NovaBone 45S5 bioglass particulate (NovaBone, Jacksonville, USA) in sheep. In addition, acute as well as chronic inflammatory reactions were associated with the use of these glass granules (Kobayashi et al. 2010). Furthermore, cylindrical porous hydroxyapatite implants were implanted in rabbit femurs. Histological analyses of bone sections with toluidine blue showed new bone formation (Damien et al. 2003). Various publications discussed the use of porous hydroxyapatite in maxillary sinuses. Histologically, the grafted sinuses exhibited a significant amount of new bone formation. The porous hydroxyapatite granules appeared integrated with the new formed bone (Browaeys et al. 2007).

To date, predominantly histological staining and immunohistochemical analyses were used to study the behaviour of bone substitute materials on bone graft healing. Only a few numbers of studies present the molecular mechanisms of bone formations associated with bone substitutes. Recently it was shown, that bovine hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) up-regulate the expression of Runx2, Alpl and osteocalcin in biopsies from human jaws (Gotz et al. 2008).

Calcium phosphate cements and sintered ceramics as well as calcium sulphate cements and bioglass have been used as BMP carriers (del Real et al. 2002; den Boer et al. 2003; Kroese-Deutman et al. 2005). Synthetic porous hydroxyapatite might be providing a delivery system for bioactive agents. It was recently shown that the supplementation of a synthetic porous hydroxyapatite with IGF-1 significantly increased new bone formation and bone mineral apposition rate compared with hydroxyapatite alone (Damien et al. 2003). In contrast, the resorption of IGF1, TGFbeta1 or bFGF onto a carrier of TCP does not enhance new bone formation (Clarke et al. 2004). A multi-centre, randomized clinical trial using recombinant human platelet-derived growth factor with β-TCP for the treatment of intra-osseous periodontal defects showed significantly higher extent of linear bone growth and per cent bone fill compared to β-TCP alone after six months (Jayakumar et al. 2011).

An example for commercially available products containing growth factors is Gem 21S™ (Osteohealth, Shirley, USA), β-tricalciumphosphate and recombinant human PDGF.

4.2 Composites with different chemical phases

Advances in biomaterial fabrication have introduced numerous innovations in designing scaffolds for tissue engineering. The combination of different bone substitution materials is an important research topic. Biomaterials on the basis of calcium phosphates are most widely used in craniofacial bone surgery and considered to be biocompatible, non-

immunogenic and osteoconductive (Ruhé et al. ; Abukawa et al. 2006; Weinand et al. 2006). It is well know that the surface topography has an influence on cell attachment. Figure 2 showed surface topographies of three different commercially available bone substitution materials, especially Cerasorb®, Straumann® BoneCeramic and NanoBone®.

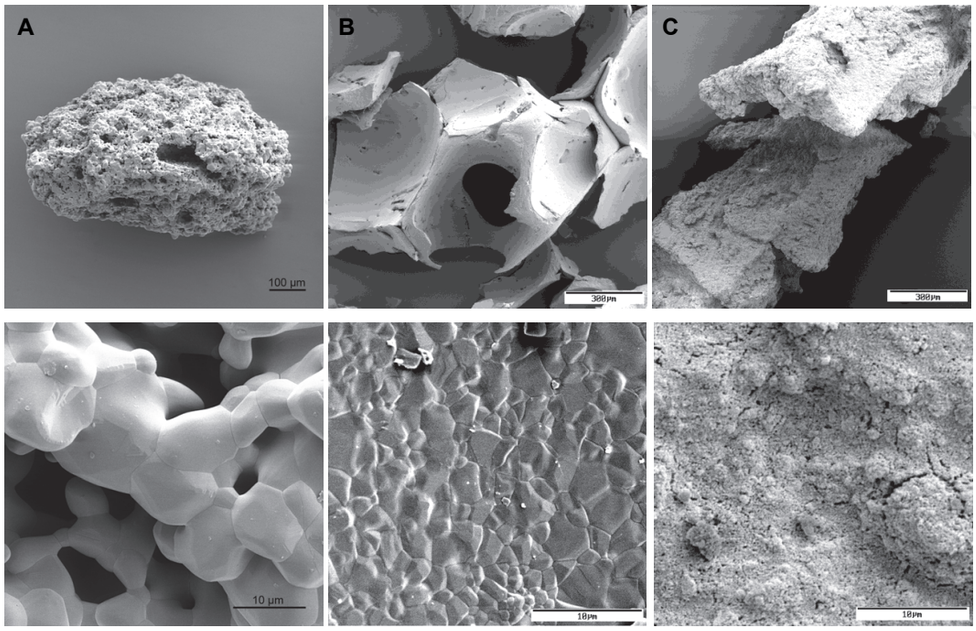

Fig. 2. Scanning electron micrographs of different biomaterials on the basis of calcium phosphates at different magnifications. (A) Cerasorb® (Curason AG, Germany), (B) Straumann® BoneCeramic (Straumann, Freiburg, Germany), (C) NanoBone® (Artoss, Rostock, Germany)

The composition of bioceramics influences the cell attachment and proliferation. It was shown that incorporation of zinc or silicate in calcium phosphate ceramics is followed by an increase of osteoblast attachment and proliferation (Ishikawa et al. 2002; Thian et al. 2005), whereas carbonate had contradictory effects on these cells (Redey et al. 2000).
Viability, proliferation and growth characteristics of fibroblasts cultured together with NanoBone® and Straumann® BoneCeramic were analysed over a period of 28 days. Fibroblast viability does not show any differences between cells incubated with the different bone graft materials and the control cells. Electron microscopy showed that fibroblast cells grew and proliferated at the surface of both bone graft materials (Kauschke et al. 2006). The same results could be observed by Reichert and coworkers (Reichert et al. 2009). They show no differences in the cellular proliferation between control cells and cells incubated with Straumann® BoneCeramic, NanoBone® and BonitMatrix® (Reichert et al. 2009). Furthermore, endothelial cells predominantly spread on BonitMatrix® and cells expressed endothelium-specific surface marker proteins (Thimm et al. 2008).
The biocompatibility of Straumann® BoneCeramic, NanoBone® and BonitMatrix® were tested using different animal models. A rapid vascularization and good integration within the peri-implant tissue was shown for BonitMatrix® after subcutaneously implantation in Wistar rats (Ghanaati et al. 2010).
In vivo studies using the mouse dorsal skinfold chamber model revealed a high biocompatibility comparable to that of cancellous bone for NanoBone®. Both NanoBone

granules and plates do not show any venular leucocyte activation after implantation. Furthermore, signs of angiogenesis could be observed (Abshagen et al. 2009). Vascularization as well as osteoneogenesis after implantation of NanoBone® was observed using guinea pigs (Punke et al. 2008). Our own group could establish a FE model of remodelling processes occurring in a bone area provided with bone graft substitutes (Gedrange et al. 2008). MicroCT images revealed specimen changes in the spongious and compact areas provided with the bone substitution material (Figure 3). The use of NanoBone® as bone substitution material seems to decrease the bone atrophy after teeth extraction in pigs as compared to untreated alveolars, but the reduction is not significant (Figure 4).

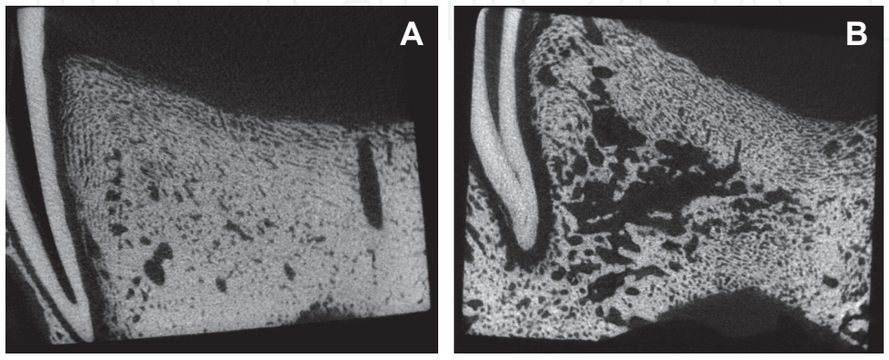

Fig. 3. MicroCT images of embedded bone specimens showing the remodelling of the area provided with NanoBone®. (A) NanoBone treated animal; (B) control animal (Gedrange et al., 2008).

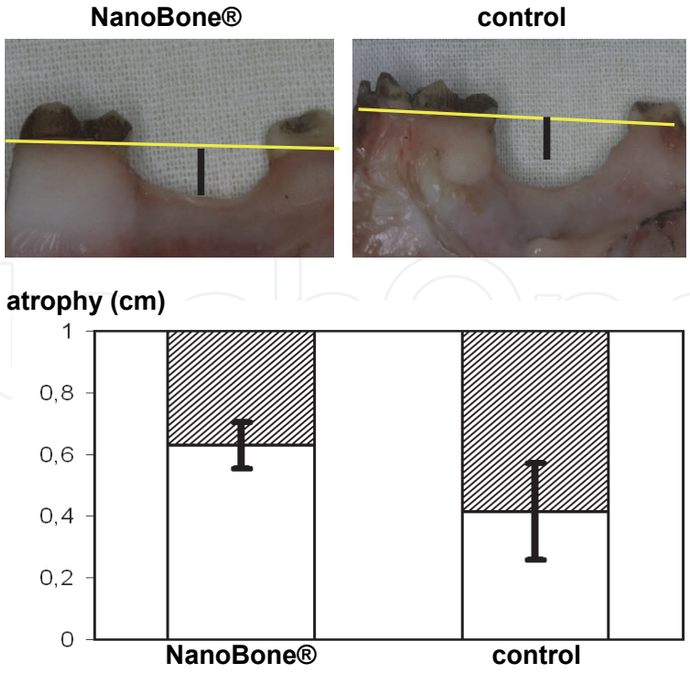

Fig. 4. Measurement of alveolar bone atrophy 70 days after teeth extraction and animal treatment with or without NanoBone®

The ideal substrate for the synthesis of bone should be able to promote the expression of the osteoblastic phenotype as well as provide a template for bone deposition (Kaufmann et al. 2000). An osteoblastic phenotype expression on the surface of hydroxyapatite ceramics, with subsequent gene expression of osteocalcin was found (Okumura et al. 1997). Recently it has been shown that OSSA NOVA and BONITmatrix® could stimulate bone regeneration of surgically created cranial defects in rats, but OSSA NOVA leads, in comparison with granular BONITmatrix®, to an accelerated more comprehensive bone regeneration (Kunert-Keil et al. 2009). Studies from our lab demonstrated an increased expression of the IGF1 mRNA in cranial defects treated with BONITmatrix® and OSSA NOVA (Gredes et al. 2010a). The increase in the IGF1 expression was followed by an accelerated, more comprehensive bone regeneration using both synthetic biomaterials consisting of calcium phosphates embedded in a silica matrix (Gredes et al. 2010a). Furthermore, Real-time RT-PCR analyses showed that the amount of the osteocalcin and Alpl mRNA was significantly increased after treatment with BONITmatrix®. It was recently shown that mesenchymal stem cells differentiated to osteoblasts within 14 days on hydroxyapatite and BONITmatrix® in expansion medium with and without osteogenic differentiation additives (Muller et al. 2008). Cells revealed a higher Alpl activity and increased mRNA expression of osteocalcin and collagen 1 (Muller et al. 2008). Another bone graft material consisting of nanocrystalline hydroxyapatite embedded in a porous silica gel matrix, Nanobone® was implanted in the mandible of minipigs. It was shown that Nanobone® is able to stimulate the differentiation of bone cells into osteoblasts and osteoclasts, because osteocalcin, alkaline phosphatase, osteopontin and BMP-2 were located in newly formed bone and focally in particles (Gerber et al. 2006). Furthermore, the matrix of all Nanobone® granules showed very strong immunoreactivity of osteocalcin and alkaline phosphatase in human jaw bone augmented with Nanobone® (Gotz et al. 2008). In addition, two other common markers for osteogenesis, mineralization and bone remodelling, namely Runx2 and Phex were analysed. Similar to the outcomes of other bone substitution materials like NanoBone® and their influence on bone healing process (Gotz et al. 2008), the expression of Runx2 gene was unaltered after application of BONITmatrix® and OSSA NOVA. Otherwise BONITmatrix® slightly increased the expression of the osteogenic transcription factor Runx2 in mesenchymal stem cells (Muller et al. 2008). In addition, it was suggested that diverse forms of hydroxyapatite nanoparticles may differentially affect the osteoblast cell function as shown on gene and protein levels (Xu et al. 2009). That NanoBone® could up-regulate the expression of Runx2, Alpl and osteocalcin was also shown in sinus lift procedures in rabbits (De Souza Nunes et al. 2010).

The degree of osteoclast activity on the most hydroxyapatite containing scaffolds depends on material qualities such as crystal size and surface roughness (Rumpel et al. 2006). Therefore the silica gel matrix granules of NanoBone® were replaced by an organic matrix by accompanying osteoblastic and osteoclastic relationships. Bio-Oss®, another type of hydroxyapatite material derived from bovine bone, is also degraded by osteoclasts with ruffled borders and acid phosphatase activity (Rumpel et al. 2006). The osteoclast-like cells are not only localized along the surface of the newly formed bone but also directly on the biomaterial. In the case of Bio-Oss® signs of osteoclastic resorption were evident in the formerly vascular osteon channels not surrounded by bone lamellae. Contrastingly, osteoclast attached to NanoBone® granules also occurred in defect regions without visible bone formation (Rumpel et al. 2006). Similar findings showed osteoclast-like cells on the surface of the NanoBone® bodies as well as on newly formed bone in human jaw bone augmented with Nanobone®. An osteoclastic as well as osteoblastic compartment could be observed after TRAP staining (Gotz et al. 2008).

Results of clinical studies using bone graft materials have recently shown that the implant success rate was 100% after three years when NanoBone® was used as grafting material. Furthermore the periotest values indicated a solid osseointegration (Heinemann et al. 2009).

4.3 Polymers

Tissue repair and regeneration by tissue engineering is dependent on the use of biodegradable polymer scaffolds which serve as a carrier matrix for bioactive substances or for incorporated cells. The adhesion of cells to bioresorbable scaffolds and the proliferation of cells on these scaffolds are important components for tissue engineering projects and play a fundamental role in regulating cell differentiation, growth and survival. Biodegradable polymers have displayed several properties of a suitable implant scaffold for the growth of osteoblast precursor cells. The ideal polymer would satisfy the following criteria: i) fill defects of various sizes and shapes, ii) mechanical and physical properties for a particular application, iii) a long shelf life, and iv) biocompatible degradation products.

Polymers are large organic macromolecules composed by many monomers in a regular pattern (structural units). Cellulose, collagen, agarose, chitin or hyaluronan are members of natural polymeric materials or so-called biological polymers. Natural polymers like collagen have been used for bone tissue engineering purposes (Hutmacher et al. 2001a). The most widely used bioresorbable materials are polymers of monocarboxylic acid derivates. A number of natural and synthetic polymers, such as poly-lactid acid (PLA), poly-glycolic acid (PGA), polyurethane (PU), and polycaprolactone (PCL), are in use as tissue scaffolds (Kiremitci and Piskin 1990; Vunjak-Novakovic et al. 1998; Hayashi et al. 2008). Recently these materials have been joined by linear polyesters of microbiological origin – polyhydroxykanoates (PHA). Local tissue responses to polymers *in vivo* depend on the biocompatibility of the polymer as well as its degradative by-products (Hollinger and Battistone 1986).

At the moment, two polymers are commercially available as a bone substitution material, first a PLA granulate (TruGraft™, Osteobiologics, San Antonio, U.S.A.) and second NovoSorb™ (PolyNovo Biomaterials, Port Melbourne, Australia).

The poly(α-hydroxy acid) polymers such as PLA, polygylcolide (PLG) and their copolymers PLGA are the most commonly used synthetic polymers to deliver BMPs (Isobe et al. 1999). Additional polymers used as BMP delivery systems include polyanhydrides, polypropylene fumurate, polyethylene glycol-PLA as well as polyphosphate (Lucas et al. 1990; Miyamoto et al. 1992; Renier and Kohn 1997; Behravesh et al. 1999). Incorpoartion of BMP-2 and BMP-7 in polycaprolactone suppressed bone marrow mesenchymal stem cell proliferation and increased the alkaline phosphatase activity (osteogenic differentiation) (Yilgor et al. 2010). An *in vivo* study determined the effects on osseointegration when polycaprolactone with BMP-2 coating was applied to bone screws. BMP-2 within the polycaprolactone coating did not stimulate osteogenesis (Niehaus et al. 2009). In contrast, using PCL scaffold, platelet-rich plasma and recombinant human BMP-2, a critical-size defect in the anterior mandible of a 71-years-old female patient was regenerated using de novo-grown bone (Schuckert et al. 2009).

4.3.1 Poly-3-hydroxybutyrate (PHB)

The most widespread and best studied polyhydroxyalkanoat (PHA) is the lipidic polymer, poly-3-hydroxybutyrate (PHB), which is found in the plasma membranes of Escherichia coli

complexted to calcium polyphosphorate (Reusch and Sadoff 1983). Different bacterial types of microorganisms produce PHB from renewable sources, e.g. sugar and molasses, as intracellular storage materials. PHB is an ideal biomaterial, because it is a biodegradable polyester and completely degrades in D,L-b-hydroxybutyrate (HB), a normal component of blood and tissue (Miller and Williams 1987; Suwantong et al. 2007). PHB is perfectly isotactic viz. the monomers have all branch groups on the same side of the polymeric chain and are oriented in the same way (Figure 5).

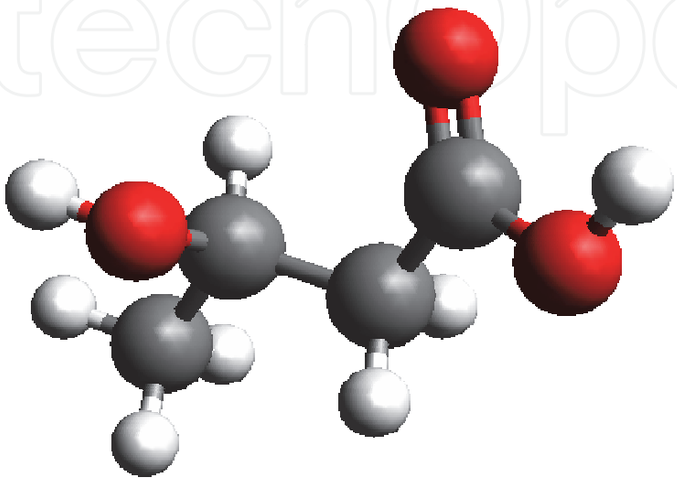

Fig. 5. Schematically illustration of a PHB monomer. The PHB monomer consists of hydroxybutyric acid ($C_4H_6O_2$ units) and a methyl group as side chain. Grey = carbon molecule; white = hydrogen molecule, red = oxygen molecule.

The biocompatibility of PHB has been confirmed *in vitro*, in cell cultures of various origins. It was found that mouse fibroblast cells and chondrocytes do not grow very well on microbial PHB films (Deng et al. 2002; Yang et al. 2002). PHB showed a high degree of crystallization and this rapid crystallization generates pores and protrusions on the PHB surface film. It was speculated that this surface could prohibit the attachment and growth of mammalian cells (Zhao et al. 2003). It is well known that biocompatibility of bio materials depends not only on the chemical structure but, to a large extent, on the size. Suwantong and coworker could show using indirect cytotoxicity assessment that PHB fibre mats were acceptable to both mouse L929 fibroblast cells and mouse Schwann cells (RT4-D6P2T) (Suwantong et al. 2007). Mouse fibroblast cells cultured with polymeric PHB microspheres revealed no cytotoxic effects which were indicated by the absence of changes in the cell morphology, cell viability and proliferative activity (Shishatskaya and Volova 2004). In addition, human osteoblast cells grow very well on PHB embroidery (Mai et al. 2006).
Several *in vivo* studies have shown that PHB may serve as a scaffold for tissue engineering due to its excellent biocompatibility as evidenced by lack of toxicity and compatibility with tissue and blood (Saito et al. 1991; Clarotti et al. 1992; Gogolewski et al. 1993; Schmack et al. 2000; Mai et al. 2006; Mack et al. 2008; Gredes et al. 2009). PHB sheets did not caused any inflammation in the chorioallantoic membrane of the developing egg (Saito et al. 1991). In mouse tissues no acute inflammation, abscess formation, or tissue necrosis was observed after subcutaneously implantation. In contrast, mononuclear macrophages, proliferating fibroblasts, and mature vascularized fibrous capsules were typically found (Gogolewski et

al. 1993). Furthermore, PHB sutures implanted intramuscularly did not cause any acute vascular reaction or inflammation, necrosis calcification of the fibrous capsule or malignant tumour formation in rats (Shishatskaya et al. 2004). On the other hand, implantation of PHB implants significantly increased the mRNA expression of VEGF (vascular endothelial growth factor), IGF1 and IGF2 (insulin-like growth factor) and decreased the GDF8 (growth differentiation factor 8; myostatin) mRNA amount in Musculus latissimus dorsi of rats (Table 1) (Gredes et al. 2009).

Tested gene	**mRNA expression (gene/β-actin)**		
	controls	**6 weeks of treatment**	**12 weeks of treatment**
VEGF	3.9 ± 1.0	6.2 ± 1.6 *	
IGF1	14.2 ± 3.0	29.0 ± 7.0 *	19.2 ± 3.0 *
IGF2	5.5 ± 1.8	8.2 ± 1.8 *	
GDF8	4.8 ± 1.6	2.9 ± 1.0 *	3.3 ± 0.8 *

Table 1. Relative expression of the VEGF, IGF1, IGF2 and GDF8 mRNA in M. latissimus dorsi of control rats and in M. latissimus dorsi of rats 6 and 12 weeks after PHB scaffold implantation. Mean ± S.E.M. are given. Students t-test * = $p < 0.05$.

In addition, PHB scaffolds showed synergistic effects with the surrounding muscle, because of an increase in the slow myosin isoform after implantation (Mack et al. 2008). Another study showed ectopic bone formation in nude rats after intramuscular implantation of PHB embroidery seeded with human osteoblast cells (Mai et al. 2006). The osteogenic potential of different PHB scaffolds was also shown in nude rats (Rentsch et al. 2009). In mini pigs PHB was used for covering defined bone defects in the anterior skull base including a Dura mater lesion. The anterior skull base bone defect was completely closed after 9 months. Furthermore no reaction or adhesions between brain and PHB or Dura mater and PHB was observed (Bernd et al. 2009).
PHB patches were used to close experimentally induced atrial septal defects in calves. Twelve months postoperatively no shunt or sign of infection was found. This experimental model thus prompted formation of regenerated endothelial tissue and complete degradation of the PHB patches (Malm et al. 1992). Recently it was shown that PHB can be used as an alternative to epineural suturing in the treatment of peripheral nerve injuries at the wrist/forearm level of the arm (Aberg et al. 2009).

4.3.2 Polylactid (PLA)

Among the family of biodegradable polyesters, polylactides have come to the fore because, they are produced from renewable resources, they are biodegradable, have high mechanical performance and very low or no toxicity. The degradable material PLA is applied in dentistry as Guided Tissue/Bone Regeneration membranes even though these membranes do not have any significant biological function such as the facilitation of cell adhesion. Polylactic acid or polylactide (PLA) is the most commonly used biodegradable, thermoplastic, aliphatic polyester derived from renewable resources in surgery especially in osteosynthesis (Ashammakhi et al. 2003). PLA can be used for biomedical purposes because

of its biodegradability in contact with biological tissues (Rimondini et al. 2005). Polylactide acid is degraded by hydrolysis or specific cleavage of oligopeptides (Drury and Mooney 2003). It is known that the degradation product of PLA, lactic acid, is normally present in the body, can enter the tricarbonxylic acid cycle and is excreted as H_2O and CO_2 (Gunatillake and Adhikari 2003). On the other hand degradation products of PLA are partially cytotoxic (Nesic et al. 2006).

In our own experiments we could show that cultivation of L929 mouse fibroblast cells with PLA membranes significantly decreased cell proliferation and increased the amount of death cells compared to control cells. Our results are in agreement with findings from Yang et al (Yang et al. 2002). They show that growth of L929 cells was poor on PLA films. The numbers of adhered cells cultured on PLA were significantly lower than those on control disks in early periods of incubation but became comparable after 21 days (Iwamatsu-Kobayashi et al. 2005). Human keratinocytes delayed growth has been shown on a PLA film with respect to culture on standard tissue culture polystyrene, even though the same plateau level was observed after 2 weeks (Garric et al. 2005). Former *in vitro* studies have shown that osteoblast cells are able to attach well to PLA or poly-glycolic acid (PGA) (Ishaug et al. 1994). We could demonstrate that primary osteoblast cells growth on PLA membranes (Figure 6).

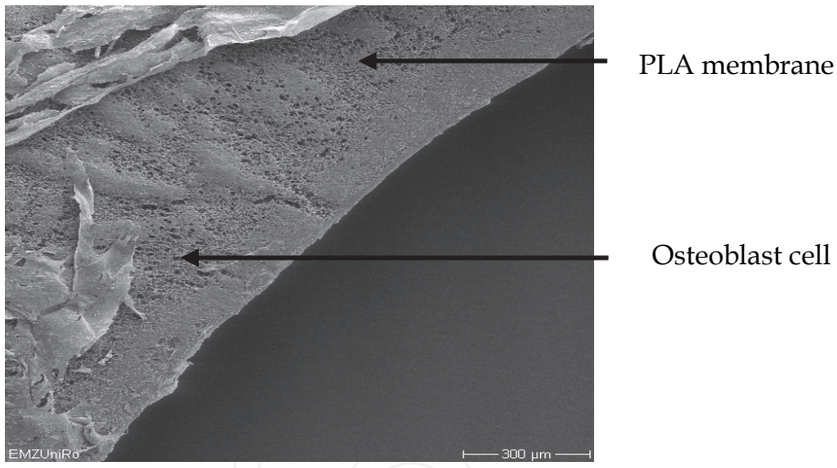

Fig. 6. Scanning electron micrograph of osteoblast cells (PO62) growing on a PLA membranes. Heterogenous morphology of these cells was observed after 10 days of cell culture. Bars = 300 µm

Collagen coating and NaOH treatments of the PLA film improved skin cell adhesion and proliferation but their extent depends usually on the culture time (Garric et al. 2005). Furthermore, an enhanced 3-dimensional porous structure of PLA coated with collagen showed a significantly higher amount of initial cell attachment than PLA porous scaffold without collagen immobilization (Fuse et al. 2009). Other PLA surface modifications with chloric acid mixture solution increased the surface wettability and with it the cell adhesion on the surface (Lee et al. 2002). In contrast, human RT-112 cells did not show any differences in the proliferation rate after incubation with PLA alone or PLA-copolymers (Holzl et al. 2000).

In vivo biocompatibility of poly(L-lactide) resorbable mesh was shown in rats. It was found that PLA induces important immunological reaction. Amongst others PLA caused mild

inflammatory response by infiltrating mononuclear and polynuclear inflammatory cells in animals (de Tayrac et al. 2008). Furthermore, stereocomplexed PLA nanofibers induced milder inflammatory reaction than poly(L-lactide) nanofibers after subcutaneous implantation in rats (Ishii et al. 2009).
To date, predominantly histological staining and immunohistochemical analyses were used to study the behaviour of bone substitute materials on bone graft healing. Only a few numbers of studies present the molecular mechanisms of bone formations associated with bone substitutes. Recently it was shown that the expression of type I and type II collagen was increased in human mesenchymal progenitor cells as well as adipose stem cells seeded on PLA (Heckmann et al. 2007; Maenpaa et al. 2010). PLA nanofibres possessed a growth inhibitory effect on human tendon derived fibroblasts and no meaningful influence on the gene expression of collagen I was observed (Theisen et al. 2010). Furthermore, PLA nanofibres tend to result in a down-regulation in bone morphogenetic protein 2 (BMP-2) and VEGF expression during the period of human mesenchymal stem cell differentiation towards osteoblasts (Schofer et al. 2009). On the other hand PLA membranes do not have any influence on the gene expression of different growth factors, collagen I and II as well as myostatin after subcutaneously implantation in rats (Gredes et al. 2010b).

4.3.3 Polycaprolactone (PCL)

Polycaprolactone (PCL), a thermoplastic polymer derived from the chemical synthesis of crude oil, has a very low melting point of about 60°C and degrades by hydrolysis of its ester linkage under physiological conditions. Polycaprolactone has also been regarded as a tissue-compatible biodegradable polymer with good mechanical properties (Khor et al. 2003). PCL has been proven to be a tissue-compatible biodegradable polymer with satisfying mechanical properties although it is not very favourable for cell growth because of its intrinsic hydrophobicity and lack of bioactive functional groups (Zhu et al. 2002). *In vitro* studies showed that human calvarial periosteal cells attached and proliferated on PCL membranes with the formation of extracellular matrix (Schantz et al. 2002). Further studies were conducted with primary human fibroblast cells. Light, environmental scanning electron, and confocal laser microscopy as well as immunohistochemistry showed cell proliferation and extracellular matrix production on the polycaprolactone surface in the first culturing week. Over a period of 3-4 weeks in a culture, the fully interconnected scaffold architecture was completely 3D-filled by cellular tissue (Hutmacher et al. 2001b). Human primary craniofacial cells proliferated on PCL as shown by DNA-assay and collagen-I staining. Short- and long-term attachment studies demonstrated the expression of osteoblast cell markers on the PCL (Gough et al. 2003). Figure 7 illustrates a natural cover of a PCL membrane with primary osteoblast cells after 10 d of cell culture.
On the other hand, decreased fibroblast cell density was present by Vance et al. after 5 days of treatment with NaOH-modified PCL (Vance et al. 2004). These findings are in agreement with our own observations that mouse fibroblast cells incubated with PCL membranes showed a significantly decreased proliferation rate and a higher amount of death cells as compared to control cells.
The ability of PCL to repair bone defects were tested in white rabbits. Bone defects of 4.5 x 12 mm in the bilateral femoral condyle were prepared and PCL cylinders implanted into the defects. After 3 to 12 months of implantation, it was shown that bone defects were filled with new bone on the PCL-surface, and no inflammatory reaction appeared. Furthermore, the bone mineral density was greater in the PCL treated animals compared to untreated control group (Aahmat et al. 2005). PCL has a good biocompatibility and high osteoinductivity.

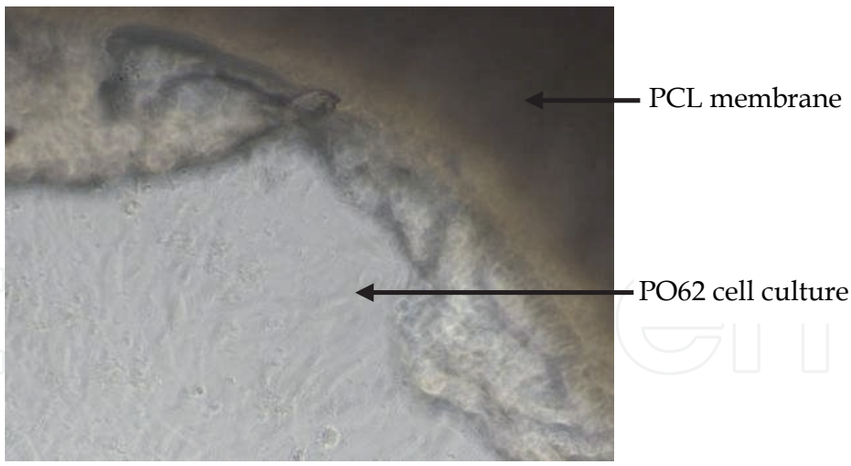

Fig. 7. Transmission microscopy of osteoblast cells (PO62) growing on a PCL membrane. Normal morphology of these cells was observed after 10 days of cell culture. Magnification: x 200.

To increase the osteoinductivity and osteoconductivity of PCL scaffolds, the PCL surface was modified. Previously, PCL-tricalcium phosphate scaffolds were developed and implanted into rat femoral defects. Histological evaluation illustrated infiltration of vascularized connective tissue and bone in treated rats, whereas the bone in-growth of untreated defects is minimal (Rai et al. 2007a). Polycaprolactone-20% tricalcium phosphate (PCL-TCP) scaffolds were assessed for the treatment of critical-sized defects of the mandible. Micro-CT measurements showed an increase in the bone volume fraction in defects grafted with scaffolds. The increase in the bone volume fraction was more pronounced after combination of the PCL-TCP scaffolds with platelet-rich plasma (Rai et al. 2007b). Another modification is the incorporation of hydroxyapatite (HA) particles in PCL scaffolds. These PCL/HA scaffolds increase the expression of osteogenic differentiation markers, such as type I collagen and osteocalcin in an in vitro model. Furthermore, the use of the PCL/HA scaffolds in a mouse calvarial model showed significantly greater amounts of new bone compared to pure PCL scaffolds (Chuenjitkuntaworn et al. 2010).

4.3.4 Collagen

Natural polymers used in bone tissue engineering include collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan (Hutmacher et al. 2001a). Many of the naturally occuring animal-derived polymers are components of the extracellular matrix. They are biocompatible, bioresorbable and can be formulated into many configurations with variable residence time using enzymatic treatment and chemical crosslinking. Collagen is the most abundant extracellular matrix protein and component of connective tissue. Collagen, in the form of elongated fibers, is mostly found in tendon, ligament and skin, as well as in cartilage and bone. The collagen used in dental procedures is readily isolated and purified from various animal species by enzyme treatment. Collagen type I is the main organic component that is originally secreted by osteoblasts, which then becomes mineralized at a later stage of bone development. Collagen has been actively investigated as a favourable artificial environment for bone ingrowth. It was shown that endothelial cells adherent, spread and proliferated on a collagen membrane (Lycoll®, Resorba, Nuernberg, Germany) (Breithaupt-

Faloppa et al. 2006). Twardowski and co-workers found that type I collagen potently stimulates angiogenesis *in vitro* and *in vivo* (Twardowski et al. 2007). Furthermore, mesenchymal stem cell osteogenic differentiation was demonstrated on collagen scaffolds (Donzelli et al. 2007; Schneider et al. 2010; Jurgens et al. 2011). Alveolar ridge augmentation was found utilizing collagen wound dressing, so called COLLACOTE® (Zimmer Dental, Carlsbad, USA) (Ceravolo et al. 1987).

Type I collagen gel matrix was used for nasal reconstruction in rats. Histological sections of the collagen implant revealed restoration of the nasal anatomy with a thin plate of immature bone (Lindsey et al. 1996; Toung et al. 1999). Recently it was shown that resorbable collagen membranes can be used for guided bone regeneration after apicectomy or around dental implants. Both studies demonstrate that quantity and quality of new bone formation were much better in bone defects covered with collagen compared to untreated bone defects (Fei et al. 2008; Dominiak et al. 2009). At the moment two collagen-based bone augmentation materials are commercially available: Foundation™ (J. Morita USA, Inc.) and COLLAPLUG® (Zimmer Dental, Carlsbad, USA). Foundation™, an absorbable atelo-collagen sponge consists of 85 to 95% bovine collagen type I and 5 to 15% bovine collagen type III and has been used with great success since 1998 in Japan. Using rats it was shown that TERUPLUG® (different brand name for Foundation™) is able to stimulate endochondral ossification within two weeks after implantation into the calf muscle. Alkaline phosphatase activity and the calcium content had increased markedly in treated rats (Shinji et al. 2003). In addition, four weeks after implantation of TERUPLUG® in rabbit cranial defects a lot of newly formed collagen fibers around the inserted material were observed. Newly formed and matured bone was found eight weeks after implantation of TERUPLUG® compared to untreated animals (Kim et al. 2008). TERUPLUG® was used for maxillary sinus augmentation in rabbits. Gradually increased new bone formation was observed after 4, 12 and 24 weeks (Marukawa et al. 2011). In contrast, little or no new bone formation was observed on the maxillary sinus floor at six months following sinus membrane elevation and support with COLLAPLUG® in humans (Ahn et al. 2011).

Despite the good *in vitro* and *in vivo* biocompatibility, collagen undergoes rapid degradation upon implantation within 4-5 weeks (Donzelli et al. 2007). Furthermore, commercial collagen sponge (COLLAPLUG®) and membrane (BioGuide®, Geistlich Pharma, Wolhusen, Germany) induced considerable cell death, impaired initial function, and generated extraordinary intracellular reactive oxygen species in attached osteoblast cells. These effects substantially ameliorated after N-acetyl cysteine pre-treatment of the collagen sponge and membrane (Yamada et al. 2010).

Collagen is very often used as matrix for application of growth factors, e.g. TGF-β or BMP-2. Intramuscular application of TGF-β-loaded collagenous matrix is resulted in bone induction in primates (Ripamonti et al. 1997; Duneas et al. 1998). TERUPLUG®, as a carrier for Escherichia coli-derived recombinant human bone morphogenetic protein-2 (ErhBMP-2), was implanted into the calf muscle of Wistar rats. After 21 days, mature bone was formed and on days 14 to 21 after implantation, alkaline phosphatase activity and calcium content increased (Shinji et al. 2003).

Examples for a commercially available product containing growth factors is INFUSE® Bone Graft (Medtronic GmbH, Meerbusch, Germany), recombinant human bone morphogenetic protein 2 in an absorbable collagen sponge.

4.4 Biocomposites containing flax fibers in a polyester matrix

The usage of barrier membranes for osteogenesis has increased in the last years. Small bony defects seem not to depend on the application of membranes to regenerate bone, but large bone defects benefit from membranes, because they reduce the indispensable resorption of bone grafts when used alone. The application of barrier membranes to promote bone regeneration was described in orthopedic research. However, the clinical potential of these membranes was recognized some years ago for periodontal regeneration (Hermann and Buser 1996; Sculean et al. 2008).

Fibre-reinforced plastics have successfully proven their value in various applications because of their excellent specific properties, such as high strength and stiffness. The use of natural fibres for fabrication of composites in combination with biodegradable polymers is an excellent approach for biomedical application. Natural fibres are of basic interest since they have the capability to replace glass, ceramics or carbon fibres (Bax and Müssig 2008). Natural fibres combined with biodegradable polymers like polyhydroxyalkanoate (PHA), polylactic acid (PLA), polycaprolactone (PCL) and starch polymers frequently used as a matrix resulted in composites called "green" because of their complete biodegradability (Mohanty et al. 2005). The influence of plant fibres like flax, jute, ramie, oil palm fibres or cellulose fibres on the mechanical properties of biodegradable polymers was tested several years ago. Wollendorfer and Bader found in increased tensile strength as well as a four times better wheat starch of the reinforced polymer compared to the polymers without fibres (Wollendorfer and Bader 1998) Composites reinforced with natural fibres have been used in several branches of industry (i.e. automobile) and medicine with special focus on tissue artificial scaffold, drug-release systems, cardiovascular patches and nerve cuffs (Williams and Martin 2002; Misra et al. 2006). Flax fibres are amongst the oldest fibre crops in the world. The use of flax for the production of linen goes back at least to ancient Egyptian times. It is known that flax fibres are stronger than cotton fibres but less elastic. Flax fibres provide natural raw materials for composites and pulp / paper (van Dam et al. 1996). Former studies have shown an increased biological reaction around linen threads compared to synthetic threads (Jarosz-Cichulska 1988). Recently, the cytotoxicity of flax fibres, oil emulsion, and seedcake extract from transgenic flax overproducing various antioxidative compounds was determined in a culture of mouse fibroblasts. No changes in the total number of fibroblasts and comparable numbers of dead cells were shown in the presence of each type of flax material (Skorkowska-Telichowska et al. 2010).

In order to improve the biochemical and mechanical properties of flax fibres, transgenic flax over-expressing the bacterial polyhydroxybutyrate (PHB) gene was produced (Wrobel et al. 2004; Wrobel-Kwiatkowska et al. 2007). Composites containing fibres from transgenic plants did not differ from the control plants in the level of major fibre constituents (i.e. cellulose, lignin and pectin content) but was significantly stronger and more elastic than those containing fibres from control flax plants (Szopa et al. 2009). Furthermore, genetically modified flax plants producing PHB showed significantly increased parameters such as strength, Young´s modulus, energy for failure of flax fibres and phenolic acid level, a reason for improved plant resistance to pathogen infection (Wrobel-Kwiatkowska et al. 2007; Wrobel-Kwiatkowska et al. 2009).

We found that composites containing flax fibres from transgenic flax plants producing polyhydroxybutyrate (M50) and control (wt-Nike) plants in a polylactid (PLA) or polycaprolactone (PCL) matrix showed a good *in vitro* biocompatibility despite the cell viability of mouse fibroblast cells treated with these composites being slightly reduced and

the amount of dead cells significantly increased compared to untreated cells after incubation for 12h to 48h. The biocompatibility of composites from transgenic flax plant fibres producing PHB did not differ from composites of non-transgenic flax plant fibres. Both linen membranes coated with PLA showed a significant increase in the proliferation rate of fibroblast cells compared to cells treated with membranes composed of PLA alone, whereas both flax membranes in the PCL matrix significantly decreased the proliferation rate of mouse fibroblast cells compared to cells treated with membranes composed of PCL alone. No differences were found between genetically modified and non-modified flax (Figure 8).

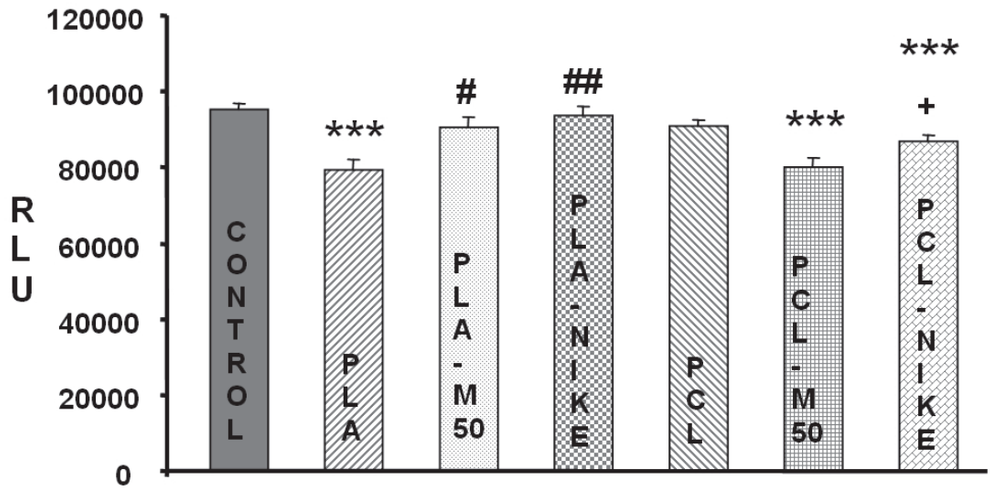

Fig. 8. Determination of cell viability by measurement of ATP. Viability of mice fibroblast cells (L929) cultured without and with membranes produced of polylactide (PLA), linen from PHB producing flax fibres coated with PLA (PLA-M50), linen from normal flax fibres coated with PLA (PLA-NIKE), polycaprolactone (PCL), linen from PHB producing flax fibres coated with PCL (PCL-M50) and linen from normal flax fibres coated with PCL (PCL-NIKE) for 24h was analysed using the CellTiter-Glo® assay. Means ± S.E.M. are given in all cases for n = 14-48 samples. Stars indicate significant differences: ***) $p < 0.005$, membrane-treated cells versus control; ##) $p < 0.01$, ###) $p < 0.005$, PLA versus linen membrane, +) $p < 0.05$, PCL versus linen membrane, unpaired t-test. RLU = relative luminescence units.

Beside macroscopically and histological examination the biocompatibility can be assessed by analyses of the biomaterial influence on the gene expression. Because of this we analysed the gene expression of growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) as well as myostatin also known as growth differentiation factor 8 (GDF8) after subcutaneous implantation. We found that subcutaneously insertion of the different biocomposites had no influence on the gene expression of the most tested genes. The constantly happening muscle contraction of the Musculus latissimus dorsi is not followed by stress-induced changes in muscle growth factor expression (Gredes et al. 2010b). The influence of the new biocomposites on bone regeneration must be checked using *in vitro* models. Cell culture experiments with primary osteoblast cells and these biocomposites showed that osteoblast cells are able to attach and proliferate on these membranes. The proliferation is much better on PLA-biocomposites compared to PCL-flax membranes (Figure 9).

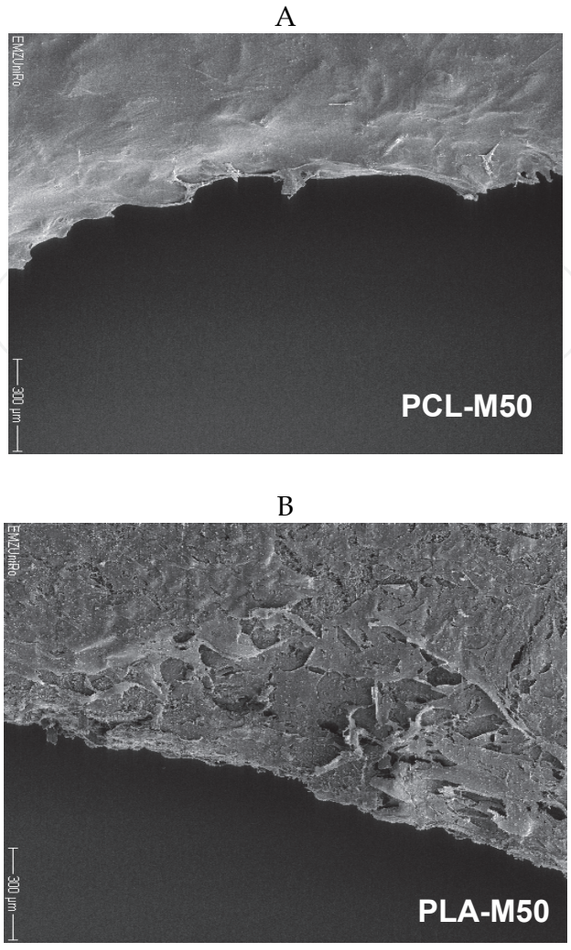

Fig. 9. Scanning electron micrographs of osteoblast cells (PO62) growing on a PCL-M50 (A) or a PLA-M50 membrane (B). After 10 days of cell culture on PCL-M50 membranes moderate natural cover with osteoblast cells could be observed, whereas culturing on PLA-M50 increased the amount of growing cells. Bars = 300 µm

4.5 Carrier for growth factors

As described earlier in this chapter bone formation is mediated by the release of local, biochemical and biophysical messengers, e.g. growth factors. In therapeutic use the bone growth factors need a carrier. Carrier requirements for most bone repairs are also simplified by the concurrent use of either internal or external fixation or coaptation, eleminating the need for mechanical strenght or resistance to motion. Furthermore, the carrier should assure local, sustained release of the growth factors which may otherwise be rapidly absorbed before instituting their effect. Bone is also highly vascular and carriers used for bone repair have to be capable of supporting vascular in-growth. Several biodegradable materials have been investigated as carriers for bone growth factors, including (1) organic materials such as inactive demineralized bone proteins, collagen, fibrin, squalene, and coral; (2) ceramics, including tricalcium phosphate, hydroxyapatite, calcium-sulphate composites, and bioactive glasses; and (3) synthetic polymers such as polylactide, polylactide-polyglycolide co-polymer, polyanhydride, and polyorthoester (Table 2).

Biodegradable materials as carriers for bone growth factors		Quality characteristics	References
organic materials	collagen	Collagen molecules assemble into heterotypic aggregates that define the biological and mechanical properties of most tissues and organs. Type I collagen, the major component of the organic bone matrix can bind osteoblasts via specific cell surface receptors, the integrins.	(Burgeson and Nimni 1992; Tzaphlidou 2005)
	fibrin	High adhesion to many biological surfaces, excellent seeding effects and good tissue development. It is not toxic, allergenic or inflammatory.	(Ye et al. 2000)
	coral	Natural coral is radiodense, it resorbs more rapidly than ceramic materials and the natural pore size of native coral facilitates osteoconduction and bone growth.	(Sciadini et al. 1997)
ceramics	tricalcium phosphate	Porous beta-tricalcium phosphate scaffolds are employed for local drug delivery in bone. It is used alone or in combination with resorbable polymer or autologous materials.	(Bansal et al. 2009; Kundu et al. 2010)
	hydroxyapatite	An identical composition to the bone tissue of living organisms and has similar physical, mechanical, and other properties. It shows high biocompatibility, does not give rise to inflammatory phenomena, and is non-toxic.	(Yarosh et al. 2001)
	calcium-sulphate composites	Calcium sulfate (plaster of Paris) is an absorbable, moldable material that has easy handling properties. It promotes osseous formation.	(Pecora et al. 1997)
	bioactive glass	This material has osteoconductive and osteo-promotive abilities in the biocompatible interface for osseous migration, and a bioactive surface colonized by osteogenic cells free in the surgical wound. It bonds to soft and osseous tissues.	(Wilson and Low 1992; Macedo et al. 2004)

synthetic polymers	polylactide (PLA)	Prosperities of PLA depend on the component isomers, processing temperature and molecular weight. In nature, polymer degradation is induced thermal activation, hydrolysis, biological activity, oxidation, photolysis, or radiolysis. In biomedical fields it is also used as bone fixation material and drug delivery microsphere.	(Zhao et al. 2004; Madhavan Nampoothiri et al. 2010)
	polylactide-polyglycolide copolymer	It promotes specific cell adhesion, differentiation and bone formation. Its degradation depends on the formulation, amorphous/crystalline structure, isomeric characteristics, molecular weight and amount of material used.	(Lu et al. 2003; Serino et al. 2003)
	polyanhydride	Polyanhydrides and their degradation products have not been found to cause significant harmful responses and are considered to be biocompatible. *In vivo*, polyanhydrides degrade into non-toxic diacid monomers that can be metabolized and eliminated from the body.	(Tamada and Langer 1992; Kumar et al. 2002)
	polyorthoester	This polymer degrades at its surface and becomes thinner with time rather than crumbling. It can cause minor moderate inflammation reaction, compared with PLA.	(Ekholm et al. 1997)

Table 2. Carrier materials for bone growth factors.

5. Conclusion

The literature has shown that early bone loss can be significantly reduced by socket grafting. The process of socket grafting requires an understanding of wound healing and an appreciation of the biological properties of the products available for socket grafting. Various types of alloplastic bone substitution materials have been created and showed ossification and new bone formation. The huge variety of biomaterials makes it difficult to decide which material is adequate for which indication. To date, tissue engineered bone is far from a routine clinical application. Clinical investigations using alloplastic bone substitution materials are very rare in the literature but, because of similar bone remodelling processes in pigs and humans, we found the same details about usage of alloplasts in dentistry. The most alloplastic materials are not popular in dentistry and, because of this, further studies are necessary.

6. Acknowledgment

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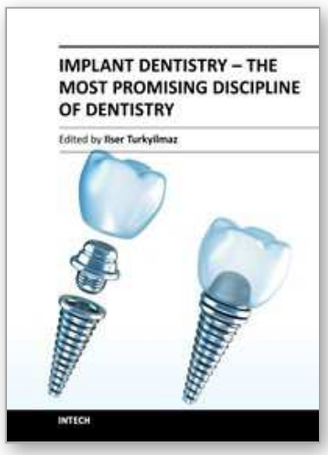

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Since Dr. Branemark presented the osseointegration concept with dental implants, implant dentistry has changed and improved dramatically. The use of dental implants has skyrocketed in the past thirty years. As the benefits of therapy became apparent, implant treatment earned a widespread acceptance. The need for dental implants has resulted in a rapid expansion of the market worldwide. To date, general dentists and a variety of specialists offer implants as a solution to partial and complete edentulism. Implant dentistry continues to advance with the development of new surgical and prosthodontic techniques. The purpose of Implant Dentistry - The Most Promising Discipline of Dentistry is to present a comtemporary resource for dentists who want to replace missing teeth with dental implants. It is a text that integrates common threads among basic science, clinical experience and future concepts. This book consists of twenty-one chapters divided into four sections.

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