Polymers **2011**, *3*, 509-526; doi:10.3390/polym3010509

OPEN ACCESS

polymers

ISSN 2073-4360
www.mdpi.com/journal/polymers

Review

Biopolymers for Hard and Soft Engineered Tissues: Application in Odontoiatric and Plastic Surgery Field

Eriberto Bressan [1], Vittorio Favero [1], Chiara Gardin [2], Letizia Ferroni [2], Laura Iacobellis [2], Lorenzo Favero [1], Vincenzo Vindigni [3], Mario Berengo [1], Stefano Sivolella [1,*] and Barbara Zavan [2,*]

[1] Department of Periodontology, School of Dentistry, University of Padova, Via Venezia 90, 35100 Padova, Italy; E-Mails: eriberto@studiobressan.com (E.B.); vittorio_favero@yahoo.it (V.F.); lorenzo.favero@unipd.it (L.F.); mario.berengo@unipd.it (M.B.)

[2] Department of Histology, Microbiology and Medical Biotechnology, University of Padova, Via G. Colombo 3, 35100 Padova, Italy; E-Mails: chiara.gardin@cribi.unipd.it (C.G.); letizia.ferroni@gmail.com (L.F.); lauraiacobellis@libero.it (L.I.)

[3] Unit of Plastic and Reconstructive Surgery, University of Padova, Via Giustiniani 2, 35100 Padova, Italy; E-Mail: vincenzo.vindigni@unipd.it (V.V.)

* Author to whom correspondence should be addressed; E-Mails: stefano.sivolella@unipd.it (S.S.); barbara.zavan@unipd.it (B.Z.).

Received: 23 December 2010; in revised form: 24 January 2011 / Accepted: 15 February 2011 / Published: 28 February 2011

Abstract: The goal of modern dentistry and plastic surgery is to restore the patient to normal function, health and aesthetics, regardless of the disease or injury to the stomatognathic and cutaneous system respectively. In recent years tissue engineering and regenerative medicine have yielded many novel tissue replacements and implementation strategies. Scientific advances in biomaterials, stem cell isolation, growth and differentiation factors and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory. Repairing of bone and skin is likely to become of clinical interest when three dimensional tissue reconstructive procedures and the appropriate supporting biomimetic materials are correctly assembled. In the present review, we provide an overview of the most promising biopolymers that may find clinical application in dento-maxillo-facial and plastic surgery.

Keywords: stem-cell; tissue engineering; scaffolds; skin; hard tissue

1. Introduction

The biomedical burden of treating diseased or injured organs continues to increase in parallel with expanding populations. Despite state-of-the-art medical and surgical therapies, clinical outcomes remain suboptimal, in part because of a lack of replacement biological parts.

Nowadays the goal of soft and hard tissue engineering is the replacement of tissue lost due to trauma, cancer, or congenital defects. In fact, while the reconstruction of small, or moderate sized tissue, defects is technically feasible, thanks to the natural ability of the body to repair tissue damage, larger volume defects remain the real problem.

The promise of unlimited bioengineered resources to replace defective or missing parts has been of continued interest, which is increasingly challenged by clinical problems requiring an ever-expanding array of reconstructive options. The past few decades have seen significant advances toward achieving these goals, with the hope of not only replacing organs but also exploiting the body's innate capacity for regeneration [1-4].

A variety of different biomaterials are currently being used as scaffold for reconstruction of soft (such as adipose tissue or skin) or hard (bone) defects. The optimal biomaterial should meet the following requirements: (1) biocompatibility with the tissues; (2) biodegradability at the ideal rate corresponding to the rate of new tissue formation; (3) nontoxicity and nonimmunogenicity; (4) optimal mechanical property; and (5) adequate porosity and morphology for transporting of cells, gases, metabolites, nutrients and signal molecules both within the scaffold and between the scaffold and the local environment [5].

Currently single and multi-phase materials (composites) have been designed as scaffolds to support cell growth and then used for *in vivo* tissue replacement.

Subsequently both kinds of scaffolds will be reported that are currently used in clinical applications for the *in vivo* reconstruction of hard and soft tissue.

2. Soft Tissue

Current strategies for soft tissue reconstruction that rely on autologous tissue grafts and synthetic implants are limited by multiple factors, including tissue resorption and implant rupture or contracture. Several cell types are used for this aim, such as adult cells (*i.e.*, keratynocites, fibroblasts, adipocytes) or adult stem cells (*i.e.*, mesenchymal stem cells) cells that are loaded in biocompatible scaffolds. Many natural biomaterials have been widely used for this purpose. They can be used alone or combined with other synthetic or inorganic materials. Table 1 lists the principal engineered dermal tissue or skin artificial tissue that have widespread clinical application. The main properties of such tissue are the special dressing and nursing care, the reduced time in grafting. However, its mechanical fragility and high cost must be taken into consideration.

Table 1. Table of scaffolds that are currently widely used in clinical applications.

Scaffolds	Trademark name	Cells
Collagen-GAG and silicone	Integra ™	Fibroblast, keratynocytes
Acellular dermal matrix Cryopreserved cadaveric skin	AlloDerm ™	Thin autograft
PGS/PLA+	DermaGraft ™	Fibroblast, keratynocytes
Hyaluronic acid+	LaserSkin ™	keratynocytes
PEO	PolyActive ™	Fibroblast, keratynocytes
Collagen gel+	ApliGraft ™	Fibroblast, keratynocytes
Collagen gel+	ORCEL ™	Fibroblast, keratynocytes
BioBrane™	TransCyte ™	Fibroblast
Hyaluronic acid	Hyalograft 3D ™	keratynocytes
Porcine skin	Permacol ™	No cells
Cryopreserved amniotic membrane	Amniograft ™	No cells
Porcine small intestine submucosa	Oasis ™	No cells

2.1. Single-Component Scaffolds

Collagen is any of a family of extracellular matrix (ECM) proteins occurring as a major constituent of connective tissue, giving it strength and flexibility. Collagen is a natural polymer widely used to synthesize dermal substitutes because it is biocompatible and permits physiological interactions with cells. Several collagen-based scaffolds are currently commercialized as dermal substitutes, in particular, in the form of hydrogels. Such biomaterials are usually obtained by encapsulating dermal fibroblasts in a collagen hydrogel at low concentration (0.66 mg/mL). However they suffer from extensive contraction by cells and weak resistance against degradation, which limits their use as permanent graft. Recently, Helary and co-workers [6] have proposed a new procedure to synthesize more concentrated collagen hydrogels at 5 mg/mL (CCH5) in order to improve hydrogel resistance and integration capability. 1 cm^2 pieces of CCH5 were implanted in sub-cutaneous pockets of rat abdomen and analyzed after 15 and 30 days. At a macroscopic level, CCH5 scaffolds were still visible at day 30 without any reduction of their area, and they appeared to be well integrated within the implant. In addition, neo-vascularization was observed and reached the core of the implant after 15 days. Thus, these novel materials show superior stability and *in vivo* integration compared to less concentrated collagen hydrogels, and appear promising for the treatment of skin lesions.

Fibrin is a protein involved in the clotting of blood and formed by polymerization of fibrinogen in presence of thrombin. Even though it is not a part of the normal ECM, it is temporarily present during wound healing. In the field of regenerative medicine, fibrin glue has been widely used as a biological tissue adhesive. Fibrin glue is composed of two separate solutions of fibrinogen derived from blood plasma and bovine thrombin. When mixed together, these agents mimic the last stages of the clotting cascade to form a fibrin clot. Fibrin glue can be applied using a double-barrel syringe or by spray application [7]. In the study of Foster and colleagues [8], fibrin glue was used as an alternative to staples in burn patients requiring wound excision and skin grafting. A total of 138 patients with burn wounds ≤40% of total body were treated with both fibrin glue and staples at two comparable test sites, and wound closure was evaluated at a macroscopic level 28 days after the surgery. Their results show that fibrin glue is safe and effective for attachment of skin grafts, with outcomes better than staple

fixation. In particular, the use of the glue is of major benefits to patients because the removal of staples is often painful and time-consuming.

Gelatin is formed from the hydrolysation of collagen. Studies have reported that gelatin activates macrophages, shows high hemostatic effect, and does not cause antigenicity [9]. Similar to fibrin glue, gelatin has been widely used as a tissue adhesive for wound closure. In the study of Liu, *et al.* [10], the adhesive is based on the cross-linking of gelatin by a microbial transglutaminase (mTG). *In vivo* experiments were performed using small (rat) and large (pig) animal models. Immediately before application, the tissue adhesive was prepared by mixing the warm gelatin (type A from porcine) and mTG solutions. The temperature of the adhesive solution was approximately 40 °C when applied to the tissue. *In vivo* tests with a rat liver wound model showed that the gelatin-mTG adhesive achieves complete hemostasis in 2.5 minutes and the gel offers substantial adhesive and cohesive strength. In a large animal porcine model, a femoral artery wound that resulted in extensive bleeding was sealed in four minutes by applying the gelatin-mTG adhesive after excess blood removal. Thus, the gelatin-mTG adhesive is found to gel *in situ* within a short time (<5 min), and to adhere to tissue in the presence of modest amounts of blood. In addition, the gelatin-mTG adhesive may provide a simple and safe surgical sealant because it requires neither reactive reagents nor external stimuli (e.g., light). Moreover, since gelatin and mTG are not derived from human blood, these components would be readily available and inexpensive, which is one of the limitations of the fibrin-based adhesive.

Hyaluronic Acid (HA) is a major component of the ECM in connective tissues. Being a polysaccharide and not a protein, it is potentially less antigenic, which is an important property when considering clinical uses. Its supportive role for cell proliferation and differentiation has been confirmed by several *in vivo* and *in vitro* studies [11]. HYAFF®11 (Fidia Advanced Biopolymers s.r.l., FAB, Abano, Italy) is a linear derivative of Hyaluronic Acid modified by complete esterification of the carboxylic function of glucuronic acid with benzyl group. This increases the hydrophobicity, the residence time *in vivo* and makes HYAFF®11 more resistant to hyaluronidase activity. Stillaert and co-workers [12] performed pilot clinical trials in humans. Preadipocytes were isolated from lipoaspirate material and seeded on HYAFF®11. The cellular construct (called ADIPOGRAFT®) and the acellular control scaffold (HYAFF®11) were implanted in the sub-umbilical area of 12 volunteers. 16 weeks after implantation, the ADIPOGRAFT® scaffolds maintained their original volume and dimension and also demonstrated the presence of a tissue-like substance within the scaffold. In contrast, the HYAFF®11 explants showed consistent volume loss with degradation of the scaffold into a gel-like substance, even eight weeks after implantation. Though HA scaffolds were stable cell carriers and had the potential to generate volume retaining tissue, no adipogenic differentiation was observed within the preadipocyte-seeded scaffolds.

2.2. Composite Scaffolds

In addition to single-component scaffolds, composites of various naturally derived polymers are also often used in the field of tissue engineering. It is commonly accepted that composite materials show an excellent balance between strengths and weaknesses, and overall exhibit improved characteristics compared with individual components [13]. In order to eliminate undesired characteristics and exploit advantages of the individual polymers, various composite scaffolds

comprising two or more polymers have often been considered. For example, the stability of polymers that are mechanically weaker can be reinforced by fabricating composites containing mechanically stronger polymers. Similarly, polymers that possess poor cell proliferation or migration properties can be remedied with the incorporation of a more bioactive polymeric phase.

In a recent work, Judith, *et al.* [14] developed an acellular provisional matrix aimed to mimic the ECM of a wound site, and evaluated its potential for *in vivo* dermal regeneration. 1.5 × 1.5 cm excisions were created on the back of female albino rats. The wounds of the animal were treated for seven days with platelet-derived growth factor (PDGF)-incorporated chitosan or PDGF-incorporated collagen-chitosan (CCP). Chitosan is a polysaccharide composed of glucosamine and N-acetyl glucosamine, and obtained from N-deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans [15]. Wound epithelialization in CCP-treated animals was completed by 10 days after the excision, whereas this took 14 days to occur in the chitosan-PDGF treated group. Therefore, the use of collagen for treatment of cutaneous wounds, in addition to chitosan and PDGF, enhances the healing process in its early phases.

Weinstein-Oppenheimer and colleagues [15] report production and preclinical studies to examine the tolerance and efficacy of an autologous cellular gel-matrix integrated implant system (IIS) for regeneration of skin damaged by burns or other severe trauma. IIS was formed by integrating autologous skin cells by means of the *in situ* gelification of fibrin into a porous cross-linked scaffold composed of chitosan, gelatin and hyaluronic acid. Full-thickness excision wounds were performed on the paravertebral skin of eight young-adult rabbits. The preclinical assays showed that the IIS was well tolerated and efficacious because there were no signs of inflammation and all the wounds healed, showing complete epithelization after 60 days. Moreover, the IIS treated animals exhibited an overall better survival, better growth over time and smaller cicatrization areas. The use of autologous cells in this system was an advantage, not only because a scar of better quality was achieved, but also because it minimized the infectious diseases transmission risk from one individual to another.

Other important results have been reported by Kahn *et al.* [16]. The authors describe a series of patients with chronic wounds reconstructed with a commercially available bilayer, acellular dermal replacement (ADR), containing a collagen-glycosaminoglycan dermal template and a silicone outer layer. A retrospective review was performed of 10 patients treated for chronic wounds with ADR and negative pressure dressing followed by split-thickness skin graft between July 2006 and January 2009. Data collected included age, gender, comorbidities, medications, wound type or location, wound size, the number of applications of ART, the amount of ADR applied (in square centimeters), the amount of time between Integra placement and grafting, complications, need for reoperation, and percentage of graft taken after five and 14 days. The mean percentage of graft taken at day 5 was 89.55%, 90% at day 14, and 87.3% at day 21. In light of such results, the author confirmed ADR can be used successfully in the treatment of chronic wounds. ADR provides direct wound coverage and can be applied to a variety of anatomical sites. The authors demonstrate that the use of ADR in treating chronic wounds results in high rates of skin graft taking.

Recently, peptides have been recognized as a valuable scientific tool in the field of tissue engineering because of their ability to improve the functional activity of the scaffold and to bypass or minimize immune rejection. Many functional domains in ECM proteins have been localized using protease-digested fragments or synthetic peptides. The study of Min, *et al.* [17] raised the hypothesis

that a laminin-5-derived peptide can promote wound healing by accelerating re-epithelialization *in vivo*. Laminin is an ECM glycoprotein that is generally present in the basement membrane. It promotes adhesion, migration, growth, and differentiation of a variety of cells [18]. To examine this hypothesis, chitin microfibrous matrices (Beschitin W®, Unitika Co., Japan) coated with a laminin-5-derived motif (PPFLMLLKGSTR) were applied in both rat and rabbit full-thickness cutaneous wound models. The application of this synthetic peptide onto chitin matrices significantly promoted early-stage wound healing by accelerating re-epithelialization, notably reduced inflammatory cell infiltration, and prominently enhanced fibroblast proliferation. In a previous work [19], the use of a hybrid peptide (SIRVXVXPG, where X: A or G), derived from laminin (SIKVAV) and elastin (VGVAPG,) has been reported. Elastin is another abundant ECM protein present in elastic fibers known to promote cell adhesion. The hybrid peptide was linked to a wound dressing made up of alginate which is a polysaccharide isolated from brown algae. One circular skin defect 10 mm in diameter was made in each ear of 66 young-adult male Japanese white rabbits. Nine days after operation, the *in vivo* effectiveness of the dressing in accelerating wound repair was evaluated. Ears with the alginate dressings linked with the hybrid peptide showed significantly greater epithelialization and a larger volume of regenerated tissue compared to those treated with unlinked alginate dressings. These results suggest that alginate dressings linked with the novel hybrid peptide could be promising materials for the treatment of healing-impaired wounds.

Along this line, Kim *et al.* [19] addressed their studies on RGD-*g*-PLLA biosynthetic scaffold for targeted EPC delivery and results successfully support the *in vitro* growth and endothelial functions of EPCs. This scaffold also appeared to be good for *in vivo* targeted delivery carriers of EPCs as it promoted vascular regeneration in murine dermal wound models. Furthermore, direct comparison with the intradermal EPC injection revealed that the targeted delivery of EPCs by using the RGD-*g*-PLLA scaffold was superior to their conventional local injection method in terms of the localization and survival/retention of the transplanted EPCs, and their vascular repairing potential. These results suggest that the development of an effective stem cell delivery system may help to maximize the tissue-repairing efficacy with a limited number of stem cells, thereby resolving the limited clinical success of current stem cell therapies that have utilized simple cell injections or infusions.

One of the key problems in tissue engineering is how to grow the engineered tissue and that it remains alive after implantation. Simple diffusion of oxygen and nutrition is not enough for these complex tissues, and adequate vascular systems must be built. Different studies have shown that HA has a crucial effect in angiogenesis: high-molecular weight HA in its native form inhibits angiogenesis [20], but short chain HA with 3–10 disaccharide units are proangiogenic [21]. The work of Perng and co-workers [22] compared the angiogenic properties between short chain and long chain HA when added to a porous scaffold with cross-linked type I collagen. The collagen-HA scaffold was implanted under the inferior epigastric flap in mice. Angiogenesis in scaffolds with short-chain HA was observed 14 days after implantation, and increased significantly at day 21 and 28. With long-chain HA, angiogenesis was not observed until day 28. The angiogenic effect of short-chain HA with 3–10 disaccharide (o-HA) units is mediated by the receptor for HA-mediated motility (RHAMM) and CD44 [23]. Both receptors are related to endothelial cell proliferation and migration. With degradation of short-chain HA cross-linked in the porous collagen scaffold, o-HA may be gradually released from the scaffold, causing endothelial cells around the scaffold to proliferate and migrate into the scaffold. This may be

the reason that scaffolds with short-chain HA revascularized faster than scaffolds with long-chain HA and collagen alone.

Another important component of extracellular matrix with widespread application on tissue engineered soft tissue, is lamin. Min *et al.* [24] addressed its studies on the PPFLMLLKGSTR motif of the human laminin-5 alpha3 chain that has been previously reported to promote keratinocyte survival; however, the *in vivo* effects of the PPFLMLLKGSTR motif have not yet been studied. These studies raised the hypothesis that a laminin-5-derived peptide can promote wound healing by accelerating re-epithelialization *in vivo*. To examine this hypothesis, they applied chitin microfibrous matrices coated with the PPFLMLLKGSTR motif in both rat and rabbit full-thickness cutaneous wound models. Compared with vehicle-treated and peptide-treated cutaneous wounds, the application significantly promoted early-stage wound healing by accelerating re-epithelialization, notably reduced inflammatory cell infiltration, and prominently enhanced fibroblast proliferation. These findings support our hypothesis that the PPFLMLLKGSTR motif acts as a very effective wound healing accelerator by enhancing re-epithelialization.

3. Hard Tissue Engineering

Bone deficiency in oral surgery and periodontology is one the most common challenges that the clinician has to face and overcome in order to succeed in a coherent treatment plan both from the functional and the aesthetical point of view; thus, bone grafting procedures have become increasingly important as valuable methods to make up for the deficiency. Grafts may derive from the patient's own body (autograft), from human donors (allograft), from animals (xenograft) or from engineered materials. The aim of bone tissue engineering is to optimize the resources offered by materials engineering and biological sciences in order to enhance the regeneration of new bone. Engineered materials include bioceramics, biopolymers, metals and composites. Each material has peculiar properties of resorption, reactivity and biocompatibility [25]. The versatility of biopolymers in oral surgery and periodontology has already given proof of itself in a wide range of clinical situations of bone deficiency, regardless of the etiology. Anorganic bovine bone (ABB) is widely used for implanto-prosthetic rehabilitation both in the maxilla and in the mandible [26-28]. Many authors report long-term stability for implants positioned after maxillary sinus lift or alveolar ridge augmentation with ABB in association with other engineered materials, such as scaffolds or membranes or with autologous or homologous grafts [29,30]. Bioceramics also provide similar results, given a correct therapeutical indication and clinical application [31-33]. Some trials and reports also support the use of biomaterials for the treatment of infrabony defects in periodontitis, describing, for Guided Tissue Regeneration (GTR) in combination with biomaterials, greater clinical attachment level gain and probing depth decrease than GTR alone [34]. Moreover, some authors also reported a successful use of ABB in peri-implantitis [35]. As far as histology and the formation of new bone are concerned, most studies describe a consistent tissutal regeneration both for ABB and bioceramics, although autologous grafts seemed to be more osteoconductive [36,37]. Anyway, the achievement of adequate osteoinductivity and osteoconductivity is strictly dependant on correct indications and the definition of a coherent treatment plan with regards to the expectations and needs of the patient.

3.1. Single-Phase Scaffolds

Having only one constituent, single-phase materials do not display properties to allow use in all implants.

Metallic alloys containing Cobalt (Co), chromium (Cr) or molybdenum (Mo) are in the majority of implants currently utilized in bone surgery. These alloys are used as artificial hip and knee joints. The study by Lewis, *et al.* [38], demonstrated a 98% survival rate with a minimum follow-up of two years for 50 patients receiving Cobalt-Chrome femoral heads in primary total hip arthroplasty. Co-Cr-Mo alloy or ultrahigh molecular weight polyethylene is used for the sockets. Co-Cr-Mo alloys are all nickel-free. Metals were chosen as suitable material thanks to their good mechanical properties, for example, stiffness, which makes them especially suitable for load-bearing implants. However, they do not match the mechanical properties of natural bone, as they are more rigid and weighty [39]. Another possible consequence is the corrosion that metals could undergo in the environment of body fluids, releasing cytotoxic or immunogenic metallic ions. To prevent this event, peptide amphiphiles nanofibers (PA) were explored to enhance bioactivity of these metal implants. A nickel-titanium alloy, that is frequently used for bone plates, was modified through covalent attachment of PA nanofibers using standard silanization and cross-linking chemistry [40,41] and in *in vivo* models did not show any adverse events.

Special attention must be put on alloys such as titanium. Chen, *et al.* [42] used titanium mesh coral composite scaffolds for jaw reconstruction. In this experiment, segmental bone grafts were engineered in a predetermined shape via seeding osteoblast precursor cells into titanium mesh-coral composite scaffolds. Then, the composites were implanted subcutaneously into the backs of nude mice and incubated *in vivo*. Two months after implantation, the animals were killed and new bone formed in the scaffolds was investigated by gross inspection, X-ray examination, histological observation and mechanical testing. The results showed that newly formed tissue was red and presented the gross appearance of bone, and kept the original shape of the column. Titanium mesh was situated on the surface of the bone graft. Histological observation demonstrated a large amount of new bone formed and integrated well with titanium mesh. Mechanical testing showed that new bone improved the mechanical property of the graft significantly. In conclusion, a titanium mesh-coral composite scaffold with osteoblast precursor cells is an efficient means to engineer segmental bone, possessing the desired shape and mechanical strength.

With the same alloy, Ryan, *et al.* [43] reports a multi-stage rapid prototyping technique that was successfully developed to produce porous titanium scaffolds with fully interconnected pore networks and reproducible porosity and pore size. The scaffolds porous characteristics were governed by a sacrificial wax template, fabricated using a commercial 3D-printer. Powder metallurgy processes were employed to generate the titanium scaffolds by filling around the wax template with titanium slurry. Three-dimensional reconstruction enabled the main architectural parameters such as pore size, interconnecting porosity, level of anisotropy and level of structural disorder to be determined. The titanium scaffolds were compared to their intended designs, as governed by their sacrificial wax templates. Although discrepancies in architectural parameters existed between the intended and the

actual scaffolds, overall the results indicate that the porous titanium scaffolds have the properties to be potentially employed in orthopaedic applications.

Bioceramics are highly biocompatible. They are prepared from calcium phosphates or sulfates. Due to the apatitic structure of natural calcified tissues, apatites appear to be the most comprehensively investigated of the available calcium orthophosphates [44]. Calcium orthophosphates are interesting hard tissue engineering biomaterials because of their similarity to the mineral component of mammalian bones and teeth. They are non-toxic, biocompatible, and, most importantly, integrate into living tissue using the same processes as healthy bone. Moreover, calcium orthophosphates support osteoblast adhesion and proliferation. Even so, major limitations to the use of calcium orthophosphates are their brittleness and poor fatigue resistance. As they cannot be used as biomedical applications in repairing large osseous defects, calcium orthophosphates are used primarily as fillers and coatings. Recently, nanosized crystal and particles have demonstrated their relevance in the formation of hard tissues in animals. Nanosized and nanocrystalline calcium orthophosphates can mimic the dimensions of constituent components of calcified tissues. Thus, they can be utilized in biomineralization and as biomaterials due to their improved biocompatibility [45]. Nanohydroxyapatite based products now commercially available for bone filling, are NanOss, Ostim and Vitoss. NanOss is a bone filler from Angstrom Medica considered to be the first nanotechnological medical device. It is mechanically strong and osteoconductive. Ostim is a ready-to-use injectable bone matrix in paste form. Vitoss, a beta-tricalcium phosphate bone, may find clinical application as a filler [46].

Calcium sulfate is an interesting salt obtained from inexpensive and abundant raw materials. It has a longer history of clinical use than most currently available biomaterials and is widely recognized as a well-tolerated material with applications in bone regeneration [47]. *In vivo* there is not a significant inflammatory response. Commercially available products are pellets for filling bone cavities in orthopedic applications. Examples of these products include PerOssal and calcium sulfate paste. PerOssal calcium sulfate pellets are mixed with nanocrystalline hydroxyapatite and a calcium sulfate paste and are available for oral use [48].

Polymers present an enormous variability in their properties. Their common feature is the compatibility with human body. Disadvantages are excessive softness and elasticity. They are also not able to carry the weight load on their own. The ideal polymer for an orthopedic application would have the following properties: (1) does not evoke an inflammatory/toxic response, disproportionate to its beneficial effect; (2) is metabolized in the body after fulfilling its purpose leaving no trace components; (3) is easily processed into the final product form; (4) has acceptable shelf life; and (5) is easily sterilized [49].

Some polymers have become interesting carriers for the delivery of bone morphogenetic proteins (BMPs). These cytokines have promising potential for clinical bone repair because of their strong effect on it. Indeed they are probably the most important growth factors in bone formation and healing. Having powerful osteoinductive properties, BMPs have become particularly interesting in orthopaedic and dentistry surgery. Current applications include recombinant human BMPs (rhBMPs) loaded in delivery systems made of synthetic or natural polymers and the differentiation of transplanted stem cells from the patient with rhBMPs for later body implantation [50]. The delivery system has the main

role of retaining these growth factors at the site of injury for a prolonged time. Cells attach to this initial support and form regenerative tissue.

There are several natural polymers that may be used as carriers for BMP delivery. These include collagen, starch-based polymers, chitosan, hyaluronans, and poly(hydroxyalkanoates). These polymers have received considerable attention to be used in tissue engineering thanks to their biocompatibility, biodegradability and hydrophilicity. Natural polymers may present risks of immunogenic reactions and disease transmission. A clinical trial by Jung *et al.* [29] reported the effectiveness of ABB in maxillary sinus lift with the association of rhBMP-2, resulting in a marginal difference of one level from baseline with a five-year follow up in 11 patients.

Collagen is a key protein for the maintenance of biologic and structural integrity of ECM. It is made up of three polypeptide strands (alpha chains) each possessing the conformation of a left-handed helix. The fibrillar type I collagen is responsible of tensile strength of the bone. Collagen's good properties are: excellent biocompatibility, easy degradation into physiological end-products, favorable influence on cellular infiltration, and suitable interaction with cells and other macromolecules. Collagen carriers have historically been and remain the primary delivery system for BMPs to clinical defects [51].

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine. Chitosan and its derivatives are very attractive candidates as scaffold composites because they apparently degrade as new tissues are formed, without inflammatory reactions or toxic degradation [52]. Kim *et al.* [5] noted that chitosan has been extensively used in bone tissue engineering because of its capacity to promote growth and mineral rich matrix deposition by osteoblasts in culture. Also, chitosan is biocompatible and biodegradable.

Alternative natural polymers potentially interesting for bone regeneration may be starch-based polymershyaluronan, hyaluronan, and poly(hydroxyalkanoates).

Starch is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. Starch-based polymers have been demonstrated to be potentially useful for tissue engineering of bone because of their interesting mechanical properties.

Hyaluronan (or hyaluronic acid) is an important extracellular matrix glicosamminoglican (GAG) composed of a linear sequence of D-glucuronic acid and N-acetyl-glucosamine. It has been used in a variety of trials as a delivery vehicle for recombinant human BMPs.

Poly(a-hydroxy acid) polymers represent a versatile class of biodegradable materials suitable for use in bone tissue engineering, particularly in combination with BMPs [51].

Bone morphogenetic protein (BMP) is known to require a suitable carrier to induce ectopic bone formation *in vivo*. To evaluate the suitability of DegraPol-foam, a degradable, elastic, and highly porous polyesterurethane foam, as a carrier for BMP-induced bone formation, a fraction containing all the active BMPs (BMP cocktail) was combined with DegraPol-foam and implanted subcutaneously into rats by Saad *et al.* [53]. DegraPol-BMP scaffolds were found to induce osteogenesis two weeks after implantation as evidenced by morphological and biochemical observations. In addition, the osteoblast-compatibility of DegraPol-foam was examined. *In vitro*, primary rat osteoblasts and osteoblasts from the human cell line (HFO1) attached and proliferated preferentially on the surface of the DegraPol-foam. Both cell types exhibited relatively high attachment and low doubling time that

resulted in a confluent cell multilayer with spindle-shaped morphology on the surface of the foam. Osteoblasts produced high concentrations of collagen type I and osteocalcin, and expressed increasing levels of alkaline phosphatase (ALP) activity. Taken collectively, both osteoblasts from rat tibia and from the human cell line HFO1 showed high cell attachment and growth, and preserved their phenotype. The geometrical structure of DegraPol is a suitable carrier for BMP for the induction of bone formation.

Use of BMP-2 for bone tissue engineering is reported by Kang *et al.* [54] who developed a multi-head deposition system (MHDS), a form of solid freeform fabrication, as a method for fabricating scaffolds. In this study, the surface of a scaffold fabricated using MHDS was coated with a mixture of fibrin and hyaluronic acid (HA) and used as a vehicle for delivery of both bone morphogenetic protein-2 (BMP-2) and adipose-derived stromal cells (ASCs). Fibrin/HA coating of the scaffold significantly enhanced initial cell attachment. Importantly, the transplantation of undifferentiated ASCs inoculated on BMP-2-loaded, fibrin/HA-coated, scaffolds resulted in improved bone formation and mineralization than did the transplantation of undifferentiated ASCs seeded on uncoated scaffolds or on fibrin/HA-coated scaffolds without BMP-2, but containing BMP-2 in the cell suspension medium. These results show that BMP-2-loaded, fibrin/HA-coated, scaffolds fabricated using MHDS may be useful in stimulating bone regeneration from undifferentiated ASCs *in vivo*.

Another growth factor important for bone reconstruction is PDGF. In this context, Hee Soon *et al.* [55] performed a study conducted to determine the effect of different kinds of bone substitutes and collagen on the concentration of platelet-derived growth factor (PDGF) and transforming growth factor beta-1 (TGF beta-1) in platelet-rich plasma (PRP). PRP was treated with thrombin, hydroxyapatite (HA), and thrombin, HA alone, collagen-grafted HA, calcium metaphosphate (CMP), and collagen-grafted CMP. The concentrations of PDGF-AB and TGF beta-1 were measured. After PRP treated with HA and CMP, the concentrations of PDGF and TGF beta-1 were not significantly different from the concentrations of them in PRP alone. The concentration of PDGF in PRP with collagen-grafted HA and collagen-grafted CMP was significantly higher than that of PRP with HA and CMP. The concentration of PDGF and TGF beta-1 in PRP with collagen-grafted CMP was higher than with collagen-grafted HA. The results of multiple regression analysis showed that PDGF increased with the use of collagen and thrombin, and was higher in native whole blood with higher platelet counts. However, PDGF decreased with the use of HA.

Having a high mechanical stability, polymers of acrylate are widely used for fixation of total joint prothesis, vertebroplasty and for craniofacial bone defects.

Other popular biodegradable synthetic polymers include poly(α-hydroxy acids), especially poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their co-polymers, poly(ε-caprolactone) (PLGA), poly(propylene fumarate), poly(dioxanone), polyorthoesters, polycarbonates, polyanhydrides and polyphosphazenes. Particularly interesting are PLA e PGA polyesters which are extensively used in biodegradable implants, tissue engineering, and drug delivery.

3.2. Composite Scaffolds

Multi-phase materials (composites) have two or more different constituents. The major advantage of composites utilization is the creation of new constructs having desirable properties distinct from original single materials. The mechanical properties of the composite material depend not only on the

type of combined materials, but also on the volume fraction and shape of the heterogeneities (particles, fibers, whiskers, platelets, *etc.*), according to which they are classified into certain groups [39].

Polymers/bioceramics are another type of composite. Beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) of chitosan-calcium phosphates (CP) bioceramics are excellent candidates for bone repair and regeneration because of their similarity in chemical composition with inorganic components of bone [5]. Nanosized hydroxyapatite (HA) crystals can anchor type I collagen framework in collagen/HA composites. The addition of nano-crystalline HA to natural polymer scaffolds has been shown to improve mechanical properties compared to polymer control scaffolds [56] and the presence of HA may potentially reduce adverse effects associated with the degradation of some synthetic polymers [57].

Several clinical trials reported the efficiency of these materials in humans. Jung *et al.* [29] reported that bovine-derived HA is an effective graft material for bone defects in the posterior mazilla and mandible both when used in association with PEG hydrogel membrane and standard collagen membrane, resulting from a randomized clinical trial of 37 patients with a six-month follow-up. De Vicente *et al.* [26] described similar results from 34 patients receiving 90 implants in rehabilitated maxillas, and evaluating the survival rate of the implants and the histologic features of the tissue found in the sinuses nine months after surgery. A prospective study with a larger number of patients was carried out by Ferreira *et al.* [27] observing implant survival rate and histologic features of new bone in 314 patients undergoing 406 maxillary sinus lifts and 1,025 implant placements. HA combined with collagen membrane led to an excelent implant survival rate, together with significant bone formation histologic signs after a three-year follow-up. In the treatment of infrabony defects in periodontal disease, Stavropulous *et al.* [34] published the results of a randomized clinical trial evaluating GTR with or without bovine derived HA with a six-year follow-up. 36 patients were available for the whole control, demonstrating that GTR with HA provided a greater clinical attachment level than GTR alone. Even though there are no published clinical trials at the moment, HA with collagen membrane has been reported to be effective also in the treatment of 20 patients affected by peri-implantitis [35] over a four-year follow-up, demonstrating a consistent probing depth reduction.

Fibers embedded in a matrix made of other proteins or proteoglycans attempt to recreate the organization of natural bone, which is basically a collagen-hydroxyapatite composite. Both scaffold components are closely associated with the inorganic component of the bone. Fibers are natural (e.g., collagen, chitosan) or synthetic (polylactide, polyglycolide and their copolymers) polymers. Promising and interesting matrices are chitosan, hyaluronic acid, polylactides and their copolymers with glycolides.

Recent important findings were obtained with nanostructured scaffolds. Gomoll *et al.* [58], for example, used a polymethylmethacrylate (PMMA-) based bone cement containing micrometer-size barium sulfate or zirconium oxide particles to radiopacify the cement for radiographic monitoring during follow-up. Considerable effort has been expended to improve the mechanical qualities of cements, largely through substitution of PMMA with new chemical structures. The introduction of these materials into clinical practice has been complicated by concerns over the unknown long-term risk profile of these new structures *in vivo*. The authors investigated a new composite with the well characterized chemical composition of current cements, but with nanoparticles instead of the

conventional, micrometer-size barium sulfate radiopacifier. The nanocomposite cement showed a two-fold increase in fatigue life compared to the conventional, microcomposite cement. In summary, nanoparticulate substitution of radiopacifiers substantially improved the *in vitro* mechanical properties of PMMA bone cement without changing the known chemical composition.

Balasundaram G. *et al.* [59] created nanometer crystalline hydroxyapatite (HA) and amorphous calcium phosphate compacts functionalized with the arginine-glycine-aspartic acid (RGD) peptide sequence. Crystalline HA and amorphous calcium phosphate nanoparticles were synthesized by a wet chemical process followed by a hydrothermal treatment for 2 h at 200 °C and 70 °C, respectively. Resulting particles were then pressed into compacts. For all materials, results showed that the immobilization of the cell adhesive RGD sequence increased osteoblast (bone-forming cell) adhesion compared to those non-functionalized and those functionalized with the noncell adhesive control peptide (RGE) after 4 h. However, surprisingly, results also showed that the adhesion of osteoblasts on non-functionalized amorphous nanoparticulate calcium phosphate was similar to conventional HA functionalized with RGD. Osteoblast adhesion on nanocrystalline HA (unfunctionalized and functionalized with RGD) was below that of the respective functionalized amorphous calcium phosphate but above that of the respective functionalized conventional HA.Results of this study suggest that decreasing the particulate size into the nanometer regime and reducing crystallinity of calcium phosphate based materials may promote osteoblast adhesion to the same degree as the well-established techniques of functionalizing conventional HA with RGD.

Ergun, *et al.* [60] focussed their attention on the biological properties of calcium phosphate-derived materials that are strongly influenced by changes in Ca/P stoichiometry and grain size, which have not yet been fully elucidated. For this reason, the objective of their *in vitro* study was to understand osteoblast (bone forming cells) adhesion on nanoparticulate calcium phosphates of various Ca/P ratios. Most importantly, results demonstrated increased osteoblast adhesion on calcium phosphates with higher Ca/P ratios (up to 2.5). In this regard, this study provided evidence that Ca/P ratios should be maximized (up to 2.5) in nanoparticulate calcium phosphate formulations to increase osteoblast adhesion; a necessary step for subsequent osteoblast functions such as new bone deposition.

4. Conclusions

Remarkable progress has been made in the field of biomaterials and the combining of scaffolds with either adult cells or stem cells gave rise to the birth of tissue engineering; the most promising tools for regenerative medicine. There remain some obstacles that could impede immediate clinical translation. Researchers will indeed need to better characterize stem cell fate following *ex vivo* manipulation and subsequent implantation. These biological challenges are further burdened by constraints of increasing health care costs, regulatory restrictions, and ethical concerns. However, the engineering of tissues and organs remains an exciting and dynamic field that requires a collaborative multidisciplinary approach [61].

References

1. Bhatia, S.K. Tissue engineering for clinical applications. *Biotechnol. J.* **2010**, *5*, 1309-1323.

2. Ripamonti, U.; Tsiridis, E.; Ferretti, C.; Kerawala, C.J.; Mantalaris, A.; Heliotis, M. Perspectives in regenerative medicine and tissue engineering of bone. *Br. J. Oral Maxillofac. Surg.* **2010**, doi: 10.1016/j.bjoms.2010.07.020.
3. Arvidson, K.; Abdallah, B.M.; Applegate, L.A.; Baldini, N.; Cenni, E.; Gomez-Barrena, E.; Granchi, D.; Kassem, M.; Konttinen, Y.T.; Mustafa, K.; Pioletti, D.P.; Sillat, T.; Finne-Wistrand, A. Bone regeneration and stem cells. *J. Cell Mol. Med.* **2010**, doi: 10.1111/j.1582-4934.2010.01224.x.
4. Wong, V.W.; Rustad, K.C.; Longaker, M.T.; Gurtner, G.C. Tissue engineering in plastic surgery: A review. *Plast. Reconstr. Surg.* **2010**, *126*, 858-868.
5. Kim, I.Y.; Seo, S.J.; Moon, H.S.; Yoo, M.K.; Park, I.Y.; Kim, B.C.; Cho, C.S. Chitosan and its derivatives for tissue engineering applications. *Biotechnol. Adv.* **2008**, *26*, 1-21.
6. Helary, C.; Abed, A.; Mosser, G.; Louedec, L.; Meddahi-Pellé, A.; Giraud-Guille, M.M. Synthesis and *in vivo* integration of improved concentrated collagen hydrogels. *J. Tissue Eng. Regen. Med.* **2010**, doi: 10.1002/term.326.
7. Thompson, D.F.; Letassy, N.A.; Thompson, G.D. Fibrin glue: A review of its preparation, efficacy, and adverse effects as a topical hemostat. *Drug Intell. Clin. Pharm.* **1988**, *22*, 946-952.
8. Foster, K.; Greenhalgh, D.; Gamelli, R.L.; Mozingo, D.; Gibran, N.; Neumeister, M.; Abrams, S.Z.; Hantak, E.; Grubbs, L.; Ploder, B.; Schofield, N.; Riina, L.H. Efficacy and safety of a fibrin sealant for adherence of autologous skin grafts to burn wounds: Results of a phase 3 clinical study. *J. Burn Care Res.* **2008**, *29*, 293-303.
9. Dhandayuthapani, B.; Krishnan, U.M.; Sethuraman, S. Fabrication and characterization of chitosan-gelatin blend nanofibers for skin tissue engineering. *J. Biomed. Mater. Res. B Appl. Biomater.* **2010**, *94*, 264-272.
10. Liu, Y.; Kopelman, D.; Wu, L.Q.; Hijji, K.; Attar, I.; Preiss-Bloom, O.; Payne, G.F. Biomimetic sealant based on gelatin and microbial transglutaminase: An initial *in vivo* investigation. *J. Biomed. Mater. Res. B Appl. Biomater.* **2009**, *91*, 5-16.
11. Solchaga, L.A.; Dennis, J.E.; Goldberg, V.M.; Caplan, A.I. Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage. *J. Orthop. Res.* **1999**, *17*, 205-213.
12. Stillaert, F.B.; Di Bartolo, C.; Hunt, J.A.; Rhodes, N.P.; Tognana, E.; Monstrey, S.; Blondeel, P.N. Human clinical experience with adipose precursor cells seeded on hyaluronic acid-based spongy scaffolds. *Biomaterials* **2008**, *29*, 3953-3959.
13. Liu, X.; Ma, P.X. Polymeric scaffolds for bone tissue engineering. *Ann. Biomed. Eng.* **2004**, *32*, 477-486.
14. Judith, R.; Nithya, M.; Rose, C.; Mandal, A.B. Application of a PDGF-containing novel gel for cutaneous wound healing. *Life Sci.* **2010**, *87*, 1-8.
15. Weinstein-Oppenheimer, C.R.; Aceituno, A.R.; Brown, D.I.; Acevedo, C.; Ceriani, R.; Fuentes, M.A.; Albornoz, F.; Henríquez-Roldán, C.F.; Morales, P.; Maclean, C.; Tapia, S.M.; Young, M.E. The effect of an autologous cellular gel-matrix integrated implant system on wound healing. *J. Transl. Med.* **2010**, *8*, 59-69.

16. Kahn, S.A.; Beers, R.J.; Lentz, C.W. Use of acellular dermal replacement in reconstruction of nonhealing lower extremity wounds. *J. Burn Care Res.* **2010**, *2*, 124-128.
17. Min, S.K.; Lee, S.C.; Hong, S.D.; Chung, C.P.; Park, W.H.; Min, B.M. The effect of a laminin-5-derived peptide coated onto chitin microfibers on re-epithelialization in early-stage wound healing. *Biomaterials* **2010**, *31*, 4725-4730.
18. Hashimoto, T.; Suzuki, Y.; Tanihara, M.; Kakimaru, Y.; Suzuki, K. Development of alginate wound dressings linked with hybrid peptides derived from laminin and elastin. *Biomaterials* **2004**, *25*, 1407-1414.
19. Kim, K.L.; Han, D.K.; Park, K.; Song, S.H.; Kim, J.Y.; Kim, J.M.; Ki, H.Y.; Yie, S.W.; Roh, C.R.; Jeon, E.S.; Kim, D.K.; Suh, W. Enhanced dermal wound neovascularization by targeted delivery of endothelial progenitor cells using an RGD-g-PLLA scaffold. *Biomaterials* **2009**, *30*, 3742-3748.
20. West, D.C.; Kumar, S. Endothelial cell proliferation and diabetic retinopathy. *Lancet* **1988**, *1*, 715-716.
21. West, D.C.; Hampson, I.N.; Arnold, F.; Kumar, S. Angiogenesis induced by degradation products of hyaluronic acid. *Science* **1985**, *228*, 1324-1326.
22. Perng, C.K.; Wang, Y.J.; Tsi, C.H.; Ma, H. *In vivo* angiogenesis effect of porous collagen scaffold with hyaluronic acid oligosaccharides. *J. Surg. Res.* **2010**, doi: 10.1016/j.jss.2009.09.052.
23. Slevin, M.; Krupinski, J.; Gaffney, J.; Matou, S.; West, D.; Delisser, H.; Savani, R.C.; Kumar, S. Hyaluronan-mediated angiogenesis in vascular disease: Uncovering RHAMM and CD44 receptor signaling pathways. *Matrix. Biol.* **2007**, *26*, 58-68.
24. Min, S.K.; Lee, S.C.; Hong, S.D.; Chung, C.P.; Park, W.H.; Min, B.M. The effect of a laminin-5-derived peptide coated onto chitin microfibers on re-epithelialization in early-stage wound healing. *Biomaterials* **2010**, *31*, 4725-4730.
25. Chen, F.M.; Zhang, J.; Zhang, M.; An, Y.; Chen, F.; Wu, Z.F. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* **2010**, *31*, 7892-7927.
26. De Vicente, J.C.; Hernández-Vallejo, G.; Braña-Abascal, P.; Peña, I. Maxillary sinus augmentation with autologous bone harvested from the lateral maxillary wall combined with bovine-derived hydroxyapatite: Clinical and histologic observations. *Clin. Oral. Implants Res.* **2010**, *21*, 430-438.
27. Ferreira, C.E.; Novaes, A.B.; Haraszthy, V.I.; Bittencourt, M.; Martinelli, C.B.; Luczyszyn, S.M. A clinical study of 406 sinus augmentations with 100% anorganic bovine bone. *J. Periodontol.* **2009**, *80*, 1920-1927.
28. Todisco, M. Early loading of implants in vertically augmented bone with non-resorbable membranes and deproteinised anorganic bovine bone. An uncontrolled prospective cohort study. *Eur. J. Oral Implantol.* **2010**, *3*, 47-58.
29. Jung, R.E.; Windisch, S.I.; Eggenschwiler, A.M.; Thoma, D.S.; Weber, F.E.; Hämmerle, C.H. A randomized-controlled clinical trial evaluating clinical and radiological outcomes after 3 and 5 years of dental implants placed in bone regenerated by means of GBR techniques with or without the addition of BMP-2. *Clin. Oral Implants Res.* **2009**, *20*, 660-666.

30. Jung, R.E.; Hälg, G.A.; Thoma, D.S.; Hämmerle, C.H. A randomized, controlled clinical trial to evaluate a new membrane for guided bone regeneration around dental implants. *Clin. Oral Implants Res.* **2009**, *20*, 162-168.
31. Froum, S.J.; Wallace, S.S.; Cho, S.C.; Elian, N.; Tarnow, D.P. Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-Month postsurgical assessment of vital bone formation. A pilot study. *Int. J. Periodontics Restorative Dent.* **2008**, *28*, 273-281.
32. Mardas, N.; Chadha, V.; Donos, N. Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: a randomized, controlled clinical trial. *Clin. Oral Implants Res.* **2010**, *21*, 688-698.
33. Cordaro, L.; Bosshardt, D.D.; Palattella, P.; Rao, W.; Serino, G.; Chiapasco, M. Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: Histomorphometric results from a randomized controlled multicenter clinical trial. *Clin. Oral Implants Res.* **2008**, *19*, 796-803.
34. Stavropoulos, A.; Karring, T. Guided tissue regeneration combined with a deproteinized bovine bone mineral (Bio-Oss) in the treatment of intrabony periodontal defects: 6-Year results from a randomized-controlled clinical trial. *J. Clin. Periodontol.* **2010**, *37*, 200-210.
35. Schwarz, F.; Sahm, N.; Bieling, K.; Becker, J. Surgical regenerative treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane: A four-year clinical follow-up report. *J. Clin. Periodontol.* **2009**, *36*, 807-814.
36. Simunek, A.; Kopecka, D.; Somanathan, R.V.; Pilathadka, S.; Brazda, T. Deproteinized bovine bone *versus* beta-tricalcium phosphate in sinus augmentation surgery: A comparative histologic and histomorphometric study. *Int. J. Oral Maxillofac. Implants* **2008**, *23*, 935-942.
37. Traini, T.; Degidi, M.; Sammons, R.; Stanley, P.; Piattelli, A. Histologic and elemental microanalytical study of anorganic bovine bone substitution following sinus floor augmentation in humans. *J. Periodontol.* **2008**, *79*, 1232-1240.
38. Lewis, P.M.; Moore, C.A.; Olsen, M.; Schemitsch, E.H.; Waddel, J.P. Comparison of mid-term clinical outcomes after primary total hip arthroplasty with Oxinium *vs.* cobalt chrome femoral heads. ORTHOSuperSite. 1 December 2008.
39. Vagaská, B.; Bacáková, L.; Filová, E.; Balík, K. Osteogenic cells on bio-inspired materials for bone tissue engineering. *Physiol. Res.* **2010**, *59*, 309-322.
40. Sargeant, T.D.; Rao, M.S.; Koh, C.Y.; Stupp, S.I. Covalent functionalization of NiTi surfaces with bioactive peptide amphiphile nanofibers. *Biomaterials* **2008**, *29*, 1085-1098.
41. Bansiddhi, A.; Sargeant, T.D.; Stupp, S.I.; Dunand, D.C. Porous NiTi for bone implants: A review. *Acta Biomater.* **2008**, *4*, 773-782.
42. Chen, F.; Feng, X.; Wu, W.; Ouyang, H.; Gao, Z.; Cheng, X.; Hou, R.; Mao, T. Segmental bone tissue engineering by seeding osteoblast precursor cells into titanium mesh-coral composite scaffolds. *Int. J. Oral Maxillofac. Surg.* **2007**, *36*, 822-827.
43. Ryan, G.E.; Pandit, A.S.; Apatsidis, D.P. Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. *Biomaterials* **2008**, *29*, 3625-3635.
44. Dorozhkin, S.V. Bioceramics of calcium orthophosphates. *Biomaterials* **2010**, *31*, 1465-1485.

45. Dorozhkin, S.V. Nanosized and nanocrystalline calcium orthophosphates. *Acta Biomater.* **2010**, *6*, 715-734.
46. Damron, T.A. Use of 3D beta-tricalcium phosphate (Vitoss) scaffolds in repairing bone defects. *Nanomedicine (Lond)* **2007**, *2*, 763-775.
47. Thomas, M.V.; Puleo, D.A. Calcium sulfate: Properties and clinical applications. *J. Biomed. Mater. Res. B Appl. Biomater.* **2009**, *88*, 597-610.
48. von Stechow, D.; Rauschmann, M.A. Effectiveness of combination use of antibiotic-loaded PerOssal with spinal surgery in patients with spondylodiscitis. *Eur. Surg. Res.* **2009**, *43*, 298-305.
49. Balasundaram, G.; Webster, T.J. An overview of nano-polymers for orthopedic applications. *Macromol. Biosci.* **2007**, *7*, 635-642.
50. Bessa, P.C.; Casal, M.; Reis, R.L. Bone morphogenetic proteins in tissue engineering: The road from laboratory to clinic, Part II (BMP delivery). *J. Tissue Eng. Regen. Med.* **2008**, *2*, 81-96.
51. Yu, N.Y.; Schindeler, A.; Little, D.G.; Ruys, A.J. Biodegradable poly(alpha-hydroxy acid) polymer scaffolds for bone tissue engineering. *J. Biomed. Mater. Res. B Appl. Biomater.* **2010**, *93*, 285-295.
52. Swetha, M.; Sahithi, K.; Moorthi, A.; Srinivasan, N.; Ramasamy, K.; Selvamurugan, N. Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering. *Int. J. Biol. Macromol.* **2010**, *47*, 1-4.
53. Saad, B.; Kuboki, Y.; Welti, M.; Uhlschmid, G.K.; Neuenschwander, P.; Suter, U.W. DegraPol-foam: A degradable and highly porous polyesterurethane foam as a new substrate for bone formation. *Artif. Organs.* **2000**, *24*, 939-945.
54. Kang, S.W.; Kim, J.S.; Park, K.S.; Cha, B.H.; Shim, J.H.; Kim, J.Y.; Cho, D.W.; Rhie, J.W.; Lee, S.H. Surface modification with fibrin/hyaluronic acid hydrogel on solid-free form-based scaffolds followed by BMP-2 loading to enhance bone regeneration. *Bone* **2011**, *48*, 298-306.
55. Cho, H.S.; Park, S.Y.; Kim, S.; Bae, S.K.; Shin, D.S.; Ahn, M.W. Effect of different bone substitutes on the concentration of growth factors in platelet-rich plasma. *J. Biomater. Appl.* **2008**, *22*, 545-557.
56. Wei, G.; Ma, P.X. Structure and properties of nano-hydoxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* **2004**, *25*, 4749-4657.
57. Smith, I.O.; Liu, X.H.; Smith, L.A.; Ma, P.X. Nanostructured polymer scaffolds for tissue engineering and regenerative medicine. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2009**, *1*, 226-236.
58. Gomoll, A.H.; Fitz, W.; Scott, R.D.; Thornhill, T.S.; Bellare, A. Nanoparticulate fillers improve the mechanical strength of bone cement. *J. Biomater. Appl.* **2008**, *22*, 545-557.
59. Balasundaram, G.; Sato, M.; Webster, T.J. Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD. *Biomaterials* **2006**, *27*, 2798-2805.

60. Ergun, C.; Liu, H.; Webster, T.J.; Olcay, E.; Yilmaz, S.; Sahin, F.C. Increased osteoblast adhesion on nanoparticulate calcium phosphates with higher Ca/P ratios. *J. Biomed. Mater. Res. A* **2008**, *85*, 236-241.
61. Wong, V.W.; Rustad, K.C.; Longaker, M.T.; Gurtner, G.C. Tissue engineering in plastic surgery: A review. *Plast. Reconstr. Surg.* **2010**, *126*, 858-868.