1

Bioactive Ceramics as Bone Morphogenetic Proteins Carriers

Sayed Mahmood Rabiee
Babol University of Technology
Iran

1. Introduction

Bone tissue is the component of the skeletal system that provides the supporting structure for the body. Bone has a complex morphology; it is a specialized connective tissue composed of a calcified matrix and an organic matrix. The tissue can be organized in either the dense (compact) or spongy form (cancellous), with pore sizes within the wide range of 1-100 μm (Lane et al., 1999). Although the shape of bone varies in different parts of the body, the physicochemical structure of bone for these different shapes is basically similar. The biochemical composition of bone is precisely composed of two major phases at the nanoscale level namely, organic and inorganic as a good example for a composite. These phases have multiple components which consist of, in decreasing proportions, minerals, collagen, water, non-collagenous proteins, lipids, vascular elements, and cells (Murugan & Ramakrishna, 2005). An overall composition of the bone is given in Table 1.

Inorganic Phases	Wt%	Bioorganic phases	Wt%
Calcium Phosphates (biological apatite)	~ 60	Collagen type I	~ 20
Water	~ 9	None-collagenous proteins	~ 3
Carbonates	~ 4	Primary bone cells	Balance
Citrates	~ 0.9	Other traces	Balance
Sodium	~ 0.7		
Magnesium	~ 0.5		
Other traces	Balance		

Table 1. The biochemical composition of bone (Murugan & Ramakrishna, 2005).

The mineral fraction of bone consists of significant quantities of non-crystalline calcium phosphate compounds and predominantly of a single phase that closely resembles that of crystalline hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Hench & Wilson, 1993; Dorozhkin, 2010a). Biological hydroxyapatite also contains other impurity ions as Cl, Mg, Na, K, and F and

trace elements like Sr and Zn (LeGeros, 2002). The apatite in bone mineral is composed of small platelet-like crystals of just 2 to 4 nm in thickness, 25 nm in width, and 50 nm in length (Dorozhkin & Epple, 2002). Bone mineral non-stoichiometry is primarily due to the presence of divalent ions, such as CO_3^{2-} and HPO_4^{2-}, which are substituted for the trivalent PO_4^{3-} ions. Substitutions by CO_3^{2-} and HPO_4^{2-} ions produce a change of the Ca/P ratio, resulting in Ca/P ratio which may vary between 1.50 to 1.70, depending on the age and bone site (Raynaud et al., 2002). When a loss of bony tissue occurs as a result of trauma or by the excision of diseased, healing requires the implantation of bone substitutes. There is a high clinical request for synthetic bone substitution materials, due to the drawbacks such as a prolonged operation time and donor site morbidity in about 10–30% of the cases associated with biological bone grafts (Giannoudis et al., 2005; Beaman et al., 2006; Chu et al., 2007). Biological grafts are generally associated with potential infections. In order to avoid the problems associated with biological bone grafting, there has been a continuous interest in the use of synthetic bone substitute materials. Bioactive ceramics such as calcium phosphates offer alternatives to synthetic bone substitute (Vallet-Regı & lez-Calbet, 2004; Best et al., 2008; Rabiee et al., 2008a). These biomateials with a porous structure not only possess good biocompatibility but also allow the ingrowth of tissues and penetration of biological fluids and form a chemical bond with bone (Lu & Leng, 2005; Rabiee et al. 2008b). Moreover, the Calcium phosphates are freely formed and easily fabricated to satisfy the demands for huge bone and large quantities of bone for bone substitute. For these reasons, the Calcium phosphates have been considered as useful materials for bone repair and replacement. To fabricate a bioactive ceramic bone substitute with various porous configuration, the evidence of tissues ingrowth and biological responses provide obvious advantages in tissue-implant fixation and controlled biodegradation rate for both short-term and long-term implantation purposes (Karageorgiou & Kaplan, 2005, Rabiee et al. 2008b). Many processing technologies have been employed to obtain porous calcium phosphates as bone filler (Rabiee et al., 2007; Best etal. 2008). For example, porous calcium phosphates can be obtained by merging the slurry with a polymer sponge-like mold or polymer beads before sintering. During the sintering, the polymer is completely burnt out, which results in a porous structure. The use of highly porous calcium phosphate induces bone formation inside the implant and increases degradation. Cortical bone has pores ranging from 1 to 100 μm (volumetric porosity 5 to 10%), whereas trabecular bone has pores of 200 to 400 μm (volumetric porosity 70 to 90%). Porosity in bone provides space for nutrients supply in cortical bone and marrow cavity in trabecular bone. Microporosity covers pores sizes smaller than 5 μm for penetration of fluids and Pores larger than 10 μm can be considered as macropores. Macroporous dimensions are reported to play a role in osteoinductive behavior of bone substitutes (Karageorgiou & Kaplan, 2005; Rabiee et al., 2009). Because of the influence of bioactive ceramics on cell behaviour, the bone forming cells are often introduced into these porous ceramics to speed-up tissue ingrowth. The surface of bioactive ceramics is a good substrate for seeding cells (Cao et al., 2010; Rungsiyakull et al., 2010). Bone Tissue engineering typically involves coupling osteogenic cells and/or osteoinductive growth factors with bioactive scaffolds (Buma et al., 2004; Mistry & Mikos, 2005). Some studies have investigated the bone forming capacities of growth factors loaded synthetic bone substitutes. In terms of growth factors, most research has focused on the use of the bone morphogenic proteins (BMPs) (Mont et al. 2004; Termaat et al., 2005). They are

signalling molecules which can induce de novo bone formation at orthotopic and heterotropic sites (Boix et al., 2005). Current examination of alternatives to grafting techniques suggests three possible new approaches to inducing new bone formation: implantation of certain cytokines such as BMPs in combination with appropriate delivery systems at the target site (Liu et al., 2007; Niu et al., 2009); transduction of genes encoding cytokines with osteogenic capacity into cells at repair sites; and transplantation of cultured osteogenic cells derived from host bone marrow (Chu et al., 2007). BMPs have crucial roles in growth and regeneration of skeletal tissues (Nie & Wang, 2007). One primary role of BMPs is to regulate the key elements in the bone induction cascade required for regeneration of skeletal tissues (Schneider et al., 2003). BMPs are bone matrix protein that stimulate mesenchymal cell chemotaxis and proliferation, and promotes the differentiation of these cells into chondrocytes and osteoblasts (Calori et al., 2009; Nie & Wang, 2007). This osteoinductive action of BMPs is well established to be beneficial during the repair bone defects (Termaat et al., 2005). BMPs act locally and therefore must be delivered directly to the site of regeneration via a carrier (Hartman et al., 2005; Chu et al., 2007). Bioactive ceramics can act as vehicle for factor delivery to the surrounding tissues. Future research should be investigated the potentials of these constructs to find a successful alternative for biological bone substitute.

2. Bioactive ceramics

Bioactive ceramics are used in a number of different applications in implants and in the repair and reconstruction of diseased or damaged body parts. Most medical applications of bioactive ceramics relate to the repair of the skeletal system and hard tissue. They include several major groups such as calcium phosphate ceramics, bioactive glasses and glass-ceramics.

2.1 Calcium phosphate ceramics

Calcium phosphate ceramics are very popular implants for medical applications because of their similarity to hard tissue. These bioceramics have been synthesized and used for manufacturing various forms of implants, as well as for solid or porous coatings on other implants. Calcium phosphate compounds exist in several phases. Most of these compounds are used as raw material for synthesis of bioactive ceramics. Different types of calcium phosphate are employed to fabricate implants to accommodate bone tissue regeneration. Table 2 lists the main Ca-P compounds for biomedical applications (Vallet-Reg1 & lez-Calbet, 2004). The atomic ratio of Ca/P in calcium phosphates can be varied between 2 and 1 to produce compounds ranged from calcium tetraphosphate(TTCP) $Ca_4P_2O_9$, hydroxyapatite (HA) $Ca_{10}(PO_4)_6(OH)_2$, octacalcium phosphate (OCP) $Ca_8H_2(PO_4)_6.5H_2O$, tricalcium phosphate (TCP) $Ca_3(PO_4)_2$ to dicalcum phosphate dihydrate (DCPD) $CaHPO_4.2H_2O$ or dicalcum phosphate anhydrus (DCPA) $CaHPO_4$. (Raynaud et al., 2002; Vallet-Reg1 & lez-Calbet, 2004; Dorozhkin, 2010b). Due to their high solubility, the calcium phosphates compounds with a Ca/P ratio less than 1 are not suitable for biological implantation. Hydroxyapatite with Ca/P ratio of 1.667 is much more stable than other calcium phosphates. Under physiological conditions, calcium phosphates degrade via dissolution–reprecipitation mechanisms (Raynaud et al., 2002).

When the dissolution of calcium phosphate is higher than the rate of mineral reprecipitation and tissue regeneration, it is not suitable as a good bone substitute. The dissolution process is dependent on the nature and their thermodynamic stability of calcium phosphate substrate, for example (in order of increasing solubility), HA > TCP > OCP > DCPD or DCPA (Bohner, 2000; Dorozhkin, 2010a). In an ideal situation, a biodegeradable bone substitute is slowly resorbed and replaced by natural bone. TCP with Ca/P ratio of 1.5 is a biodegradable and more resorbed than HA. The use of a mixture of HA and β-TCP, as biphasic calcium phosphate (BCP), has been attempted as bone substitute. The dissolution and resorption rate of BCP can be controlled with ratio of β-TCP/HA (Detsch et al., 2008; De Gabory et al., 2010).

Name	Ca/P	Formula	Acronym
Calcium Dihydrogen Phosphate	0.5	$Ca(H_2PO_4)_2H_2O$	MCP
Dicalcum phosphate dihydrate	1	$CaHPO_4.2H_2O$	DCPD
Dicalcum phosphate anhydrous	1	$CaHPO_4$	DCPA
Octacalcium phosphate	1.33	$Ca_8H_2(PO_4)_6.5H_2O$	OCP
Tricalcium phosphate	1.5	$Ca_3(PO_4)_2$	TCP
Hydroxyapatite	1.67	$Ca_{10}(PO_4)_6(OH)_2$	HA
Tetracalcium phosphate	2	$Ca_4O(PO_4)_2$	TTCP

Table 2. Varius calcium phosphate with their respective Ca/P atomic ratios (Vallet-Regı & lez-Calbet, 2004).

The major limitation to use calcium phosphates is their mechanical properties. Calcium phosphates are used primarily as fillers and coatings (Ooms et al., 2003) because they are brittle with poor fatigue resistance (Teoh, 2000).

2.2 Calcium phosphate cements

Calcium phosphate cements (CPCs) are of interest for bone tissue engineering purposes. Different studies with CPCs have shown that they are highly biocompatible and osteoconductive materials, which can stimulate tissue regeneration (Bohner, 2000; Carey et al., 2005; Ginebra et al., 2006). The main difference between cements when compared to other bioactive ceramics, in the form of ceramic granules or bulk materials, is the injectability and in-situ hardening. Calcium phosphate cements consist of a powder phase and an aqueous liquid, which are mixed together to form a paste that sets after being implanted within the body. Brown and Chow prepared the first CPBC in 1985 contained TTCP and DCPA or DCPD as the solid phase (Brown & Chow, 1985). After mixing with water, the cement components results precipitation of apatite (AP: $Ca_{10-x}(HPO_4)_x(PO4)_{6-x}(OH)_{2-x}$, where $0 \leq x \leq 2$) (Ginebra et al., 2006; Rabiee et al., 2010). There are a variety of different combinations of calcium compounds which are used in the formulation of these bone cements. In general there are two types of CPC: apatite cements and brushite cements. Brushite cement has a lower mechanical strength but a faster biodegradability than the apatite cement. Both types of cement can be applied for bone tissue engineering purposes. (Carey et al., 2005; Rabiee et al., 2010). CPCs as drug delivery systems, where the drugs can be incorporated throughout the whole cement volume. CPCs are suitable materials for local

delivery systems in osseous tissue since they can simultaneously promote bone regeneration and prevent infectious diseases by releasing therapeutic agents. Recent advances in CPC technology have resulted in the enhancement of the handling, application and osteoconductive properties of these cements. These improvements have permitted CPCs to be assayed as carriers for local delivery of drugs and biologically active substances (Ginebra et al., 2006). Drugs, such as antibiotics, antitumors, and growth factors, have been administered to defect regions to induce therapeutic effects (Ginebra et al., 2006; Chu et al. 2007). The success of this idea was favored by the easy incorporation of pharmaceutical and biological substances into the cement solid or liquid phases, the intimate adaptation of the cement paste to bone defects and permits the release of the entrapped substance to the local environment.

2.3 Bioactive glasses & glass-ceramics

Bioactive glasses and glass-ceramics have the ability to bind to hard tissues as was discovered by Hench in 1969 (Hench, 2006). They are used as implants to repair or replace parts of the body; long bones, vertebrae, joints, and teeth. Their clinical success is due to formation of a stable, mechanically strong interface with bone (Hench & Wilson, 1993; Cao et al., 2010). Bioactive materials are typically made of compositions from the Na_2O-CaO, MgO-P_2O_5-SiO_2 system. The composition of the first bioglass Hench made was in weight percent 25% Na_2O, 25% CaO, 5% P_2O_5 and 45% SiO_2 and noted as Bioglass 45S5. Melting and sol- gel processing are two methods for producting glasses. Sol-gel processing has been successfully used in the production of a variety of materials for both biomedical and nonbiomedical applications (Hench, 2006; Ravarian et al., 2010). Sol-gel processing, an alternative to traditional melt processing of glasses, involves the synthesis of a solution (sol), typically composed of metal-organic and metal salt precursors followed by the formation of a gel by chemical reaction or aggregation, and lastly thermal treatment for drying, organic removal, and sometimes crystallization (Saravanapavan & Hench, 2003). Sol-gel-derived bioactive glasses were used because they exhibit high specific area, high osteoconductive properties, and a significant degradability. The sol-gel approach to making bioactive glass materials has produced glasses with enhanced compositional range of bioactivity. When in contact with body fluids or tissues, bioactive glasses develop reactive layers at their surfaces resulting in a chemical bond between implant and host tissue (Hench, 2006). Hench has described a sequence of five reactions that result in the formation of a hydroxy-carbonate apatite (HCA) layer on the surface of these bioactive glasses (Hench, 2006). The dissolution of the glass network, leading to the formation of a silica-rich gel layer and subsequent deposition of an apatite-like layer on the glass surface, was found to be essential steps for bonding of glass to living tissues both through in vivo and in vitro studies (Cao et al., 2010). The use of bioactive glass for load-bearing applications is restricted because of its brittleness. One possibility to overcome this drawback is to crystallize the glass to obtain a glass-ceramic. Glass- ceramics are polycrystalline ceramics made by transformation of the glass into ceramic. The formation of glass ceramics is influenced by the nucleation and growth of small crystals. The nucleation of glass is carried out at temperatures much lower than the melting temperature. Professor Kokubo and his coworkers developed a glass-ceramic containing apatite and wollastonite in a glass matrix (Kokubo et al., 1986). Apatite-wollastonite (A-W) glass-ceramic is one of the most important glass ceramics for use as a bone substitute. The apatite crystals form sites for bone growth; the long wollastonite

crystals reinforce the glass (Liu et al. 2004). Drug and growth factor loading of bioactive glasses and glass ceramics is possible using the sol–gel method. Ziegler et al. introduced Growth factors into a bioactive glass and observed an initial burst of 10%, followed by a delayed boost between day 3 and 8, depending on the type of growth factor (Ziegler et al., 2002).

3. Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) induce new bone formation by directing mesenchymal stem cells. They are biologically active osteoinductive cytokines that with significant clinical potential. The key steps are proliferation of cells, and finally differentiation into cartilage and then bone. Proliferation was maximal on day 3, chondroblast differentiation was on day 5, and chondrocytes were on day 7. The cartilage hypotrophied on day 9 with vascularization and osteogenesis. On days 10 to 12 maximal alkaline phosphatase activity, a marker of bone formation was observed. Hematopoietic differentiation was observed in the ossicle on day 21. BMP were first characterized in 1965 by Urist as a biologically activator and he has led to various studies for identification of a variety of growth factors that play roles in osteogenesis. The most studied of these are the insulin-like growth factor (IGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and the transforming growth factor (TGF) group, of which, the BMPs form a subgroup. There are 15 members of BMPs family in table 3 and Among members of the BMPs, BMP2, 4, and 7 possess a strong ability to induce bone formation (Termaat et al., 2005; Nie & Wang, 2007; Calori et al., 2009).

BMP designation	**Generic Name**
BMP1	bone morphogenetic protein 1
BMP2	bone morphogenetic protein 2
BMP3	bone morphogenetic protein 3 (osteogenic)
BMP4	bone morphogenetic protein 4
BMP5	bone morphogenetic protein 5
BMP6	bone morphogenetic protein 6
BMP7	bone morphogenetic protein 7 (osteogenic protein 1)
BMP8A	bone morphogenetic protein 8a
BMP8B	bone morphogenetic protein 8b (osteogenic protein 2)
BMP9	growth differentiation factor 2 (GDF2)
BMP10	bone morphogenetic protein 10
BMP11	growth differentiation factor 11 (GDF11)
BMP12	growth differentiation factor 7 (GDF7)
BMP13	growth differentiation factor 6 (GDF6)
BMP14	growth differentiation factor 5 (GDF5)
BMP15	bone morphogenetic protein 15

Table 3. The BMPs Family. (Termaat et al., 2005).

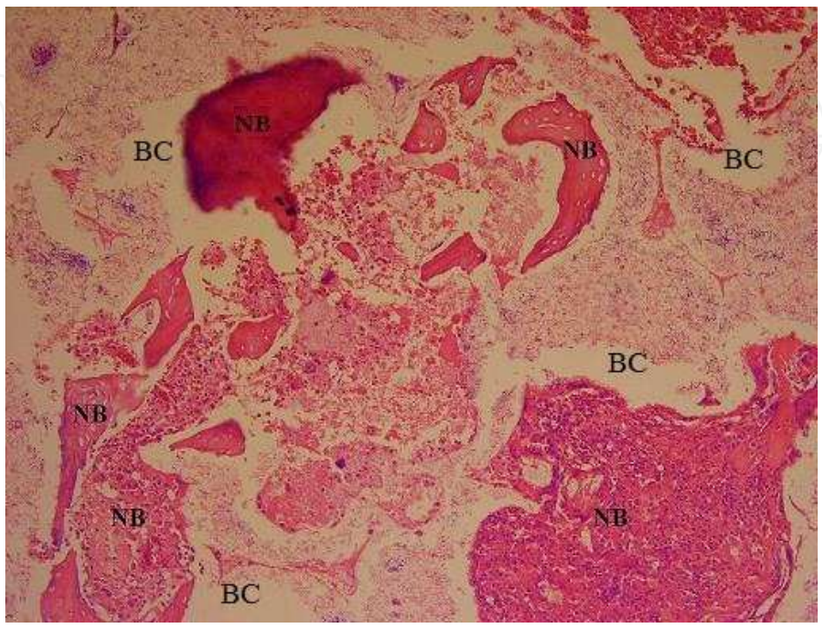
4. Bioactive ceramic as carrier for bone marrow cells: case study

This experiment focuses on a tissue engineering strategy for bone regeneration using bone marrow carried by a bioactive ceramic scaffold. To fabricate a bioactive ceramic with porous configuration, the evidence of tissues ingrowth and biological responses provide obvious advantages in tissue-implant fixation and controlled biodegradation rate for both short-term and long-term implantation purposes (Klein et al., 1984; Rabiee et al., 2008c). Many processing technologies have been employed to obtain porous bioceramics as bone substuitute. The method of casting foams has shown suitability to manufacture strong and reliable macro-porous bioceramics that have great potential to replace bone tissue (Rabiee et al., 2007, 2008c). Results obtained with bone substitutes are currently less reliable than with autologous cancellous bone grafting which remains the preferred method for healing bone defects. Bone marrow stromal cells haved proved their ability to induce bone formation (Liu et al., 2007b). So the association of autologous bone marrow and porous bioceramic might be a successful hybrid biomaterial for bone substitute (Liu et al., 2007). The porous sample was fabricated by polyurethane foam reticulate method. The macrostructure of the scaffold was controlled by the porous structure of the polymer substrate. After sintering the ceramic resembled the polymer matrix texture, giving rise to a structure characterized by several macropores, whose size (100 μm <macropores size<200 μm) can assure osteoconduction after implantation (Fig. 1). The total porosity of the porous body was evaluated from the density value calculated as weight/volume and amounted to 64±5%. Details of the preparation method can be found in Ref. (Rabiee et al., 2009).

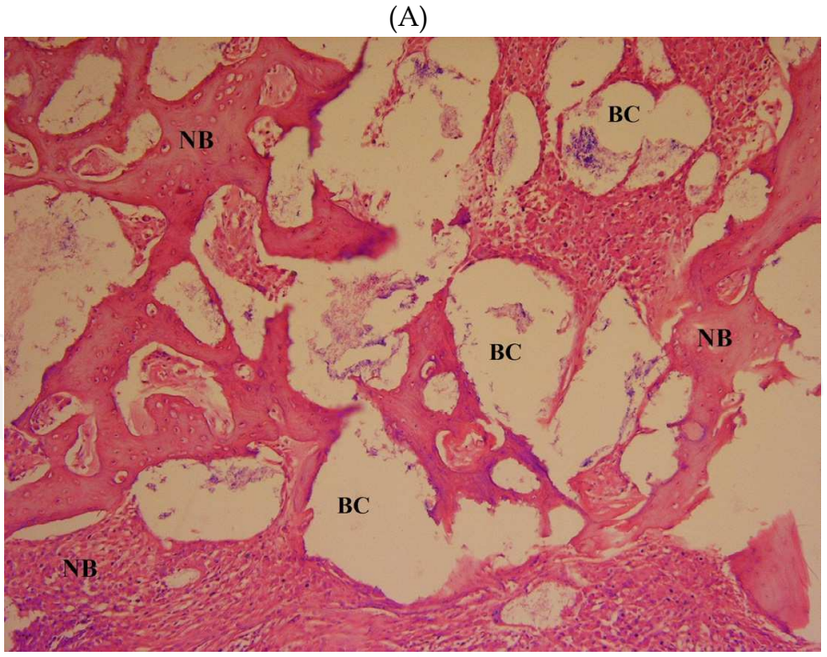
Fig. 1. SEM micrograph of a macropore in sintered bioactive ceramic.

Synthetic porous ceramic were supplied in the form of cylindrical specimens with a mean diameter of 3.4 ±0.5 mm and a mean length of 6.3±0.7 mm. Under general anesthesia, bone marrow was harvested from one medullar midshaft of the rabbit femur and diluted with 1

cc of saline. The porous ceramic were immersed in the solution for 5 min before implantation. A cavity of 3.5 mm in diameter and 7 mm in depth was drilled manually in the femoral condyles under general anaesthetic conditions and antibiotic protection. After carefully washing with a physiological saline solution, the cavities were filled with porous bioactive ceramic (BC) on one side and with porous bioactive ceramic contain Bone marrow



(A)



(B)

Fig. 2. Histological section of implants were harvested 3 months after implantation and stained with hematoxylin and eosin at 100x magnification. (A) bioactive ceramic, (B) bioactive ceramic with bone marrow cells. BC=bioactive ceramic, NB= newly formed bone.

(BCBM) on the other side. After 1, 2, 3 and 6 months, animals were killed by an overdose of thiopental sodium and the femoral condyles were removed. Experiments were performed according to the European Guidelines for Care and Use of Laboratory Animals (European Directive 86/609/CEE). During the experiment, all rabbits remained in good health and did not show any wound complications. No inflammatory signs or adverse tissue reaction could be observed. After 3 months, revealed the bridging of the BC and BCBM by host bone. Fig. 2 shows in vivo test results after 3 months. Histological investigations show a higher presence of newly formed bone and a higher osteogenesis in BCBM compared to BC after 3 & 6 months. In general, osteoblasts occurred evidently one month postoperatively, bone marrows began to develop in new bone tissues two months postoperatively, and bone tissues tended to be mature with the development of osteocytes and bone marrows over three months postoperatively.

Ideally, an implant, when placed in an osseous defect, should induce a response similar to that of fracture healing, where by the defect is initially filled with a blood clot which is invaded by mesenchymal cells, osteoblasts and fibroblasts within 2 weeks, followed by extensive bone and osteoid formation at 6 weeks, with complete healing/repair of the cancellous structure by 12 weeks (Orr et al., 2001). [

An equivalent amount of host bone was found in the BC and BCBM treated sites (Fig. 3). No significant difference was seen between BCBM and BC, at month 1 and month 2, but in Group 3 and 6 months, osteoid surface was higher in BCBM than in BC alone ($p<0.05$). BCBM have a stable biomechanical environment conducive to the formation of callus. Data from several sources show the exact effect of bone ingrowth on compressive strength and elastic modulus (Orr et al., 2001; Rabiee et al., 2008b). The porous implant with tissue ingrowth acts a composite structure. The implanted block consists of the mineral matrix of the block, fibrovascular tissue and bony tissue. All of these parameters effect on the compressive strength and modulus.

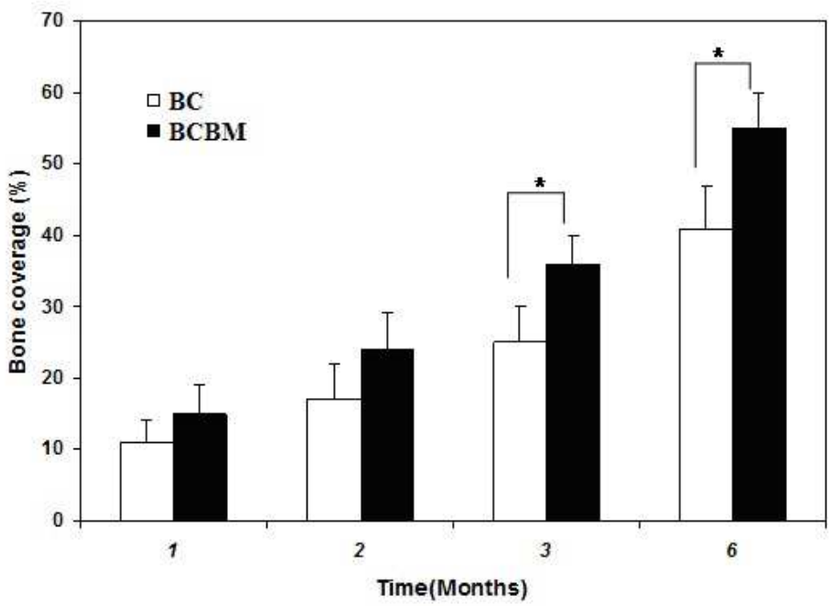


Fig. 3. Histomorphometry of the amount of bone coverage in BC and BCBM at 1, 2, 3 and 6 months after implantation. *$p<0.05$.

Results from mechanical compressive strength and elastic modulus of implanted specimens are presented in Table 4. BC specimens possessed an elastic modulus of 299±21 MPa prior to implantation. Elastic moduli of BC and BCBM became weaker after implantation (Table 4). BC moduli were significantly higher than those of the control at all time points, but no significant difference was apparent between BCBM and control 3 and 6 months after implantation. One example of the influence of a high modulus of elasticity of an implant material on surrounding bone is the dramatic bone loss around certain joint replacement prostheses. This bone loss has been attributed to the stress shielding resulting from the large disparity between the stiffness of the implant and the host bone (Orr et al., 2001).

Time of implantation	Compressive strength (MPa)		Elastic modulus (MPa)	
	BC	BCBM	BC	BCBM
Before implantatiom	6(0.5)	-	299(21)	-
1 months	5.2(0.94)	5.73(1)	268(33)	232(21)
2 months	4.8(0.9)	5.56(1.02)	232(20)	205(19)
3 months	5(0.3)	5.96(0.62)	206(22)	188(16)
6 months	5.1(0.5)	5.76(1.05)	195(17)	171(14)
AC	4.7(0.6)		169(23)	

Table 4. Mechanical properties of BC and BCBM. BC= Bioactive ceramic without bone marrow; BCBM= Bioactive ceramic with bone marrow; AC= anatomic control.

After implantation, BC and BCBM were partly degraded and their compressive mechanical properties decreased or remained at the same level. This could have resulted from two opposing reactions, with the matrix degrading slowly at the same time the amount of bone related to the reduced implant size was increasing. The first results of in vivo tests on rabbits showed good biocompatibility and osteointegration of the synthetic bioactive ceramic with bone marrow, with higher osteoconductive properties and earlier bioresorption, compared to similar synthetic bioactive ceramic without bone marrow samples. Bone marrow improved mechanical properties and bone growth. Bone ingrowth and degradation of the bioactive ceramic allow bone remodeling, which is a prerequisite for a good bone substitute.

5. Acknowledgments

The author would like to thank Prof. Moztarzadeh and Dr Mortazavi for their technical assistance, and Dr Sharifi for organizing the surgery.

6. References

Beaman, F.D., Bancroft, L.W., Peterson, J.J. & Kransdorf, M.J. (2006). Bone Graft Materials and Synthetic Substitutes. *Radiologic Clinics of North America*, Vol. 44, No. 3, pp. 451-461.

Best, S.M., Porter, A.E., Thian, E.S. & Huang, J. (2008). Bioceramics: Past, present and for the future. *Journal of the European Ceramic Society*, Vol. 28, No. 7, pp. 1319-1327.

Bohner, M. (2000). Calcium orthophosphates in medicine: from ceramics to calcium phosphate cements. *Injury*. Vol. 31, pp. 37-47.

Buma, P., Schreurs, W. & Verdonschot, N. (2004). Skeletal tissue engineering from in vitro studies to large animal models. *Biomaterials*, Vol. 25, No. 9, pp.1487–95.

Boix, T., Gómez-Morales, J., Torrent-Burgués, J., Monfort, A., Puigdomènech, P. & Rodríguez-Clemente, R. (2005). Adsorption of recombinant human bone morphogenetic protein rhBMP-2m onto hydroxyapatite. *Journal of Inorganic Biochemistry*, Vol. 99, No. 5, pp. 1043-1050

Brown,W.E., Chow,L.C. (1985). Dental restorative cement pastes. U. S. Patent No. 4518430.

Calori, G.M., Donati, D., Bella C.D. & Tagliabue, L. (2009). Bone morphogenetic proteins and tissue engineering: future directions. *Injury*, Vol. 40, pp. 67-S76

Cao, B., Zhou, D., Xue, M., Li, G., Yang, W., Long, Q. & Ji, L. (2010). Study on surface modification of porous apatite-wollastonite bioactive glass ceramic scaffold. *Applied Surface Science*, Vol. 255, No. 2, pp. 505-508.

Carey, L.E., Xu, H.H.K., Simon Jr, C.G., Takagi, S. & Chow, L.C. (2005). Premixed rapid-setting calcium phosphate composites for bone repair. *Biomaterials,* Vol. 26, pp. 5002–5014.

Chu, T.M.G., Warden, S.J., Turnera, C. H. & Stewart, R.L. (2007). Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2. *Biomaterials,* Vol. 28, pp. 459–467.

De Gabory, L., Bareille, R., Stoll, D., Bordenave, L. & Fricain, J. (2010). Biphasic calcium phosphate to repair nasal septum: The first in vitro and in vivo study. *Acta Biomaterialia*, Vol., No. 3, pp. 909-919.

Detsch, R., Mayr, H. & Ziegler, G. (2008). Formation of osteoclast-like cells on HA and TCP ceramics, *Acta Biomaterialia*, Vol. 4, No. 1, pp. 139-148.

Dorozhkin, S.V. & Epple, M. (2002). Biological and medical significance of calcium phosphates. *Angewandte Chemie International Edition*, Vol. 41, pp. 3130–3146.

Dorozhkin, S.V. (2010a). Amorphous calcium (ortho)phosphates. *Acta Biomaterialia*, Vol. 6, No. 12, pp. 4457-4475.

Dorozhkin, S.V. (2010b), Bioceramics of calcium orthophosphates. *Biomaterials,* Vol. 31,Vol. 7, pp. 1465-1485.

Giannoudis, P.V., Dinopoulos, H. & Tsiridis, E. (2005). Bone substitutes: An update. *Injury,* Vol. 36, No. 3, pp. S20-S27.

Ginebra, M.P., Traykova, T.& Planell, J.A. (2006). Calcium phosphate cements as bone drug delivery systems: A review. *Journal of Controlled Release*, Vol. 113, No. 2, pp. 102-110.

Hartman, E.M., Vehof, J.W.M., Spauwen, P.H.M. & Jansen, J.A. (2005). Ectopic bone formation in rats: the importance of the carrier. *Biomaterials,* Vol. 26, No. 14, pp. Pages 1829-1835.

Hench, L.L. (2006). The story of Bioglass*, Journal of Materials Science: Materials in Medicine*, Vol. 17, pp. 967-978.

Hench, L.L. & Wilson J. (1993). An introduction to bioceramics, World Scientific Publishing Co.; Singapore. pp. 245-251.

Karageorgiou, V. & Kaplan, D. (2005). Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials,* Vol. 26, No. 27, pp. 5474-5491.

Klein, C., Driessen, A.A. & De Groot, K. (1984). Relationship between the degradation behavior of calcium phosphate ceramics and their physical chemical characteristics and ultra structural geometry. *Biomaterials*, Vol. 5, pp. 157–160.

Kokubo, T., Ito, S., Sakka S. & Yamamuro, T. (1986). Formation of a high-strength bioactive glass-ceramic in the system MgO-CaO-SiO_2-P_2O_5, *Journal of Materials Science*, Vol. 21, No. 2, pp. 536–540.

Lane, J. M.; Tomin, E., & Bostrom, M.P.G. (1999). Biosynthetic bone grafting. *Clinical Orthopaedics and Related Research*, Vol.65, pp. 107–117.

LeGeros, R.Z. (2002). Properties of osteoconductive biomaterials: calcium phosphates. *Clinical Orthopaedics and Related Research*, Vol. 395, pp. 81–98.

Liu, X., Ding, C. & Chu, P.K. (2004). Mechanism of apatite formation on wollastonite coatings in simulated body fluids. *Biomaterials*, Vol. 25, No. 10, pp. 1755-1761.

Liu, Y., Enggist, L., Kuffer, A.F., Buser, D. & Hunziker, E.B. (2007a). The influence of BMP-2 and its mode of delivery on the osteoconductivity of implant surfaces during the early phase of osseointegration, *Biomaterials,* Vol. 28, pp. 2677–2686

Liu, Y., Cooper, P.R., Barralet J.E. & Shelton, R.M. (2007b). Influence of calcium phosphate crystal assemblies on the proliferation and osteogenic gene expression of rat bone marrow stromal cells. *Biomaterials,* Vol. 28, No. 7, pp. 1393-1403.

Lu, X. & Leng, Y. (2005). Theoretical analysis of calcium phosphate precipitation in simulated body fluid. *Biomaterials*, Vol. 26, pp.1097-1108.

Mistry, A.S. & Mikos, A.G. (2005). Tissue engineering strategies for bone regeneration. *Advances in Biochemical Engineering/Biotechnology*, Vol. 94, pp.1–22.

Mont, M.A., Ragland, P.S., Biggins, B., Friedlaender, G., Patel, T., Cook S, Etienne, G., Shimmin, A., Kildey, R., Rueger, D.C. & Einhorn, T.A. (2004). Use of bone morphogenetic proteins for musculoskeletal applications. An overview. (2004). *Journal of Bone and Joint Surgery*, Vol. 86, pp. 41–55.

Murugan, R. & Ramakrishna, S. (2005). Development of nanocomposites for bone grafting. *Composites Science and Technology* Vol.367, pp. 2385–2406.

Nie, H., & Wang, C. (2007). Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA. *Journal of Controlled Release*, Vol. 120, No. 1-2, pp. 111-121.

Niu, X., Feng, Q., Wang, M. Guo, X. & Zheng, Q. (2009). Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. *Journal of Controlled Release*, Vol. 134, No. 2, pp. 111-117.

Ooms, E.M., Wolke, J.G.C., Van de Heuvel, M.T., Jeschke, B. & Jansen, J.A. (2003). Histological evaluation of the bone response to calcium phosphate cement implanted in cortical bone. *Biomaterials,* Vol. 24, No. 6, pp. 989–1000.

Orr, T.E., Villars, P.A., Mitchell, S.L., Hsu, H.P. & Spector, M. (2001). Compressive properties of cancellous bone defects in a rabbit model treated with particles of natural bone mineral and synthetic hydroxyapatite. *Biomaterials*, Vol. 22, pp. 1953-1959.

Rabiee, S.M., Moztarzadeh, F., Salimi-Kenari, H. & Solati-Hashjin, M. (2007). Preparation and properties of a porous calcium phosphate bone graft substitute, *Materials Science- Poland*. Vol. 25, No. 4, pp. 1019-1027.

Rabiee, S. M., Moztarzadeh, F., Solati-Hashjin, M. & Salimi-Kenari, H. (2008a). Porous tricalcium phosphate as a bone substitute. *American Ceramic Society Bulletin*, Vol. 87, No.2, pp.43-45.

Rabiee, S.M., Mortazavi, S.M.J., Moztarzadeh, F., Sharifi, D., Sharifi, Sh., Salimi-Kenari, H., Solati-Hashjin, M. & Bizari, D. (2008b). Mechanical behavior of a new biphasic calcium phosphate bone graft. *Biotechnology and Bioprocess Engineering*. Vol. 13, pp. 204-209.

Rabiee, S.M., Moztarzadeh, F., Salimi-Kenari, H., Solati-Hashjin, M. & Mortazavi, S.M.J. (2008c). Study of Biodegradable Ceramic Bone Graft Substitute, *Advances in Applied Ceramics,* Vol.107, No.4, pp.199-202.

Rabiee, S.M., Mortazavi, S.M.J., Moztarzadeh, F., Sharifi, D., Fakhrejahani, F., Khafaf, A., Houshiar Ahmadi, S.A., Ravarian, R. & Nosoudi, N. (2009). Association of a Synthetic Bone Graft and Bone Marrow Cells as a Composite Biomaterial. *Biotechnology and Bioprocess Engineering*. Vol. 14, pp. 1-5.

Rabiee, S.M., Moztarzadeh, F. & Solati-Hashjin, M. (2010). Synthesis and characterization of hydroxyapatite cement. *Journal of Molecular Structure*, Vol. 969, pp. 172–175.

Ravarian, R., Moztarzadeh, F., Solati-Hashjin, M., Rabiee, S.M., Khoshakhlagh, P. & Tahriri, M. (2010). Synthesis, characterization and bioactivity investigation of bioglass/hydroxyapatite composite, *Ceramics International*, Vol. 36, No. 1, pp. 292-297.

Raynaud, S., Champion, E., Bernache-Assollant, D. & Thomas, P. (2002). Calcium phosphate apatites with variable Ca/P atomic ratio I. Synthesis, characterisation and thermal stability of powders. *Biomaterials,* Vol. 23, pp. 1065-72.

Rungsiyakull, C., Li, Q., Sun, G., Li,W. & Swain, M.V. (2010). Surface morphology optimization for osseointegration of coated implants. *Biomaterials,* Vol. 31, No. 27, pp. 7196-7204.

Schneider, A., Taboas, J.M., McCauley L.K. & Krebsbach, P.H. (2003). Skeletal homeostasis in tissue engineered bone. *Journal of Orthopaedic Research*, Vol. 21, No. 5, pp. 859-864.

Saravanapavan, P. & Hench, L.L. (2003). Mesoporous calcium silicate glasses. II. Textural characterisation. Journal of Non-Crystalline Solids, Vol. 318, No. 1-2, pp. 14-26.

Teoh, S.H. (2000). Fatigue of biomaterials: a review. *International Journal of Fatigue*, Vol. 22, No. 10, pp. 825-837.

Termaat, M.F., Den Boer, F.C., Bakker, F.C., Patka, P. & Haarman, H.J. (2005). Bone morphogenetic proteins. Development and clinical efficacy in the treatment of fractures and bone defects. *Journal of Bone and Joint Surgery*, Vol. 87, No. 6, pp.1367–78.

Vallet-Regı, M., lez-Calbet, J.M.G. (2004). Calcium phosphates as substitution of bone tissues, *Progress in Solid State Chemistry*, Vol. 32, pp. 1–31.

Ziegler, J., Mayr-Wohlfahrt, U., Kessler, S., Breitig, D. & Günther K.P. (2002). Adsorption and release properties of growth factors from biodegradable implants. *Journal of Biomedical Materials Research,* Vol. 59, pp. 422–428.

Biomaterials Applications for Nanomedicine

Edited by Prof. Rosario Pignatello

ISBN 978-953-307-661-4
Hard cover, 458 pages
Publisher InTech
Published online 16, November, 2011
Published in print edition November, 2011

These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentialities of different synthetic and engineered biomaterials. Contributions were selected not based on a direct market or clinical interest, but on results coming from a very fundamental studies. This too will allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessary imposed by industrial or profit concerns. Biomaterial constructs and supramolecular assemblies have been studied, for example, as drug and protein carriers, tissue scaffolds, or to manage the interactions between artificial devices and the body. In this volume of the biomaterial series have been gathered in particular reviews and papers focusing on the application of new and known macromolecular compounds to nanotechnology and nanomedicine, along with their chemical and mechanical engineering aimed to fit specific biomedical purposes.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sayed Mahmood Rabiee (2011). Bioactive Ceramics as Bone Morphogenetic Proteins Carriers, Biomaterials Applications for Nanomedicine, Prof. Rosario Pignatello (Ed.), ISBN: 978-953-307-661-4, InTech, Available from: http://www.intechopen.com/books/biomaterials-applications-for-nanomedicine/bioactive-ceramics-as-bone-morphogenetic-proteins-carriers

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen