Journal of Biomimetics, Biomaterials and Tissue Engineering Vol.2 (2009) pp 39-55
Online available since 2009/May/18 at www.scientific.net

doi:10.4028/www.scientific.net/JBBTE.2.39

New Generation of Synthetic, Bioresorbable and Injectable Calcium Phosphate Bone Substitute Materials: Alpha-bsm®, Beta-bsm™ and Gamma-bsm™

A. Tofighi[1,a], A. Rosenberg[1,b], M. Sutaria[1,c], S. Balata[1,d] J. Chang[1,e]

[1]ETEX Corporation, University Park at MIT, 38 Sidney Street, Cambridge, MA 02139, USA
[a]atofighi@etexcorp.com, [b]arosenberg@etexcorp.com, [c]msutaria@etexcorp.com,
[d]sbalata@etexcorp.com, [e]jchang@etexcorp.com

Keywords: Calcium phosphate cement; Apatite; Critical size defect; Competitors.

Abstract. Alpha-bsm® is a first generation self-setting, injectable and moldable apatitic calcium phosphate cement (CPC) based on amorphous calcium phosphate (ACP). ACP was prepared using low temperature double decomposition technique, from a calcium solution (0.16 M), and phosphate solution (0.26 M) in a basic (pH~13) media. ACP was than stabilized using three crystal growth inhibitors (CO_3^{2-}, Mg^{2+}, $P_2O_7^{4-}$), freeze-dried, and heated (450 ºC, 1h) to remove additional moisture and some inhibitors. Dicalcium phosphate dehydrate (DCPD) was also prepared using wet chemistry at room temperature from calcium and phosphate solution, respectively, 0.3 M and 0.15 M.

ACP and DCPD powder were combined at a 1:1 ratio and ground to produce Alpha-bsm® bone cement. The cement is supplied as a powder and when mixed with an appropriate amount (0.8 ml/g) of physiological saline at room temperature, forms an injectable putty-like paste. The paste has a working time of about 45 minutes at room temperature, when stored in a moist environment.

The setting reaction proceeds isothermically at body temperature (37°C) in less than 20 minutes, forming a hardened, porous (total porosity 50 to 60%), low crystalline (40% comparing with HA), apatitic calcium phosphate cement with a compressive strength range of 10 to 12 MPa.

Extensive pre-clinical studies (rabbit radius critical sized defect, canine tibia osteotomy, sheep tibia, primate fibula fracture healing, and primate fibula critical size defect) demonstrate that Alpha-bsm® undergoes remodeling in conjunction with new bone formation.

The next generation of Bone Substitute Materials (Beta-bsm™ and Gamma-bsm ™) are formulated based on the Alpha-bsm® chemistry but differ in powder processing (e.g. milling) technique. These materials are also self-setting, injectable and/or moldable apatitic calcium phosphate cements with improved handling and mechanical properties. The setting & hardening reaction of these new CPCs proceeds isothermically in less than 5 minutes at 37°C and once hardened demonstrate a compressive strength of 30 to 50 MPa. The final product (after full conversion) is a low crystalline (40% compared with Hydroxyapatite), calcium deficient (Ca/P atomic ratio = 1.45) carbonated apatite similar to the composition and structure of natural bone mineral (crystal size: length = 26 nm, width thickness = 8 nm). A desirable feature of these cements is their high surface chemistry (with specific surface area of about 180-200 m^2/g) which is ideal for remodeling and controlled release of growth factors. A pilot rabbit critically sized femoral defect study comparing the three synthetic family products demonstrate that they share similar remodeling and resorption characteristics up to 52 weeks. Physico-chemical and mechanical performance of these next generation CPCs are favorable when compared with existing CPCs in the market, specifically material working time (at room temperature), cohesivity in a wet environment and fast setting & hardening rate (at body temperature).

Introduction

Calcium phosphates (CaP) are the principal constituent of hard tissues (bone, cartilage, tooth enamel and dentine). Bone mineral is made of about 70 w/w % nanometer-sized of poorly crystalline hydroxyapatite (PCHA) [1]. The composition and structure of bone mineral is significantly different and distinguished from the high crystalline structure and stoechiometric hydroxyapatite (HA), and may be represented by [2]: $Ca_{8.3}(PO_4)_{4.3}(HPO_4,CO_3)_{1.7}(OH,CO_3)_{0.3.}$

Bone mineral nonstoichiometry is primarily due to the presence of divalent ions (CO_3^{2-} and HPO_4^{2-}), which substitute trivalent PO_4^{3-} resulting in calcium to phosphorous atomic ratio that varies from 1.45 to 1.75, depending on the kind, age and/or anatomical site of the bone.

Autogenous iliac bone grafting (AIBG) has long been the "gold standard" for treatment of bony defects caused by trauma or disease. However, natural bone grafts are associated with problems such as limited availability, painful harvesting procedures, risks of viral transmission, and immune reaction to allograft bone from a cadaver.

Synthetic, injectable, self-setting and biocompatible calcium phosphate bone substitutes play an important role in a variety of surgical procedures to replace autograft and/or allograft. Designing a synthetic bone substitute material with desirable properties such as biocompatibility, structural integrity, bioresorbability, and remodeling of new bone is a challenging endeavor. Large scale manufacturability, cost-effectiveness and ease of use for the clinician are also important considerations for the product.

The first generation of synthetic bone substitute materials (BSM) was initially investigated in the mid 1970s using HA as a biomaterial for remodeling of bone defects [3]. The concepts established by calcium phosphate cement (CPC) pioneers [4, 5] in the early 1980s were used as a platform to initiate a second generation of BSM for commercialization [6]. Since then, advances have been made in composition, performance and manufacturing.

CPCs are generally based on a combination of two or more CaP precursors reacted together in an aqueous media to form a paste with putty-like consistency. These cements have the ability to precipitate different end products (e.g. HA, PCHA, DCPD or brushite, etc.). Based on the type of chemical reaction, these materials may be classified as: acid-base cements, monocomponent cements, and hydrolysable cements [7].

As of today, various CPC essentially based on acid-base reaction have been developed [6, 8, 9, 10]. Our first generation of synthetic bone substitute material (alpha-bsm®), is an injectable, self-setting and resorbable CPC, based on the rapid hydrolyzing of amorphous calcium phosphate (ACP) to PCHA [11, 12].

The performance of cement in mediating bone healing has been studied in various critical sized defects of rabbit, canine, sheep and monkey models [13, 14, 15, 16]. Clinical studies [17, 18, 19, 20, 21] have demonstrated the effectiveness of alpha-bsm® as a bone void filler. A prospective multicenter randomized clinical study of AIBG vs. alpha-bsm® demonstrated the efficacy of this material, proposing alpha-bsm® as a new gold standard in tibial plateau repair [22].

Recently, two new types of synthetic, injectable, moldable, fast setting & hardening and bioresorbable CPCs (beta-bsm™ and gamma-bsm™) were designed [23]. Beta and gamma were formulated based on the same chemistry as alpha-bsm® using mechano-chemical high energy powder processing [24, 25].

The purpose of this paper is to investigate *in-vitro* and *in-vivo* performance of alpha-bsm®, and compare with two new generations of nano-crystalline apatitic CPCs (beta-bsm™ and gamma-bsm™). In addition we report compared physico-chemical and mechanical performance of synthetic family with CPCs in the current market.

Experimental Methods

Wet Chemistry and Powder Processing. ACP was prepared using low temperature double decomposition technique, by adding rapidly a calcium solution (0.36 M), to phosphate solution (0.16 M) in a basic (pH~13) media. The amorphous phase was then stabilized using three crystal growth inhibitor ions (CO_3^{2-}, Mg^{2+} and $P_2O_7^{4-}$), freeze-dried, and heated (450 ºC, 1h) to remove additional moisture and some crystal growth inhibitors.

DCPD was also prepared using wet chemistry by adding rapidly a calcium solution (0.30 M), to phosphate solution (0.15 M) in a slightly acidic (pH~5-6) media. During precipitation, the chemical composition of DCPD was controlled to approximately 10 to 25% (w/w) apatite. DCPD wet cake was then vacuum dried (6h at 37 °C), and milled to achieve particle size less than 125 µm.

ACP and DCPD powder were combined at a 1:1 ratio and ground to produce alpha-bsm® cement.

Physico-Chemical and Mechanical Characterization Techniques. Alpha-bsm® is a powder that when mixed with an appropriate amount (0.8 ml/g) of aqueous media (physiological saline) at room temperature, forms a moldable paste easily injectable through a 16-gauge needle. The cement was evaluated for physico-chemical (FTIR, XRD, BET, TGA, SEM, Ca/P atomic ratio, particle size and porosity) characterization. The hardening kinetics of the paste was monitored using a texture analyzer technique based on needle penetration assay into plastic zone (Gilmore Needle) at different temperatures (25-37°C). The compressive strength was determine using a uniaxial force applied on harden block cylinder (θ=6 mm, h=12 mm).

Accelerated aging stability testing was also conducted.

In-Vivo Studies of Alpha-bsm®. Efficacy of alpha-bsm® as a bone graft substitute has been established in various pre-clinical studies (rabbit radius critical sized defect, canine tibia osteotomy, sheep tibia, primate fibula fracture healing, primate critical size defect in fibula).

New Cement Design and Characterization. Two new types of synthetic CPC entitled: beta-bsm™ and gamma-bsm™ were designed. These cements were formulated based on the same chemistry as alpha-bsm® but ground by using mechano-chemical high energy powder processing technique in order to improve their mechanical properties.

ACP and DCPD powders (at a 1:1 ratio) were high-energy dry ball milled using a 10 mm diameter high-density ZrO_2 ball for 3 hours residence time. The process is performed in a rotating ceramic jar that agitates the balls (media) into a random state of motion of internal porosity called kinematic porosity [26]. The process first breaks the particles into small size and then beats and compacts them together to form agglomerates. In this expanded condition, the media and particles are free to move, collide, and impinge upon each other. This generates high shear and powerful impact in order to produce an array of CPC materials.

The CPC powders were then mixed with hydration media (physiological saline) to produce an injectable and/or moldable putty using liquid to powder ratio (L/P) respectively 0.5 and 0.4 ml/g. The above cements were evaluated by the same technique of characterization that has been performed for alpha-bsm®.

In-Vivo Studies comparing alpha-bsm® and beta-bsm®. *In vivo* performance of the beta-bsm® in mediating bone healing was compared to alpha-bsm® in a rabbit femoral defect model. The objective of this pilot study was to compare the biocompatibilities and relative resorption rates of these two cements in surgically created bilateral femoral core defects. A total of 17 skeletally mature New Zealand white rabbits (greater than 10 months old with average weight 4.5 ± 0.25 kg) were used. Through a lateral incision, the lateral femoral condyle was exposed. Defects (5.0 mm diameter and 10 mm deep) were created in each lateral femoral condyle by drilling under constant saline irrigation. alpha-bsm® or beta-bsm™ paste was implanted in each defect, or the defect was left empty (as a negative control), according to the randomized implantation chart.

Animals were sacrificed at 6, 12, 26, 38, and 52 weeks, and the femora were harvested and evaluation (of the surgical graft implantation for all groups) was conducted using digital images of plastic embedded sections (of rabbit femoral defects) at magnifications: 1, 10 and 20X.

Commercial Products Performance Comparison. Product literature was obtained to compare the physico-chemical and mechanical performance of commercially available products.

Results

In Vitro Characterization

The chemical composition and structure of synthetic family powders are presented in the Figures 1 and 2.

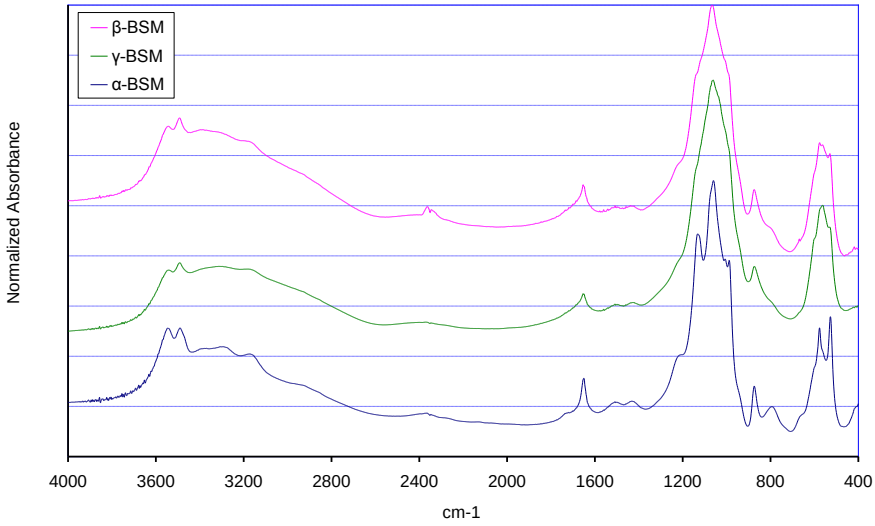

Figure 1. FTIR Spectra comparing α-BSM®, β -BSM™ and γ -BSM™ powder.

Decreasing of $PO4^{2-}$ bond (526, 580, 987 and 1133 cm^{-1}) and CO_3^{2-} bond (794 cm^{-1}) results of amorphization phenomena was clearly observed on beta-BSM® and gamma-bsm® spectra.

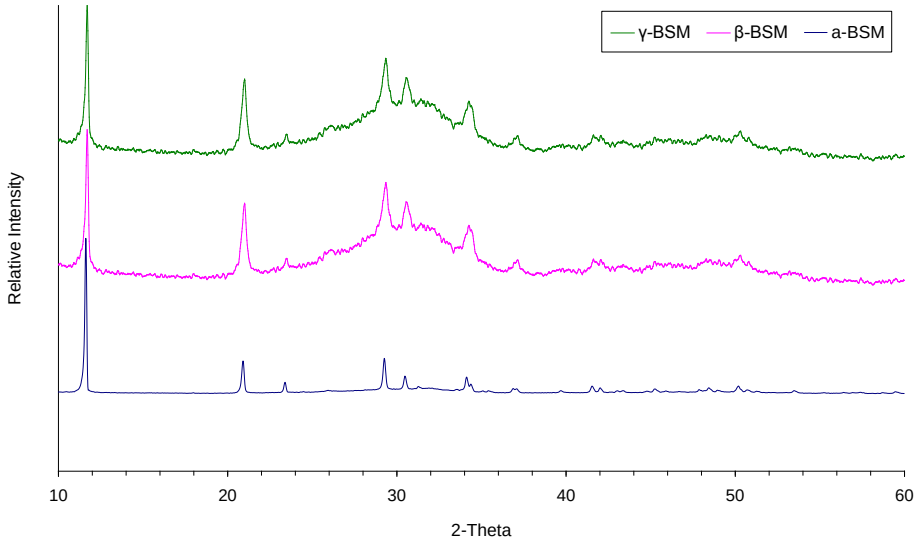

Figure 2. X-Ray Diffraction (Cu-Kα) comparing α-BSM®, β -BSM™ and γ -BSM™ powder.

The major DCPD peaks (2θ: 11.6, 23.4, 29.2, 30.5 and 36.3) are present and coexist with amorphous phase. Amorphization phenomena (with broad peaks) initiate for beta and gamma cement.

Particle size distribution of synthetic family powders (Figure 3) confirms a mono-model form, with major particle ranging from 1 to 100 µm.

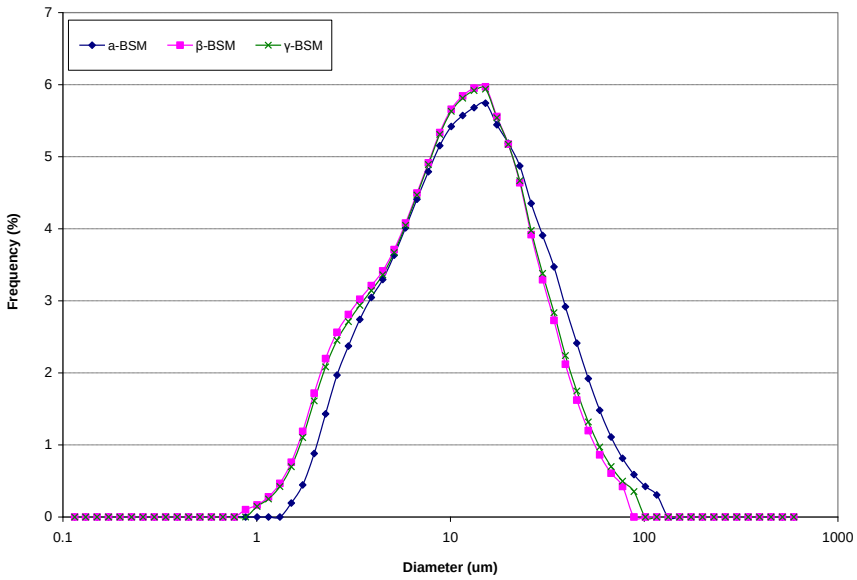

Figure 3. LASER particle size distribution of synthetic family powders.

Alpha-bsm® powder is currently mixed through a (closed) mixing bulb, beta-bsm™ through a (closed) syringe to syringe mixing method and gamma-bsm™ through an open mixing technique using a bowl and spatula. The mixing time (after addition of saline to the powder) is about one minute. The liquid to powder ratio (L/P) for proper hydration of alpha-BSM®, beta-bsm™ and gamma-bsm™ are respectively 0.8, 0.5 and 0.4 ml/g.

The kinetics of setting & hardening of synthetic family pastes at room temperature (25 °C) are presented in Figure 4.

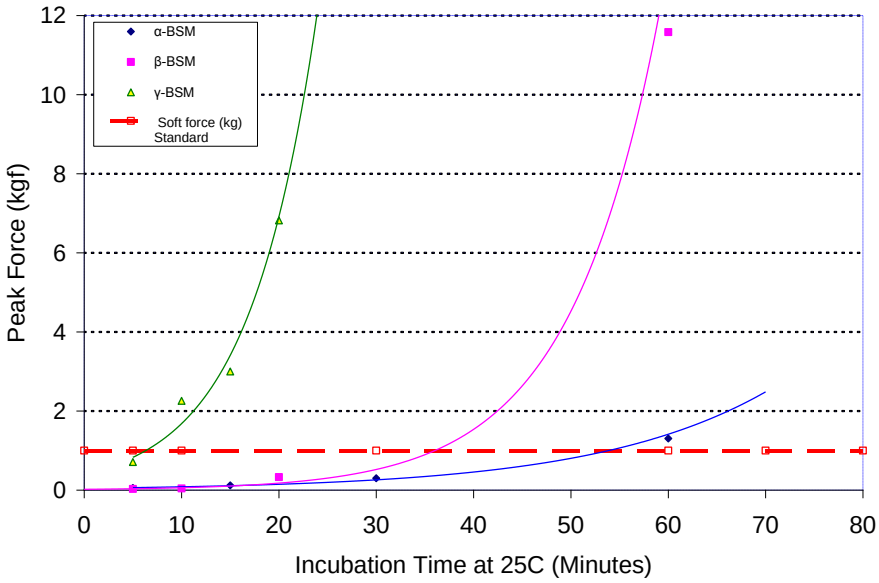

Figure 4. Hardening kinetic of synthetic family at 25 °C.

In the closed mixing & delivery system, the paste working time is longer (up to 50 min). For the open mixing system (gamma-bsm™), the evaporation rate decreases the paste working time. However, the consistency of the paste can be always readjusted by adding hydration media.

The kinetics of setting & hardening of the synthetic family pastes at body temperature (37 °C) is presented on the Figure 5.

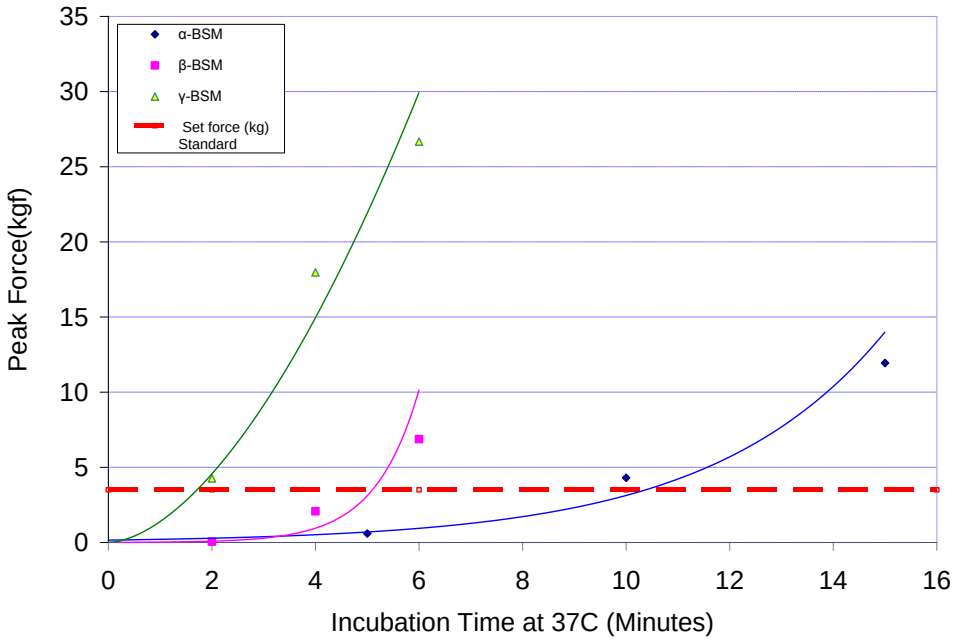

Figure 5. Hardening kinetics of synthetic family at 37 °C.

The setting & hardening reaction proceeds isothermically at 37°C in less than 3, 5 and 15 minutes respectively for gamma-bsm™, beta -bsm™ and alpha-bsm®.

Fully converted cements (after adding physiological saline and incubation at 37 °C for about 15h), confirm the chemical composition and structure of low crystalline apatite, similar to the structure of bone mineral (Figure 6).

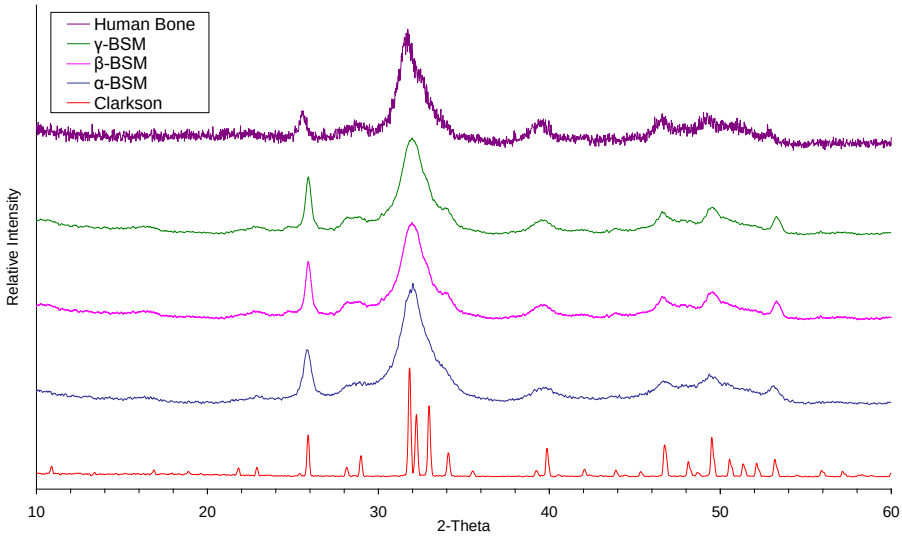

Figure 6. X-Ray Diffraction (Cu-Kα) comparing HA, final product of α-BSM®, β-BSM™ and γ-BSM™, and human bone mineral.

For both bone and the synthetic family, the apatitic phase appears as broad peaks (2θ: 32), which are indicative of the low crystalline nature of the four materials as compared to the corresponding sharp peaks observed with stoichiometric crystalline HA.

It is important to note that the final product of the synthetic family is low crystalline (with a crystallinity index of about 40%) calcium deficient (Ca/P = 1.45) carbonated apatite, having nano-sized crystal (Length = 26 nm and Width-Thickness = 8 nm). These crystal sizes are analogous to human bone mineral which is reported to be: Length = 23-32 nm and Width-Thickness = 6.7-8.0 nm [12].

The specific surface area of blended synthetic family powder (37 m^2/g) is decreased within the first three hours of high energy milling confirming a compacting effect of ball milling process. On the other hand, all of the fully converted products present high specific surface area (180 to 200 m^2/g).

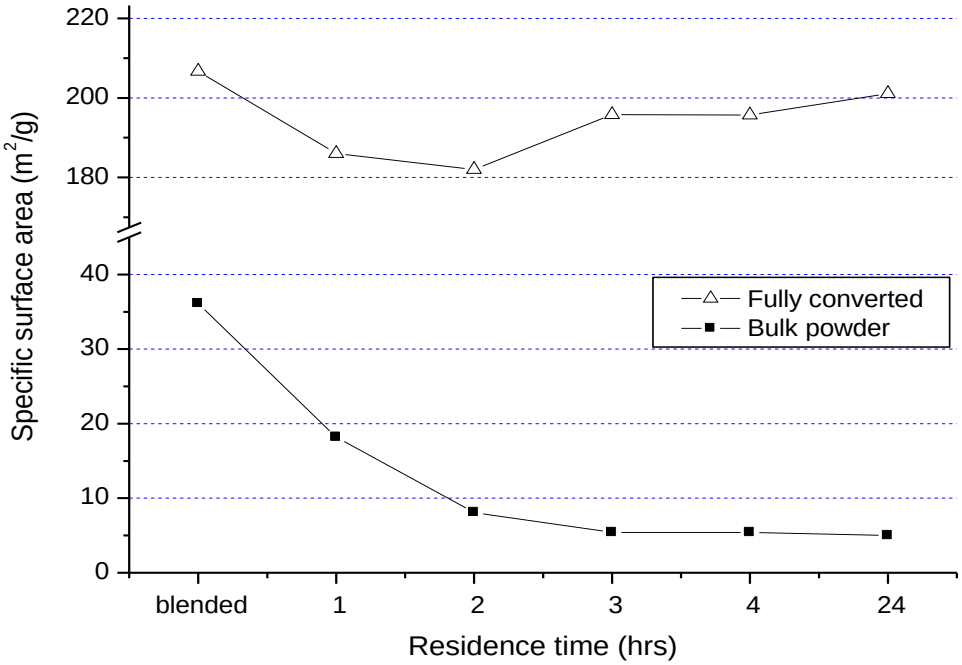

Figure 7. Specific surface area (single-point BET) of blended synthetic family powder and after fully conversion to an apatitic phase.

The peak compressive strength of the synthetic family is obtained after 2h incubation of the paste at 37 °C (Figure 8).

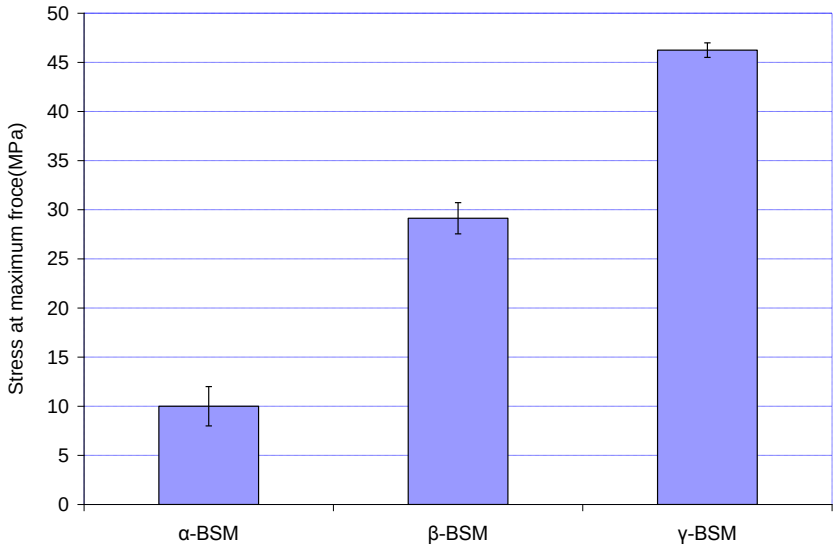

Figure 8. Compressive strength comparison of synthetic family.

It is confirmed that beta-bsm™ and gamma-bsm™ has improved mechanical performance compared to alpha-bsm®. The difference in compression value between beta (about 30 MPa) and gamma (about 46 MPa) is due to the L/P ratio (respectively 0.5 and 0.4 ml/g). In fact, lowering the L/P ratio on the same formulation of cement results in higher paste density, therefore higher compressive strength.

In Vivo Studies

Canine Femoral Slot Model. Femoral slot defects in 28 canines (4 to 8 for each time point) were filled with either autologous bone implant (ABI) or alpha-bsm®. Sections of the femoral bone defect site from animals sacrificed at 3, 4, 12 and 26 weeks were used to compare remodeling to the new bone formation and resorption rate (Figure 9).

Defect filling with new bone occurred by the third week for both treatment groups; the boundary between new and old bone at this time point was still clearly visible**.** By week 12, it was difficult to identify the boundaries of the defect site. Over this time, the medullary canal underwent remodeling with restoration of normal looking marrow tissue without evidence of residual cement or autologous bone fragments remaining. By week 26, restoration of the native bone morphology was virtually complete, the defect was filled with normal cortical bone and the defect edges were not clearly delineated by any of the staining methods used. Trabeculae present in the newly formed bone often extended into residual alpha-bsm® without discontinuity**.** When residual material was observed from later time points, it was found to be integrated within the healed bone.

Rat Dorsal Subcutis Model. Alpha-bsm® powder was hydrated using a buffer containing either 0.02 or 0.2 mg/ml rh-BMP-2. The paste was then shaped into a disk and incubated in 100% relative humidity at 37°C for one hour in-vitro. The hardened disks were then implanted into the rat dorsal subcutis for 2 and 4 weeks.

Ectopic bone formation was observed at both 2 and 4 weeks in animals receiving the highest dose of rh-BMP-2. The amount of cement remaining at the implant site was similar among the animals that did not form ectopic bone. However, at 4 weeks, in those animals where ectopic bone was present, there was a roughly eightfold reduction in residual alpha-bsm®**.**

Canine Tibia Osteotomy Model. This study was designed to compare the healing rate of 1 mm lower tibia osteotomies in response to injection of alpha-bsm® with rhBMP-2 vs. alpha-bsm® alone. Bilateral diaphyseal tibia osteotomies were created via open approach in healthy adult canines (n=8 per group) and stabilized with an external fixator for 8 weeks. The torsional stiffness of the alpha-bsm®-rhBMP-2 treated tibia (23.8 Nm) was identical to the strength of an intact tibia (Figure 10).

Cynomologus Monkey Fibula Fracture Healing. Bilateral fibula osteotomies in adult cynomologus monkeys were used to evaluate acceleration of healing with percutaneous injection of alpha-bsm®-rhBMP-2 at 1, 2, 4, and 8 weeks. The radiograph comparison of alpha-bsm® with and without rhBMP-2 at 8 weeks (Figure 11) demonstrate acceleration of fracture healing.

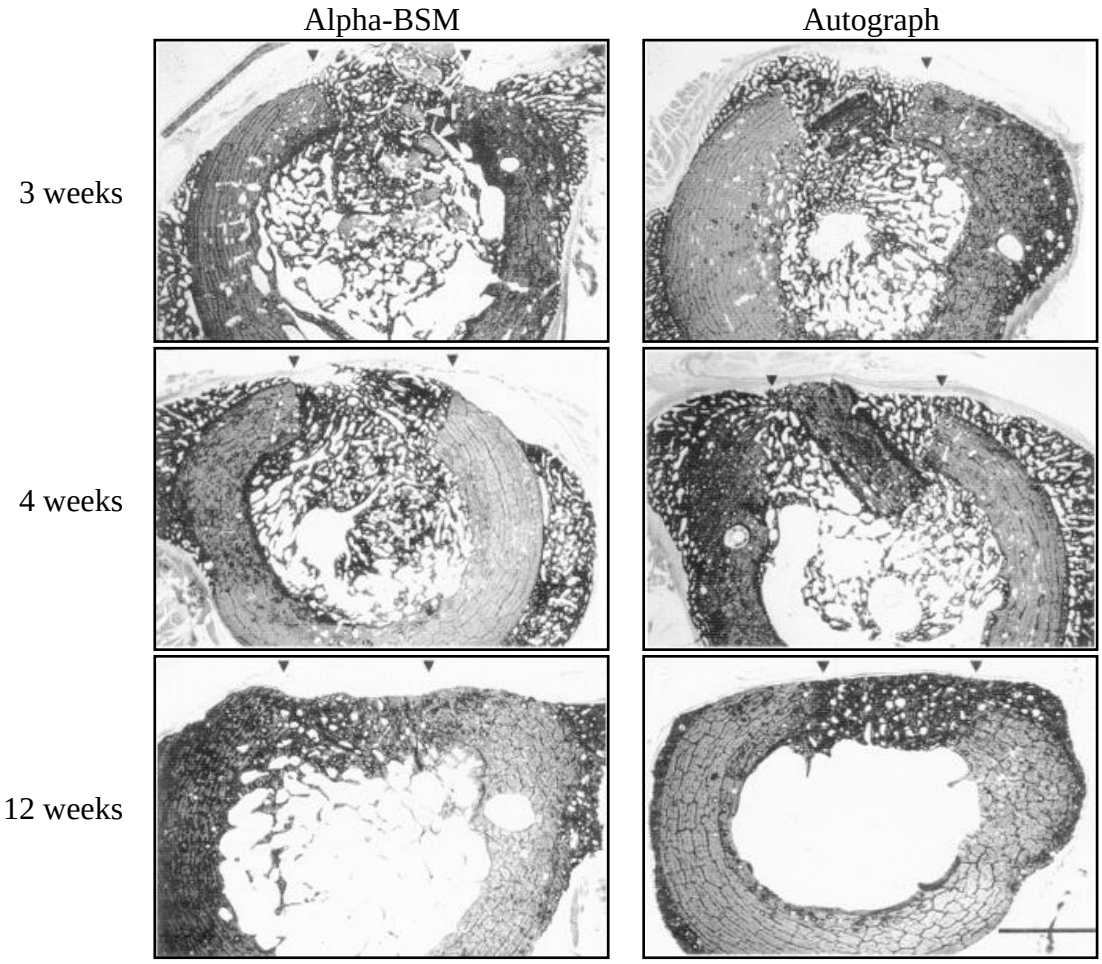

Figure 9. Photomicrographs of cross sections through the femoral slot bone defect comparing α-BSM® (left panels) to autologous bone implants (right panels).

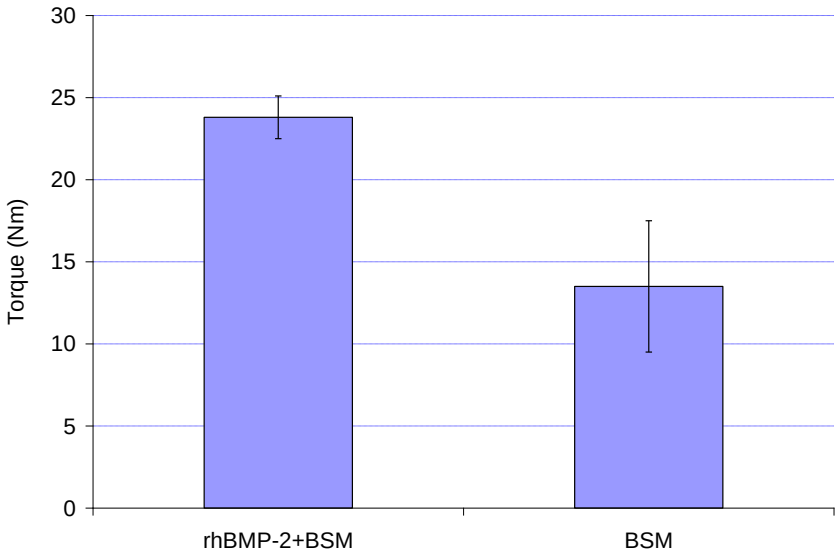

Figure 10. The torsional stiffness comparing α-BSM® with combination of α-BSM®-rhBMP-2 at 8 weeks.

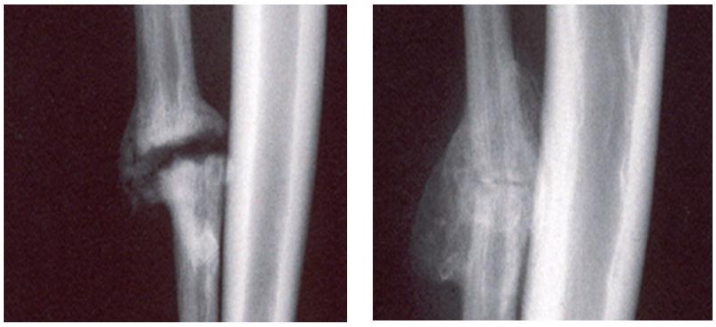

Figure 11. Fibula osteotomy defect healing comparing a) α-BSM®-rhBMP-2 with b) empty defect (control) at 8 weeks time point.

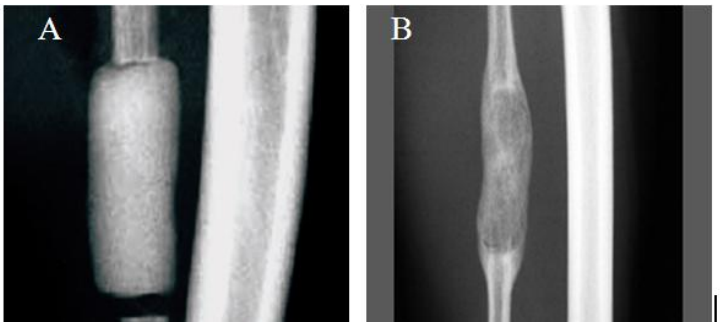

Figure 12. Radiograph comparison of α-BSM®-rhBMP-2: a) Post operation and b) 12 Weeks implantation in Fibula Critical Size Defect of adult Cynomologus Monkeys.

Cynomologus Monkeys Fibula Critical Size Defect. Critical size defects (3 cm) in the fibula of adult cynomologus monkeys were used to evaluate the ability of combination of alpha-bsm®-rh-BMP-2 (cylindrical paste) to heal a segmental defect at 1, 2, 4, 12 and 16 weeks. Remodeling and initial bone induction was observed by 4 weeks. At 12 weeks (Figure 12), the implanted material was fully remodeled and replaced with trabecular bone.

Rabbit Radius Critical Size Defect. Rabbit radius critical sized defects (2 cm length) have been used in several studies to investigate the osteopromotive effects of alpha-bsm® with and without rh-BMP-2 (11 μg and 58 mg). The defects were implanted with alpha-bsm® alone (as control) and alpha-bsm® combined with rh-BMP-2. All animals were euthanized at 8 weeks and histological evaluation and histomorphometric analysis were conducted.

At 8 weeks time (figure 13), in the unfilled defect, healing is not observed**.** When the defect is filed with alpha-bsm® alone, the defect is bridged but differentiation of the cortex and medullary canal is not apparent. When the defect is filled with a combination of alpha-bsm® and rh-BMP-2, not only the defect is bridged but differentiation of the cortex and medullary canal are also visible**.**

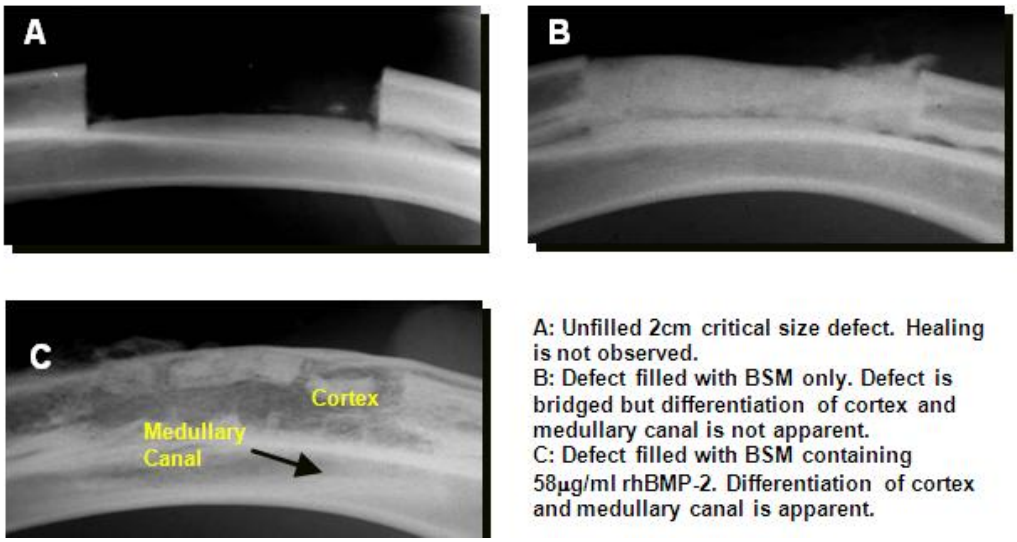

Figure 13. Radiograph of Rabbit radius critical sized defect at 8 weeks.

Rabbit Femoral Defect Comparing alpha-bsm® and beta-bsm™. *In vivo* performance of beta-bsm® was compared to alpha-bsm® in a rabbit femoral defect model. Defects (θ=5.0 mm, h=10 mm) were created in each lateral femoral. alpha-bsm® or beta-bsm™ paste was implanted in each defect. Empty defects were used as a negative control. At 6 weeks (Figure 14-A), alpha-bsm® (1X image) appears to extend beyond the cortical bone. The 10 and 20X images indicate active new bone formation on the eroded surface. Osteoclasts are present on the necrotic and new bone surfaces and appear to be remodeling these tissues by encasing alpha-bsm® fragments into new woven bone.

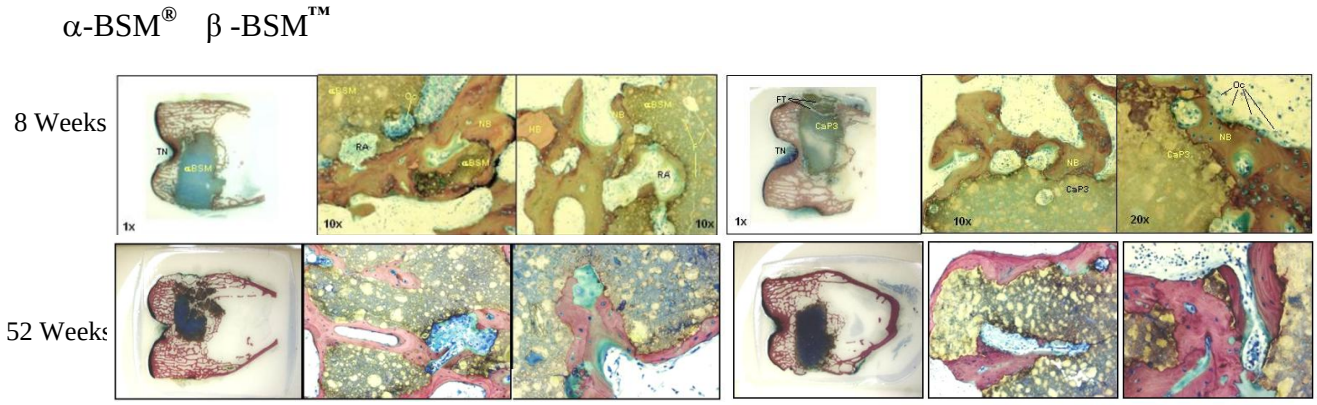

Figure 14. Photomicrographs of cross sections through the femoral slot bone defect comparing α-BSM® (left panels) to β-BSM® (right panels).

At 6 weeks, beta-bsm™ (1X image) also appear to extend beyond the cortical bone. The 10X and 20X images demonstrate prior osteoclastic surface erosion which is covered with active new bone formation. Osteoclasts are also present and appear to be actively remodeling tissue.

At 52 weeks, significant amounts of new bone with extensive surface remodeling cover much of the surface of alpha-bsm® and encircle fragments of the material while remaining in continuity with the host trabecular bone. At 52 weeks, new bone covers most of the remodeled implant surfaces of beta-bsm™. Particularly, the surface of the beta-bsm™ adjacent to the joint communication is devoid of new bone formation and cellular elements. As with the alpha-bsm® specimens, most beta-bsm™ remodeling occurred at the margins of the cortical window.

This pilot model demonstrates no evidence of chronic inflammation associated with either implant material: the two products share similar resorption characteristics. With both implant materials, more intensive remodeling and replacement with new bone occurred adjacent to the cortical bone window and marrow spaces. The model appears to be a critical size defect.

Commercially Available Products Comparison

Physico-chemical and mechanical performance of the synthetic family compared with commercially available products is presented in Table 1.

Table 1. Physico-chemical and mechanical performance of the synthetic family compared with commercially available products.

Products	**α-BSM®**	**β-BSM™**	**γ-BSM™**	**BoneSource® [5, 28]**	**HydroSet® [27, 28]**	**Norian SRS® [6, 28]**
Starting Materials	ACP + DCPD	n-ACP + DCPD	n-ACP + DCPD	TTCP + DCPA	TTCP+DCPD + Tri –Na Citrate	alpha-TCP + $CaCO_3$ + MCPM
Hydration Media	Physiological Saline	Physiological Saline	Physiological Saline	PBS	PBS+Polyvinyl Pyrrolidone	PBS
Working Time at 25 ºC (min)	45-60	10-15	10-15	4-6	2.5-4.5	2
Setting Time at 37 (min)	15-20	3-5	2-3	8-12	8-10	10
Compressive Strength (MPa)	10-12	28-32	45-47	60-65	15.9	55
Delivery	Injectable (8-16G)	Injectable (8-16G)	Moldabale	Not Injectable	Injectable (8-10 G)	Injectable (8-14 G)
Sterilization	Gamma Rad.	Gamma Rad.	Gamma Rad.	Gamma Rad.	Ethylene Oxide + Gamma Rad.	Gamma Rad.
End Product	Carbonated Apatite	Carbonated Apatite	Carbonated Apatite	HA	HA	Carbonated Apatite

The new synthetic generation (beta-bsm™ and gamma-bsm™) are fast setting & hardening, with high compressive strength and having reasonable working time. The end product of all synthetic family is a low crystalline carbonated apatite which also has greater degradation rate comparing with high crystalline and stoichiometric HA.

Discussion

In the high energy dry mechano-chemical milling technique, the media and calcium phosphate (CaP) powder are free to move, collide, impinge upon each other and generate shear. The simultaneous media-powder collision and friction, generating (local) heat to hygroscopic CaP, causes an inter-diffusion of particles, and produces structural (amorphization) and chemical changes. After 3h of milling, new nano-crystalline CPC with faster setting and greater strength are produced.

These products are provided in powder form that is hydrated with physiological saline to form a workable putty. The paste is injectable through a 16-gauge needle, or remains formable and moldable (in the case of gamma-bsm™) at room temperature. The paste is self-setting and hardening at 37°C. The paste sets under relatively benign conditions (isothermically, neutral pH, and body temperature), and is compatible with the range of different hydration media (water, saline, physiologically buffers, etc).

Alpha-bsm®, beta-bsm™ and gamma-bsm™ are chemically pure calcium phosphate cements; their formulations do not contain any additive such as organic matrix, porogenic and/or cohesiveness agents. Addition of organic additives may disturb biocompatibity of the products and may result in *in-vivo* side effects and complications.

Beta-bsm™ and gamma-bsm™ are mechanically fast setting and high strength cements that are chemically similar in formulation and function to alpha-bsm®. It was demonstrate that the fast-hardening reaction could be related to the rapid hydrolysis (about 1 hr) of unstable ACP into apatite [12]. DCPD is a template and hydrolysis lasts longer (over 10 hrs). The existence of an epitaxial relationship [29] between DCPD and apatite probably favors nucleation of apatite.

In β and γ cement formulation, an ACP with lower Ca/P atomic ratio (less than 1.5) was used. It is believed that lowering the Ca/P of CPC results in fast hydrolysis of raw material (ACP), therefore, providing greater resorbability of the product. These CPC are not freely soluble in aqueous solution and hardened cements display limited solubility at 37°C. Solubility characteristics were confirmed by in vitro mass loss studies at both neutral and acidic pH.

The control of total porosity with appropriate pore size and pore distribution plays a crucial role for cellular activities and *in-vivo* remodeling of CPC. Generally the CPC hardening reaction forms elongated, entangled crystals. However, the presence of very reactive non-apatitic environments at the surface of poorly crystalline apatite's crystal exhibits a strong ionic mobility which is related to direct crystal-crystal interaction. We have shown [30] that such interactions have been evidenced in the formulation of calcium phosphate ceramics at low temperature and it's believed to be related to the crystal fusion phenomenon described in biological apatites.

The specific structure of these products, with high surface reactivity and existence of a reactive non-apatitic environment enables the CPC to be biocompatible, bioactive, resorbable and have the ability to be mixed with growth factors and bioactive molecules.

Because of their interesting surface chemistry and biocompatibility, the synthetic family can be an excellent vehicle candidate for the controlled delivery of biologically active agents (biomolecules, growth factors etc.). We have shown [11] that the alpha-bsm® setting reaction has numerous favorable characteristics that make it an attractive vehicle for therapeutic agent delivery. These bone substitute materials are clearly distinguished from hydroxyapatite (HA) and other ceramics (bi-aphasic HA/TCP, or beta-TCP granules) by their handling characteristics (injectability and/or moldability), self-setting nature and fast hardening.

Histology evaluation illustrates active remodeling of alpha-bsm®. Specifically, trabeculae are observed which travel between new bone and residual alpha-bsm® without discontinuity. Bone forming cells are frequently observed within fissures and lacunae present in the residual alpha-BSM®, and cell clusters within residual cement are generally well vascularized. The retention of biological activity in a protein which was subjected to the material hardening reaction was confirmed in a study which the enzyme alkaline phosphatase (AP) was added to a Tris buffered saline solution, which was then used to prepare alpha-bsm® paste from the precursor powder.

Following one hour incubation at 37°C in wet environment, the hardened cement was found to contain active AP [31]. The clinical performance criteria of these products can be presented in the Table 2.

Table 2. Clinical performance criteria of synthetic family products.

Products	**α-BSM®**	**β-BSM™**	**γ-BSM™**
Formulation	Convert to Bone Mineral	Convert to Bone Mineral	Convert to Bone Mineral
Mixing System	Closed Bulb Mixing	Closed Syringe to Syringe Mixing	Open Mixing System
Delivery	Injectable and Moldable	Injectable and Moldable	Moldable Putty
Cohesive	Can be Irrigated after Setting	Can be Irrigated after Setting	Can be Irrigated after Setting
Drillability	During & after Setting	During & after Setting	During & after Setting
Remodeling	Cell Mediated Resorption	Cell Mediated Resorption	Cell Mediated Resorption

The performance of CPC can be further enhanced by combining with DBM (Demineralized Bone Matrix) and/or growth factors, offering both osteoconductivity and osteoinductivity. There is significant evidence that these combination products will remodel faster and form bone at superior quality [32].

Clinical trials for alpha-bsm® have been performed in both Europe and United States, follow-up included radiographs, clinical function tests, and wound healing assessments. To date, there have been no adverse events associated with use of this product.

Bioresobable calcium phosphate materials, such as alpha-bsm®, appear to be a better choice than AIBG for the treatment of subarticular defects associated with tibial plateau fractures. A prospective multicenter randomized clinical study (AIBG vs. alpha-bsm®) demonstrated the efficacy of this material, proposing alpha-bsm® as a new gold standard in tibial plateau repair [22].

Conclusion

A new generation of synthetic, injectable & moldable, fast hardening and resorbable CPCs beta-bsm™ and gamma-bsm™ were designed, prepared and characterized *in-vitro & in-vivo*. These cements were formulated based on the same chemistry as alpha-bsm® but ground by using a high energy ball milling process in order to improve their handling and mechanical properties.

High energy dry mechano-chemical processing of hygroscopic CaP material produces simultaneous media-powder collision and local heat generation that causes an inter-diffusion of particles, leading to structural and chemical changes. This processing difference yields cements that harden four times faster, and exhibit a compressive strength four times greater than alpha-bsm®. These performance increases indicate the material has undergone significant structural changes and the process can be used as a method to produce single and/or multi-component CPC.

The end product of all synthetic family products is a low crystalline carbonated apatite similar to the composition and structure of natural bone. The low crystallinity nature and carbonated substitute apatite are characteristic of a greater degradation rate compared with highly crystalline and stoichiometric HA. Following conversion of alpha-bsm®, beta-bsm™ and gamma-bsm™ (after adding hydration media and incubation at 37°C for 15h), the specific surface area of the material increases to 180-200 m^2/g. This is evidence that, despite the initial mechanical (fast hardening & compressive strength) differences, the materials obtain the same identity, both chemically and

structurally, following conversion to an apatitic phase similar to the composition and structure of natural bone mineral.

Because of their interesting surface chemistry, resorption profile and biocompatibility, alpha-bsm®, beta-bsm™ and gamma-bsm™ can be excellent vehicles for the controlled delivery of biologically active agents (biomolecules, growth factors and antibiotics). In-vivo comparison of synthetic family in a rabbit femoral defect model at 52 weeks demonstrates that the three cements share similar resorption characteristics.

References

[1] F.C. Besic, C.R. Knowles, M.R. Wiemann and O. Keller: J. Dental. Res., Vol. 48, (1) (1969), p. 131.

[2] R. Legros, N. Balmain and G. Bonel: J. Chem. Res., Vol. S1, (1986), p. 8.

[3] M. Jarcho, J.F. Kay, H.P. Drobeck and R.H. Doremus: J. Bioeng., Vol. 1, (1976), p.79.

[4] R.Z. LeGeros, A. Chohayeb and A. Shulman: J. Dental Res., Vol. 61, (1982), p. 343.

[5] W.E. Brown and L.C. Chow: J. Dental Res., Vol. 62, (1983), p.672.

[6] B.R. Constantz, I.C. Ison and M.T. Fulmer et al.: Science, Vol. 267, (1995), p.1796.

[7] C. Rey, A. Tofighi, S. Mounic, C. Combes and D. Lee: Actualites en Biomateriaux Edition Romillat, Paris, Vol. 6, (2002), p. 27.

[8] C.D. Friedman, P.D. Costantino, S. Takagi and L.C. Chow: J. Biomed. Mater. Res. Vol. 43 (1998), p. 428.

[9] A.A. Mirtchi, J. Lemaitre and N. Terao: Biomaterials, Vol. 10, (1989), p. 475.

[10] Z. Hatim, M. Freche, A. Kheribech and J.L. Lacout: Ann. Chim. Sci. Mat., Vol. 23, (1998), p. 65.

[11] D. Lee, A. Tofighi and M. Aiolova et al.: Clinical Orthopaedics and Related Research, 367 (Suppl), (1999), p. S396-S405.

[12] A. Tofighi, S. Mounic, P. Chakravarthy, C. Rey and D. Lee: Key Engineering Materials, Vol. 192-195, (2001), p. 769.

[13] H.D. Zegzula, D.C. Buck, J. Brekke, J.M. Wozney and J.O. Hollinger: The Journal of Bone & Joint Surgery, Vol. 79A, (1997), p. 779.

[14] J. Schmitt, D.C. Buck, S.P. Joh, S.E. Lynch and J.O. Hollinger: J. Periodontal Vol. 68, (1997), p. 1043.

[15] J.O. Hollinger, J. Schmitt and D.C. Buck: J. Biomed Mater Res., Vol. 43, (1998), p. 356.

[16] J.O. Hollinger, D.C. Buck and J. Schmitt: Trans Orthop. Res. Soc., Vol. 22, (1999), p. 189.

[17] P. Patka, H.J. Th. M. Haarman and F.C. Bakker: Ned Tijdschr Geneeskd., Vol. 142, No.16, (1998), p. 893.

[18] M.R. Sarkar, I.P. Hoellen, J.F. Shao, N. Wachter, L. Kinzl and Wenz R: Transactions of the Society for Biomaterials, Vol. 22, (1999), p. 92.

[19] W. Linhart, D. Briem, N.D. Schmitz, M. Priemel, W. Lehmann and J.M. Rueger: Unfallchirurg Vol. 106, No.8, (2003), p. 618.

[20] T.A. Russell, R.K. Leighton, R.W. Bucholz, C.N. Cornell, S. Agnew and R.F. Ostrum: 20th Orthopedic Trauma Association (OTA) Ottawa, CA, Vol. 10, (2004), p. 109

[21] A. Trenholm, S. Landry, K. McLaughlin, K.J. Deluzio, J. Leighton and K. Trask: J Orthop. Trauma, Vol. 19, No.10, (2005), p. 698.

[22] T.R. Russell, R.K. Leighton and R.W. Bucholz et al.: Vol 90, (2008), p.2846-2847.

[23] A. Tofighi and R. Palazzolo: Key Engineering Materials, Vol. 284-286, (2005), p. 101.

[24] A. Tofighi, R. Palazzolo, A. Rosenberg, K. Schaffer and S. Woods: Orthopaedic Research Society (ORS) & AAOS, No.1563, (2007), p. 124.

[25] A. Tofighi and C. Rey: U.S. Patent 7,318,841. (2008)

[26] A. Just and M. Yang: Powder & Bulk Solids Technology Union Process Inc., Akron, Ohio, USA (1997).

[27] L.C. Chow and L. Takagi: J. Res. Natl. Stand. Technolo. Vol. 106 (2001), p. 1029.

[28] P.J. Catalano, G. Insley and B. Hess: Key Engineering Materials, Vol. 330-332, (2007), p. 799.

[29] M.D. Francis and N.C. Webb: Calcif. Tissue, Res., Vol. 6, (1971), p. 335.

[30] S. Sarda, A. Tofighi, M.C. Hobatho, D. Lee and C. Rey: The Journal for Inorganic Phosphorus Chemistry, Phosphorus Research Bulletin, Vol. 10, (1999), p. 208.

[31] D. Knaack, M. Aiolova, A. Tofighi, A. Catalano and D. Lee: *CIMTEC-World Forum on New Materials*, Materials in Clinical Applications, edited by P. Vincenzini, Vol. 9, (1999), p. 55.

[32] A.H. Reddi: The Journal of Bone & Joint Surgery Vol. 83-A, Sup. 1, Part 1, (2001), p. S1-1.

Journal of Biomimetics, Biomaterials and Tissue Engineering Vol.2
doi:10.4028/www.scientific.net/JBBTE.2

New Generation of Synthetic, Bioresorbable and Injectable Calcium Phosphate Bone Substitute Materials: Alpha-bsm®, Beta-bsm^{TM} and Gamma-bsm^{TM}
doi:10.4028/www.scientific.net/JBBTE.2.39

References

[1] F.C. Besic, C.R. Knowles, M.R. Wiemann and O. Keller: J. Dental. Res., Vol. 48, (1) (1969), p. 131.

[2] R. Legros, N. Balmain and G. Bonel: J. Chem. Res., Vol. S1, (1986), p. 8.

[3] M. Jarcho, J.F. Kay, H.P. Drobeck and R.H. Doremus: J. Bioeng., Vol. 1, (1976), p.79.

[4] R.Z. LeGeros, A. Chohayeb and A. Shulman: J. Dental Res., Vol. 61, (1982), p. 343.

[5] W.E. Brown and L.C. Chow: J. Dental Res., Vol. 62, (1983), p.672.

[6] B.R. Constantz, I.C. Ison and M.T. Fulmer et al.: Science, Vol. 267, (1995), p.1796.
doi:10.1126/science.7892603
Can't connect to PubMed

[7] C. Rey, A. Tofighi, S. Mounic, C. Combes and D. Lee: Actualites en Biomateriaux Edition Romillat, Paris, Vol. 6, (2002), p. 27.

[8] C.D. Friedman, P.D. Costantino, S. Takagi and L.C. Chow: J. Biomed. Mater. Res. Vol. 43 (1998), p. 428.
doi:10.1002/(SICI)1097-4636(199824)43:4<428::AID-JBM10>3.0.CO;2-0
Can't connect to PubMed

[9] A.A. Mirtchi, J. Lemaitre and N. Terao: Biomaterials, Vol. 10, (1989), p. 475.
doi:10.1016/0142-9612(89)90089-6
Can't connect to PubMed

[10] Z. Hatim, M. Freche, A. Kheribech and J.L. Lacout: Ann. Chim. Sci. Mat., Vol. 23, (1998), p. 65.
doi:10.1016/S0151-9107(98)80024-X
Can't connect to PubMed

[11] D. Lee, A. Tofighi and M. Aiolova et al.: Clinical Orthopaedics and Related Research, 367 (Suppl), (1999), p. S396-S405.
doi:10.1097/00003086-199910001-00038
Can't connect to PubMed

[12] A. Tofighi, S. Mounic, P. Chakravarthy, C. Rey and D. Lee: Key Engineering Materials, Vol. 192-195, (2001), p. 769.
doi:10.4028/www.scientific.net/KEM.192-195.769
Can't connect to PubMed

[13] H.D. Zegzula, D.C. Buck, J. Brekke, J.M. Wozney and J.O. Hollinger: The Journal of Bone & Joint Surgery, Vol. 79A, (1997), p. 779.

[14] J. Schmitt, D.C. Buck, S.P. Joh, S.E. Lynch and J.O. Hollinger: J. Periodontal Vol. 68, (1997), p. 1043.

[15] J.O. Hollinger, J. Schmitt and D.C. Buck: J. Biomed Mater Res., Vol. 43, (1998), p. 356.
doi:10.1002/(SICI)1097-4636(199824)43:4<356::AID-JBM3>3.0.CO;2-7
Can't connect to PubMed

[16] J.O. Hollinger, D.C. Buck and J. Schmitt: Trans Orthop. Res. Soc., Vol. 22, (1999), p. 189.

[17] P. Patka, H.J. Th. M. Haarman and F.C. Bakker: Ned Tijdschr Geneeskd., Vol. 142, No.16, (1998), p. 893.

[18] M.R. Sarkar, I.P. Hoellen, J.F. Shao, N. Wachter, L. Kinzl and Wenz R: Transactions of the Society for Biomaterials, Vol. 22, (1999), p. 92.

[19] W. Linhart, D. Briem, N.D. Schmitz, M. Priemel, W. Lehmann and J.M. Rueger: Unfallchirurg Vol. 106, No.8, (2003), p. 618.
doi:10.1007/s00113-003-0628-3
Can't connect to PubMed

[20] T.A. Russell, R.K. Leighton, R.W. Bucholz, C.N. Cornell, S. Agnew and R.F. Ostrum: 20th Orthopedic Trauma Association (OTA) Ottawa, CA, Vol. 10, (2004), p. 109

[21] A. Trenholm, S. Landry, K. McLaughlin, K.J. Deluzio, J. Leighton and K. Trask: J Orthop. Trauma, Vol. 19, No.10, (2005), p. 698.
doi:10.1097/01.bot.0000183455.01491.bb
Can't connect to PubMed

[22] T.R. Russell, R.K. Leighton and R.W. Bucholz et al.: Vol 90, (2008), p.2846-2847.

[23] A. Tofighi and R. Palazzolo: Key Engineering Materials, Vol. 284-286, (2005), p. 101.
doi:10.4028/www.scientific.net/KEM.284-286.101
Can't connect to PubMed

[24] A. Tofighi, R. Palazzolo, A. Rosenberg, K. Schaffer and S. Woods: Orthopaedic Research Society (ORS) & AAOS, No.1563, (2007), p. 124.

[25] A. Tofighi and C. Rey: U.S. Patent 7,318,841. (2008)

[26] A. Just and M. Yang: Powder & Bulk Solids Technology Union Process Inc., Akron, Ohio, USA (1997).

[27] L.C. Chow and L. Takagi: J. Res. Natl. Stand. Technolo. Vol. 106 (2001), p. 1029.

[28] P.J. Catalano, G. Insley and B. Hess: Key Engineering Materials, Vol. 330-332, (2007), p. 799.
doi:10.4028/www.scientific.net/KEM.330-332.799
Can't connect to PubMed

[29] M.D. Francis and N.C. Webb: Calcif. Tissue, Res., Vol. 6, (1971), p. 335.
doi:10.1007/BF02196214
Can't connect to PubMed

[30] S. Sarda, A. Tofighi, M.C. Hobatho, D. Lee and C. Rey: The Journal for Inorganic Phosphorus Chemistry, Phosphorus Research Bulletin, Vol. 10, (1999), p. 208.

[31] D. Knaack, M. Aiolova, A. Tofighi, A. Catalano and D. Lee: CIMTEC-World Forum on New Materials, Materials in Clinical Applications, edited by P. Vincenzini, Vol. 9, (1999), p. 55.

[32] A.H. Reddi: The Journal of Bone & Joint Surgery Vol. 83-A, Sup. 1, Part 1, (2001), p. S1-1.