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The micro macroporous biphasic calcium phosphate concept for bone reconstruction and tissue engineering

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SUMMARY

Developping calcium phosphate ceramics and other related biomaterials for bone grafts required better control of biomaterial resorption and bone substitution processes. The biphasic calcium phosphate ceramics (BCP) concept is determined by an optimum balance between the more stable HA phase and the more soluble TCP. The material is soluble and gradually dissolves in the body, seeding new bone formation as it releases calcium and phosphate ions into the biological medium.

The main attractive feature of BCP ceramic is its ability to form direct bone bonding with host bone, resulting in a strong interface. The formation of this dynamic interface is the result of a sequence of events involving interaction between biological fluid and cells, as well as the formation of carbonate hydroxyapatite (CHA), which is similar to bone mineral, by means of dissolution/precipitation processes. Associating micro and macroporosity with the BCP chemical concept resulted in high osteogenicity and osteoinductive properties. At the present time, micro macroporous scaffolds are commercially available in blocks, particulates and customised designs, and specific matrices have been developed for combination with bone marrow or mesenchymal stem cells for tissue engineering (hybrid bone). The search for the ideal scaffold for tissue egineering and bone reconstruction in low trophic areas or large bone reconstruction remains a challenge, as those currently available are not appropriate.

In addition, the need for material for Minimally Invasive Surgery (MIS) has led to the development of a concept combining specific granules with polymer or self setting calcium phosphate cement for injectable/mouldable bone substitutes. Different types of injectable/mouldable bone substitutes have been developed: a) injectable biomaterial without initial hardening, where BCP granules are associated with a hydrosoluble polymer; b) the association of MBCP and fibrin sealant, c) the association of synthetic self hardening polymers and d) new generation macroporous calcium phosphate cements.

The purpose of this paper is to review the fundamental properties of biphasic calcium phosphate bioceramics, their biological properties, the development of new technologies for tissue engineering, and examples of clinical applications.

INTRODUCTION

In 1920, Albee reported the first successful application of a calcium phosphate reagent for the repair of a bone defect in humans [1]. More than 50 years later, clinical use of a "tricalcium phosphate" preparation in surgically-created periodontal defects in animals was reported by Nery *et al.* [2] and the use of dense hydroxyapatite HA as an immediate replacement for tooth root was reported by Dennnissen [3]. Largely through the separate efforts of Jarcho, de Groot and Aoki in the early 1980s [4-7], synthetic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), became commercially available as bone substitute materials for dental and medical applications. The BCP concept has been widely developed by Daculsi and Legeros since the 1990s [8-11].

Developping BCP ceramics and other related biomaterials for bone grafts required controlling the processes of bioceramic resorption and bone formation at the expense of the biomaterial. Synthetic bone graft materials are available as alternatives to autogeneous bone for repair, substitution or augmentation.

The BCP concept [10] is based on an optimum balance between the more stable phase (HA) and the more soluble phase (β-TCP). BCP bioceramics are soluble and gradually dissolve *in vivo*, seeding new bone formation as it releases calcium and phosphate ions into the biological medium. Commercial BCP bioceramics consist of a mixture of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ and beta-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$ of varying HA/β-TCP ratios (Table 1).

The main attractive feature of bioactive bone graft materials such as BCP ceramic is its ability to form a strong direct bond with host bone resulting in a strong interface compared to bioinert or biotolerant materials which form a fibrous interface [9-15]. The formation of the dynamic interface between bioactive materials and host bone is believed to be the result of a sequence of events involving interaction with cells and the formation of carbonate hydroxyapatite (CHA), which is similar to bone mineral, by means of dissolution/precipitation processes [12,15].

At the present time, commercial BCPs are sold in Europe, the US, Brazil, Japan, Korea, Taiwan and China as bone-graft or bone substitute materials for orthopaedic and dental applications under various trade marks. Currently, BCP bioceramics are recommended for use as an alternative or additive to autogeneous bone for orthopaedic and dental applications. These commercial BCPs are available in blocks, particulates (granules), and custom-designed

shapes like wedges for Tibial Opening Osteotomy, cones for spine and knee, and inserts for vertebral cage fusion.

Exploratory studies have demonstrated the potential uses for BCP ceramic as a scaffold for tissue engineering in bone regeneration, gene therapy, and drug delivery. More recently, the BCP granule concept has been applied to the development of a new generation of injectable, mouldable bone substitutes [16,17]. BCP granules are combined with various polymers, natural (e.g. fibrin sealant), or synthetic (e.g. hydrosoluble polymer), to develop an injectable bone substitute, IBS [17], or calcium phosphate cement to improve macroporosity and provide greater osteoconduction. Three new products have been developed recently: MBCP Gel® (an injectable, non-self hardening biomaterial composed of BCP granules combined with a hydrosoluble polymer); Tricos® (a combination of BCP and fibrin sealant as bone substitute biomaterials); and MCPC® (a micro- and macro-porous calcium phosphate cement containing BCP granules that produces a cement with interconnecting macroporosity [18]).

The present review focuses on the main physical and chemical properties of various BCP bioceramics such as micro and macrostructure, role and performance of different HA/TCP ratios, new developments in bioceramics for injectable, mouldable BCP bioceramics and the clinical relevance of such bioceramics.

1/ The fundamental physical, chemical and biological properties of biphasic calcium phosphate bioceramics

Introduction to macroporosity and microporosity

Two physical properties of bioceramics were considered to be very important for optimum biological performance in bioceramic-cell interaction, bioceramic resorption, the bioceramic-tissue interface and new bone formation. These fundamental physical properties are interconnecting macroporosity and appropriate microporosity [19,20].

Macroporosity in BCP ceramic is introduced by incorporating volatile materials (e.g. naphthalene, hydrogen peroxide or other porogens), heating at temperatures of less than 200 ^{o}C and subsequent sintering at higher temperatures [19, 21-25]. Macroporosity is formed as a result of the release of the volatile materials (Fig. 1). Microporosity is a consequence of the temperature and duration of sintering [20]: the higher the temperature, the lower the microporosity content and the lower the specific surface area (Fig. 2).

At present, commercial BCP products of different or similar HA/β-TCP ratios are manufactured in many parts of the world and their successful use in medicine and dentistry has been reported [11,26-31]. The total porosity (macroporosity plus microporosity) of these products is reported to be about 70 % of the bioceramic volume. Current BCP commercial products with HA/β-TCP ratios ranging from 60/40 to 75/25 (Table 1), present similar macroporosity percentages (50 to 60 %), but microporosity percentages are very different, varying from 3 % to 25 %.

A low microporosity percentage and low surface area can result in lower bioactivity and lower dissolution properties. Microporosity of at least 20 % with a specific surface area of more than 2 m^2/g is required for optimal BCP efficacy.

Ideally, pore size for a bioceramic material should be similar to that of bone. It has been demonstrated that microporosity (diameter < 10 μm) allows body fluid circulation whereas macroporosity (diameter > 100 μm) provides a scaffold for bone-cell colonisation.

Significant improvements in the method for introducing macroporosity/microporosity have recently been developed in the production of micro- macro-porous BCP (MBCP2000®, Biomatlante, France) [32]. In this method, CDA is mixed with a combination of selected particles of naphthalene and sugar. After isostatic compaction, the CDA block is subjected to a specific process of sublimation/calcination. The BCP obtained using the classic naphthalene porogen (MBCP) compared to that using a mixture of porogens, naphthalene and sugar (MBCP2000), resulted in differences in density, Specific Surface Area (SSA) of the crystal, compression strength and total porosity (Table 2). The permeability after incubation in bovine serum of MBCP2000 was twice as high as that of MBCP, and MBCP2000 showed a 30 % increase in absorption compared to MBCP. The considerably higher permeability of MBCP2000 compared to MBCP cannot be explained by any difference in total porosity but may be attributed to differences in pore size, particularly mesopores.

Physical and chemical properties

As β-TCP is more soluble than HA [33], the extent of dissolution of BCP ceramics of comparable macroporosity and particle size will depend on the HA/β-TCP ratio: the higher the ratio, the lower the extent of dissolution [8,10,13]. The dissolution properties are also affected by the methods used in producing the BCPs: whether from a single calcium–deficient apatite phase (BCP1) or from a mechanical mixture of two unsintered calcium phosphate preparations (BCP2): BCP2 has a higher extent of dissolution than BCP1 [33]. In some cases,

BCP ceramic with similar HA/β-TCP ratios could present different dissolution rates [34]. This phenomenon may be caused by processing variables (sintering time and temperature) that may affect total macroporosity and microporosity: the greater the macroporosity and microporosity, the greater the extent of dissolution. *In vivo*, dissolution of BCP ceramics is manifested by a decrease in crystal size and an increase in macro- and microporosity [9-12].

Mechanical properties

It is logical for the pore size and percentage of macroporosity of the BCP ceramic to affect the mechanical properties [22,35]. The preparation method has also been found to have a significant influence on compressive strength. BCP ceramic prepared from a single calcium-deficient apatite phase is reported to have higher compressive strength (2 to 12 MPa) than BCP ceramic prepared by mixing two unsintered calcium phosphate preparations (2MPa): one which, after sintering at 1200^{o}C, results in only HA and the other which results in only β-TCP [34]. Initial mechanical property is not the best criterion for evaluating the efficacy of bone ingrowth. For example, BCP with high mechanical properties because of low microprosity (as a result of a high sintering temperature) may have reduced bioresorption and bioactivity. On the contrary, it has been demonstrated that the initial mechanical property of BCP increased two or three times (2 to 6 MPa) in a few weeks after implantation thanks to the physical and chemical events of dissolution and biological precipitation into the micropores [12].

Bioactivity, osteogenic properties

Bioactivity is described as the property of a material to form carbonate hydroxyapatite (CHA) on its surface *in vitro* [15, 36] or *in vivo* [9, 37-39]. Osteoinductivity or osteogenic property is the property of the material to induce bone formation *de novo* or ectopically (in non-bone forming sites). Bioceramics (calcium phosphates, bioactive glass) do not usually have osteoinductive properties [19]. However, several reports have shown the osteoinductive properties of certain calcium phosphate bioceramics such as coralline HA (derived from coral) or those observed in certain studies using BCP [40-41]. Reddi [42] explains these apparent osteoinductive properties as the ability of particular ceramics to concentrate bone growth factors that are circulating in the biological fluids, and these growth factors induce bone formation. Ripamonti [43] and Kuboki [44] independently postulated that the geometry of the material is a critical parameter in bone induction. Others have speculated that low oxygen tension in the central region of implants might provoke a dedifferentiation of pericytes from blood microvessels into osteoblasts [45]. It has been also postulated that the

nanostructured rough surface or the surface charge of implants might cause the asymmetrical division of stem cells into osteoblasts [46].

Surface microstructure appears to be a common property of the materials that induce ectopic bone formation. Recent studies have indicated the critical role played by micropores on ceramic-induced osteogenesis. For example, it has been reported that bone formation occurred in dog muscle inside porous calcium phosphate ceramics with surface microporosity but bone was not observed inside the dense surface of macroporous ceramics[47]. It has also been reported that metal implants coated with a microporous layer of octacalcium phosphate could induce ectopic bone in goat muscle, while a smooth layer of carbonated apatite on these porous metal implants was not able to induce bone formation [48]. In all the previous experiments, ectopic bone formation occurred inside the macroporous ceramic blocks.

It has been demonstrated that sintering temperature has a drastic effect on the microporosity of calcium phosphate ceramics: the higher the sintering temperature, the denser the ceramic surface. To evaluate microporosity, how it is produced and the role it plays, we precipitated calcium-deficient apatite (CDA) prepared to provide BCP with an HA/β-TCP ratio of 60/40 $\pm$ 2, then sintered between 1000 °C and 1200 °C; discs (25 mm diameter) were prepared for an *in vitro* test and machined into implants (3 mm diameter) for *in vivo* implantation. The discs were distributed into five groups: D1, D2, D3, D4 and D5, and sintered using various conditions (heating rate and temperature) (Fig.2). All groups were subjected to the same rate of temperature rise (5 °C/min), cooling rate (1 °C/min) and total sintering period (5 h). The discs in groups D2, D3 and D4 were heated to 900 °C then allowed to remain at this temperature for 3 h (D2) or 12 h (D3 and D4). The final temperature was 1050 °C for groups D1, D2 and D3, and 1200 °C for groups D4 and D5. XRD and FTIR analyses of the sintered discs showed only the BCP phase with an HA/β-TCP ratio of 60/40. Surface area, microporosity %, cell coverage and disc properties are summarised in Table 2.

Composition: XRD analysis showed the final HA/βTCP ratios for all BCP specimens to be 60/40 ± 2, independent of the sintering conditions. No differences in relative intensities or broadening of the diffraction peaks were observed in any of the BCP specimens. In the FTIR spectra, only absorption bands attributed to the OH group for the HA and to the PO_4 groups for the HA and the β-TCP were observed. An apparent decrease in the intensity of the OH absorption bands, loss of resolution and broadening of the OH and PO_4 absorption bands were observed in the FTIR spectra of BCP specimens D4 and D5 compared to those of D1, D2, and D3.

Dissolution properties: Dissolution experiments in an acidic buffer (0.1M KAc, pH 6, 37 °C) showed differences in the extent of dissolution as reflected by the concentration of Ca ions released into the acidic buffer over time. Maximum dissolution (maximum change in Ca concentration in the buffer) was observed in the first 10 minutes after exposing the BCP discs to the acidic buffer, and no significant change in Ca concentration was observed after 60 minutes. The BCP discs in groups D1 and D2 behaved almost identically followed by D3, indicating greater solubility than the BCP discs in groups D4 and D5.

Cell proliferation and colonization: An established mouse fibroblast cell line L929 was used. After 7 days of culture, it was observed that percentage cell coverage on the BCP disc surfaces differed: much higher percentage coverage was observed on the surfaces of D1 and D2 discs (80 and 60 %, respectively) than on the surfaces of D3, and even lower for surfaces of D4 and D5 discs (about 10 to 20 %), as shown in Table 2. The cells on BCP discs D1 and D2 were observed to have proliferated and to be present all over the surface of the discs while the cells on the D4 and D5 discs showed much less proliferation and were observed only on the areas where they were originally deposited. The cells on the surface of the D1 and D2 discs had polygonal shapes while the cells on D4 and D5 appeared to be more rounded and contracted. Furthermore, the cells on D5 were characterised by long filopodia. The surfaces of the D1 and D2 discs appeared grainy and rougher while the surfaces of the D5 discs appeared smooth and similar to the surface as it was before the *in vitro* experiment.

In vivo experiment: The right and left epiphyses of rabbits were implanted, and two defects created, one in the upper part (cancellous bone) and one in the diaphysis (no bone trabeculae, only bone marrow). After 3 weeks, the implants were processed for histology. All the implanted BCP discs showed good biocompatibility. However, bone growth and bone contact were very different for the discs implanted in the cancellous bone of the epiphysis compared to those in bone marrow: the higher the density, the lower the osteogenicity, particularly for the discs implanted in the bone marrow site. Moreover, the higher the density (D5 discs), the lower the amount of newly-formed bone in direct contact with the implant surface (Figs 3, 4, 5).

Current FDA and/or CE-approved commercial BCP products (e.g., *MBCP®, Biosel®, TCH®, Calciresorb®, Triosite®, Tribone® 80, OpteMix®, Hatric®*, etc) with similar HA/βTCP ratios and similar percentage macroporosity (60 to 70 %) can vary in the interconnectivity of their macroporosity and in their percentage microporosity. Differences in the porogens used to provide the macroporosity, plus differences in sintering temperature and conditions, all affect percentage microporosity. It is therefore not surprising that these products may show different

in vivo performances. For the BCP bioceramic to have osteogenic/osteoinductive properties, high percentage microporosity and macroporosity are required. These data showed that higher osteogenic properties were observed with BCP discs with lower density (produced at a lower sintering temperature). These results confirm that microporosity promotes osteogenic/osteoinductive properties in BCP bioceramics.

The properties of ceramic, such as composition, geometry, porosity, size and microstructure, should be considered as critical parameters for bone induction. These properties play a more important role in bone induction than in mechanical stability [49].

We can explain such osteogenic/osteoinductive properties for BCP ceramics by the formation of microcrystals with Ca/P ratios similar to those of the bone apatite crystals observed after implantation of MBCP. The abundance of these crystals was directly related to the initial ß-TCP/HA ratio in the BCP: the higher the ratio the greater the abundance of the microcrystals associated with the BCP crystals. Using high resolution TEM, we demonstrated that the formation of these bone apatite-like microcrystals after implantation of calcium phosphates (HA, BCP) was non-specific, i.e., not related to implantation site (osseous or non-osseous sites), subject of implantation, or type of CaP ceramics.

The coalescing interfacial zone of biological apatite and residual BCP ceramic crystals (mostly HA), provides a scaffold for further bone-cell adhesion and stem cell differentiation into osteogenic lines, and further bone ingrowth. The bone repair or bone regeneration process involves dissolution of calcium phosphate crystals and then a precipitation of needle-like carbonate hydroxyapatite (CHA) crystallites in micropores close to the dissolving crystals. The coalescing zone forms the new biomaterial/bone interface, which includes the participation of proteins and CHA crystals originating from the BCP ceramic crystals. This has been described as a coalescing zone and dynamic interface (50).

If we consider that the coalescing zone (chemical bonding) caused by precipitation of the biological apatites is related to dissolution of the calcium phosphate (higher for b-TCP) [10,15], we can suspect that the kinetics of bone ingrowth at the expense of the bioceramics is directly related to the HA/TCP ratio of the biphasic calcium phosphate. In 1998, we reported using TEM the difference at the crystal level between HA, TCP and various types of BCP. But we have never published data at the bony tissue level. To determine the influence of ratio on tissue ingrowth and bioceramic resorption, we used different types of BCP (pure HA, pure β-TCP, HA/TCP 27/75, 50/50 and 75/25) for filling maxillofacial defects in dog mandibulars (only granules with micropore content), and critical size defects in dog femoral epiphyses (6 mm in diameter filled with a cylindrical implant that was both micro and macroporous). The

implantation period was 4, 6 or 12 weeks. From the two studies it appears that no statistical difference was measured for either bone ingrowth or bioceramic resorption in the BCP samples. Large bone remodelling appears in both BCP samples (Fig 6) with well-architectured bone (lamellar bone), and osteoconduction was evidenced. For the pure HA and pure β-TCP, significant differences were measured for bioceramic resorption, the higher resorption over time from 4 to 12 weeks was reported for TCP. However, in spite of the considerable resorption for TCP, there was no more bone ingrowth than for HA, the TCP appeared to have been resorbed without osteoconduction, and cells and unmineralised tissue had taken the place of the TCP (figure 7). For HA, as there was no apparent resorption, the space for bone ingrowth was still limited. On the contrary, for both types of BCP we observed an equilibrium between bioceramic resorbtion and bone ingrowth. At 12 weeks no significant difference was measured in the 3 types of BCP tested, in spite of what appeared to be a slight increase in bioceramic resorption for the higher HA/TCP ratio (75/25).

2/ Bioceramics: new developments

At present, granules and particles are used increasingly in mouldable, injectable or resorbable composites. However, the biological behaviour of the particles can be influenced not only by chemical composition and crystalinity, but by several other parameters such as granulometry and microporosity. The influence of microporosity on new-bone formation, though apparently one of the more complicated variables, has rarely been studied, unlike macroporosity. A recent study by O. Malard *et al.* [51] is reported. Two different porosities of biphasic calcium phosphate granules were prepared (20 % and 40 % porosity) and evaluated in rat critical size defects. This study sought to specify the role of microporosity in an *in vivo* experiment, as well as examine the amount and kinetics of newly-formed bone ingrowth, and the biodegradation of BCP ceramic. LP (20 %) and HP (40 %) microporous granules were prepared from calcium-deficient apatite (CDA) sintered at 1050 °C which resulted in a biphasic calcium phosphate BCP of 60 % HA and 40 % β-TCP.

Physical and chemical characterisation: XRD showed BCP content without trace of carbonate, with an HA/β-TCP ratio of 60/40. No difference was observed between the HP and LP granules.

SEM image analysis showed that porosity was 17.6 % ± 3.6 for LP granules and 39.7% ± 10.3 for HP granules ($p<0.001$). LP granules were regular rounded granules and HP granules were more irregular in shape, with a sharper surface.

Density of the LP was 3.096 gram per cm3 ± 0.003 and was 3.093 gram per cm3 ± 0.002 for the HP (no significant difference, p=0.18). SEM image analysis showed that the LP granules had a mean diameter granulometry of 780 µm ± 148 and the HP samples of 814 µm ± 207 (no significant difference, p=0.13).

After 3 and 6 weeks of implantation, newly-formed bone was observed in both samples. Bone ingrowth was less apparent at 3 weeks than at 6 weeks and seemed to develop faster after implantation of high porosity granules. In the early stages, it consisted of woven bone containing many osteocytes. Newly-formed bone was observed mainly in the deep zones of the implanted defects from the surface to the core, initially in close contact with the BCP particles (without fibrous interposition) but always close to the bordering bone. Osteoid appeared around the ceramic particles and was observed at the early time of 3 weeks. Using SEM, bone ingrowth at 3 weeks in the LP group was 5 % ± 0.3 of the implanted defect, and was greater than in the HP group (1.6% ± 0.4, p<0.0001). At 6 weeks, in the LP group, the amount of newly-formed bone was 4.3% ± 0.5 of the implanted defect, and was lower than in the HP group (8.6% ± 1.6, p=0.044). Between 3 and 6 weeks, the amount of newly-formed bone increased in the HP group (p<0.0001). During the same interval, the amount of newly-formed bone did not significantly increase in the LP group (p=0.25). The BCP surface area in the implanted bone defects at 3 weeks was not statistically different between the 2 groups (p=0.1), and no more significant at 6 weeks (p=0.8). The decrease in the BCP surface area was not significant between 3 to 6 weeks in the LP group (p=0.4) or in the HP group (p=0.3). With the LP granules, new bone formation was significantly greater at 3 weeks than with HP. This could be related to higher macrophagous activity with regard to the release of large particles (as confirmed by histological examination), in addition to the release of Ca and P ions. Between 3 and 6 weeks, newly-formed bone did not significantly increase in the LP granules, whereas it did with the HP (5-fold increase), confirming previous studies about high initial inflammation, and macrophagous activity acting as a booster for octeogenic cell differentiation [52]. At the later time of 6 weeks, there was an inversion in the amount of newly-formed bone, with more newly-formed bone in the HP implants confirming the osteo-inductive property of the micropores as described in other animal models. The reason why granules that are less porous can induce faster bone ingrowth is not clear and needs further experiments. It has not been clearly elucidated why higher porosity is related to a huge increase in new bone formation after 3 weeks.

Micropores for calcium phosphate bone substitutes are one of the most important parameters for promoting or inducing bone formation. In addition to macropores and chemical nature,

control of micropore size and distribution must be taken into account when developping high osteogenic scaffolds for tissue engineering.

BCP-based macroporous cements

The need for a material for Minimally Invasive Surgery (MIS) prompted the development of a concept for injectable, mouldable calcium phosphate cement (CPC) as bone substitutes. Currently, several calcium phosphate bone cements are commercially available and more are being investigated. The concept was first introduced by LeGeros *et al.* in 1982 [52] and the first patent was obtained by Brown and Chow [53] in 1986. All the current CPCs are reported to have good mechanical properties and reasonable setting times. However, after setting, these materials remain dense and do not provide rapid bone substitution because of the lack of macroporosity. Numerous studies have reported the applications of currently available commercial calcium phosphate cements [54,55]. A new BCP-based calcium phosphate cement has recently been developed (patent) [56]. The MCPC consists of multiphasic calcium phosphate phases, including BCP, and *in vivo*, the components of the cement resorb at different rates allowing the formation of interconnecting macroporosity, thus facilitating bone ingrowth and substitution of the cement with the newly-forming bone [18].

The powder component is essentially made of a settable and resorbable matrix (which includes alpha-TCP, stabilised amorphous calcium phosphate (s-ACP) and monocalcium phosphate monohydrate, MCPM). A sieved fraction of macroporous biphasic calcium phosphate (BCP) granules ranging between 80 and 200 μm in diameter are incorporated into the matrix. The cement liquid is an aqueous solution of Na_2HPO_4.

After setting MCPC in distilled water at 20 °C, the mechanical properties in compression of such materials were 10 Mpa ± 2 at 24 h and 15 MPa ± 2 after 48 h. The cohesion time for injectability was reached after 20 min. Animal models using critical size defects in rabbit epiphyses or goat vertebral bodies demonstrate the performance and efficacy of this concept of calcium phosphate cement. MBCP granules act as a scaffold for bone osteoconduction, and resorption of the ACP content of the cement allowed macroporosity and bone ingrowth between and at the surface of the BCP granules, extending to the core of the implanted site. The cement matrix dissolved as was expected, forming an open structure and interconnecting porosity.

SEM analyses showed that organised bone trabeculae were well differentiated from the residual granules. After 12 weeks, few granules were fully integrated into the new cortical bone and deeper into the core, spongious bone was formed. Bone remodelling was in

evidence at both 6 and 12 weeks in rabbits and less extensively in the goat model, in spite of 6 months of implantation (Fig. 8). These differences could be explained by the mechanical strain and the osteogneic properties of the implantation sites. X-ray microtomography (microCT) demonstrated bone ingrowth at the expense of the cement and surrounding the residual BCP granules. Bone trabeculae were observed coming from the spongious bone to the implant site. Resorption of the BCP granules was evident from 6 to 12 weeks.

How to improve the radiopacity of bioactive injectable bone substitutes:

In the context of bone healing, biomaterials need to have certain specific characteristics: providing bone regeneration, being resorbable, having mechanical or rheological properties [57]. Nowadays, for Minimally Invasive Surgery (MIS), X-ray contrast performance also provides important data, as the radiopacity of materials is required during the implantation process to ensure the location of the filled material [58]. Various radiopaque agents (RA) such as barium sulfate, zirconium oxide or iodine have been tested (mechanical properties, cytotoxicity effects) [59]. In vertebroplasty, the non resorbable acrylic bone cements (polymethyl methacrylate, PMMA) contain barium sulphate [60]. To obtain a resorbable injectable bone substitute with rheological properties and x-ray contrast characteristics, Serge Baroth et al [61] have developed "rounded" calcium phosphate granules loaded with an RA such as barium salt or with rare earth elements containing bismuth, lutetium and gadolinium. The 4 RA produced were: barium sulphate ($BaSO_4$), lutetium oxide (Lu_2O_3), bismuth oxide (Bi_2O_3) and gadolinium phosphate ($GdPO_4$). CDA (BCP 60-40) were mixed with RA (20 % w/w) to obtain radiopaque calcium phosphate composites. The composites were shaped in 80-200 µm "rounded" granules or pellets (10 mm in diameter, 1 mm thickness). The materials were sintered according to specific processes. Steam sterilisation (121 °C, 30 minutes for pellets) or dry sterilization (180 °C, 4 hours for granules) were used for *in vitro* and *in vivo* tests.

X-ray diffraction (XRD) and infra-red spectroscopy (FTIR) showed integration of the Ba into the CaP crystal lattice, while no substitution was noticed for the other RA used. SEM observation of the composite surfaces showed that the addition of our RA to the calcium phosphate matrix had an influence on crystal shape, crystal size and microporosity. With the addition of $BaSO_4$, large crystals were obtained with low microporosity and a smooth surface. With the addition of Bi_2O_3, crystals grew in needle shapes with significant microporosity. With Lu_2O_3 and $GdPO_4$, the microporosity was significant with small crystals and a number of fragments present on the BCP/Lu_2O_3 matrix.

RA cytotoxicity was quantified *in vitro* according to ISO 10993-5 on extracted using osteoblastic cells MC3T3-E1. The RA cytotoxicity test showed no adverse effect (when MTS activity decreased more than 20 %) on $BaSO_4$ and Bi_2O_3, whereas Lu_2O_3 was cytotoxic for osteoblastic cells:

Materials	Plastic control	Act D	BCP/Ba	BCP/Bi	BCP/Lu
MTS activity (%) at 72 h	100	10	100	98	55

Whatever the matrix type (chemical composition or surface topography), it had an influence on cell behaviour. The MC3T3-E1 had classic osteoblastic morphology (long pseudopodia with good spreading), except for the Bi_2O_3 matrix, where the cell morphology and spreading were modified. Proliferation was the parameter most affected by the different types of pellet, it was greater with BCP/Ba, lower but equal with BCP/Lu and Gd, and lower still with BCP/Bi.

In situ radiopaque evaluation of our different materials showed that the BCP/Ba and BCP/Bi had the best contrast intensity when compared to the bone environment (Fig 9).

Histological results after *in vivo* implantation in rat epiphyses revealed newly-formed bone and granule resorption, and the quality of the bone ingrowth in contact with the materials. BCP/Ba and BCP/Gd materials had the most bone ingrowth quantitatively and qualitatively speaking, when compared to the BCP/Bi and BCP/Lu implanted materials. [61].

Improved visualisation is necessary when bone defects are filled. The BCP/Ba composite has given promising results for developping a radiopaque, resorbable, injectable bone substitute designed for MIS, particularly in spine surgery for vertebroplasty.

BCP for resorbable osteosynthesis

Following a loss of osseous substance of tumoral or traumatic origin, it is often necessary to restore the osseous structure associating osteosynthesis and bone substitute. However a second operation is required to remove the osteosynthesis after wound healing, otherwise the osteosynthesis (such as titanium or Peek) will remain in place definitively. Resorbable osteosynthesis has been developed using resorbable polymers for many years [62-64]. Resorption control and higher osteogenic properties have improved using a combination of calcium phosphate granules, generally b-Tricalcium phosphate. PL DLLA polymers are resorbable by means ofhydrolysis but are not well controlled over time. After 6 months, the hydrolysis appears in all the samples and suddenly the mechanical properties disappear. This

uncontrolled process can appear in 6 or 12 months, often before bone ingrowth healing and mechanical stability have been attained. For osteosynthesis, we must have better control of mechanical stability –and thus promote bone ingrowth at the expense of the implant. The advantage of a composite with PL DLLA and PCa is that it is possible to control the hydrolysis over time, to maintain the initial mechanical properties during bone ingrowth and then have long term mechanical properties from bone ingrowth at the expense of the implant. The development of composites combining PL DLLA and PCa have proved from the use of interference screws that the hydrolysis is controlled and delayed over time until wound healing. A recent study [67,68] reported the resorption kinetics of a composite using PL DLLA (Poly [L-Lactide-co-D,L-Lactide] acid) charged with PCa granules and the interaction with an injectable substitute such as MBCP gel. The injectable biomaterial was non self hardening, the biomaterial consists of BCP granules associated with a hydrosoluble polymer. The material was shown to be perfectly biocompatible and potentially resorbable and, thanks to its initial plasticity, it assumes the shape of the bone defects very easily, eliminating the need to shape the material to adjust it to the implantation site. MBCP gel has no mechanical properties and must be associated with osteosynthesis during the bone ingrowth process. However, bone cells are able to invade the spaces liberated by the disappearance of the polymer carrier. Bone ingrowth takes place all around the granules and at the expense of the resorption of the BCP granules. In time, mechanical properties could be observed due to the presence of the newly-formed bone [17,69-71]. Moreover, the 3-dimensional structure of the network favourably influenced recruitment, cellular differentiation, angiogenesis and the formation of bone tissue.

PL DLLA tubes charged with 20 % BCP granules (6 mm in diameter and 8 mm in length with an open internal channel of 4 mm) were sterilised by irradiation at 25 KGrays. The tubes were implanted in rabbit femoral epiphyses. Some implants were kept empty, and others were filled with MBCP gel®. After 6 months, both samples were well-integrated into newly-formed bone. The composite showed considerable resorption on the surface and bone ingrowth into the implant with osteoconduction at the surface of the BCP granules (Fig. 10). The shape of the implant was significantly changed by bone ingrowth at the expense of the implant. As a result, secondary mechanical properties appeared thanks to bone ingrowth at the expense of the composite, contrary to the pure polymer. In the hole of the defect, large bone ingrowth was observed at the expense of the MBCP gel.

Bone trabeculae and direct bone formation were observed between and at the surface of the BCP granules of the MBCP gel. The thickness of the newly-formed bone at the surface was around

300 µm. Bone architecture and bone remodelling were observed only after 6 months of implantation. The bone trabeculae were perpendicular to the shell of the newly-formed bone directly in contact with the composite. All the surfaces evidenced by the 3D reconstruction were osteoconductive and supported bone formation (fig11).

Resorbable polymers such as PLLA (poly lactide acid) and poly DL lactide co-glycolide (PDLG) have been used in clinical applications for many years [72]. It is often reported that such polymers cannot have bioabsorption kinetics that are adapted to bone reconstruction. The addition of a calcium phosphate like β-TCP, or calcium carbonate, improves resorption control, probably thanks to the release of Ca and PO_4 ions, neutralising the acid released as a result of the hydrolysis of the polymer. In the composite using biphasic calcium phosphate granules, we obtained bone growth at the expense of the composite. Histology and micro CT were performed to examine the bone ingrowth and the interaction with the injectable bone substitute. The cooperation of the two materials for the mechanical performance of the composite and high osteogenic bone substitute can promote new surgical technology.

The hydrolysis of the PL DLLA /CaP composite had no incidence on the bone ingrowth at the expense of the MBCP gel. It appears that the lactic acid released by the hydrolysis had no effect on the osteogenic properties of the MBCP Gel. After 26 weeks, the composite was not fully substituted, only the surface at 200 to 300 µm appeared to have been transformed and invaded by newly-formed bone. The surface of the composite was osteoconductive without fibrous encapsulation.

This efficiency can be attributed to the calcium phosphate granules integrated into the PL DLLA. Certain authors attribute this process to a buffered effect of the calcium phosphate particles [73]. PL DLLA /CaP composites will provide suitable resorbable osteosynthesis associated with a non hardening injectable bone substitute. The PL DLLA /CaP composite can play a part in developping a concept for resorbable osteosynthesis and dedicated bone substitutes such as MBCP gel. The synergy of the two medical devices can contribute to new, less invasive, surgical technology, suppressing the need for a second operation to remove the osteosynthesis. Initial mechanical stability will be attained thanks to the PL DLLA /CaP composite and major bone ingrowth with secondary mechanical properties similar to those of natural bone will be achieved over time.

BCP scaffolds for bone tissue engineering

Reconstruction of large segmental bone defects is still a challenge in either orthopaedics or oral oncology situations. Ceramics alone have failed to provide enough new bone formation. Most models associate isolation from whole bone marrow aspirates, *ex vivo* expansion and then an attachment of mesenchymal stem cells to the biomaterial. However there are several critical questions regarding the quality of cell population isolation, the preservation of stem cell properties after expansion, and, given the complexity of the model, its reproducibility in clinical and surgical applications [74]. Moreover it is still a challenge in tumoral therapy, and few studies have focused on the use of ceramics in irradiated bone. We have previously reported good results for bone substitution with BCP and bone marrow grafts in pre-radiation or post-radiation conditions in animal studies [75,76]. Recently Jegoux *et al.* [77] reported the association of MBCP® granules with a 20/80 HA/TCP ratio, a porcine collagen membrane, and bone marrow for reconstruction in an irradiatepreclinical model.

Bone implantations were performed in rabbits. Segmental defects 2 cm in length were surgically removed from the middle height of the femur. Osteosyntheses were performed by two superposed steel plates. A 30x40 mm resorbable porcine collagen membrane was placed around the defect and then completely filled with MBCP® granules (Fig. 12).

External fractionated radiation delivery was initiated 2 weeks after implantation and performed at ONCOVET (59650 Villeneuve d'Ascq, France). The delivered doses were calculated to be equivalent to those used in the treatment of squamous cell carcinoma in rabbits. Irradiation was strictly localised on the hind legs. Irradiation was delivered by low-energy photons from an X-ray source with energy of 300KVp (PANTAK, THERAPAX DXT 300, Gulmay Medical, UK). A total cumulative dose of 32 Gy was delivered at a rate of 2 Gy per day, 4 days per week, for 4 weeks. One week after radiation, an autologous BM graft was injected into the implanted site. One ml of BM was removed from the right humeral epiphysis with an 18 G needle previously heparinated (50 mg of heparin in 1000 ml of physiologic serum dilution) and were then immediately injected transcutaneously into the centre of the implants under radioscopic control.

Eighteen weeks after the BM injection, the implants were analysed. Ilium aspirate was technically more difficult to perform than in other sites in both species, owing to the orientation of the cortical surface that was also covered in thick muscles. Physiological bone marrow cells were found in all samples and cell counts confirmed that the grafts enclosed the physiological bone marrow without blood recovery. The global abundance of haematopoietic

cells was significantly lower in tibial sites than in the ilium or humeral sites in dogs ($p<0.05$), whereas no difference was observed between any sites in rabbits. No difference was found between the ilium or humerus with respect to global haematopoietic cell abundance and count for every lineage. The overall quantity of haematopoietic cells was significantly lower in dogs than in rabbits for all sites ($p<0.05$).

3D imaging made possible an overall examination of the implants and showed a bony formation bridging the whole length of the defect in all implants. 2D imaging showed that newly-formed bone repartition was homogeneous in 3 implants and relatively heterogeneous in others. In the latter, new bone was observed essentially around the implant at the initial collagen membrane location. The new bone formation was observed both around the MBCP® granules and in the macropores with direct contact (Fig. 13). With both polarised light microscopy and SEM using BSE, the bone appeared well-mineralised and organised. Lamellar bone was observed directly in contact with the MBCP granules without fibrous interposition. From each host bone, the new bone formation had developed bone extremities from the defect to the core with osseous continuity. However, the bone ingrowth was not homogeneous according to the repartition in the axial section. In 3 implants, new bone formation was formed both in the centre and the periphery of the implant, and the MBCP granules appeared totally integrated. In the other 3, newly-formed bone was observed mainly on the periphery of the osseous defects. The border of the defect was filled with compact bone with dense, lamellar structures with few spaces for vessels and soft tissue. The centre of the defect for these 3 implants was filled with few osseous trabeculae, but large amounts of haematopoietic precursors and blood cells were observed. Some granules had no close contact with new bone formation but were associated with the considerable cellular content of the haematopoietic cells. This trend for peripheral trabecular bone formation was also observed in the first 3 implants in which the MBCP granules were filled with both compact bone at the border, and trabecular bone within the granules. Neither acellular, avascular areas nor fibrous encapsulation was observed in either sample. No statistical difference was observed between the centre and the quarters of the length defect according to the ceramic and newly-formed bone calculated with SEM image analysis.

As autologous bone grafts or biomaterial alone have failed to reconstruct large defects, BM cells have been proposed in this indication. BM is composed of haematopoietic cells and mesenchymal stem cells (MSC). The latter are multipotent and can differentiate, among others, into an osteoblastic lineage. BM has been reported to contain almost 1-2 % of cells with the potential for osteoblastic differentiation [78] and MSC osteoinduction properties

have been well demonstrated [79,80]. Some studies have reported several differences in the quantity and quality of bone marrow MSC depending on the location of the bone, especially between orofacial and long bone. There are also limitations for collecting sufficient BM samples because of the limited size or inaccessibility of certain bones. Therefore, determining an appropriate technique and most favorable site for BM collecting could be critical for completing experimental models. This study revealed that BM samples are significantly less rich in the tibia than in the humerus and ilium in dogs while no significant difference was observed in rabbits. BM samples are significantly less rich in dogs than in rabbits ($p<0.05$). The humerus collecting technique appeared to be more reproducible than that of the ilium, essentially because it was relatively easier to approach. These significant differences in relation to bone location and species are consistent with the need to better define experimental models in BM-based tissue engineering. Moreover, comparisons of studies conducted in different animal models should be made with caution.

Few studies have focused on large segmental defects in high weight bearing bones. The viability of a critical size segmental defect in a rabbit femur is a challenge in itself as osteosynthesis must support physiological loading and the defect should not heal spontaneously until the end of the implantation delay. Two cm defects in rabbit femurs have been described as critical size defects at 16 weeks [81,82]. The presented defect was considered to be critical and for ethical reasons no control group was constituted. Potential use of faster resorbable ceramics in bone tissue engineering have been suggested [82] and we chose a bioceramic with a 20/80 HA/TCP ratio for this study. As periosteum was removed, a porcine collagen membrane was used to maintain the granules in the defect.

External radiotherapy after major bone removal and reconstruction is a common situation either in orthopaedic and orofacial oncology. The effects of radiation on normal bone are well known: BM is deprived, and there is less vascularisation, bone ingrowth and bone remodeling [84-88]. The adjunction of an osteogenic component to the ceramic thus appears to be necessary when bone formation limitation factors (large and segmental defect, maximal biomechanical stimulation, radiation) are cumulated. Although MSC adjunction has proved its efficacy in improving bone ingrowth in these critical conditions, there are still several questions regarding donor site, isolation and expansion methods, and stem cell behaviour. Moreover such demanding protocols are less likely to be reproducible in tumoral surgery owing to waiting times and high costs. The experimental results indicated successful bone ingrowth of the composite associating MBCP®+collagen membrane+ post radiation total BM graft in a critical size defect in rabbit femurs. Bridging of the defect with lamellar and well-

organised bone was achieved in all animals. These observations are consistent with a biomechanical stimulated implant due to chosen osteosynthesis. The quantities of bone and ceramic were identical at the different levels of the implant, which is unusual in macroporous calcium phosphate bioceramics where centripetal bone colonisation is classic. These observations suggest that bone marrow grafts in the centre of a defect may have osteoinductive properties. Although the whole axial plane of the defect was not completely filled with newly-formed bone, a tendency for periosteum-like formation was observed in most animals. The collagen membrane is a biocompatible barrier that also acts as a resorbable healing scaffold that can lead to periosteum-like tissue formation on the external bony surface [89].

This study allowed us to better define bone tissue engineering models by determining the most favorable donor site – which is the humeral site in both dog and rabbit models - and to achieve optimal outcomes in further irradiated bone regeneration studies. These findings show that a composite associating a collagen membrane filled with MBCP® granules with a total autologous bone marrow graft injection can successfully repair a critical segmental defect in irradiated bone. This has significant implications for the bone tissue engineering approach to patients with cancer-related segmental bone defects.

Tissue engineering for bone regeneration involves the seeding of osteogenic cells (e.g., mesynchymal stem cells, MSC) on to appropriate scaffolds and subsequent implantation of the seeded scaffolds into the bone defect. Bone marrow-derived mesenchymal stem cells (MSCs) are multipotential cells that are capable of differentiating into, at the very least, osteoblasts, chondrocytes, adipocytes, tenocytes, and myoblasts [90-92]. From a small volume of bone marrow, MSCs can be isolated and culture-expanded into large numbers due to their proliferative capacity, and they maintain their functionality after culture expansion and cryopreservation [93]. MSCs are thus thought to be a readily available and abundant source of cells for tissue engineering applications. Several reports have shown the efficiency of BCP scaffolds of different HA/β-TCP ratios [94,95].

BCP scaffolds with mesenchymal stem cells (MSC)

Arinzeh *et al.* [95] reported a comparative study of BCP with different HA/β-TCP ratios as scaffolds for human mesenchymal stem cells (hMSC) used to induce bone formation. The study was designed to determine the optimum HA/ β-TCP ratio in BCP in combination with MSCs that would promote rapid and uniform bone formation *in vivo*. Their study demonstrated that the BCP scaffold with the lower HA/β-TCP ratio (20/80) loaded with

hMSCs promoted the greatest amount of bone and the new bone formed was uniformly distributed throughout the porous structure of the BCP scaffold. Scaffolds made from 100 % HA, higher HA/β-TCP ratios and 100 % TCP stimulated lesser amounts of bone formation at 6 weeks post-implantation. In this *in vitro* study of hMSC differentiation on 60/40 HA/β-TCP *versus* 20/80 HA/ β-TCP, hMSCs had expressed osteocalcin, a specific bone marker, when grown on the 20/80 HA/TCP, without the presence of the osteoinductive media, by 4 weeks. The enhanced amount of bone formation for hMSC-loaded 20/80 HA/TCP *in vivo* and apparent differentiation into the bone cell phenotype, as characterised by the expression of osteocalcin *in vitro* under normal culture conditions, may be due in part to the rate of degradation, the degradation products, and surface chemistry of 20/80 HA/β-TCP in relation to the other BCP compositions. The concentration of degradation products and hMSC interaction with the surface and its varying chemistries may be responsible for the optimal bone formation exhibited by the 20/80 formulation.

The rate of degradation or resorption of HA/β-TCP ceramics *in vivo* can be accelerated by increasing the amount of the more soluble phase, TCP. In order to design a scaffold that supports bone formation while gradually being replaced by bone, an optimum balance between the more stable HA phase and the more soluble β-TCP phase must be achieved.

BCP scaffolds for growth plate chondrocyte maturation.

Recently, Teixera *et al.* [96] reported the efficiency of an MBCP scaffold for cartilage regeneration. The purpose of the study was to create an *in vitro* cartilage template as the transient model for *in vivo* endochondral bone formation. This study reported successful growth and maturation of chondrocytes (isolated from chick embryonic tibia on macroporous BCP (MBCP®). The thickness of the chondrocyte and extracellular matrix layer increased in the presence of retinoic acid. Alkaline phosphatase activity and expression, proteoglycan synthesis, cbfa1 and type I collagen mRNA levels also increased in the presence of retinoic acid. This study demonstrated for the first time the proliferation and maturation of chondrocytes, and matrix depositing on MBCP, suggesting the potential for such scaffolds in tissue engineering via the endochondral bone formation mechanism.

BCP granules and polymers for injectable bioceramics

The need for a material for minimally invasive surgery (MIS) has led to the development of BCP granules combined with polymers, producing injectable / mouldable bone substitutes. We have developed three types of injectable / mouldable bone substitutes.

MBCP Gel™ is a non self-hardening injectable biomaterial. It is composed of BCP granules associated with a hydrosoluble polymer. These materials have been shown to be perfectly biocompatible and potentially resorbable and, thanks to their initial plasticity, they assume the shape of bone defects very easily, eliminating the need to shape the material to adjust it to the implantation site. MBCP gel does not have the mechanical properties of hydraulic bone cements. However, bone cells are able to invade the spaces created by the disappearance of the polymer carrier (Fig. 14). Bone ingrowth takes place all around the granules at the expense of the resorption of the BCP granules. In time, the mechanical properties are increased due to the presence of the newly-formed bone [97-100]. Numerous reports both *in vitro* and *in vivo* have confirmed the efficacy and performance of this concept for an injectable bone substitute used in bone reconstruction [101-105]

IBS 2 [106,107] is a self-hardening composite. The BCP granules are associated with silanised hydrogel HPMC-Si. The guiding principle of silanised hydrogel HPMC-Si is its hydrophilic and liquid property (it is viscous before being mixed with the calcium phosphate load and injection) and its pH-controlled reticulation process. The silanized hydrogel/calcium phosphate composite presented self reticulation obtained by the change in pH as a catalyst and with an exothermic effect. Once in the implantation site, in contact with the biological buffer liquids, a chemical reaction without additive and without any catalyst allows bridging and reticulation between the various macromolecular chains. This reaction is triggered by the change in pH.

The advantage of ready-to-use mixtures is their easiness of use and the reproducibility of the final material. Their kinetics for osseous reconstruction can be fast because of the many inter-granular paths. These materials have relatively few intrinsic primary mechanical properties, even if the vehicles used harden by reticulation. Achieving mechanical properties is secondary as it is obtained thanks to rapid, physiological bone ingrowth. The use of active substances or growth promoters locally released by these mixtures will make possible fast, biological secondary hardening.

BCP/fibrin glue

The association of bioceramics (Tricos®) and fibrin sealants may develop the clinical applications of composite bone substitutes [108]. As far as the calcium phosphate granules are concerned, they are not easy to handle. They are limited to filling bone cavities and are not available for bone apposition. In addition, improved performances of bioceramics can be

made by adding bioactive factors. In this context, the adjunction of a binding agent such as fibrin glue facilitates the stability of the granules at the site of implantation and provides the scaffold effect of bioceramics with additional osteogenic property. Fibrin-calcium phosphate composite was obtained by mixing Baxter's fibrinogen, the thrombin components of fibrin sealant (Tisseel® Baxter BioSciences BioSurgery) and TricOs® granules. Macroporous Biphasic Calcium Phosphate TricOs® is a mixture of HA/β-TCP in a 60/40 ratio. Granules of 1 to 2 mm in diameter presenting both macroporosity (50-55 %) and microporosity (30-35 %) were used. To enhance the working time, a low thrombin concentration (4 U) was used. The Tisseel/TricOs volume ratio was 1 for 2. Numerous preclinical studies have been performed in rabbits, and goats, both for biocompatibility and biofonctionality using, for example, sinus lift augmentation, and bone filling in long bone. Histology, histomorphometry and X-ray microtomography have shown the osteogenic properties of the composite [108,109] (Fig. 15).

BCP granules for drug delivery:

Calcium phosphate bioceramics have frequently been proposed for the adsorption of bioactive factors and Drug Delivery Systems. However recently, Smucker *et al.* [110] reported for the first time a study demonstrating enhanced posterolateral spinal fusion rates in rabbits using a synthetic peptide (B2A2-K-NS) coated on to microporous granules of BCP with a 60/40 HA/TCP ratio. Different concentrations of the peptide (a synthetic receptor-targeted peptide that appears to amplify the biological response to *rh*BMP-2) were tested. This study provided more evidence of mature/immature bone ingrowth across the inter-transverse process spaces than the controls did. Microporous macroporous biphasic calcium phosphate granule bioceramics for peptide adsorption and local delivery seem to be a good compromise for future associations of osteoconductive/osteogenic properties for such bioceramics and the osteoinductive properties of peptides and growth factors.

Other kinds of drug which can be delivered are antibiotics. It is common practice for surgeons to mix antibiotics with bone grafts when treating infected bone defects or for preventing infection after surgery [111]. Local delivery of antibiotics is both pharmacologically more effective, and safer. Bioactive cements have been shown to be an ideal carrier for antibiotics for local delivery if properly formulated [112,113]. A new calcium phosphate cement has been specifically engineered to have micro-porosity, macro-porosity and resorbability for optimal cell adhesion, cell migration, and bone formation. Recently, the MCPC® reported in this paper was associated with gentamycin [114].

The gentamycin release profiles from the cement samples with different setting times were quite similar. Both cement groups showed an initial burst of gentamycin release in the first 24 hours. After the initial burst, the release rate slowed significantly, and stayed relatively constant after day 7 up to the day 28 endpoint. The amount and rate of the initial burst release was affected by the cement setting time. The release of gentamycin from the cement set for 1 hour showed greater variation than the cement allowed to set for 24 hours. Within the first 24 hours, approximately 72 % of the gentamycin was released from the cement with a 1 hour set time, compared to slower release of approximately 51 % of the gentamycin from the cement with a 24 hour set time. By 28 days, around 87 % and 76 % of the gentamycin had been released from the cements that had set for either 1 hour or 24 hours respectively. The gentamycin release rates from both the 1 hour and 24 hour set-time samples were almost constant after day 7, averaging 59 µg/day for the cement with a 1 hour set time, and 87 µg/day for the 24 hour set time. In our release system, therefore, these constructs are capable of producing gentamycin concentrations of 12 µg/ml and 17 µg/ml on a daily basis for the 1 hour and 24 set–time cement samples, respectively. This is more than one order of magnitude greater than the Minimum Inhibitory Concentration (MIC) for reference strains of S. aureus, which is in the range of 0.12 – 0.25µg/ml [115].

It was interesting to note that the cement without gentamycin showed a decrease in ultimate compressive strength during setting from 24 to 48 hours in phosphate buffered saline at 37 °C. The ultimate compressive strength dropped from 5.5 MPa to 3.87 MPa indicating that the cement had probably dissolved. When the gentamycin was present, the cement showed an increase in both the strength and modulus when the set time was extended from 24 to 48 hours. It appeared that the addition of gentamycin may have delayed the dissolution of the cement while allowing it to continue to set to further increase the mechanical properties.

Owing to its unique preparation method and bioresorbability, the bioactive cement employed in this study may be effective as both a bone graft substitute and as a carrier for the local delivery of antibiotics to prevent or treat infections. An ideal bioactive cement can release a clinically effective amount of antibiotics initially, maintain a steady release of a safe dose over an extended period, and retain no residual amount of antibiotics after the desired treatment time is over. As demonstrated in this study, the MCPC™ bioactive cement released over 50 % of the loaded gentamycin per cylinder, i.e. 7.5 mg, in the first 24 hours. A steady release of a therapeutically significant amount of gentamycin, i.e. about 60 to 90 µg of gentamycin per day, was maintained up to 28 days. As the MCPC™ is engineered to bioresorb and quickly develop a macroporous structure, the remaining amount of 2 to 4 mg of

gentamycin per set-time sample is expected to discharge completely as the bioactive cement resorbs. The MCPC™ resorbable bone substitute has demonstrated its potential to be used as a carrier for the local delivery system for gentamycin. Future studies will expand the investigation to evaluate the release profile and mechanical properties of this bioactive cement when loaded with other antibiotics such as tobramycin and vancomycin.

CLINICAL APPLICATIONS

BCP bioceramics of various sizes and shapes are widely used all over the world in maxillofacial surgery, dentistry, ENT surgery, and orthopaedics. Here, we report some examples of clinical applications for MBCP.

Applications in Dentistry

Dental applications for BCP include prevention of bone loss after tooth extraction; repair of periodontal defects, and sinus lift augmentation [116,117].

Prevention of bone resorption: Bone loss occurs after tooth extraction causing reduction of alveolar ridge height and width resulting in difficulty in fitting dentures or placing dental implants. BCP granules with an HA/TCP ratio of 60/40 or 20/80 (MBCP® and Tribone 80®, respectively) were placed in the alveolar cavity immediately after tooth extraction and followed up radiographically from 0 to 5 years, with biopsies taken at different time periods from 6 months to 5 years [118,119]. The radiographs revealed newly-formed bone with higher density and residual BCP granules. After 6 months, we observed a lesser amount of BCP granules with 20HA/80TCP compared to 50HA/40TCP. In addition, during drilling, clinicians reported higher bone density without interference from residual granules. Organised and well-mineralised bone ingrowth was observed using micro CT and light microscopy. In all cases, the radiopacity of the implantation sites decreased with time indicating that resorption and bone ingrowth proceeded at the expense of the BCP granules. Observation after 1 and 5 years showed that alveolar ridge height had been maintained, compared to the control (no BCP) which showed a decrease in alveolar ridge height of 2 to 5 mm. Five years after implantation, the resorption of the BCP was 78 % for the 60/40 and 87 % for the 20/80, and bone ingrowth 38 % and 32 %, respectively. Resorption and bone ingrowth were not significantly different for the BCP of different HA/TCP ratios.

Sinus lift augmentation: The problems involved in delivering MBCP granules into tooth sockets has discouraged many dental surgeons. A recently developed product composed of

MBCP granules in a polymer carrier provides a ready-to-use injectable bone substitute (MBCP Gel™) [105-112]. The osteoconductive potential of this innovative biomaterial has already been demonstrated for clinical applications in an animal model with the quantification of each component, BCP, bone and soft tissue [17]. Macroporous BCP in a polymer carrier has been shown to be effective in filling dental sockets after tooth extraction because it maintained the alveolar bone crest, supported bone healing and was gradually substituted by bone tissue.

In vivo resorption, just like *in vitro* dissolution, depends on chemical composition and particle size [120]. MBCP Gel™ with 40 to 80 µm BCP granules was used for bone regeneration around dental implants placed in fresh extraction sockets in a dog model [103]. Three months after implantation, the BCP granules were no longer visible using SEM. In the same animal model and after the same implantation time, most of the BCP granules (200 to 500 µm granules) were still present. In the case of nanoparticles (BCP particle size smaller than 10 µm), complete and fast resorption of the BCP granules was observed, but so was significant inflammation [52]. The particle size of the BCP (or any resorbable biomaterials) should thus be adapted to the clinical situation. For pre-prosthetic surgery large granule size compatible with injection should be used out of preference and for pre-implantation surgery, small granule size compatible with acceptable levels of inflammation is recommended

Applications in orthopaedics

BCP has been used in orthopaedic applications for the last 20 years. Its efficacy has been demonstrated in numerous preclinical and clinical studies [26-31,121-123]. Below are brief descriptions of selected clinical situations using specific shaped blocks (custom-designed) for spine arthrodesis (cage insert) and wedges for tibial valgisation osteotomy.

Cervical Spine Arthrodesis: Several studies have been published using bioceramic inserts for filling cage fusion [121-125]. Mousselard [126] recently reported a clinical study of a prospective, comparative, multicentre and randomized study comparing iliac grafts and a macroporous BCP.

Anterior cervical fusion with PEEK cage: Peek cervical radiolucent fusion cages provide immediate mechanical support after anterior cervical discectomy. The aim of this study was to compare the clinical efficiency and quality of the fusion after reconstruction with an anatomically-shaped PEEK cage associated with an iliac crest autograft or with MBCP in the surgery of cervical disc.

Iliac disease: The addition of an iliac autograft makes possible an excellent fusion rate but is associated with increased morbidity and persistent pain at the donor site. Clinical reports by Scareo prospectively comparing the two techniques has shown the clinical advantage of using MBCP and avoiding bone graft harvesting. 58 patients were selected in a multicentre, comparative and prospective study with 24-month follow-up. The patients undergoing anterior cervical decompression and fusion were randomised for autologous graft or MBCP. After 24 months, cervical X rays showed 87 % complete fusion, 13 % uncertain fusion and 0 % real pseudarthrosis in the autograft group versus 86 %, 10 % and 4 %, respectively, in the MBCP group. No implant failures were recorded. These results suggest that the use of an insert associated with an anterior cage allows better recovery for patients while achieving a fusion rate similar to that of ACDF with a tricortical graft, and does not have the associated complications. Using an MBCP insert is safe and avoids potential graft site morbidity and pain in comparison with an autologous graft procedure.

High Tibial Valgisation Osteotomy (HTO):

Many surgical procedures have been described for high tibial valgisation osteotomy (HTO) as a treatment for medial femorotibial arthritis with genu varum deformity. Filling the cavity created by the opening has remained a problem, although various osteosynthetic solutions have been proposed. Bone substitutes have been used in a number of different cases [127-129].

A single centre prospective study [130] from December 1999 to December 2002 was completed involving 42 patients (13 females and 29 males, average age 46 years) who underwent HTVO with medial addition for various types of deformity using custom-designed wedges made of micro-macroporous biphasic calcium phosphate bioceramic bone substitute and an orientable locking screw plate (Numelock II®, Stryker). After one year, correction was unchanged in 99.5 % of cases. Histological analysis showed MBCP resorption and bone ingrowth into the pores and at the expense of the bioceramic. Residual MBCP fragments showed ingrowth of trabecular and/or dense lamellar bone both on the surface and in the macropores. X-ray radiography and microCT revealed a well-organised and mineralised structure of newly-formed bone [132]. In spite of a certain number of fractures in the MBCP wedges during implantation, or proximal screws fractured without compromising the stability or post-operative correction angles, high bone ingrowth was reported. This study indicated that MBCP wedges in combination with orientable locking screws and plates are a simple, safe, and fast surgical technique for HTO.

CONCLUSION

The concept of biphasic calcium phosphate ceramics (BCP) is determined by an optimum balance between the more stable HA phase and the more soluble TCP. The material is soluble and gradually dissolves in the body, seeding new bone formation as it releases calcium and phosphate ions into the biological medium. As a means of promoting these events, and in order to develop calcium phosphate ceramics and other related biomaterials for bone grafts, we need a better control of the biomaterials resorption and bone substitution processes.

The main attractive feature of BCP ceramic is its ability to form a direct bond with the host bone, resulting in a strong interface. The formation of this dynamic interface is the result of a sequence of events involving interaction with cells and the formation of carbonate hydroxyapatite CHA (similar to bone mineral) by means of the dissolution/precipitation processes. Associating micro and macroprosity with the BCP concept has resulted in high osteogenicity and osteoinductive properties. At the present time, MBCP is commercially available in blocks, particulates and customised designs. Specific matrices have been developed for combination with bone marrow or mesenchymal stem cells for tissue engineering (hybrid bone). The need for a material for Minimally Invasive Surgery (MIS) has led to the development of a concept for BCP granules combined with a polymer or calcium phosphate cement to create an injectable/mouldable bone substitute.

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References

1. Albee FH (1920). Studies in bone growth: Triple calcium phosphate as a stimulus to osteogenesis. Ann Surg 71: 32-36.
2. Nery EB, Lynch KL, Hirthe WM, Mueller KH (1975). Bioceramic implants in surgically produced infraboney defects. J. Periodontol. 63: 729-735.
3. Denissen, H.W. (1979) PhD Thesis, Amsterdam, Vrije Universiteit .
4. Jarcho M. (1981). Calcium phosphate ceramics as hard tissue prosthetics. Clin Orthop 157: 259-278.
5. De Groot K.(1983). Ceramics of calcium phosphate : preparation and properties, In: Bioceramics of Calcium Phosphates. CRC Press, Boca Raton,100-114.
6. Metsger SD, Driskell TD, Paulsrud JR(1982). Tricalcium phosphate ceramic: a resorbable bone implant: Review and current uses. J Am. Dent. Assoc 105:1035-1048.
7. Aoki , H, Kato, K (1975). Study on the application of apatite to dental materials. Jpn. Ceram. Soc. 10:469.
8. Daculsi G. (1998) Biphasic calcium phosphate concept applied to artificial bone, implant coating and injectable bone substitute. Biomaterials, 19:1473-1478.
9. LeGeros RZ, Nery E, Daculsi G, Lynch K, Kerebel B.(1988). In vivo transformation of biphasic calcium phosphate of varying b-TCP/HA ratios: ultrastructural characterization. Third World Biomaterials Congress (abstract no. 35).
10. Daculsi G., LeGeros RZ, Nery E., Lynch K , Kerebel B. (1989). Transformation of biphasic calcium phosphate ceramics: ultrastructural and physico-chemical characterization. J Biomed Mat Res 23: 883-894
11. Daculsi G., Passuti, N.(1990). Effect of macroporosity for osseous substitution of calcium phosphate ceramic. Biomaterials **11:**86-87.
12. Trecant, M., Delecrin, J., Royer, J. Goyenvalle, E., Daculsi, G. (1994). Mechanical changes in macroporous calcium phosphate ceramics after implantation in bone. Clin Mater. **15:** 233-240
13. LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP (2003). Biphasic calcium phosphates: Preparation and properties. J. Mater. Sci.: Mat in Med 14:201-210
14. Hench L.L., Splinter R.J., Allen W.C. Greelee T.K.(1978) Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 2: 117-141.
15. Daculsi G., LeGeros RZ., Heugheaert M., Barbieux. I. (1990). Formation of carbonate apatite apatite crystals after implantation of calcium phosphate ceramics. Calcif Tissue Int 46: 20-27.
16. Daculsi G., Biphasic calcium phosphate Granules concept for Injectable and Mouldable Bone Substitute:(2006).Advances in Science and Technology Volume 49, Trans Tech Publications, Switzerland, pp 9-13
17. Daculsi G, Weiss P, Bouler JM, Gauthier O, Aguado E Bcp/hpmc composite : a new concept for bone and dental substitution biomaterials. Bone, 1999, 25:59-61
18. Daculsi G, Khairoun I, LeGeros RZ, Moreau F, Pilet P, Bourges X, Weiss P, Gauthier O (2006). Key Engineering Materials 330-332: 811-814

19. LeGeros RZ (2002). Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthopaed Rel Res 395:81-

20. Goyenvalle E., Aguado, E., Legeros R., Daculsi G., (2007). Effect of Sintering Process on Microporosity, and bone growth on Biphasic Calcium Phosphate Ceramics. Key Engineering Materials vols 333-334, in press, Trans Tech Publication Switzerland

21. Bouler JM, Trécant M, Delécrin J, Royer J, Passuti N, Daculsi G Macroporous Biphasic Calcium Phosphate Ceramics : Influence of Five Synthesis Parameters on Compressive Strength J. Biomed. Mater. Res., 1996, 32 : 603-609

22. LeGeros RZ (2002). Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthopaed Rel Res 395:81-

23. Goyenvalle E., Aguado, E., Legeros R., Daculsi G., (2007). Effect of Sintering Process on Microporosity, and bone growth on Biphasic Calcium Phosphate Ceramics. Key Engineering Materials vols 333-334, in press, Trans Tech Publication Switzerland

24. Hubbard, W.G. (1974). PhD Thesis, Milwaukee, Marquette University

25. Schmitt, M (2000). PhD Thesis, Nantes, University of Nantes .

26. Daculsi, G., Bagot D'arc, M., Corlieu , P., Gersdorff, M. (1992). Macroporous Biphasic Calcium Phosphate efficiency in mastoid cavity obliteration. Ann Orol. Rhinol Laryngol **101:**669-674

27. Gouin, F., Delecrin, J., Passuti, N., Touchais, S., Poirier, P, Bainvel, J.V. (1995). Comblement osseux par céramique phosphocalcique biphasée macroporeuse : A propos de 23 cas. Rev Chir Orthop 81:59-65

28. Ransford A.O., Morley T., Edgar M.A., Webb P., Passuti N., Chopin D., Morin C., Michel F., Garin C., Pries D. (1998). Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients." J Bone Joint Surg Br **80**(1): 13-18.

29. Cavagna, R., Daculsi, G., Bouler, J-M., (1999). Macroporous biphasic calcium phosphate: a prospective study of 106 cases in lumbar spine fusion. Long term Effects Med Impl **9 :** 403-412

30. Soares EJC, Franca VP, Wykrota L, Stumpf S. (1998). Clinical evaluation of a new bioaceramic opthalmic implant. In LeGeros RZ, LeGeros JP (Eds) Bioceramics 11, Singapore, World Scientific, pp 633-636..

31. Bagot d'Arc M, Daculsi G, Emam N. Biphasic ceramics and fibrin sealant for bone reconstruction in ear surgery. Ann Otol Rhinol Laryngol. 2004 Sep;113(9):711-20

32. Daculsi G., (2006). High performance of new interconnected MicroMacroporous Biphasic Calcium Phosphate matrices MBCP2000 for bone tissue engineering Proceedings 20th European Conference on Biomaterials, Nantes France

33. Bouler JM, Trécant M, Delécrin J, Royer J, Passuti N, Daculsi G Macroporous Biphasic Calcium Phosphate Ceramics : Influence of Five Synthesis Parameters on Compressive Strength J. Biomed. Mater. Res., 1996, 32 : 603-609

34. LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP (2003). Biphasic calcium phosphates: Preparation and properties. J. Mater. Sci.: Mat in Med 14:201-210

35. Bouler JM, Trécant M, Delécrin J, Royer J, Passuti N, Daculsi G Macroporous Biphasic Calcium Phosphate Ceramics : Influence of Five Synthesis Parameters on Compressive Strength J. Biomed. Mater. Res., 1996, 32 : 603-609

36. Hench LL (1994). Bioceramics: From concept to clinic. J Am Ceram Soc 74:1487-1510.

37. Basle M.F., Chappard D., Grizon F., Filmon R., Delecrin J., Daculsi G., Rebel A. Osteoclastic Resorption of CaP biomaterials implanted in rabbit bone. Calcif. Tiss. Int., 1993, 53 : 348-356.

38. Gauthier O., BoulerJ-M., Aguado,E., Pilet P, Daculsi G (1998). Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth. Biomaterials. 1998 Jan-Feb;19(1-3):133-9..

39. Kokubo T, Takadama H., How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 2006 May;27(15):2907-15.

40. LeGeros RZ (2002). Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthopaed Rel Res 395:81-

41. Le Nihouannen D, Daculsi G, Saffarzadeh A, Gauthier O, Delplace S, Pilet P, Layrolle P. 2005 Ectopic bone formation by microporous calcium phosphate ceramic particles in sheep muscles. Bone. Jun;36(6):1086-93

42. Reddi AH (2000). Morphogenesis and tissue engineering of bone and cartilage: Inductive signals, stem cells and biomimetic biomaterials. Tissue Eng 6:351-359.

43. Ripamonti U. (1991) The morphogenesis of bone in replicas of porous hydroxyapatiteobtained by conversion of calcium carbonate exosk eletons of coral. J Bone Joint Surg Am 1991;73:692-703.

44. Kuboki Y., Takita H., Kobayashi D (1998). BMP-induced osteogenesis on the surface of hydroxyapatite with geometrically feasible and nonfeasible structures: topology of osteogenesis. J Biomed Mater Res 39:190-199.

45. Diaz-Flores L., Gutierrez R., Lopez-Alonso A., Gonzalez R. and Varela. H. (1992) Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop Relat Res. 1992 Feb;(275):280-6..

46. Habibovic P, Yuan H, van der Valk CM, Meijer G, van Blitterswijk CA, and De Groot K. (2005) Microenvironment as essential element for osteoinduction by biomaterials. Biomaterials 26:3565-75

47. Yuan H., Kurashina K., Joost de Bruijn D., Li Y., de Groot K., Zhang X. (1999), A preliminary study ofn osteoinduction of two kinds of calcium phosphate bioceramics. Biomaterials 20:1799-1806.

48. Barrere F, van der Valk CM, Dalmeijer RA, Meijer G, van Blitterswijk CA, de Groot K, Layrolle P. (2003). Osteogenicity of octacalcium phosphate coatings applied on porous titanium. J. Biomed Mater Res 66A:779.

49. Daculsi G ; LeGeros R. Z; Grimandi G.; Soueidan A., LeGeros J., (2007) Effect o f Sintering Process of HA/TCP (BCP) Bioceramics on Microstructure, Dissolution and Cell Proliferation. Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

50. Daculsi G., Dard M. Bone-Calcium-Phosphate ceramic interface Osteosynthese International, 1994 , 2 : 153-156

51. Malard O., Gautier H., Daculsi G. (2007), In vivo demonstration of 2 types of Microporosity on the kinetic of bone ingrowth and biphasic calcium phosphate bioceramics resorption, Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

52. LeGeros RZ, Chohayeb A, Shulman A (1982). Apatitic calcium phosphates: possible restorative materials. J Dent. Rs 61 (spec issue):343.

53. Brown WE, Chow LC (1987). "A new calcium phosphate water-setting cement" in Brown PW (ed). Cement Research Progress 1986. Americin Ceramic Society, Westerville, OH pp. 352-379.

54. Niwa S, LeGeros RZ (2002). "Injectable calcium phosphate cements for repair of bone defects". In: Lewandrowski K-U, Wise D.L., Trantolo DJ., Gresser J.D. Tissue Engineering and Biodegradable Equivalents. Scientific and Clinical Applications. Marcel Dekker, New York, pp. 385-400. .

55. Constanz B.R., Ison I.C., Fulmer M.T., Poser R.D., Smith S.T. Van Wagoner M. Ross J. Goldstein S.A. (1995) Science 267: 1796-1799

56. Khairoun I, LeGeros RZ, Daculsi G, Bouler JM, Guicheux J, Gauthier O (2004). macroporous, resorbable and injectable calcium phosphate-based cements (MCPC) for bone repair, augmentation, regeneration and osteoporosis treatment.Provisional patent no. 11/054,623

57. O. Gauthier et al. (2005) Biomaterials 26, Issue 27: 5444-43

58. M.P. Ginebra, et al. (2002) Biomaterials 23, Issue 8: 1873-82

59. Catharina S. J. van Hooy-Corstjens et al. (2004) Biomaterials 25, Issue 13: 2657-67

60. S.M. Kurtz et al. (2005) Biomaterials 26, Issue 17: 3699-712

61. Baroth S., Bourges X., Fellah B., Daculs G. (2005) Radiopaque Strategy for Bone Injectable Substitute, Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

62. Lajtai G., Schmiedhuber G., Unger F., Aitzetmuller G., Klein M., Noszian I., Orthner E., (2001) Arthroscopy 17: 597-602

63. Barber F.A., Boothby M.H., (2007) Arthroscopy 23:476-81

64. Rotunda AM, Narins RS (2007), Dermatol Ther. 2006 May-Jun;19(3):151-8.

65. Jouan G., Goyenvalle E., Aguado E., Cognet R.,. Moreau F., Bourges X., Daculsi G. (2007) PL DLLA calcium phosphate composite combined with MBCP gel® for new surgical technologies: Resorbable Osteo Synthesis and Bone Substitute. Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

66. Jouan G., Goyenvalle E., Aguado E., Cognet R.,. Moreau F., Bourges X., Daculsi G. (2007) PL DLLA calcium phosphate composite combined with Macroporous Calcium Phosphate Cement MCPC® for new surgical technologies combining Resorbable Osteo Synthesis and Injectable Bone Substitute. Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

67. Gauthier O, Bouler Jm, Aguado E, Legeros Rz, Pilet P, Daculsi G. J Of Mater Sc : Mat In Med,1999,10:199-204.

68. Gauthier O, Bouler Jm, Weiss P, Bosco J, Daculsi G, Aguado E. J Biomed Mater Res, 1999,47(1) : 28-35.

69. 68 Gauthier O., Bouler J.M., Weiss P., Bosco J., Aguado E., Daculsi G.. Bone. 1999 Aug;25(2 Suppl):71s-74s.

70. Magarelli N., Savastano M.A., Palmieri D., ZAppacosta R., Lattanzio G., Salini V., Orso C.A., Guglielmi G., Colosimo C., (2007), Int. J. Immunopathol. Pharmacol. 20:207-11

71. Cotton N., (2007) www:smith-nephew.com

72. Miura M, Miura Y, Sonoyama W, Yamaza T, Gronthos S, Shi S. Oral Dis. 12(6) (2006),514-22.

73. Lerouxel E, Weiss P, Giumelli B, Moreau A, Pilet P, Guicheux J, et al.. Biomaterials. 27(26) (2006),4566-72.

74. Malard O, Guicheux J, Bouler JM, Gauthier O, de Montreuil CB, Aguado E, et al.. Bone. 36(2) (2005),323-30

75. Jegoux F., Aguado E., Cognet R, Malard O., Moreau F., Daculsi G., Goyenvalle E. Repairing segmental defect with a composite associating collagen membrane and MBCP® combined with total bone marrow graft in irradiated bone defect: an experimental study in rabbit. Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

76. Olmsted-Davis EA, Gugala Z, Camargo F, Gannon FH, Jackson K, Kienstra KA, et al. Proc Natl Acad Sci U S A. 100(26) (2003),15877-82.

77. Ohgushi H, Kitamura S, Kotobuki N, Hirose M, Machida H, Muraki K, et al.. Yonsei Med J. 45 Suppl(2004),61-7.

78. Ohgushi H, Miyake J, Tateishi T.. Novartis Found Symp. 249(2003),118-27; discussion 27-32, 70-4, 239-41.

79. Crigel MH, Balligand M.. Vet Comp Orthop traumatol. 15(2002),158-63.

80. Hollinger JO, Kleinschmidt JC.. J Craniofac Surg. 1(1) (1990),60-8.

81. Livingston TL, Gordon S, Archambault M, Kadiyala S, McIntosh K, Smith A, et al. J Mater Sci Mater Med. 14(3) (2003),211-8.

82. Dudziak ME, Saadeh PB, Mehrara BJ, Steinbrech DS, Greenwald JA, Gittes GK, et al.. Plast Reconstr Surg. 106(5) (2000),1049-61.

83. Matsumura S, Jikko A, Hiranuma H, Deguchi A, Fuchihata H.. Calcif Tissue Int. 59(4) (1996),307-8.

84. Nathanson A, Wersall J.. Scand J Plast Reconstr Surg. 12(2) (1978),139-49.

85. Nussenbaum B, Rutherford RB, Krebsbach PH.. Laryngoscope. 115(7) (2005),1170-7.

86. Sabo D, Brocai DR, Eble M, Wannenmacher M, Ewerbeck V.. J Bone Joint Surg Br. 82(2) (2000),276-82.

87. von Arx T, Broggini N, Jensen SS, Bornstein MM, Schenk RK, Buser D.. Int J Oral Maxillofac Implants. 20(6) (2005),843-53.

88. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.

89. Caplan AI, Fink DJ, Goto T, Linton AE, Young RG, Wakitani S, Goldberg V, Haynesworth SE (1993).In Jackson DWetal (ed), The Anterior Cruciate Ligament: Current and Future Concepts, New York, Raven Press, 405-417.

90. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP(1997). Osteogenic differentiation of purified culture-expanded human mesenchymal stem cells in vitro.,J Cell Biochem 64: 295-312.

91. Bruder SP, Jaiswal N, Haynesworth SE (1997): Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 64: 278-294.

92. Kadiyala S, Jaiswal N, Bruder SP (1997). Culture-expanded, bone marrow-derived mesenchymal stem cells can regenerate a critical-sized segmental bone defect. Tissue Engineering 3: 173-185.

93. Livingston Arinzeh T., Peter S, Archambault M, Van Den Bos C, Gordon S, Kraus K, Smith A, Kadiyala S (2003). Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. Journal of Bone and Joint Surgery American 85-A: 1927-1935,.

94. Teixeira CC, Nemelivsky Y, Karkia C, LeGeros RZ (2006). Biphasic calcium phosphate: a scaffold for growth plate chondrocytes. Tissue Engineering 12:2283-2289.

95. Daculsi G., Weiss P., Bouler J.M., Gauthier O., Aguado E. (1999) Biphasic Calcium Phosphate hydrosoluble polymer composites: A new concept for Bone and dental substitution Biomaterials, Bone, 25:59-61

96. Gauthier O, Bouler JM, Aguado E, Legeros Rz, Pilet P, Daculsi G. (1999) Elaboration conditions influence physicochemical properties and in vivo bioactivity of macroporous biphasic calcium phosphate ceramics. J of Mater Sc : Mat in Med, ,10:199-204

97. Gauthier O, Bouler Jm, Weiss P, Bosco J, Daculsi G, Aguado E. (1999). Kinetic study of bone ingrowth and ceramic resorption associated with the implantation of different injectable calcium-phosphate bone substitutes. J Biomed Mater Res, 1999,47(1) : 28-35

98. Gauthier O., Bouler J.M., Weiss P., Bosco J., Aguado E., Daculsi G.. (1999) Short-term effects of mineral particle sizes on cellular degradation activity after implantation of injectable calcium phosphate biomaterials and consequences for bone substitution. Bone, 25:71-74

99. Millot F., Grimandi G., Weiss P. Daculsi G.(1999) Preliminary in vivo studies of a new injectable bone substitute. Cells and Mat. 9:21-30.

100. Dupraz A, Nguyen TP, Richard M, Daculsi G, Passuti N. (1999) Influence of a cellulosic ether carrier on the structure of biphasic calcium phosphate ceramic particles in an injectable composite material. Biomaterials, ,20:663-673.

101. Gauthier O, Boix D, Grimandi G, Aguado E, Bouler Jm, Weiss P, Daculsi G .. (1999) A new injectable calcium phosphate for immediate bone filling of extraction sockets : a preliminary study in dogs. J Periodontol,;70:375-383.

102. Gauthier O, Bouler Jm, Weiss P, Bosco J, Daculsi G, Aguado E. (1999). Kinetic study of bone ingrowth and ceramic resorption associated with the implantation of different injectable calcium-phosphate bone substitutes. J Biomed Mater Res, 1999,47(1) : 28-35

103. Gauthier O., Bouler J.M., Weiss P., Bosco J., Aguado E., Daculsi G.. (1999) Short-term effects of mineral particle sizes on cellular degradation activity after implantation of

injectable calcium phosphate biomaterials and consequences for bone substitution. Bone, 25:71-74

104. Lapkowski M, Weiss P, Daculsi G Et Dupraz A. Patent WO 97/05911 Déposant: CNRS, Composition pour biomatériau, procédé de préparation II Date de dépôt : 20 Février 1997 Inventeur :

105. Fellah BH, Weiss P, Gauthier O, Rouillon T, Pilet P, Daculsi G, Layrolle P (2006) Bone repair using a new injectable self-crosslinkable bone substitute, J Orthop Res. Apr;24(4):628-35.

106. Le Guehennec L, Layrolle P, Daculsi G (2004). A review of bioceramics and fibrin sealant. Eur Cell Mater. 2004 Sep 13;8:1-11;

107. LeNihouannen DL, Saffarzadeh A, Aguado E, Goyenvalle E, Gauthier O, Moreau F, Pilet P, Spaethe R, Daculsi G, Layrolle P.J (2007). Osteogenic properties of calcium phosphate ceramics and fibrin glue based composites. J Mater Sci Mater Med. 2007 Feb;18(2):225-235

108. Smucker J.D;, Aggarwal D., Zamora P.O., Atkinson B.L., Bobst J.A., Nepola J.V., Fredericks D.C., proceedings AAOS 2007, 12-14 February San Diego USA

109. A.D. Hanssen: Clin. Orthop. Relat. Res. Vol. (2005), p. 91-6

110. T. Sasaki, Y. Ishibashi, H. Katano, A. Nagumo, and S. Toh: J. Arthroplasty Vol. 20 (2005), p. 1055-9

111. P. Frutos, S. Torrado, M.E. Perez-Lorenzo, and G. Frutos: J. Pharm. Biomed. Anal. Vol. 21 (2000), p. 1149-59

112. McNally A., Sly K., Lin S., Bourges X., Daculsi G. (2007) Release of Antibiotics from Macroporous Injectable Calcium Phosphate Cement. Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

113. J.M. Andrews: J. Antimicrob. Chemother. Vol. 48 Suppl 1 (2001), p. 5-16

114. Nery EB, Lynch KL, Hirthe WM, Mueller KH (1975). Bioceramic implants in surgically produced infraboney defects. J. Periodontol. 63: 729-735.

115. Denissen, H.W. (1979) PhD Thesis, Amsterdam, Vrije Universiteit .

116. Clemencia Rodríguez M, Jean A., Kimakhe S., Sylvia Mitja S., Daculsi G., (2007). Five years clinical follow up bone rgeneration with CaP Bioceramics. Proceedings IADR 2007, New Orleans

117. Clemencia Rodríguez M, Jean A., Kimakhe S., Sylvia Mitja S., Daculsi G., (2007). Five Years Clinical Follow up Bone Regeneration with CaP Bioceramics Key Engineering Materials vols 333-334 (2007), in press, Trans Tech Publication Switzerland

118. LeGeros RZ (1993). Biodegradation and bioresoprtion of calcium phosphate ceramics. Clin. Mater. **14 :** 65-88.

119. Daculsi, G. Passuti, N. Martin, S., Deudon, C. LeGeros, R.Z. (1990). Macroporous calcium phosphate ceramic for long bone surgery in human and dogs. J Biomed. Mater. Res. **24:** 379 396

120. Delecrin J, Takahashi S, Gouin F, Passuti N (2000). A synthetic porous ceramic as a bone graft substitute in the surgical management of scoliosis: a prospective, randomized study. Spine 25:563-569

121. Wykrota LL, Garrido CA, Wykrota FHI (1998). Clinical evaluation of biphasic calcium phosphate ceramic use in orthopaedic lesions. In LeGeros RZ, LeGeros JP (Eds) Bioceramics 11, Singapore, World Scientific, pp 641-644

122. Shima T., Keller J.T., Alvira M.M., Mayfield F.H., Dunker S.B. Anterior cervical discectomy and interbody fusion. An experimental study using a synthetic tricalcium phosphate. J. Neurosurg. 1979, 51 : 533-8

123. Toth J.M., An H.S., Lim T.H., Ran Y., Weiss N.G., Lundberg W.R., Xu R.M., Lynch K.L. (1995) Evaluation of porous biphasic calcium phosphate ceramics for anterior cervical interbody fusion in a caprine model. Spine, 20(20), 2203-10

124. Zdeblick T.A., Cooke M.E., Wilson D., Kunz D.N., McCabe R. (1993) Anterior cervical discectomy, fusion and plating. A comparative animal study. Spine, 18(14) : 1974-83

125. Zdeblick T.A., Ghanayem A.J., Rapoff A.J., Swain C., Basset T., Cooke M.E., Markel M. (1998) Cervical interbody fusion. An animal model with and without bone morphogenetic protein. Spine, 23 : 758-65

126. Pascal-Moussellard H., Catonné Y., R. Robert R., Daculsi G., (2007) Anterior cervical fusion with PEEK cages: clinical results of a prospective, comparative, multicenter and randomized study comparing iliac graft and a Macroporous Biphasic Calcium Phosphate. Proceedings ESB 20, Nantes France

127. Lascart T, Favard L, Burdin P, Traoré O. Utilisation du phosphate tricalcique dans les ostéotomies tibiales de valgisation par addition interne. Ann Orthop Ouest 1998;30:137-141.

128. Bonnevialle P, Abid A, Mansat P, Verhaeghe L, Clement D, Mansat M. Ostéotomie tibiale de valgisation par addition médiale d'un coin de phosphate tricalcique. Rev Chir Orthop 2002;88:486-492.

129. Koshino T, Murase T, Saito T. Medial opening-wedge high tibial osteotomy with use of porous hydroxyapatite to treat medial compartment osteoarthritis of the knee. J Bone Joint Surg [Am.] 2003;85-A:78-85.

130. Rouvillain J.L. (2007) MBCP™ Wedges Performance During Open Medial Tibial Osteotomy. Proceedings ESB 20, Nantes France

Table 1: Commercial BCP and BCP composites

HA/β-TCP ratio	
60/40	MBCP® (Biomatlante, France)
20/80	MBCP2000® (Biomatante France)
20/80	Tribone 80® (Strycker Europe)
55/45	Eurocer 400® (FH France)
60/40	Osteosynt® (Einco Ltd, Brazil)
60/40	Triosite® (Zimmer, IND)
60/40	4Bone® (MIS Israel)
60/40	SBS® (Expanscience France)l)
60/40	4Bone® (MIS Israel)
60/40	Kainos +® (Signus Germay)
60/40	Hatric® (Arthrex Germany, US)
60/40	OptiMX® (Exactech USA
65/35	BCP® (Medtronic)
65/35	Eurocer 200® (FH France)
BCP cement	MCPC™ (Biomatlante France)
Composites	
BCP/Collagen	Allograft (Zimmer, IN)
BCP/HPMC	MBCP Gel ® (Biomatlante France)
BCP/Fibrin:	Tricos® (Baxter BioSciences BioSurgery)
BCP/Silicon	FlexHA (Xomed, FL)

Table2

	density	crystal size	SSA	compression strength	Total porosity
MBCP	0.83 g/cc	1.5 μm	1.7 m^2/g	6 MPa	69 %
MBCP2000	0.75 g/cc	0.5 μm	1.6 m^2/g	4 MPa	73 %

(*SSA: specific surface area)*

Table 3

	SSA $m^2/g \pm 0.01$	% micropores	% cell coverage	dissolution ppm Ca, 60 min
D1	3.5	80	35 ± 1.2	7.8
D2	3.2	60	35 ± 1.0	7.9
D3	3.1	50	28 ± 1.5	6.9
D4	0.3	10	20 ± 2.0	3.8
D5	0.8	10	16 ± 2.0	3.1

Captions:

Figure 1: Macroporosity of MBCP observed with SEM

Figure 2: SEM of discs sintered at 1050 °C (D1, D2, and D3) and at 1200 °C (D4, D5)

Fugure 3: Disc D3 implanted in rabbit epiphysis cancellous bone, polarised light microscopy

Figure 4 : Disc D1 implanted in the bone marrow site of a rabbit epiphysis

Figure 5 : Disc D5 implanted in the bone marrow site of a rabbit epiphysis

Figure 6 : a) BCP in mandibular area with HA/TCP ratio of 20/80 and b) ratio of 80/20

Figure 7 : Pure b-TCP implanted in a dog mandibular defect showing no bone contact with the granules and extensive resorption at the surface of the grain (arrow)

Figure 8 : MCPC implantation in a rabbit epiphysis femoral defect, SEM observation

Figure 9 : Radiographies of rat femoral epiphyses containing radiopaque granules after 3 weeks of implantation

Figure 10: Micro CT image analysis showing the bone ingrowth architecture at the expense of both the composite and MBCP gel

Figure 11: MicroCT 3D reconstruction showing the hollow implant filled with MBCP Gel and bone trabeculae forming a shell at the surface of the implant with bone bridges perpendicular to the implant surface.

Figure 12: Collagen membrane containing MBCP® granules in the defect area.

Figure 13 SEM observations showing the newly-formed bone in close contact with MBCP macropores.

Figure 14: MBCP Gel after 4 weeks of implantation in rabbit femoral epiphyses, SEM using BSE showing the BCP particles (white) closely associated with bone trabeculae

Figure 15: Tricos

Figure 1

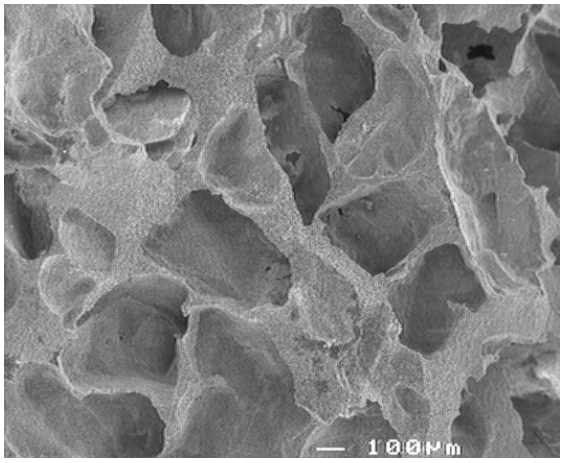
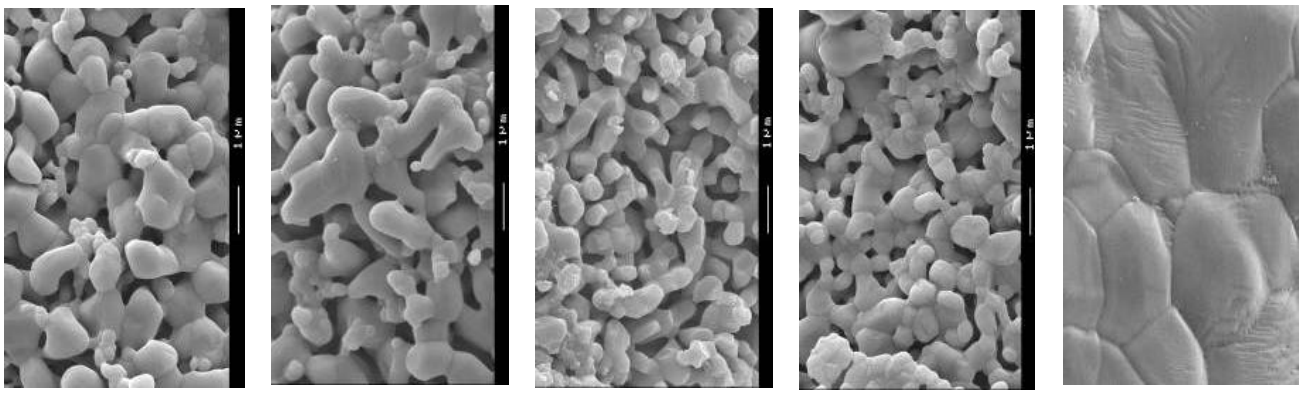


Figure 2



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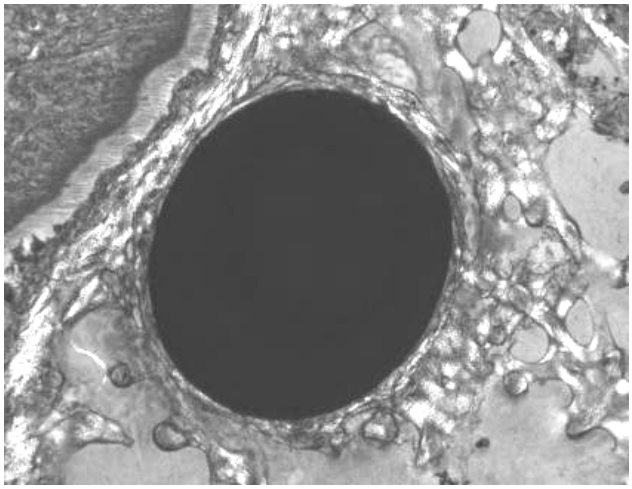

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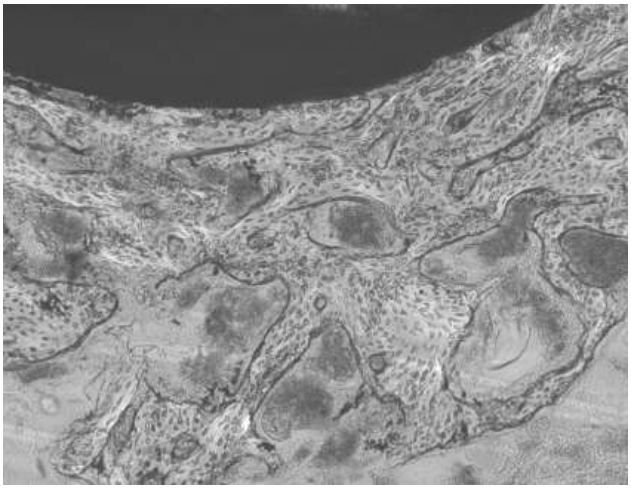

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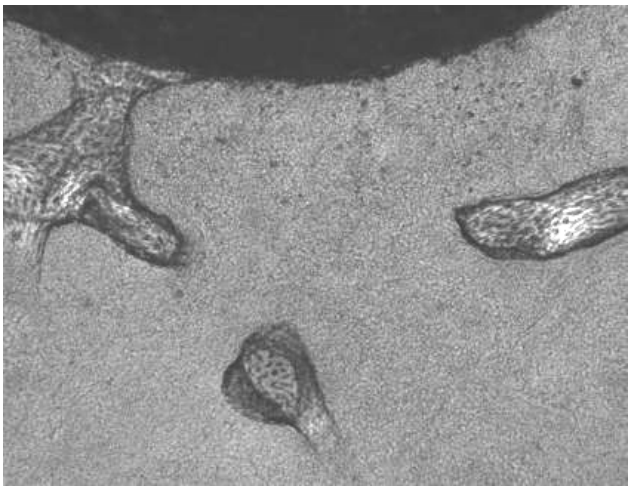


Figure 6 a

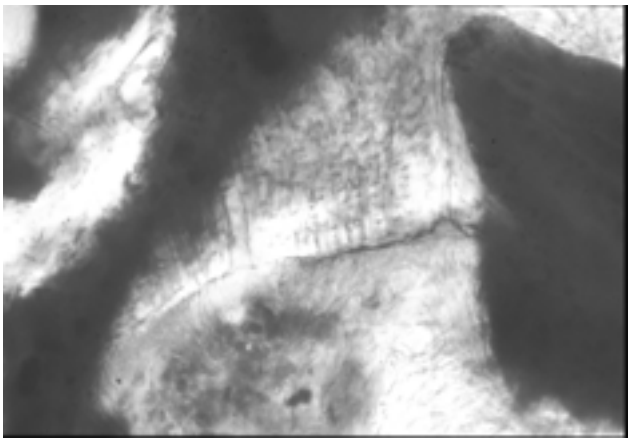
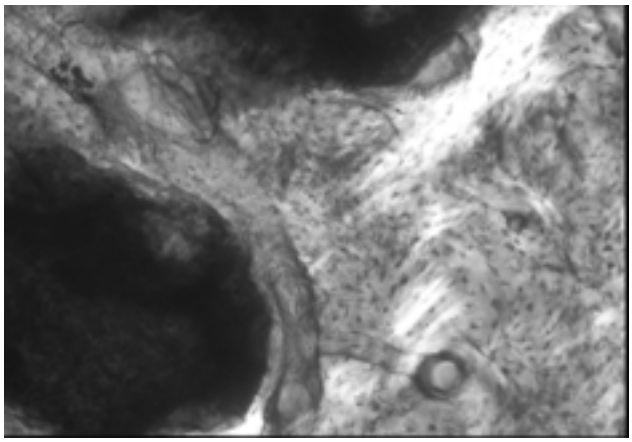


Figure 6 b



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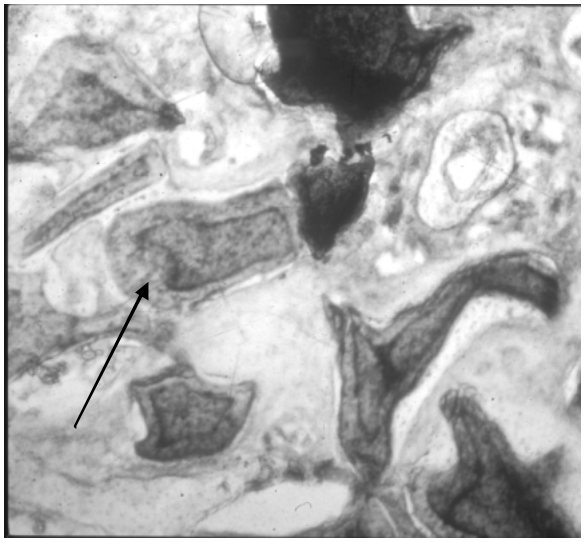

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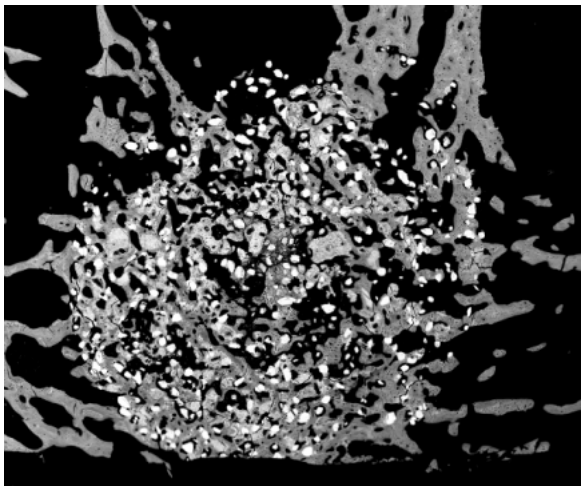

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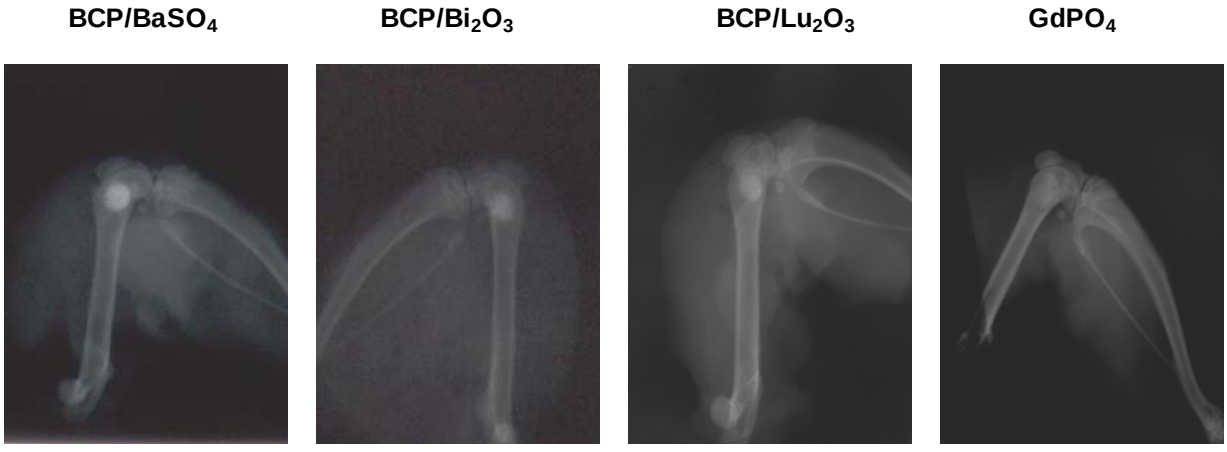

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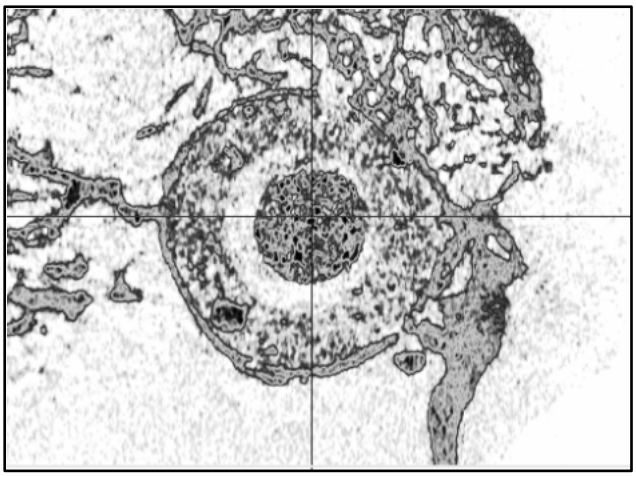

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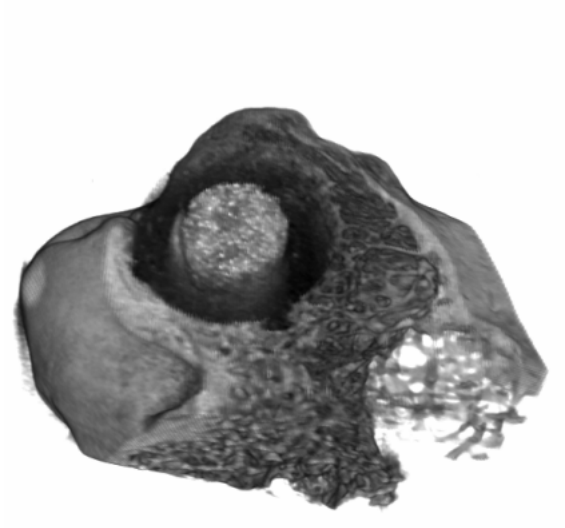

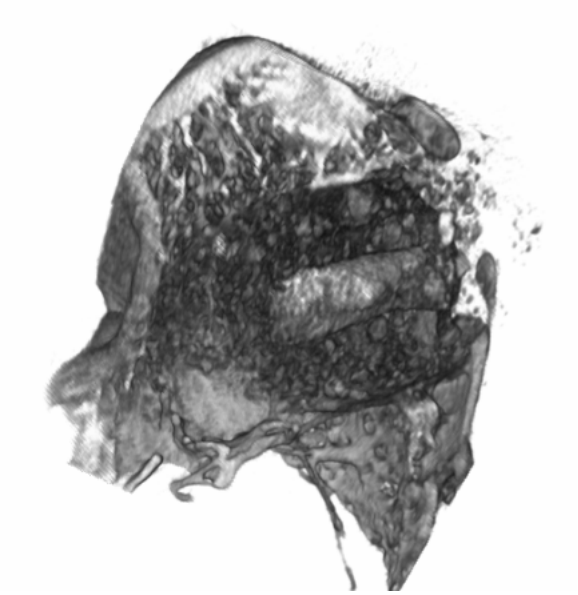

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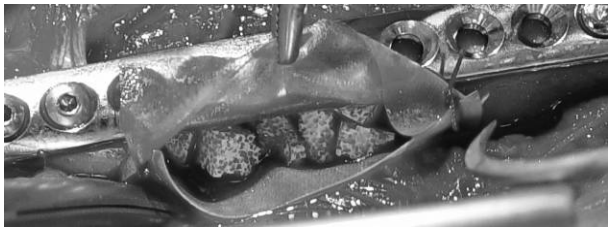

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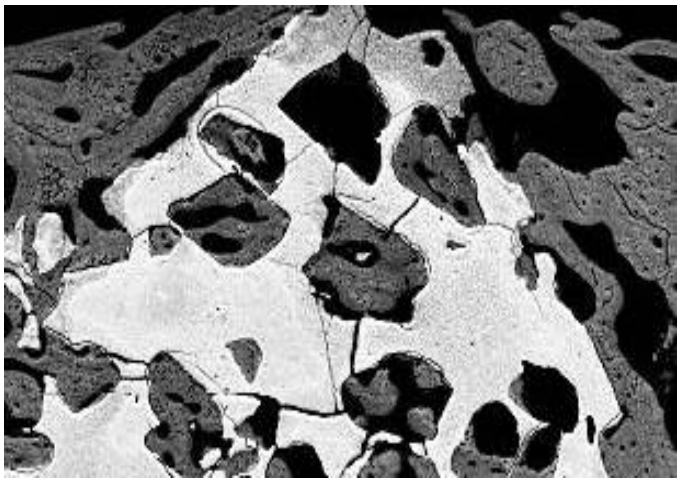

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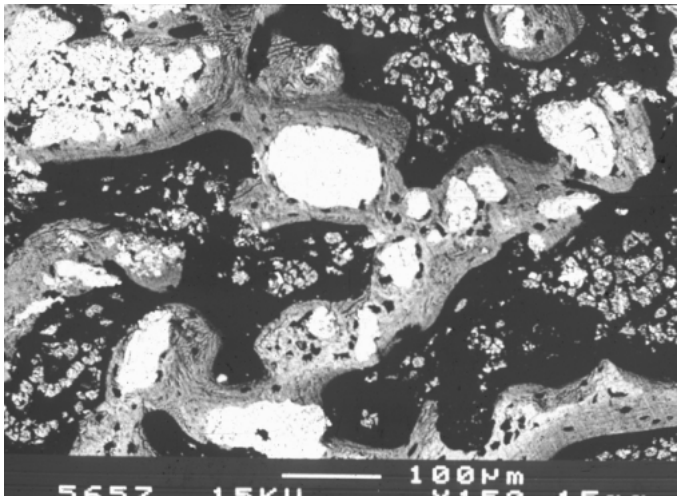

Figure 15