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In Vivo Comparative Study of Two Injectable/Moldable Calcium Phosphate Bioceramics

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Abstract. Calcium phosphate bioceramic granules associated with hydrosoluble polymers formed putties currently more used in clinical applications as they are easy to handle (injectability, moldability). In this study, 2 kinds of materials were tested in rabbit bone defects. The first one is In'Oss™ (Biomatlante), a microporous biphasic CaP granules (BCP, HA/TCP mixture) with polysaccharidic hydrogel; and the second one is Actifuse ABX™ (Baxter/Apatech), pure hydroxyapatite granules containing silicate (HA-Si) with blocks copolymer hydrogel (poloxamer), . The aim of this study was to compare osteogenic properties of two kinds of CaP putties containing HA-Si versus BCP and the kinetic of resorption of their hydrogel. Data have demonstrated that both hydrogels increase the handling properties. Bone regeneration was observed in the two types of sample, however at 3 weeks, Actifuse ABX hydrogel was not totally absorbed, while In'Oss hydrogel was no longer observed. The second difference observed was osteoconduction. Newly formed bone over the time period studied was moreover in close contact with BCP granules than with HA-Si granules. Larger granules resorption on time was observed for BCP compared to HA-Si. Resorption of Actifuse ABX remains limited and explains the faster kinetic of absorption for In'Oss. This study demonstrates biocompatibility, absorbability and bone ingrowth at the expense of the two types of putty injectable/moldable bioceramic used for bone regeneration.

Introduction

Calcium phosphate bioceramic granules associated with hydrosoluble polymers are currently more used. Different combination were proposed with polymers as gelatine, collagen, fibrin, DBM, hyaluronic acid or synthetic polymer like poloxamer, cellulose derivative etc.

To date, several injectable biomaterials have been developed. Some of these injectable bone substitutes are made of CaP hydraulic cements that harden in the bone defect [1, 2]. Others are composed of CaP granules suspended in hydrogel as they are the most interesting carriers actually used for the development of injectable bone substitute. The first material tested in this study is an injectable resorbable bone substitute consisting of microporous BCP granules in suspension associated with a hydrosoluble polymer of derivative cellulose [3, 4]. The second one is based on a suspension of hydroxyapatite containing silicate granules and a hydrogel poloxamer [5]. The purpose of this study was to compare the biocompatibility and the kinetic of absorption in critical size defect in rabbit long bones.

Materials and Methods

Both putty materials were implanted in rabbit bone defect femoral epiphyses according ISO10993.
The first putty (P1) is In'Oss™ (Biomatlante), a microporous biphasic CaP granules (BCP, HA/TCP intimate nanoscale melting of hydroxyapatite and beta tri calcium phosphate) with a polysaccharidic hydrogel carrier. The second putty (P2) is Actifuse ABX™ (Baxter/Apatech), pure hydroxyapatite granules containing silicate (HA-Si) with blocks copolymer hydrogel (poloxamer).

	In'Oss (Putty 1)	Actifuse ABX (Putty 2)
Granules size	0.08-1 mm	1-2 mm
Macropores	yes	yes
Micropores	Yes > 70%	No
Chemical nature	HA 60%, β-TCP 40% molecular mixture	HA with Si content
Polymer	Polysaccharidic hydrosoluble hydrogel	copolymer hydrogel (poloxamer)
Granules/hydrogel weight ratio	1/1	1/1
Polymer % in hydrogel (weight)	4%	25 to 30%

Nine New Zealand rabbits were implanted in femoral epiphysis (critical size defects of 6 mm in diameter and 8 mm length). The animals were maintained during 3, 6, and 9 weeks. The epiphyses were processed for microtomography µCT (Microscanner Skycan 1072). 3-D qualitative imaging and quantitative image analyses of the bone ingrowth was performed.. After µCT acquisition, the implants were embedded in PMMA resin, sectioned and polished, coated with gold palladium and examined in scanning electron microscopy (SEM) using backscattered electron (BSE) (LEO 1450VP). 2-D image analyses were performed using specific software Quantimet Leica Q500. Sections prepared with a diamond saw microtome were observed with light microscopy using polarized light. Thin sections of 7 µm were stained with Movat's pentachrome and observed in light microscopy.

Results

Before implantation the BCP granules of P1 showed grain size < 1 µm and high micropore content (fig 1a). The HA-Si granules of P2 showed less micropores and higher density with numerous grain boundaries (fig 1b).

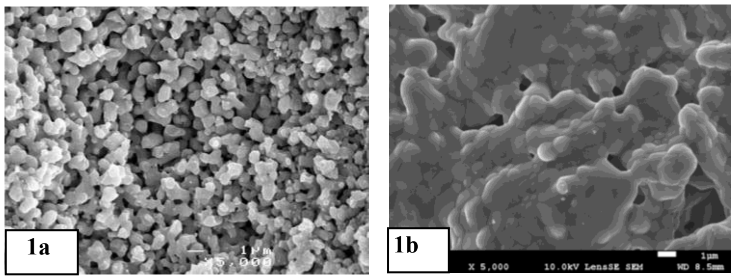

Figure 1: SEM In'Oss (1a) and Actifuse ABX (1b) CaP granule microporosity

At 3 weeks, bone regeneration was observed in both materials. In putty 1, the hydrogel was no longer observed but only bone trabeculae, MBCP granules, and bone marrow (fig 2a). On the contrary (fig.2b), in Putty 2 hydrogel was not totally absorbed.

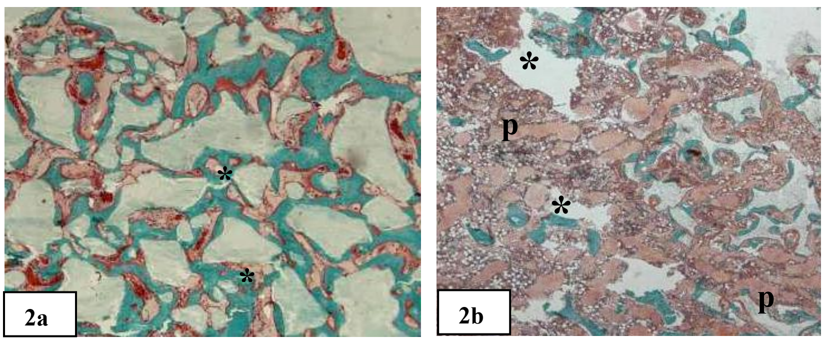

Figure 2: a, In'Oss, bone trabeculae (green) appeared in direct contact with the MBCP granules (*) and in all intergranular spaces. b, Actifuse ABX polymer is still present (p) between bone trabeculae (green) and cellular bone marrow, HA Si granules (*)

SEM observation (only mineralized tissue can be visualized) indicated large differences on osteoconduction of the bioceramic content. Newly formed bone was largely in close contact with BCP granules while numerous HA-Si granules remain without direct bone apposition (fig. 3a, 3b).

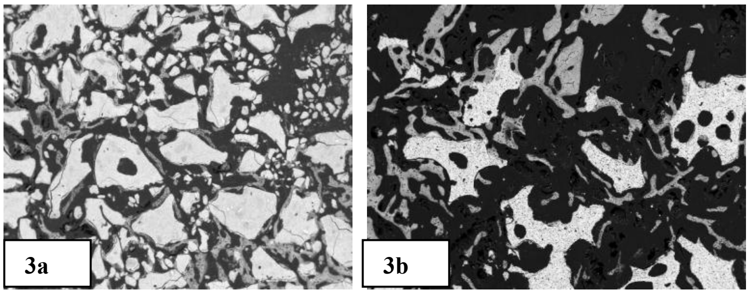

Figure 3: a, P1 BCP granules were closely associated to bone. b, less direct contact was observed for P2 HA Si.

After 6 weeks, the P2 polymer was no longer observed; the intergranular spaces were occupied by bone trabeculae (fig. 4b). For P1, bone regeneration appeared both into the intergranular spaces and in close contact to the BCP granules surface (fig. 4a).

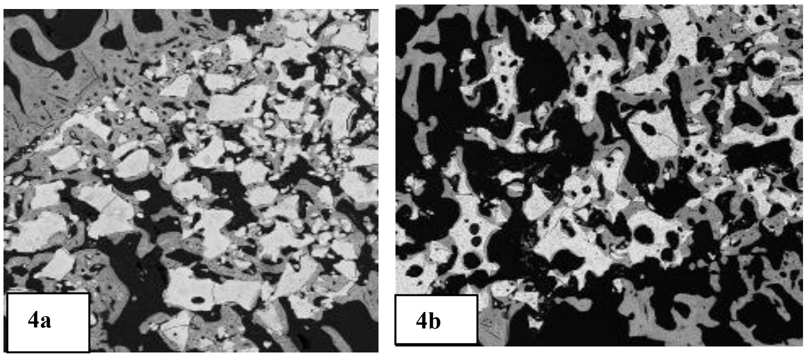

Figure 4: a,P1 . b, P2

In polarized light microscopy, we evidenced lamellar bone architecture essentially (no woven bone) in both samples. Higher bone inter granular trabeculae was observed in P1 with macroporous BCP granules (fig. 5a). For P2 with HA-Si granules, not all the macropores were invaded by newly formed bone (fig. 5b).

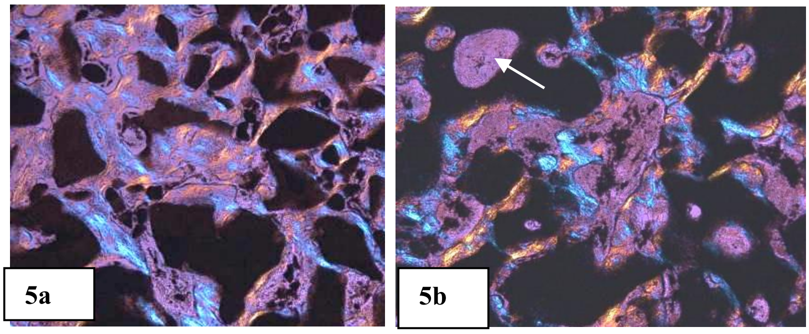

Figure 5: Polarized microscopy, bone ingrowth (blue or yellow) . a, P1 . b, P2 macropores (arrow)

At twelve weeks, bone remodelling was observed in both samples. MicroCT evidenced the bone ingrowth at the expense of the putties, with a regeneration of the spongious bone or the cortical bone at the surface (fig. 6a and 6b). The radio density of the HA-Si of Actifuse ABX appeared higher compared to MBCP granules.

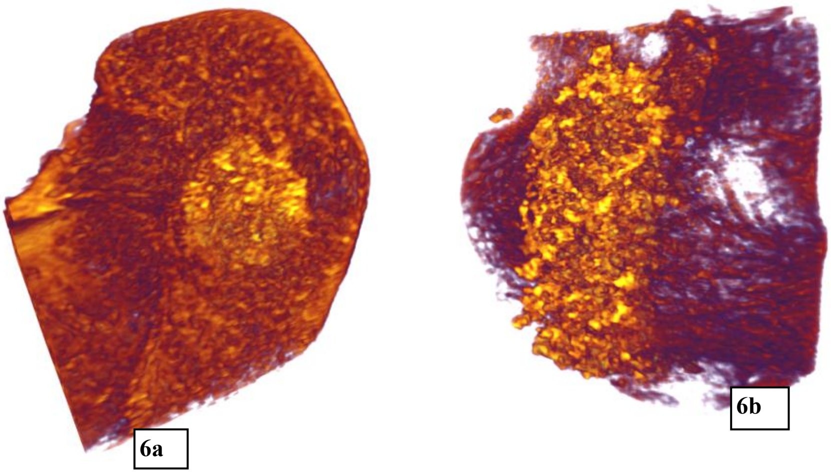

Figure 6: a, P1, b, P2

After 9 weeks, the absorption of the BCP granules for P1 was 76% and 40% for HA-Si of P2. The bone ingrowth was 46% at the expense of IP1 and 29% for P2. The kinetics from 0 to 9 weeks are reported on figure 7. The evolution of absorption (resorption of the bioceramic content and polymer) are faster for In'Oss compared to Actifuse ABX.

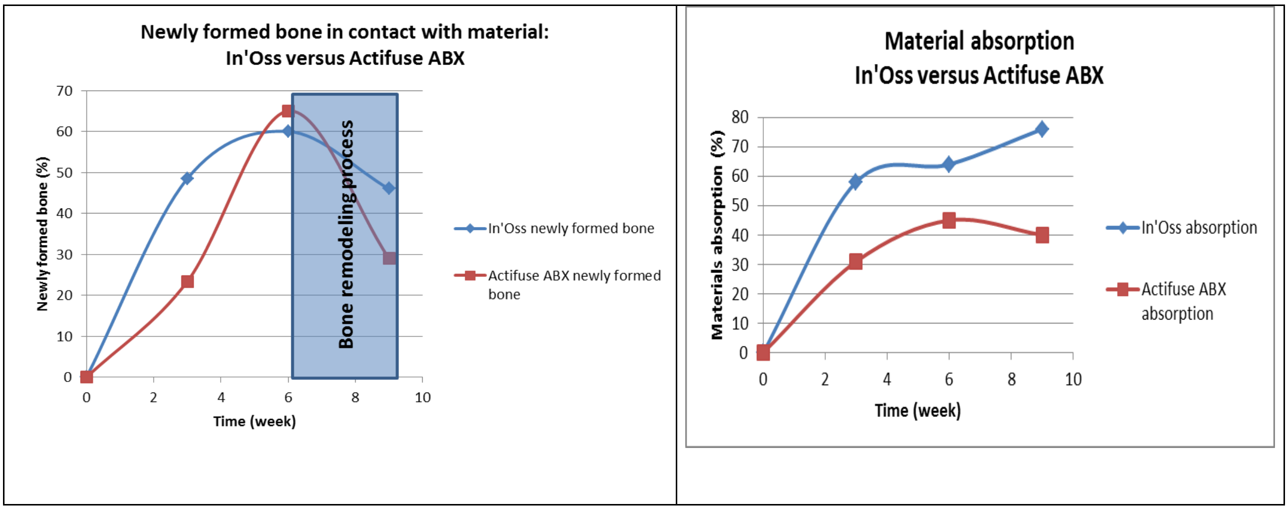

Figure 7: Newly formed bone and absorption of In'Oss and Actifuse ABX

Discussion

Both biomaterials In'Oss and Actifuse ABX are produced in a sterile ready-to-use cartridge form, as the products are not prepared during surgery, the risk of infection is minimal. The addition of polymers to bioceramics granules improved the handling properties. The initial plasticity makes it possible to fill complex shaped bone defects very easily. The materials does not have mechanical strength like the hydraulic calcium phosphate cements but it leads to have rapid and abundant bone growth due to theirs intrinsic porosities. In this concept of Injectable Bone Substitute, bone cells are able to invade the spaces created by the disappearance of the polymer carrier. Bone ingrowth can take the place all around the granules, and at the expense of their resorption. However the kinetic depends on the dissolution of the polymer carrier and the resorption of the bioceramic granules [6, 7]. This study revealed differences on the absorbability and kinetic of bone ingrowth at the expense of the both putties tested.

HA bioceramics are well known for their low kinetic of resorption and very limited ability to be absorbed and re-used by the osteoblast for promoting bone mineralization [7]. Silicate ions were suspected from the Bioglass concept of Larry Hench to be involved in mineralization and osteoblastic activation, however any relevant study have proven this hypothesis. Si content in HA decrease the crystal size, and was considered to have a better kinetic of resorption than pure HA. However there is contradictory literature on this matter [8, 9].

On the contrary, β-TCP is largely recognized for his resorption and adsorption properties compared to HA [7]. BCP, mixture of HA and TCP at the molecular level have high properties of adsorption as it was demonstrated in preclinical studies and human application [10, 11], also osteoinductive property have been reported [12]. Combined with HPMC, the properties of resorption and promotion of bone ingrowth were enhanced [13, 14]

The concentration of the polymer represent an important property to prevent washed out after implantation in bleeding site for injectable bone substitute, to maintain the bioceramic granules on site in unclosed cavities, however the reticulation, too high concentration or gelling delayed the biological fluid diffusion and cell colonization [6]. Similar results have been described with fibrin glue combined to BCP granules [15], hydrogel cross linked by silane [6].

Bone regeneration was observed in the two types of putties, however at 3 weeks, Actifuse ABX hydrogel was not totally absorbed, while In'Oss hydrogel was no longer observed. The second difference observed was osteoconduction. Newly formed bone over the time period studied was largely in close contact with BCP granules than with HA-Si granules. Larger granules resorption on time was observed for BCP compared to HA-Si. Resorption of HA-Si remains limited and explain the faster kinetic of absorption for HA/TCP granules of In'Oss concept. The difference with Actifuse ABX will be due to the high temperature of sintering of HA Si reducing the microporosity as evidenced in SEM (figure 1b), in addition the large amount of hydrogel (granules/polymer ratio) of P2, reducing the kinetic of absorption, then cells, tissue and vascularization colonization comparing to the P1 polysaccharidic polymer content. The kinetic of bone ingrowth was higher and faster for In'Oss compared to Actifuse ABX. Explanation was the lower kinetic of resorption of the polymer (high concentration of polymer into the hydrogel) and less resorbability of HA bioceramics compared to BCP concept containing high amount of TCP. The comparative study demonstrates good biocompatibility and bone ingrowth at the expense of the two types of materials.

Conclusion

This study demonstrates biocompatibility, absorbability and bone ingrowth at the expense of the two types of putty injectable/moldable bioceramic used for bone regeneration. Some differences were observed such as osteoconduction property. Newly formed bone over the time period studied was largely in close contact with BCP granules than with HA-Si granules. Kinetics of cell colonization and bone tissue ingrowth was faster with In'Oss due to faster absorption of the polymer carrier and the greater resorption/absorption of the BCP (HA/TCP) granules.

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