Performance for bone ingrowth of Biphasic calcium phosphate ceramic versus Bovine bone substitute

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Abstract: Calcium phosphate bioceramics and bovine bone xenograft with or without sintering are more or less used in orthopaedics or in maxillofacial surgery. In this study we compare in a rat femoral epiphysis model after 3 weeks of implantation the bone ingrowth at the expense of granules of same size of micro macroporous biphasic calcium phosphate MBCP, sintered bovine bone and unsintered BioOss.

Introduction

Calcium-phosphate ceramics are currently largely used in orthopaedic as bone void filler [1-2]. Calcium phosphate remains the largest bone graft substitute family used in the world for spine and orthopaedic applications, while in dentistry (periodontics, implantology) theirs use are in competition with the use of xenograft from bovine origin like BioOss [3]. For implantology, it is necessary to have enough bone, with good architectural properties to support dental implant. Surgeons used autograft, particularly dense cortical bone in place of spongious bone too resorbable, in spite of the problem encountered for autograft. To supply refusal of the patient for cranial autograft, bone substitutes from synthetic or bovine origin are currently more and more used by the surgeons. The main attractive feature of bioactive bone graft materials such as BCP ceramic is their ability to form a strong direct bond with the host bone resulting in a strong interface compared to bio inert or bio tolerant materials which form a fibrous interface [4]. The formation of this dynamic interface result from a sequence of events involving interaction with cells; formation of carbonate hydroxyapatite CHA (similar to bone mineral) by dissolution/precipitation processes. For allograft or bovine xenograft such bone bonding was neither observed and no comparative study has been published about kinetic of bone ingrowth at the expense of these biomaterials. The purpose of this study was to compare the chemical nature, the structure and the biological performance in term of resorption and bone ingrowth at the expense of the implant.

Materials and methods

All implants were granules of 0.5 to 1mm in diameter. The materials were:

- MBCP™ Biphasic Calcium Phosphate constituted of 70% total porosity and 60/40 Hydroxyapatite HA/Beta Tricalcium Phosphate β-TCP (Biomatlante France). The total porosity was composed of 65% of macropores (mean size 400microns) and 35% of micropores (less than 10microns)
- BioOss™. Anorganic Bovine bone granules, unsintered 60% porous (Geistlich). The two types of granules from spongious or cortical origin were used
- BoneAp Anorganic Bovine Bone, high temperature sintered 60%, high crystalline HA (Legeros New York).

Drilled hole of 3 mm were created in femoral epiphysis of rats and filled with the granules without addition of blood or bone marrow. Three weeks after implantation, all the animals were sacrificed. Implanted area were removed and fixed in neutral formalin solution. Samples were processed in 3D Xray tomography (microscanner SkyScann) then and embedded for histology (without staining for polarized light microscopy, others were stained with Movats pentachrome), and quantitative image

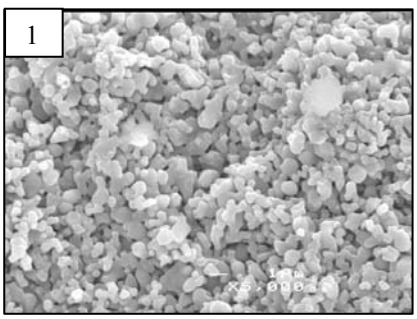
analysis were performed in scanning electron microscopy SEM using Backscattered Electron BSE. Bone ingrowth and particles resorption was evaluated in the cavity

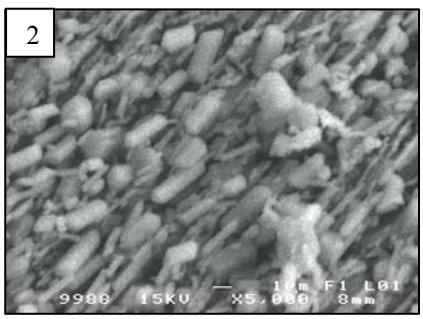
Results

The characteristics of the 3 materials are resumed in the following table:

	MBCP	**BonAp**	**BioOss**
	Fig. 1 and 4	Fig.2 and 5	Fig.3 and 6a and 6b
Chemical nature	HA 60%//TCP 40%	HA 100%	Non stochiometric HA
Crystal size	0.5-1µm	> 1µm	<0.5µm
MicroPorosity	35%	< 10µm	-
Macroporosity	65%	60-70%	-
Total porosity	70%	70%	60%

The crystal size is totally different between sintered materials (MBCP and BonAp) and non sintered material as xenograft BioOss. Higher is the temperature of sintering, higher is the crystal size and the crystallinity (fig1, 2 and 3).





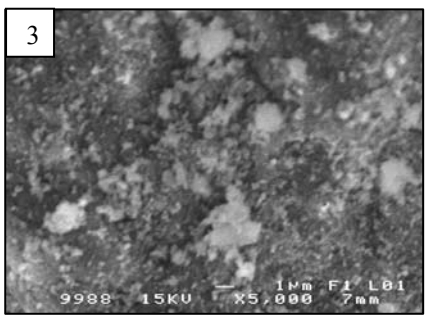
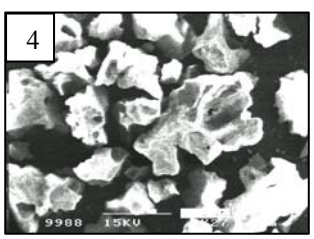
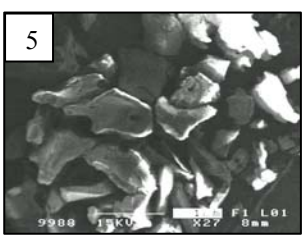
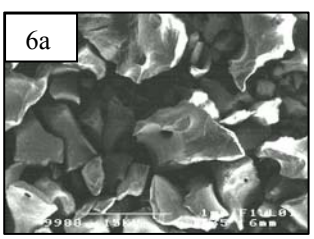


Fig.1: MBCP observed in SEM showing micropores and crystal size
Fig.2: BonAp observed in SEM showing micropores and crystal size
Fig.3:, BioOss spongious particles

Both spongious of cortical granules of BioOss, or BonAp are more dense without macropores (fig.4, 5, 6a and 6b) than MBCP granules of the same size.







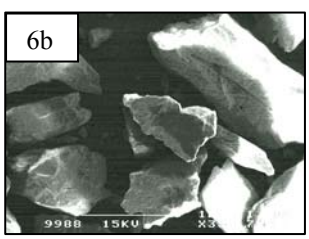
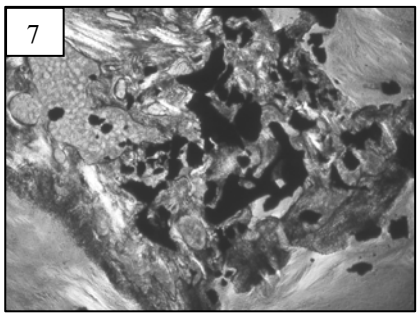
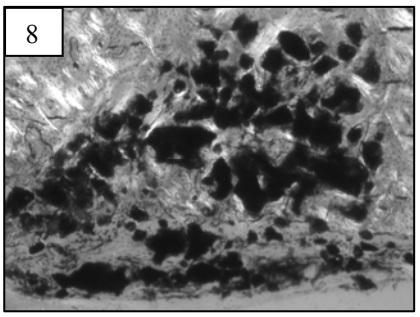


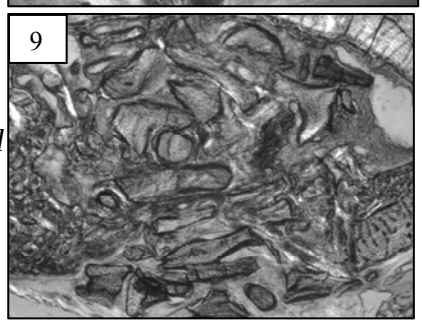
Fig.4: MBCP granules
Fig.5: BonAp sintered bovine bone granules
Fig. 6: a, BioOss spongious – b, BioOss cortical

In light microscopy of stained thin sections, the 4 types of particles elicit responses from cells coming from the implantation site. These materials allow cell attachment, proliferation and osteogenic expression without sign of foreign body reaction. Some multinucleated giant cells macrophages or osteclastics like cells were observed. Light microscopy examination showed bone ingrowth with, osteoblasts, and osteoid or bony formation between and at the surface of the residual granules. Higher amount of giant cells were observed in BioOss particles (both spongious or cortical).

The amount of bone ingrowth with well organized collagen mineralized fibers observed in polarized light microscopy were found in MBCP and Bonap group. In BioOss the granules appeared more packed with less spaces between granules and very limited newly formed bone at the surface and between the granules.
However the amount of resorption is very limited for all the bovine bone substitutes, both sintered (BonAp) and unsintered (BioOss spongious and cortical particles) in spite of evidence of higher amount of macrophagous cells in BioOss. In contrary, the biodegradation of the MBCP is associated to bone ingrowth with numerous bone trabeculae between the granules and at the surface of the residual materials. SEM observations using BSE confirm bone ingrowth and close contact (osseo-coalescence) between newly formed bone and particles implants.







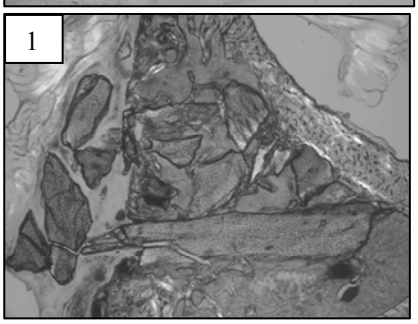


Fig 7: MBCP, polarized light microscopy
Fig 8: Bonap, polarized light microscopy
Fig 9: BioOss spongious, polarized light microscopy
Fig 10:BioOss cortical, polarized light microscopy

Image calculation of bone ingrowth and implants particles resorption are resumed in following table.

	MBCP	**BonAp**	**BioOss**	**Control**
% newly formed bone	23%	13%	14%	25%
% implants particles	35%	48%	51%	-
Total mineralized tissue	58%	61%	65%	25%
Total Total soft tissue	42%	39%	35%	75%

Microscanner revealed bone architecture of physiological bone ingrowth in all samples, it was more difficult in unsintered bovine bone substitute to differentiate host bone and the bone implant.

Discussion and conclusion

The development of calcium phosphate ceramics and other related biomaterials for bone graft involved a better control of the process of biomaterials resorption and bone substitution. Synthetic bone graft materials are available as alternatives to autogeneous bone for repair, substitution or augmentation. Synthetic biomaterials include essentially special glass ceramics described as bioactive glasses; calcium phosphates (calcium hydroxyapatite, HA; tricalcium phosphate, TCP; and biphasic calcium phosphate, BCP). The other family of bone substitutes are Xenografts. Xenograft bone could be processed to be safe for transplantation in a human host [5]. Xenograft has the same inherent problems as allografts, and being from a different species, it may cause even more pronounced immunological problems. Human allograft materials are considered more

effective and more widely available compared to xenografts at the present [6]. For these reasons, the future will be more and more the use of synthetic bone substitute.

The results from this comparative study confirm osseo-conduction properties of both bone substitutes, however the kinetic of bone ingrowth was higher for synthetic micro and macroporous calcium phosphate due to higher resorption and newly formed bone at the expense of the resorption. The sintered bovine bone revealed, in spite of this limited animal models, low bone formation in close contact to the surface probably due to high crystalline content and Hydroxypatite chemical nature. For unsintered bovine bone, in spite of microcrystalline nature, the resorption still limited and bone ingrowth appears delayed compared to synthetic MBCP. The higher content of macrophagous cells compared to sintered material (both MBCP and Bonap) will be due to remaining denaturated proteins present in unsintered bovine bone.

Properties of BCP bioceramics relating to their medical applications include: macroporosity, microporosity, compressive strength, bioreactivity (associated with formation of carbonate hydroxyapatite on ceramic surfaces in vitro and in vivo), dissolution, and osteoconductivity. Due to the preferential dissolution of the β-TCP component, the bioreactivity is inversely proportional to the HA/β-TCP ratio. Hence, the bioreactivity of BCP bioceramics can be controlled by manipulating the composition (HA/β-TCP ratio) and/or the crystallinity of the BCP.

Currently, BCP bioceramics is recommended for use as an alternative or additive to autogeneous bone for orthopedic and dental applications. It is available in the form of particulates, blocks, customized designs for specific applications and as an injectable biomaterial in a polymer carrier.

In addition, recently osteoinduction have been largely demonstrated for micro macroporous biphasic calcium phosphate [7], while mineralized xenograft was neither associated to osteoinduction contrarily to demineralized Bone Matrix DBM.

BCP ceramics and others bioactive bone graft materials (HA, β-TCP, Bioglass, bone-derived or coral-derived HA), are considered osteoconductive but not osteoinductive. Osteoconductive materials provide the appropriate scaffold or template which would allow “vascular ingress, cellular infiltration and attachment, cartilage formation and calcified tissue deposition”. Osteoinductive materials (e.g., bone morphogenetic proteins) “stimulate uncommitted cells (e.g., mesenchymal stem cells) to convert phenotypically to chondroprogenetor and osteoprogenitor cells”, Ripamonti [8] is one of the pionners of the osteoinduction concept [9]. The recent data demonstrate what synthetic calcium phosphate able to be resorbed (as BCP) associated to high microporosity structure have the best osteogenic performance in non osseous area or irradiated implantation site [10].

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