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Impact of Calcium Phosphate Particle Morphology on Osteoconduction: an *in vivo* study

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Abstract. Apatite/ β -TCP particles exhibiting non-conventional *urchin-like morphology* were prepared by hydrothermal synthesis. Their implantation in the rat calvarium was followed during 60 days. A total absence of osteoconduction was observed despite a favorable chemical composition, stressing the fundamental role of *particle morphology* on bone regeneration. Results are discussed in relation with other literature data. Possible explanations include the disfavored accumulation of biological mediators due to the acicular shape of the particles and/or a limited accessibility for cells.

Introduction

Calcium phosphates (CaP) are major constituents of hard tissues. As such, CaP ceramics are widely used for bone regeneration applications [1,2], either as compacts, granules or 3D porous scaffolds. In particular, mixtures of hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP) are extensively used today for the preparation of such scaffolds, where the presence of β -TCP permits to artificially increase the overall resorption rate of the scaffold after implantation thanks to its higher solubility [3].

Due to their chemical composition involving calcium and phosphate ions, such CaP compounds are often considered as good osteoconductive materials. However, in addition to the chemical composition, several reports have stressed the impact on osteoconduction or osteoinduction of other physico-chemical parameters such as surface topology or particle size on bone repair [4,5]. Some authors have underlined the potential role of such parameters on various biological performances of CaP-based biomaterials (extent of bone formation [5], inflammatory response [6]), indicating that the tridimensional structure of biomaterials was a key factor in bone regeneration processes. The presence of micropores on the walls of macroporous scaffolds was also shown to play a major role in activating bone formation [7,8,9] and similar conclusions were also reached for other systems (e.g. titanium [10]).

Although the exact function of these physico-chemical parameters on bone repair has not been unambiguously unveiled, some tentative explanations have been proposed in the literature. In particular, the effect of microporosity was linked to the increased capability to adsorb proteins and store growth factors such as BMP (bone morphogenetic protein), making it possible to reach the threshold needed for bone formation activation [7-10]. Also, a greater specific surface area is thought to boost ion exchange processes with body fluids, leading to the enhanced formation of a bone-like apatite layer on the surface of the implanted biomaterial [8]. Other explanations are based on cell behavior [9], as cells are thought to develop better in a confined system (e.g. inner pores).

Despite the wide use of such CaP compounds for bone regeneration, and in particular of HA/ β -TCP biphasic bioceramics, the roles of particle morphology and surface state on biological performances remain unclear. In order to shed some more light on these aspects, we provide and discuss in this contribution some original results obtained *in vivo* after implantation, in the rat calvarium, of non-conventional *urchin-like particles* composed of apatite/ β -TCP.

Materials and Methods

Materials synthesis. *Urchin-like* biphasic apatite/ β -TCP particles were prepared in situ by partial hydrolysis of β -TCP (free of magnesium impurity) in the presence of water vapor, through hydrothermal treatment at 160 °C for 24 h using the custom-built setup shown in Figure 1. The samples were then sieved in order to retain only the particle size fraction in the range 100-180 μm , and sterilized by gamma rays (32 kGy).

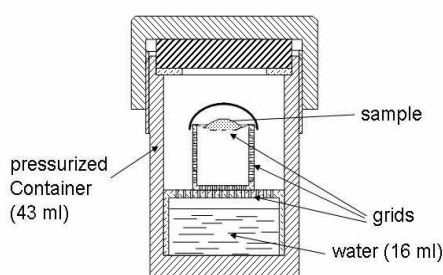
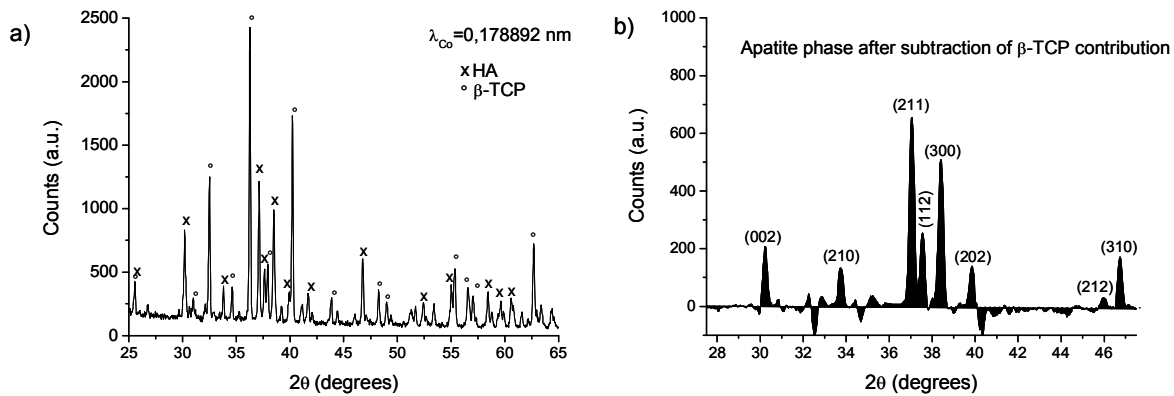


Figure 1: Custom-built setup for hydrothermal preparation of urchin-like HA/ β -TCP particles

Surgical procedure and histology. *In vivo* experiments were performed on eight Wistar male rats aged 4-5 months (350-400 g). The rats were randomly split into two subgroups: experimental and control. Anesthesia was performed through injection of ketamine sulfate (1 ml/100 g body weight). A midparietal transosseous calvarial defect (6-mm diameter) was created. In the experimental subgroup, the lesion was filled with the apatite/ β -TCP material. In the control subgroup, no material was placed in the bone defect. In all cases, the periosteum and skin were repositioned and sutured. The osteoconduction ability was evaluated through histological analyses carried out 10, 20, 30 and 60 days after surgical operation, on both subgroups.

Results

The partial hydrolysis of pure β -TCP carried out by hydrothermal synthesis led to biphasic apatite/ β -TCP particles as shown by XRD (Fig. 2a). FTIR analyses confirmed this conclusion and showed that the apatite phase was highly hydroxylated as shown by the presence of intense bands at 3565 and 632 cm^{-1} in proportions close to those of HA. Comparison of the XRD pattern with that of known mechanical mixtures of HA and β -TCP indicated that the sample composition approached 30% apatite / 70% β -TCP (weight %). A closer investigation of the apatite phase was made possible by subtracting, from the overall XRD pattern, the contribution of the β -TCP phase (Fig. 2b). For this subtraction, the XRD pattern of the initial β -TCP was used so as to keep similar microstrain and size effects on peak broadening. Despite baseline artifacts around major β -TCP peak positions, the apatite phase can clearly be identified by its main diffraction lines. This phase exhibits a high degree of crystallinity as indicated by the sharp definition of the peaks, and lattice parameters were evaluated to $a = 9.41 \pm 0.02 \text{ \AA}$ and $c = 6.87 \pm 0.03 \text{ \AA}$, which are close from the values ($a = 9.418 \text{ \AA}$, $c = 6.884 \text{ \AA}$) reported for stoichiometric HA (JCPDS 09-432). SEM analyses performed on such apatite/ β -TCP particles indicate an unusual *urchin-like morphology* (Fig. 3a) with a core composed of non-hydrolyzed β -TCP and packed surface apatite needles (average length 20-30 μm). The apparent density of the sample, without compaction, was found to be 0.35 g/cm^3 .



**Figure 2: a) XRD pattern of urchin-like HA/β-TCP particles
b) XRD pattern of apatite phase after subtracting β-TCP contribution**

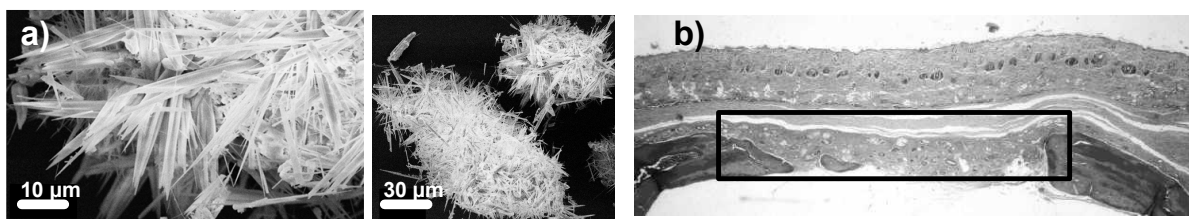


Figure 3: a) Urchin-like HA/β-TCP particles (SEM), b) histological observation at day 60

Histological analysis performed after implantation of these urchin-like apatite/β-TCP particles showed the absence of inflammatory wells. The implant was well tolerated *in vivo* during the whole duration of the experiment. The implantation sites showed in all cases the physical filling of the bone defect by the material, preventing surrounding soft tissues to fall off in the lesion. However, no signs of bone regeneration could be noticed throughout the experiment (Fig. 3b), although the material was still clearly visible in the lesion. Histological observations indicate that the defect is progressively occupied by a poorly-vascularized conjunctive fibrous tissue. In the control subgroup, no bone formation was observed, and soft tissues have progressively invaded the defect leaving a thin layer of conjunctive fibrous tissue.

Discussion

Although no adverse effects were evidenced after implantation, a total absence of bone regeneration was interestingly observed during the 60-day period. Histological analyses revealed that the particles remained observable throughout the study. This can be linked to the physico-chemical characteristics of the apatite phase (high crystallinity, high hydroxylation, lattice parameters close from HA) indicating that this phase is closer from stoichiometry than nanocrystalline biological apatites or immature synthetic analogues [11,12], which implies a greater chemical stability. The absence of bone repair therefore cannot be explained by an early resorption.

Literature data generally report the high bone formation ability of sintered HA/β-TCP mixtures (e.g. 3D porous scaffolds) [1,2], with an even higher crystallinity. However, in this work, the biphasic particles exhibited a non-conventional morphology (urchins), and the absence of osteoconduction stresses thus the impact of 3D architecture on bone regeneration. This matter was already pointed out by several authors in the case of various materials [4-10], where the strong influence of surface roughness and pore size, in particular, were unveiled.

These apatite/β-TCP particles show a prominent *convex morphology*, with needles spreading outward. As such, they could be regarded in some way as the “negative” or “complementary” morphology of 3D porous scaffolds (Fig. 4), often used in bone repair, which exhibit a pronounced concavity accessible to cells. The micropores in the latter could be seen as micro-reservoirs for

biological mediators such as growth factors, for which adequate concentration thresholds able to trigger bone formation could be reached, enabling proper cell stimulations. Such thresholds (e.g. for BMP) and their implication in bone regeneration were commented in some works [7-10]. Similarly, CaP dissolution-reprecipitation processes could be favored in micropores where the concentration in released ions could remain locally elevated. In contrast, the convex urchin morphology is likely to disfavor such molecular or ionic accumulations, at least in areas easily reachable for cells. The limited cell accessibility linked to the presence of close-packed intermingled needles (20-30 μm in length) at the basis of the particles could also directly contribute to the absence of bone repair.

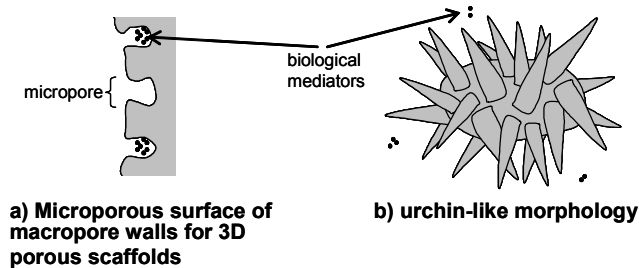


Figure 4 : Schematic morphology of a) 3D micro/macroporous scaffolds, and b) urchin-like particles

Conclusions

This work reports the absence of bone regeneration after implantation in the rat calvarium of apatite/ β -TCP *urchin-like particles*, despite the favorable chemical composition. This morphology might disfavor local accumulation of biological mediators, in contrast to microporous systems, and a limited cell accessibility probably also contributes to the absence of bone formation. This work also shows the need to control bioceramics surface topology during their whole processing, as such acicular morphologies might also be encountered after wet sterilization of hydrolysable β -TCP-containing phases, especially HA/ β -TCP ceramics, or after aqueous treatments of plasma-sprayed coatings carried out to improve their crystallinity.

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