

# Intramembranous bone tissue response to biodegradable octacalcium phosphate implant

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## Abstract

Previous studies showed that synthetic octacalcium phosphate (OCP) enhances bone formation coupled with its own osteoclastic biodegradation more than non-biodegradable hydroxyapatite (HA), including sintered HA ceramic, when implanted in animal bone defects. The present study was designed to investigate whether synthetic OCP in granule form has biodegradable characteristics when implanted in the subperiosteal area of mouse calvaria in comparison with non-sintered stoichiometric HA, especially in relatively short periods after implantation. OCP crystals exhibited plate-like morphology, whereas HA crystals had a sphere-like structure. Both crystals had large pore volumes >75% in total, with micropores within the granules. Direct bonding of newly formed bone was discernible in HA until 35 days after implantation by element analysis for calcium and phosphorus. However, histomorphometric analysis demonstrated that bone formation was facilitated on OCP surfaces with greater alkaline phosphatase activity than on HA up to 21 days. The surfaces attacked by tartrate-resistant acid phosphatase positive osteoclast-like cells were significantly greater than those of HA. OCP became encapsulated and replaced with new bone with prolonged implantation periods up to 180 days. The results suggest that the enhanced bone formation in mouse calvaria could be associated with the biodegradable nature of OCP, and that OCP could be used in augmenting intramembranous bone volume.

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## 1. Introduction

The chemical nature of the first mineral formed in vertebrate biomineralization remains controversial. Some studies suggested that octacalcium phosphate ( $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ; OCP) is a precursor of biological apatite crystals in bone [1,2]. Other studies suggested that very small poorly crystalline apatite can be formed directly in the initial bone mineralization [3,4]. Apart from the chemical nature of the first mineral formed in bone, recent

intensive studies on the experimental application of synthetic OCP have shown that it has the potential to enhance new bone formation [5–11].

The osteoconductive nature of synthetic OCP was found first by its subperiosteal implantation in mouse calvaria in comparison with synthetic hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ; HA) [5,12]. Several studies have been conducted to investigate the possible use of synthetic OCP as a bone regenerative scaffold in various forms, such as coatings on metallic implants [8–10,13], microscaffold self-assembled [14,15] and granules [5,7,11,12,16]. Recent in vitro studies disclosed that OCP facilitates osteoblastic cell differentiation [11,14,15], and that marked increase in

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osteoblast-related gene expression, such as osterix and alkaline phosphatase (ALP), was observed depending on the dose of OCP [17]. It has been shown that synthetic OCP is converted into HA both in vivo [5,11,12,18] and in vitro [11,19–23]. Previous studies showed that a process of OCP–HA conversion involves exchanges of calcium and phosphate ions with surrounding tissue milieu [11,24,25] and is involved in promoting osteoblastic cell differentiation [11,17] and bone regeneration [5,11,12]. OCP can be converted topotaxially without changing its original morphology [21,26,27] even in vivo, where bone formation was accelerated by its implantation [28] thereby providing a scaffold for osteoblast attachment, proliferation and subsequent differentiation.

It is known that solubility at physiological pH decreases in the order of OCP,  $\beta$ -tricalcium phosphate ( $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>;  $\beta$ -TCP) and HA [24].  $\beta$ -TCP is widely accepted as a biodegradable bioceramic and used clinically [29–31]. Thus, OCP is the most soluble salt among them, so a lot of attention has been paid to the use of synthetic OCP with the expectation of it acting as potential loci for the nucleation of bone induction in orthotopic sites, which could be replaced with a significantly higher volume of newly formed bone compared with the other calcium phosphate phases such as HA [29,30] or amorphous carbonated apatite [13]. It has been explained that the biodegradable characteristics of OCP are acquired via its resorption by osteoclast-like multinucleated giant cells (MNGCs) in bone marrow spaces [6,16,32] after a larger amount of new bone deposition compared with the amount by HA [16], in addition to its soluble nature in physiological condition. However, it is still uncertain whether the enhanced bone formation is induced coupled with osteoclastic resorption of OCP in not only bone marrow spaces but also in an environment near to intramembranous bone, such as the calvaria. The subperiosteal region of intramembranous bone is considered to be a less reactive site compared with the bone marrow site regarding bone formation [33].

The present study was designed to investigate whether synthetic OCP stimulates bone deposition on its surfaces coupled with osteoclastic cell resorption and/or biodegradation compared with synthetic HA when implanted in the subperiosteal region of mouse calvaria. The characteristics of OCP as a bone substitute material were compared with non-sintered HA histochemically and histomorphometrically. It was reported that synthetic OCP enhances bone formation more than commercially available sintered porous HA ceramics in rabbit bone marrow [16] and in rat critical-sized calvaria bone defects [34]. In the present study, synthetic non-sintered HA was used as a control for OCP because it is known that non-sintered HA composed of nano-particles is relatively soluble compared with dense sintered HA ceramic [30]. Therefore, this type of HA could be a control material for OCP regarding their capability to induce bone deposition and be replaced with new bone over time.

## 2. Materials and methods

### 2.1. Materials and their characterization

OCP and HA were prepared according to methods described previously [5]. Briefly, OCP was synthesized by mixing Ca solution with phosphate solution at 70 °C and pH 5–6 [5]. HA was synthesized by mixing Ca solution with phosphate solution at 80 °C by adjusting pH 8.5–9.5 by ammonium water [5,35]. The synthetics were well washed with water, filtered and then dried at 120 °C. The dried cakes were ground using a pestle and mortar, and the ground granules between 16 and 32 mesh sizes (granule sizes 0.5–1.0 mm) were used for implantation. The crystalline structures of OCP and HA were characterized by X-ray diffraction (XRD). Powder XRD patterns were recorded using step-scanning at 0.2° intervals from 3.5° to 80°, with Cu K<sub>α</sub> X-rays on a diffractometer (Mini Flex; Rigaku Electrical Co. Ltd., Tokyo, Japan) at 30 kV and 15 mA. The range of  $2\theta$  included the primary peak (100) reflection of OCP  $\sim$ 4.7°. The XRD patterns of OCP and HA were compared with the original OCP recorded by the Joint Committee on Powder Diffraction Standards. The diameter of pores, porosity, pore volume, bulk and skeletal densities, and specific surface area (SSA) were estimated by mercury intrusion porosimetry (Yuasa Ionics PoreMater 60, Osaka, Japan) using the pieces of the dried cake before grinding.

### 2.2. Animals and implantation

All animal experiments were performed under the approval of the Institutional Review Board. The sieved granules were sterilized by heating to 120 °C for 2 h and implanted into the subperiosteal area of the calvaria of 7-week-old BALB/c strain mice under anesthesia by diethyl ether. After the capilli of the parietal region was removed using a depilatory agent, the skin and periosteum of the facies parietal were aseptically incised in a sagittal direction for a length of  $\sim$ 4 mm. Furthermore, the periosteum of the parietal region was flayed with a raspatorium. Three spoonfuls of granular OCP or HA ( $\sim$ 30 pieces) were placed adjacent to the exposed region of the parietal bone of each mouse using a stainless steel microspatula, and then the cranial skins of mice were sutured, leaving the periosteum as it had been flayed. Finally, the sutured region of the cranial skin was sterilized using Nobecutane spray (Mitsubishi Tanabe Pharma Corp., Osaka, Japan). OCP and HA granules were implanted for 7, 10, 14 and 21 days. Furthermore, prolonged experiments were performed for OCP for 30, 77, 90 and 180 days, and for HA for 35 days. Five mice were used for OCP and HA implantation, respectively.

### 2.3. Undecalcified and decalcified tissue preparations

Undecalcified tissues were prepared for histological and histomorphometrical analyses. The tissues, including

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