Octacalcium phosphate/gelatin composite: the effect of synthesis and crystal elongation on rabbit tibia bone repair

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Introduction: Synthetic octacalcium phosphate (OCP) has become recognized as a highly osteoconductive and biodegradable material if implanted in various bone defects, such as murine calvaria bone defects [1,2]. It was apparent that the osteoconductivity of OCP is markedly controlled depending on the preparation condition that makes the morphology of the plate-like OCP change to elongate toward specific direction of the crystals[3,4]. We have recently developed an OCP co-precipitated gelatin composite (OCP/Gel) which enhances bone regeneration more than other OCP-polymer composites[5]. This study suggested that the growth of OCP crystals along the specific face is regulated due to the presence of gelatin molecules in the preparation of the composite. The present study was designed to investigate how the synthesis condition affects the crystal growth of OCP in the presence of gelatin molecules and whether the OCP/Gel composite with a particular crystal characteristics has an effect on the bone regeneration in rabbit tibia defect model. Special attention was paid to the relationship between the degree of supersaturation (DS) of the OCP-precipitating solutions with gelatin molecules and the crystal characteristics.

Methods:

1. Preparation of OCP co-precipitated Gel composites under different degree of supersaturation

The direct precipitation from various concentrations of calcium and phosphate solutions was performed in a 70°C hot solution in the absence or the presence of 0.5wt% to 2.0wt% Gel molecules (isolated from porcine skin with an acidic process. The OCP/Gel precipitates were recovered, and then lyophilized for the crystal characterization. The disks of OCP/Gel (40wt% OCP) were molded (5 mm in diameter, 4 mm in thickness) and subjected to a dehydrothermal treatment in a vacuum drying oven for in vivo study.

The DS value can be expressed by dividing ionic product with solubility product of OCP[6]. Ca^{2+} and PO_4^{3-} concentrations, and pH in the solution were used for the calculation. The ion pairs considered were $CaH_2PO_4^+$, $CaHPO_4^0$, $MgHPO_4^0$, $CaHCO_3^+$, and $MgHCO_3^+$.

2. Characterization of OCP crystals precipitated

The length and aspect ratio of OCP crystals grown in the absence or the presence of Gel molecules were characterized by scanning electron microscopy (SEM).

3. In vivo evaluation of OCP/Gel composite

A rabbit tibia defect model was used. Four male Japanese white rabbits weighing 2.8-3.2 kg were used. All procedures were approved by the Animal Research Committee of Tohoku University. Under intravenous anesthesia, periosteal bone defect (5 mm in diameter) was created in the tibia near condoyle by using trephine drill. OCP/Gel disk with the specific crystal characteristics was implanted into the bone defect. At 2 weeks, the experimental rabbits were sacrificed, and the tibia bone, including the implant, was retrieved. The tissue was fixed in 4% paraformaldehyde, decalcified with EDTA, and embedded in paraffin. Four-μm-thick serial sections were prepared coronally and stained with hematoxylin and eosin (H-E).

Results:

With increasing the mixing molar ratio of calcium solution and phosphate solution from 1.0 to 1.67, DS value with respect to OCP decreased from 97.6 to 23.1. The aspect ratio was markedly decreased from 17.7 to 4.0 with decreasing DS values. DS of OCP 97.6 was used for OCP/Gel preparations due to our previous data of the oseoconduction of single use of OCP. Table 1 shows the characteristics of OCP crystals prepared.

Table 1 Characteristics of OCP crystals grown in the presence and the absence of gelatin molecules

Gelatin wt%	OCP crystals		
	Length, μm	Aspect ratio	
		With Gelatin	Without Gelatin
0.5	4.0	37.0	17.7
1.0	3.6	24.2	17.7
2.0	2.3	22.8	17.7

Figure 1 shows the histological section showing the sufficient repair of the defect by the OCP/Gel composite accompanied by chondrogenesis. The control (defect only) did not repair the defect completely.

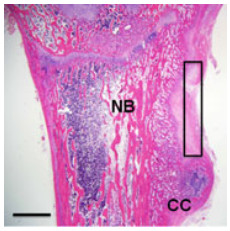

Figure 1. Decalcified histological section of an OCP/Gel implanted in rabbit tibia defect stained with H-E at 2 weeks. NB, new bone; CC, Chondrocyte. Rectangle: Defect site created. The defect was repaired by cortical bone formation. The OCP/Gel composite was almost resorbed. Bar=2mm.

Conclusions:

An OCP/Gel composite including OCP crystals having the aspect ratio 37 provided an osteoconductive property in rabbit long bone defect. OCP/Gel composite could be a material for substituting autologous bone.

References:

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