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Calcium Phosphate Biomaterials Osteoinduce Bone in Small Animals

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Abstract

Despite the fact that the osteoinduction mechanism of calcium phosphate (CP) ceramics is yet unknown, a number of crucial characteristics, including chemical composition, pore size and porosity, have been described. In this work, mice's leg muscles and rabbits' dorsal muscles were implanted with calcium phosphate powder, biphasic calcium phosphate ceramic powder (BCP, group 2), and entire BCP rods (group 3). For biological and histological investigation, samples were taken one month and three months following implantation. At the third month, mice showed new bone tissues in 9/10 samples in group 3, 3/10 samples in group 2, and 10/10 samples in group 1, but not in rabbits. Osteogenic differentiation was seen when human mesenchymal stem cells (hMSCs) were cultivated in vitro with traces of CaP and BCP powder. Our findings suggested that pore structure would promote increased bone formation and that chemical composition is a prerequisite for osteoinduction.

Keywords: Calcium Phosphate Powder; Biphasic Calcium Phosphate Ceramic; Osteoinduction

Introduction

Calcium phosphate (CP) ceramics, which have demonstrated good biocompatibility, osteo conductivity, and osteoinductivity, are promising artificial bone replacement materials. These attributes include chemical composition, sintering temperature, sintering duration, pore structure, and other physical and chemical characteristics [1]. Hydroxyapatite (HA) and tricalcium phosphate (TCP), which are both components of bone it, make up the majority of the CP ceramics. It is well known that TCP has a higher solubility and can release rich Ca and P ions for new bone formation, whereas HA has a more stable phase that can support ceramic hardness. According to Yuan et al., TCP caused greater bone growth in sheep's ilium than HA or BCP, or at least equally as much. Auto graft successfully completed In contrast to the more stable 100% HA, HA/TCP (76/24, 63/37, 56/44), and 100% TCP, Arinzeh et al. found that 20HA/80TCP had the highest impact on stem-cell-induced bone formation. CP ceramics typically sinter between 1100 and 1300°C; HA would significantly reduce surface area or porosity to negatively impact biocompatibility at temperatures below 1000°C and become unstable over 1300°C. Wilson et al. [2]. examined 10 different types of CP ceramics and discovered that they increased bone growth in the following order: the sintering temperature is low sintering temperature for BCP and high sintering temperature for HA BCP is superior to high-temperature HA. Pore structure would also have an impact on ontogenesis. According to research done in 1997 by, the ideal pore range of bio ceramics that allowed ectopic bone growth was 300-400 m, and the pores needed to be connected. Interconnectivity enables the development of blood vessels necessary for nourishing growing bones and removing waste [3]. Microspores are also required for a substance to be osteoinductive in addition to macrospores. Other osteogenic factors also point to ontogenesis, with BMP-2 being regarded as a crucial component. For instance, alkaline phosphatase (ALP) is a marker that can distinguish between cells that do not deposit calcium and those that do. Runx2 is a transcription factor that affects skeletal morphogenesis and osteoblast development. One of the most prevalent, non-collagenous proteins in mineralized adult bone is osteocalcin, and bone sialoprotei n (BSP) is a key structural protein. Collagen type I is the main collagen in mineral bone (>95%) and accounts for around 12% of the non-collagenous proteins in human bone [4].

We discovered osteoinduction in this study using calcium phosphate powder (Ca3(PO4)2, CaP), biphasic calcium phosphate ceramic powder (BCP), and intact BCP rods implanted in the muscles of mice, indicating that chemical composition is a prerequisite in osteoinduction and pore structure would promote more bone formation [5].

Materials and Methods

The Ca/P ratio of 1.5 in the commercially available CaP powder (Sigma-Aldrich, USA) employed in this investigation. Our partner, the National Engineering Research Center for Biomaterials at Sichuan University in China, delivered cylindrical BCP rods (35 mm). In a nutshell, wet-synthetic calcium phosphate powder with a 60:40 HA/-TCP ratio was used to create porous ceramics [6]. The green bodies were then foamed with 5–10% H_2O_2 at 70–80°C and dried. The green bodies were then sintered in air for two hours at 1100°C. This bio ceramics' porosity was around 50%, and its pores ranged in size from 300 to 500 m. With a Ca/P ratio and a pestle, BCP ceramics were pulverised into a powder that still had micropores, not macropores [7]. This CaP Prior to use, powder and BCP powder were filtered using a 200 mesh screen. Table 1 provides a summary of the material characteristics. Materials were sterilised at 120°C for 30 minutes, and before implantation, 10 g or 20 g of CaP and BCP powders were wetted with sterile PBS. The low-glucose Dulbecco's modified Eagle's medium (DMEM) with 10% foetal bovine serum (FBS) and 1% antibiotic/antimycotic was used to cultivate the human mesenchymal stem cells (hMSCs). Adherent cells were separated by 0.25% trypsin at 90% confluence and subcultured in a six-well plate. Equal amounts of 0.5 g CaP or BCP powder were added to the medium when the cells had reached 80% confluence in order to stimulate osteogenic differentiation in the cells [8].

At the same time, the osteogenic control group's medium was changed to one containing 10 mmol/L-glycerol phosphate, 108 mol/L

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dexamethasone, and 50 mg/L ascorbic acid 2-phosphate. At days 7 and 14, hMSCs were stained with Alizarin Red, and the medium was replaced every two days. Following 3, 7, 11, 14, and 21 days of culture, ALP activity was then measured using a kit for detecting ALP activity after cells were separated for protein extraction [9]. The Laboratory Animal Center at Sichuan University provided us with twelve male New Zealand white rabbits and forty male BALB/c mice that were both 6 weeks old. Previous descriptions of transplantation in mice have been published. Animals were briefly intraperitoneally sedated and had their legs cleaned. Ten grammes of wet CaP powder were implanted into each animal's left and right leg muscles in group 1 (n= 15), ten grammes of wet BCP powder were implanted into each animal's left and right leg muscles in group 2 (n= 15), and one BCP ceramic rod was implanted into each leg muscle in group three (n= 10) of additional ten mice. Additionally, rabbits were given intraperitoneal anaesthesia before having their dorsal cleaned and their skin prepped. There were 6 muscular pouches. each animal's dorsal muscles, and 20 g of wet CaP powder, wet BCP powder, or one wet BCP ceramic rod was inserted into each animal's muscle pouches, if appropriate (n = 4). The Sichuan University Animal Care and Use Committee gave the study their approval.

Animals were slaughtered at the first and third months, and the samples were either preserved for histology and electron microscopy analysis or harvested for total RNA and protein extraction.

Samples were fixed for two hours at room temperature in 3% glutaraldehyde. The specimens were dehydrated in an alcohol gradient and dried at the critical point for one hour prior to scanning electron microscopy (SEM) (Analysis and Testing Center, Sichuan University, China). Finally, samples were coated with gold-palladium and examined using a scanning electron microscope (SEM) at the National Engineering Research Center for Biomaterials, Sichuan University, China. Transmission electron microscope (TEM) tissue blocks were rinsed in 0.1 mol/L phosphate buffer for 4 hours at 4 degrees Celsius, then fixed in 1% osmic acid for 2 hours, followed by another 5 minutes of buffer rinsing, dehydration in gradient alcohol and acetone, soaking, embedding, and polymerization, and finally observation under TEM of 80 nm ultrathin sections [10]. We isolated and separated the proteins using a 10% polyacrylamide gel. Protein was transferred to a nitrocellulose membrane, which was then blocked with a 5% defatted milk solution, probed with mouse monoclonal antibodies against ColI and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:5000, Chemicon), and finally probed with a secondary antibody using ALP conjugated anti-mouse IgG. Blots were then developed using an ECL plus kit (GE, USA) our findings demonstrated the osteoinductivity of CaP powder, BCP powder, and whole BCP rods [11].

Conclusion

In this study, we employed real-time PCR and Western blot to determine the expression of osteogenesis genes and protein in vivo, which was rarely reported. The findings revealed that group 1 had higher levels of osteogenesis gene and protein expression than group 2, which was unexpected. The osteoinduction phenomena varies by species; it was frequently seen in large animals, like pigs, goats, dogs, etc., but only infrequently in tiny animals, like mice and rabbits. It was never discovered in rats, though. Our study used mice and rabbits as animal models, however the bone induction phenomena was only shown in mice. This may be because the phenomenon only occurs in mice.

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