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Enhanced healing of rabbit segmental radius defects with surface-coated calcium phosphate cement/bone morphogenetic protein-2 scaffolds

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ABSTRACT

Large osseous defects remain a difficult clinical problem in orthopedic surgery owing to the limited effective therapeutic options, and bone morphogenetic protein-2 (BMP-2) is useful for its potent osteoinductive properties in bone regeneration. Here we build a strategy to achieve prolonged duration time and help inducting new bone formation by using water-soluble polymers as a protective film. In this study, calcium phosphate cement (CPC) scaffolds were prepared as the matrix and combined with sodium carboxymethyl cellulose (CMC-Na), hydroxypropylmethyl cellulose (HPMC), and polyvinyl alcohol (PVA) respectively to protect from the digestion of rhBMP-2. After being implanted in the mouse thigh muscles, the surface-modified composite scaffolds evidently induced ectopic bone formation. In addition, we further evaluated the in vivo effects of surface-modified scaffolds in a rabbit radius critical defect by radiography, three dimensional micro-computed tomographic (μCT) imaging, synchrotron radiation-based micro-computed tomographic (SRμCT) imaging, histological analysis, and biomechanical measurement. The HPMC-modified CPC scaffold was regarded as the best combination for segmental bone regeneration in rabbit radius.

1. Introduction

Bone tissue engineering has become an attractive and potential approach for repairing large defects caused by tumors, trauma, surgical resection and congenital malformation [1]. Since the autografting bone substitute has limitations and disadvantages such as insufficient donors and donor site morbidity [2,3] and the potential risk of transmitting infectious for allogeneic bone exists [4], more attentions have been paid on the prosthetic scaffolds for implantation [5,6]. For bone tissue engineering, these scaffolds must require desirable mechanical properties, osteoconductivity, biocompatibility, biodegradability and non-cytotoxicity [7,8].

Since its first discovery in the 1980s, calcium phosphate cements (CPCs) have attracted great interest as bone substitutes owing to their excellent biocompatibility and osteoconductivity [9–11]. Moreover, the final composition of hardened CPCs is more similar to the bone-like apatite in vivo than sintered hydroxyapatite ceramics [12], thus exhibiting potential applications in bone regeneration, such as bone augmentation and spinal vertebroplasty [13–16]. However, despite the above advantages, the lack of osteoinductivity is one of the critical drawbacks of CPCs, which might result in bone nonunion, especially in the reconstruction of large defect. Incorporation with bioactive growth factors might be an efficient strategy endowing the osteoinductive activity.

Bone morphogenetic protein-2 (BMP-2), which is a member of transforming growth factor-β (TGF-β) superfamily, can affect cell differentiation and plays important roles in early embryonal development in adult and organisms [17–19]. As the most representative bone growth factor, recombinant human BMP-2 (rhBMP-2) has been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for the treatment of spinal fusion and tibial fractures combined with a matrix like collagen or decalcified bone [20–22]. However, rhBMP-2 has a short half-life and always appears bolus release at a very early period which may impair the bone regeneration. To overcome these problems, a number of carriers, including natural and synthetic biomaterials, have been explored in the past decade to maintain BMP retention and provide sustained delivery at the defect site [23–26].

Inorganic calcium phosphate-based scaffolds such as hydroxyapatite (HAP) and tricalcium phosphate (TCP) have already been investigated as carriers of BMP individually. Previous studies demonstrated that the incorporation of BMP into these ceramics greatly accelerates the bone formation [27–30]. CPC can either act as valid carriers of rhBMP-2. When combined with CPCs, the retention of rhBMP-2 can be prolonged to avoid enzymolysis of protease [31,32]. In addition, for the sake of clinic application and prevention of denaturation when

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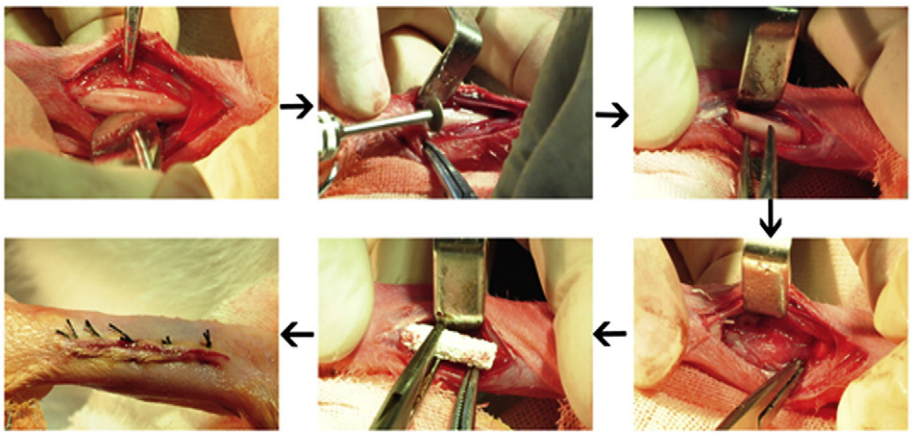

Fig. 1. The surgical procedures.

exposed to various organic solvents and undesirable nonphysiologic conditions, BMP-2 is always loaded by physical adsorption instead of conjugation or encapsulation. A series of problems arise due to the weak integration between rhBMP-2 and CPC matrix. The undesirable rapid diffusion and higher initial burst release would cause potential pathological risk.

Herein, we present a facile and convenient method to fabricate CPC-based composite scaffolds containing rhBMP-2. Three kinds of hydrophilic excipients, hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (CMC-Na) and polyvinyl alcohol (PVA) were selected for surface-coating. Optimized screening was conducted through the investigation of ectopic bone formation in mouse hindlimb pocket. Finally, a critical-sized defect model was created in a rabbit radius to evaluate the healing capacity of large bone defect. X-ray examination and micro-computed tomographic and synchrotron radiation-based micro-computed tomographic observations were performed. Histological analysis and biomechanics evaluation were also carried out. We hypothesized that via surface-coating, the rhBMP-2-impregnated CPC composite scaffold had enhanced osteogenic efficacy for segmental bone repairing.

2. Materials and methods

2.1. Materials

The CPC powder prepared in our laboratory was composed of tetracalcium phosphate (TECP) and dicalcium phosphate anhydrous (DCPA) in an equivalent molar ratio, using preparation methods according to the previous literature [33]. rhBMP-2 was denoted from Shanghai Rebone Biomaterials Co., Ltd. (Shanghai, China). Hydroxypropylmethyl cellulose (HPMC) was purchased from Shandong Liaocheng A Hua Pharmaceutical Co., Ltd. (Shandong, China). Sodium carboxymethyl cellulose (CMC-Na) was purchased from Shanghai Changwei Pharmaceutical Tech. Co., Ltd. (Shanghai, China). Polyvinyl alcohol (PVA) was obtained from Acros organics (USA).

2.2. Fabrication of porous CPC scaffold

CPC scaffolds were prepared by a particulate-leaching method [34]. Briefly, the TECP and DCPA powders were mixed with aqueous disodium hydrogen phosphate (4 wt.%) using a spatula at a powder/liquid mass ratio of 3:1 to form a paste. Sodium chloride particles sieved with diameters of 400–500 μm as porogen were added into the CPC paste. The mixture of CPC/NaCl was placed in stainless steel molds and the mixture was molded under a pressure of 2 MPa. Two different sizes of cylinder scaffolds (Φ3 × 4 mm, Φ4 × 15 mm) were preformed, one is used for ectopic bone formation and the other is applied in segmental radius repairing. After incubating in a constant temperature over at 37 °C and 100% relative humidity, the samples were then immersed in deionized water to leach out the porogen. Finally, they were vacuum-dried to obtain sponge-like scaffolds.

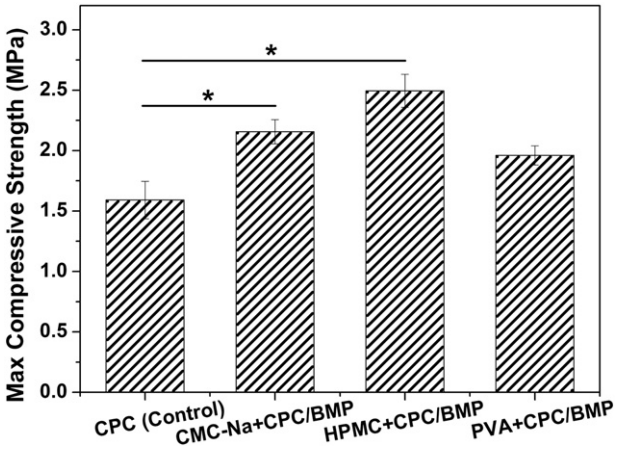

Fig. 2. The maximum compressive strength of CPC scaffolds (*$p < 0.05$, significant difference in the maximum strength as compared to the control group).

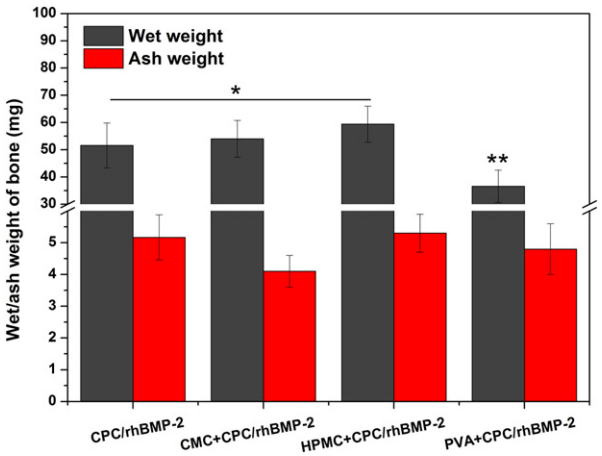

Fig. 3. Wet weights and ash content of the ectopic bone induced by rhBMP-2 in vivo for 4 weeks. (*$p < 0.05$, significant differences were found between HPMC-modified scaffolds and original ones; **$p < 0.05$, significant differences were found between PVA-modified scaffolds and the other three groups.).