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Development of BMP-2 immobilized polydopamine mediated multichannelled biphasic calcium phosphate granules for improved bone regeneration

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ABSTRACT

In the present study, the bone morphogenetic protein-2 (BMP-2) was loaded onto a polydopamine-coated biphasic calcium phosphate (BCP) granule system which could be effectively used as an injectable bone substitute (IBS) for improved bone regeneration. The surface of BCP granules was first coated with polydopamine (PD-MCG) and then BMP-2 growth factor was immobilized onto the PD-MCG by a conventional method. The results showed that BMP-2 was successfully immobilized into polydopamine-coated granules and about 87% of BMP-2 was retained on the granular surface for 30 days. *Invitro* and *invivo* studies showed enhanced cell viability and superior bone formation conferred by B-PD-MCG granules compared with PD-MCG and MCG. The percent healing capacity (BV/SV) of the three samples tested was found to be 1.64 ± 0.04, 1.4 ± 0.02 and 1.12 ± 0.03 for B-PD-MCG, PD-MCG and MCG, respectively.

1. Introduction

Despite the merits associated with the use of allograft and autograft for repairing bone defects, issues concerning their usage have limited their application, thus leading to the development of engineered bone substitutes [1]. Calcium phosphate ceramics have been studied extensively due to their biocompatibility with human hard tissue [2–4]. Among these, biphasic calcium phosphate (BCP) is one of the best suited materials for a synthetic bone substitute because it benefits from the combination of HA stability and β-TCP reactivity [3–5]. The porosity in the BCP structure further accelerates the circulation of body fluids and bone growth rate [4].

Surface modification of synthetic bone substitute with desired biochemical coating along with osteoinductive biomolecules is a promising approach to enhance specific cell responses, osteogenesis potential etc. and inturn speed up the new bone formation [5,6]. Polydopamine (PD) is simple, safe, cost effective and therefore has gained increasing attention with respect to surface modification. It has also been known to promote the adhesion and osteogenic differentiation [6,7].

An osteoinductive growth factor, BMP-2, has immense potential for inducing bone growth. However, large volume defects usually require a controlled release of BMPs to allow optimum signaling for efficient bone healing [8]. Therefore, there is a need for an efficient bone substitute system with proper surface modification accompanied by sustained release of BMPs for enhancement of bone regeneration.

This study describes a new injectable bone substitute system which was developed by surface modification of BCP granules with BMP-2 loaded polydopamine. The morphological characterization and the *invitro* and *invivo* biocompatibility of the B-PD and PD coatings on BCP granules were examined.

2. Materials and methods

2.1. Surface modification of BCP granules

Multichannel biphasic calcium phosphate granules (MCG) were used as starting material procured from Innobone Pvt Ltd., S.Korea. MCGs were thoroughly washed with deionized water (DI) before coating. Polydopamine (PD) solution was prepared by mixing dopamine hydrochloride (4 mg/mL, Sigma) with tris(hydroxymethyl)amino-methane (10 mM, Sigma). MCGs (1 g/mL) were then added to the prepared polydopamine solution. BMP-2 solution (500 mg/mL, Sigma) was dropped onto 1 g of PD-MCG and incubated at 4 °C overnight. PD-MCG loaded with BMP-2 (B-PD-MCG) was washed and freeze dried.

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2.2. Characterization

The surface morphologies of MCG and PD-MCG were examined using scanning electron microscopy (SEM, JSM-635F, JEOL, Japan). Phase analysis of MCG was analyzed using X-ray diffraction (XRD, D/MAX-250, Rigaku, Japan) with Cu Kα radiation. The absorption spectra were characterized by Fourier transform infrared spectroscopy (FTIR, Magna-IR-550, Nicolet, USA) using OMNIC version 7.3 software.

For *in vitro* studies, pre-osteoblast MC3T3-E1 cells were obtained from American Type Culture Collection (subclone 4, ATCC, USA). Cell viability was evaluated using the MTT assay per the standard protocol [ISO-10993-5:20099E-0]. MCG, PD-MCG and B-PD-MCG samples (triplicate in each) were seeded with cells ($1x10^5$cells/ml) and incubated for 1, 3, and 7 days. After each time period, 100 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide (MTT, Gibco, CA) solution was added to each well. Optical density (O.D.) was measured at a wavelength (595 nm) using ELISA reader (EL-312, Biokinetics, Bio-Tek instruments). For cell proliferation, samples were fixed with 4% paraformaldehyde (Sigma), and blocked with 1% bovine serum albumin (BSA, Sigma). The cytoskeletons of cells and cell nuclei were stained using a Fluorescein isothiocyanate (FITC) conjugated Phalloidin (25 μg/ml, Sigma) and 1 μg/ml of HOECHST 33342 (Sigma) respectively and analyzed using a confocal microscope (Olympus, FV10i-W).

The *invivo* studies, using New Zealand white rabbits, (~3 kg) were approved by the Institutional Animal Care Committee of Sonchunhyang University, S.Korea. All samples (three in each) were press-fitted into the defect (8 mm diameter, 5 mm depth) made on femur head. Micro-CT analysis was conducted after 3 and 6 weeks using apparatus (model-1172, Skyscan, Belgium). Images were reconstructed over the region of interest (ROI) using CTAn and CTVol (Skyscan) software. Percent bone volume (BV/SV, %) was calculated from sample volume (SV) and bone volume (BV). For staining, the decalcified bone sections were embedded in paraffin and cut sections (~5 μm) were placed on a slide. Sections were stained with hematoxylin & eosin (H&E) or Masson's trichrome and analyzed with an optical microscope (BX53, Olympus, Japan).

2.3. Statistical analysis

The statistical analysis was performed using two-way analysis of variance (ANOVA) in GraphPad Prism 5 and statistical significance was considered when p value < 0.05.

3. Results and discussions

3.1. Structural studies

As-received MCGs appeared as a porous cylindrically shaped interconnected network. The polydopamine coating transformed the characteristic white color of MCG to a deep yellow with no processing defects (Fig. 1a). SEM images of MCG showed the hemispherical type granular structure with pores within (Fig. 1b). PD-MCG displayed a slightly blurred surface with the presence of fibers, indicating a rougher morphology (Fig. 1c). X-ray spectra of MCG constituted of hydroxyapatite (HA, ICDD No: 01-072-1243) and tricalcium phosphate (β-TCP, ICDD No: 01-072-7587) phases (Fig. 1d). The HAP and TCP phase ratio was calculated to be 60:40, corresponding to their relative peak intensities and suggesting the formation of BCP ceramics [5]. MCG showed vibrations of PO_4^{3-} from 960 cm^{-1} to 1035 cm^{-1} and P-O at 1106 cm^{-1} (Fig. 1e) [5]. The successful coating of MCG with polydopamine was confirmed by the appearance of a broad absorption band at 3379 cm^{-1} corresponding to —OH and N—H stretching. The characteristic peaks (1500 cm^{-1}–1600 cm^{-1}) were assigned to various N—H bands. Bands in the range of 1627 cm^{-1}, 1512 cm^{-1} and 1296 cm^{-1} were attributed to C═O, C═N and C—O respectively [7].

3.2. Invitro studies

The immobilization of BMP-2 on the surface of PD-MCG was confirmed by fluorescent micrograph which exhibited positive

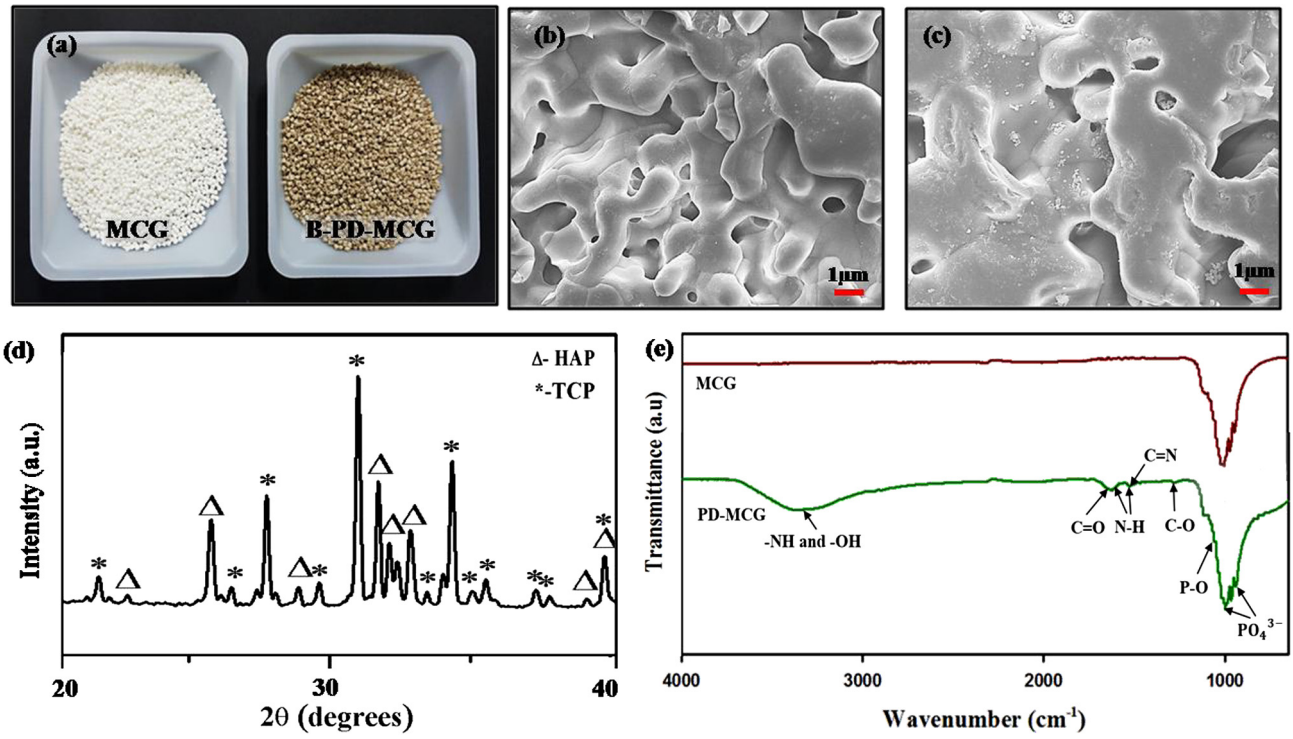

Fig. 1. (a) Pictorial view, SEM images (b) MCG, (c) PD-MCG, (d) XRD spectra of MCG and (e) FTIR spectra.