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Macroporous biphasic calcium phosphate scaffolds reinforced by poly-L-lactic acid/hydroxyapatite nanocomposite coatings for bone regeneration

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ABSTRACT

Three-dimensional (3D) interconnected porous scaffolds with biomimetic hierarchical architectures and mechanical characteristics are critical for the success of bone regeneration. In this paper, biphasic calcium phosphate (BCP) scaffolds were coated with medical-grade poly-L-lactic acid/hydroxyapatite (mPLLA/HA) nanocomposites to create controlled surface roughness while remaining the interconnected porous structures. Such mPLLA/HA coatings substantially improved the compressive strength of the scaffolds to 3.17–3.95 MPa, in contrast to 0.31 MPa for the as-prepared bare porous BCP scaffolds. Moreover, these mPLLA/HA-coated porous scaffolds were demonstrated to provide excellent support to the growth and proliferation of human barrow mysenchymal stem cells (hBMSCs). The hBMSCs-seeded mPLLA/HA-coated scaffolds were implanted to large necrotic lesions in the rabbit femoral head. New bone tissues were observed after two months, followed by gradient new bone formation over months according to H&E and Masson staining analysis. These results suggest that the mPLLA/HA-coated BCP scaffolds with improved mechanical strength and osteogenesis may be applied for bone regeneration.

1. Introduction

Millions of people are suffering from bone defects arising from trauma, tumor or bone related diseases [1], which may cause joint collapse and secondary osteoarthritis [2]. Autogenic and allogenic bone grafts have been in clinical use [3], but with source limitation for autogenetic bone grafts and immunological problems for allogenic bone grafts [4–6]. Bone tissue engineering has become a promising strategy for the repair of bone defects. Three-dimensional (3D) scaffolds with appropriate physical and mechanical properties, as well as biomimetic hierarchical structures, are desired to support or even stimulate osteogenesis and the formation of new bone [7–12].

Bioceramics have been widely recognized as most promising scaffolding materials for bone regeneration [13]. In particular, biphasic calcium phosphate (BCP) composed of HA (hydroxyapatite) and β-TCP (β-tricalcium phosphate) has showed excellent osteoinductivity, osteoconductivity, and biodegradability according to *in vitro* and *in vivo* evaluations [14–21]. Conventional fabrication methods, including dual-phase mixing method [22,23], gas forming method [24] and polymeric sponge method [25], could produce macroporous BCP scaffolds with channel-shaped pores. However, such macroporous BCP scaffolds are usually brittle, which remains a limit for bone tissue engineering [26]. To enhance the mechanical properties of ceramic scaffolds, the struts of scaffolds were coated with biocompatible polymers, while remaining the macropores intact and open [27–33]. For example, poly-L-lactide (PLLA) was used to coat BCP scaffolds because of its remarkable toughness and good biocompatibility [34]. On the other hand, in contrast to polymer materials, composite materials could exhibit improved bioactivity and mechanical properties [35]. For example, PLLA/HA

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composite scaffolds [36] enhanced the osteoblast adhesion and growth, in comparison to pure PLLA scaffolds. Our previous study has showed that the PLLA/HA nanocomposite coating on BCP scaffolds could improve the adhesion and proliferation of human bone mesenchymal stem cells [37]. Moreover, we have tried to improve the compressive strength of BCP scaffolds by increasing the layer numbers of PLLA/HA nanocomposites [38]. However, some of the pores were blocked and thus became less favorable to mass transport in the scaffolds [37]. It is known that interconnective porous structures are important for nutrients and wastes transportation for the generation and ingrowth of new bones into scaffolds. Therefore, it is desired to remain the interconnective porous structures while improving the mechanical properties by using biocompatible and biofunctional composite coatings. Herein, this is achieved by optimizing the coating formulations. Such optimized porous scaffolds have been demonstrated to nicely support the osteogenesis by using animal models.

In this paper, medical-grade poly-L-lactide/hydroxyapatite (mPLLA/HA) nanocomposites were coated on the internal surfaces of hierarchically macroporous BCP scaffolds to improve the mechanical properties and bioactivity. The porous structures, mechanical properties, cell adhesion, and biological degradation were systematically investigated as a function of the nanocomposite coating formulation. Finally, the coated scaffolds seeded with human bone mesenchymal stem cells (hBMSCs) were implanted into critical-sized rabbit femur head defect models for bone regeneration over months, and the osteogenesis was evaluated by histological examination.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA; polymerization degree ~ 1799 and hydrolysis degree ~ 99%) was obtained from Sigma–Aldrich Chemical Reagent Co. Ltd. Medical-grade poly-L-lactide powders (mPLLA; M_w = 200,000) were purchased from Dai Gang Biology Co. Ltd. Diammonium hydrogen phoshate ($(NH_4)_2HPO_4$, A.R.) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, A.R.) were obtained from Sinopharm Chemical Reagent Co. Ltd. Ammonia (A.R.) was obtained from Wuhan Chemical Reagent Co. Ltd.

2.2. Preparation of HA and BCP nanoparticles

HA nanoparticles were synthesized by using a precipitation reaction of the aqueous mixture of $(NH_4)_2HPO_4$ and $Ca(NO_3)_2{\cdot}4H_2O$ (Ca/P ratios: 1.67) at room temperature for 4 h. The solution was kept at pH 7 by using ammonia. The resultant white product was washed several times with distilled water to remove the ammonia. After drying at 80 °C, the obtained powders were heated at 450 °C to remove residual solvent and ammonia. The HA powders were suspended in water and dropped on a copper grid for imaging by using a transmission electron microscope (TEM, FEI, Hillsboro, USA) operated at 200 kV.

BCP nanoparticles were prepared by using an aqueous precipitation reaction as previously described [22]. Briefly, $(NH_4)_2HPO_4$ and $Ca(NO_3)_2{\cdot}4H_2O$ solutions were mixed at room temperature with the pH adjusted to 11. The resultant product was washed several times with distilled water, and the obtained powders were calcinated at 1100 °C for 1 h. The powders were crushed in a mortar and sieved to obtain BCP nanoparticles (200 mesh sieve).

2.3. Fabrication of macroporous mPLLA/HA nanocomposite coated BCP scaffolds

Macroporous BCP scaffolds were prepared by a replication method [38]. The prepared BCP powders were mixed in PVA solution (0.1 mol/L) to make a slurry (30 wt%), which was then templated by fully reticulated polyurethane foam. Subsequently, the PU foam was calcinated to produce porous BCP scaffold. The as-prepared BCP scaffolds were cleaned with ethanol, and dipped into sonicated mPLLA/HA slurry for 1 min, followed by drying for 3 days at 37 °C. The coated scaffolds were designated as HP2, HP3 and HP4, with the mPLLA/HA weight ratios of 10/2, 10/3, and 10/4, respectively.

2.4. Characterizations

2.4.1. Morphology

The porous scaffolds before and after coating with mPLLA/HA composites were fractured and the fracture surfaces were sputter coated with a thin gold layer for imaging by using a scanning electron microscope (SEM, FEI, Hillsboro, USA) at 20 kV. The average pore size was statistically analyzed on more than 20 pores from SEM pictures of five random areas.

2.4.2. Fourier-transform infrared spectroscopy (FTIR)

Attenuated total reflectance (ATR) FTIR spectra were collected within the range between 4000 and 500 cm^{-1} by using a VERTEX 70 FTIR spectrometer (Bruker, Germany) with a resolution of 1 cm^{-1}.

2.4.3. X-ray diffraction (XRD)

The samples were analyzed by X-ray diffraction (X-Pert PRO, PANalytical B.V.) scans at 40 kV and 40 mA with Cu-Kα radiation (1.54 Å) in the interval $10^\circ \le 2\theta \le 90^\circ$ at 2°/min.

2.4.4. Porosity

The open porosity (P) of scaffolds was measured by using a liquid displacement method. A sample was submerged in a known volume (V_1) ethanol, and a series of brief evacuation–repressurization cycles were conducted to force the liquid into the pores of the scaffold. The volume of the sample-impregnated liquid was V_2. When the liquid-impregnated scaffold was removed, the remaining liquid volume was V_3. Thus, the open porosity was calculated as: $P = (V_1 - V_3)/(V_2 - V_3)$.

2.4.5. Mechanical properties

The porous scaffolds (1 cm × 1 cm × 1 cm) were subjected to unconfined compression tests by using a testing machine (SANSCMT4503, China) at a crosshead speed of 2 mm/min. Five samples were tested for each group.

2.5. Biodegradation of scaffolds in simulated body fluid (SBF)

The *in vitro* biodegradation experiments were performed by soaking the scaffolds in SBF at 37 °C [38]. The SBF was prepared as previously reported [39]. The scaffolds were immersed in SBF solution (solid/liquid ratio: 50 mg/mL) in sealed plastic flasks. At day 5, 10, 15, 20, and 25, the samples were filtrated, rinsed with distilled water and dried for weighing by using a 0.1 mg balance (TG328D, Shanghai, China). The percentage weight loss with respect to the initial weight was calculated. On the other hand, the pH value of SBF was measured over time. Five samples were used for each group. The results are reported as mean ± SD.

2.6. Cellular assessment

The cellular assessments were performed *in vitro* by using the hBMSCs (obtained from the Orthopedic Department, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology) by following a procedure reported previously [38]. Briefly, the hBMSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) at 37 °C in a 5% CO_2 humidified environment. The