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Full Length Article

A mesoporous silica composite scaffold: Cell behaviors, biomineralization and mechanical properties

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ABSTRACT

Mesoporous structure is beneficial to cellular response due to the large specific surface area and high pore volume. In this study, mesoporous silica (SBA15) was incorporated into poly-L-lactic acid (PLLA) to construct composite scaffold by selective laser sintering. The results showed that SBA15 facilitated cells proliferation, which was mainly attributed to its unique intrinsic mesoporous structure and the released bioactive silicon. Moreover, the hydrolyzate of soluble mesoporous silica can adsorb ions to form nucleation sites that promote biomineralization, leading to improve biological activity of the composite scaffold. In addition, the compressive strength, compressive modulus and Vickers hardness of the scaffold were increased by 47.6%, 35.5% and 29.53% respectively with 1.5 wt.% SBA15. It was found that the particle enhancement of uniform distributed SBA15 accounted for the mechanic reinforcement of the composite scaffold. It indicated that the PLLA-SBA15 composite scaffold had potential applications in bone tissue engineering.

1. Introduction

Mesoporous silica attracts keen attention in tissue engineering due to its unique intrinsic structure, bioactivity and biodegradability [1–3]. Santa Barbara Amorphous-15 (SBA15), an amorphous mesoporous silica material, has two-dimensional hexagonal via-hole and cubic symmetries structure [4,5]. Its large specific surface area and pore volume can provide target sites for cell adhesion. Moreover, SBA15 can effectively improve the expression of I collagen protein and the secretion of extracellular signal regulated kinases (ERK), which stimulate osteoblast differentiation [6,7]. Furthermore, as bioactive material, it has the ability to biologically bond artificial bone to living bone. It was attributed that the hydrated silica gel layer produced by the condensation reaction between silanol groups could adsorb ions to form nucleation sites that promote biomineralization. [8–10].

Poly-L-lactic acid (PLLA), a biodegradable polyester material, was widely used in tissue engineering owing to its biocompatibility and favorable processability [11–14]. And its final hydrolyzate was carbon dioxide and water, which was harmless to tissue [15,16]. In addition, it can be fabricated into various structures with geometrical shape and architecture as demanded for various tissue engineering applications [17,18]. Nevertheless, for tissue engineering, its major drawback is lack of the ability to stimulate adhesion, proliferation and differentiation of cells and facilitate biomineralization [19,20].

Several works have been performed to use the bioactive materials as additives in PLLA [21–25]. Aitor Larranaga et al. fabricated bioactive glass (BG)/PLLA scaffolds by solvent casting/particulate leaching and found that the differentiation and ALP activity of adipose-derived stem cells were improved [26]. Fei Peng et al.

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prepared a hydroxyapatite (HA)/PLLA (20/80 wt.%) nanofibrous scaffolds by adding HA particles in PLLA via electrospinning and discovered that the biocompatibility of nanofibrous scaffold was better than pure PLLA scaffold [27]. Tao Lou et al. prepared β-tricalcium phosphate (β-TCP)/PLLA nanofibrous scaffolds by salt leaching techniques and found that the compressive modulus of the scaffold with 10 wt.% β-TCP increased by 50% [28].

In this study, interconnected porous PLLA-SBA15 composite scaffolds with mesopore/macropore were prepared by selective laser sintering (SLS). The morphology of cells adhered to scaffolds were characterized by SEM. Moreover, the proliferation and differentiation abilities of the cells were investigated by microculture tetrazolium test (MTT) and alkaline phosphatase (ALP) activities assay, respectively. In addition, the ability of the composite scaffolds to form bone-like apatite was evaluated in SBF.

2. Materials and methods

2.1. Materials

PLLA raw powder (Mw 10 000 Da; inherent viscosity 1.46 dl/g in chloroform at 25 °C, Tg = 60-65 °C, Tm = 175–185 °C) was purchased from Jinan Daigang Biomaterial Co., Ltd. (Jinan, China). SBA15 initial powder (pore diameter 5–10 nm, BET (m^2/g): 550–600) was obtained from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China).

2.2. Preparation

PLLA-SBA15 mixed powder preparation process showed in Fig. 1. First, a predetermined proportion of PLLA and SBA15 powders were poured into a 300 ml beaker and dissolved with absolute ethanol. Subsequently, the solution was treated with ultrasonic cleaner and a planetary high energy ball mill for 30 min, respectively. Then, the solution was filtered using qualitative filter paper. The filtered blended powder was placed in a constant temperature drying oven at 55 °C for 12 h. Finally, the dried mixed powder was mechanically ground using an agate mortar to obtain a powder usable for SLS processing.

The PLLA-SBA15 composite scaffolds with SBA15 content of 0 wt.% (PLLA-0SBA15), 0.5 wt.% (PLLA-0.5SBA15), 1 wt.% (PLLA-1SBA15), 1.5 wt.% (PLLA-1.5SBA15), 2 wt.% (PLLA-2SBA15) and 3 wt.% (PLLA-3SBA15) were manufactured via layer-by-layer selective laser sintering (SLS). During fabrication, the laser beam selectively scanned over the powder according to the cross-sectional profile of scaffold. The laser energy increases the temperature of powder to melting point so that the powder melting and rapidly solidify. The sintering platform was then moved down a single powder layer thickness and the sintering process was repeated until the scaffold was fully prepared. The unsintered powders were removed after the sintering was completed and the scaffold was cleaned with compressed air. A columnar (φ20 × 10 mm) interconnected porous PLLA-SBA15 composite scaffold was exhibited in Fig. 1.

2.3. Characterization of scaffold

The compressive strength of all scaffolds was determined by compression tests using a universal mechanical testing machine with a 100 N static compression load cell. The speed was set at 0.5 mm/min and five specimens were tested for each group. The compressive strength and modulus was determined through data analysis on the basis of the test data recorded.

The phase constituent of SBA15 and scaffolds was characterized via X-ray Diffraction (XRD, D8- ADVANCE/Max-40KV diffractometer, Cu-Ka radiation). Morphology of the scaffolds, bonelike hydroxyapatite, and cells were observed through scanning electron microscopy (SEM, JEOL Co., Japan) with a 20 kV field emission gun.

2.4. Biomineralization

All scaffolds were soaked in simulated body fluid (SBF, PH = 7.4) for 14 and 28 days at 37 °C to assess their bioactivity by detecting bonelike apatite formation. Scaffolds were immersed in a beaker filled with absolute ethyl alcohol and processed with ultrasonic cleaning device. The scaffolds were then incubated in a 12-well Petri dish containing SBF and the medium was changed every two days. Scaffolds were collected at regular intervals and rinsed three times with deionized water. Finally, these scaffolds were dried at room temperature for 24 h. The microstructure of the dried scaffolds was analyzed by SEM.

2.5. Cellular compatibility

MG-63 cells have similar properties to osteoblasts with the bone matrix synthesis and mineralization capacity. Thus, in this study, MG-63 cells were used to evaluate the osteoblastic responses to the PLLA-1.5SBA15 scaffolds. Cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere in DMEM supplemented with sodium pyruvate and 10% FBS plus 1% penicillin/streptomycin sulfate. Prior to cell seeding, all scaffolds were sonicated in absolute ethyl alcohol and sterilized via dry heat at 100 °C for 30 min and then transferred to tissue culture plates. The seeded concentration of cells on each scaffold was 4×10^5 cells, and the medium was replaced every other day. Cultivated for 1, 3 and 5 days, the scaffolds were taken out and fixed for 30 min in 3% glutaraldehyde, and then rinsed with PBS and dehydrated using ethanol. After they were completely dried, gold-sputtering was performed and observed by SEM.

The proliferation of MG-63 cells on the scaffolds was evaluated quantitatively via MTT, which was on the basis of the changes in absorbance at a specific wavelength. The yield of purple formazan in the osteoblast cultures with the scaffolds was measured. 100 μl MTT solution was immitted into each well after cell cultivated for 1, 3 and 5 days and incubated at 37 °C for 4 h. And then, the formazan was dissolved in DMSO for detecting at 570 nm using an enzyme-labeled apparatus (Amersham, UK). The culture medium was used as a control group to correct the test results. Each group consisted of 5 parallel experiments to statistically evaluate cell proliferation.

The ALP activity of the scaffolds was determined with MG-63 cells using a LabAssayTM ALP kit (Wako, Osaka, Japan) following the manufacturer's specification. Briefly, after 1, 3 and 5 days of osteogenic induction culture, 100 μl of a 0.25% trypsin solution was used to remove the cells from the scaffolds. And then, the cells were shifted to a new 96-well plate with 1.0 ml of culture solution to adhesion. 24 h later, the cells were washed thrice with PBS, and then fastened with formalin for 30 s. Thereafter, the cells were rinsed with deionized water twice, dyed using a staining reagent for an hour and photographed via a microscope (TE2000U, Nikon, Japan).

2.6. Statistical analysis

Experimental data were analyzed using One Way ANOVA and were expressed as mean ± standard error. When $P < 0.05$, a significant difference was expressed as*.

3. Results and discussion

3.1. Mechanical properties

The compressive strength, compressive modulus and Vickers hardness of PLLA-SBA15 composite scaffolds were evaluated, as