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Synthesis of biomedical composite scaffolds by laser sintering: Mechanical properties and *in vitro* bioactivity evaluation

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ABSTRACT

In this study, biomedical composite materials were employed to fabricate bone scaffolds using a self-developed rapid prototyping (RP) apparatus. The slurry formed by combining hydroxyapatite (HA), silica sol, and sodium tripolyphosphate (STPP) was heated by a CO_2 laser. Under appropriate processing parameters, a biocomposite green body was subsequently fabricated. Its mechanical properties, including surface roughness, bending and compression strengths, volume shrinkage rate, and surface microstructure, were analyzed after heat treatment to 1200 °C, 1300 °C, and 1400 °C. The results showed that after heating the specimen to 1200 °C, its compression and bending strengths increased significantly to 43.26 MPa and 1.28 MPa, respectively; the surface roughness was 12 μm; and surface pores were of size 5–25 μm. Furthermore, the results of WST-1 and LDH assay indicate that the biocomposites showed no cytotoxicity on 3T3 fibroblast. An optical density (OD) of 1.1 was also achieved, and the specimen was suitable for the adhesion and growth of osteoblast-like cells (MG63). Therefore, the biocomposite bone scaffolds fabricated in this study have potential to be bone implants for developing hard tissue.

1. Introduction

With increasing incidence of bone deterioration and cases of bone damage caused by accidents among the aging population, materials used for bone repairs and implants have recently attracted much research interest. Every year, many surgeries are performed for repair of damaged or broken bones. However, traditional repair methods involve mostly autogenic, allogeneic, and artificial bone transplants [1,2], all of which have demerits. While bone sources for autogenic transplantation are limited and recovery time is prolonged, patient receiving allogeneic transplants are prone to contract diseases from the bone donor, not to mention increased likelihood of rejection or viral infection. Moreover, traditional artificial bones are primarily made from metallic materials, which must remain in the human body, are not metabolized over time, and carry the risk of abrasion and ion release [3].

To solve the issues of bone shortage, disease infection, and metal ion release, tissue engineering (TE) has been under rapid development. TE is an emerging technology that involves various disciplines, such as biomedicine, material science, and engineering, with the goal of creating biological tissues and organs capable of replacing those of the human body [4]. This technology involves the implanting of cells from a lesion site onto a scaffold outside the human body. Subsequently, after the cells grow to a certain level, the scaffold is then transplanted into the lesion site to facilitate the repair of damaged tissue [5].

Most traditional bone implants are fabricated by biometals or bioceramics after mechanical processing or mold forming. If these traditional methods are applied to artificial bone transplantation, a surgical operation must be performed at the lesion site to ensure the success of implantation, which increases the risks of surgery. Hence, to fabricate bone scaffolds of similar shape as the lesion site and to decrease the risks of surgery, a rapid prototyping (RP) technique, also called 3D printing (3DP), was employed [6,7].

In recent years, several works on bone scaffolds fabricated via RP technologies have been conducted. For example, Fielding et al. [8] examined the mechanical and biological properties of 3D-printed tricalcium phosphate (TCP) scaffolds. Gbureck et al. [9] reported the resorbable dicalcium phosphate bone substitutes prepared by 3DP. Wu et al. 10] studied the preparation, characterization and *in vivo* osteogenesis for $CaSiO_3$ ceramic scaffolds fabricated by fused deposition modeling (FDM). Rai et al. [11] investigated the *in vitro/vivo* bone-forming efficacy of human mesenchymal stem cells cultured on PCL-TCP scaffolds. Kolan et al. [12] fabricated a bioactive glass scaffold for bone TE using indirect selective laser sintering (SLS). Simpson et al. [13] developed a poly(L-lactide-co-glycolide)/hydroxylapatite (HA) and β-TCP scaffold using SLS for bone replacement.

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The purpose of this study is to produce the biomedical composite scaffolds for bone repair and implant using an additive manufacturing (AM) technology. In addition, the *in vitro* bioactivity of the bone scaffold is evaluated by a microculture tetrazolium test (MTT) assay to validate its application to TE.

2. Materials and methods

2.1. Biocomposite slurry

The main elements of bone are calcium and phosphorus [14]. To fabricate a scaffold with contents similar to those of a human bone, micro-sized (average particle size of 1 μm) HA, nano- sized (average particle size of 25 nm) silica sol, and a small amount of sodium tripolyphosphate (STPP) were used as the raw materials. STPP is a type of surfactant, which when added to the slurry acts as a dispersant that increases the suspension effect. The HA, silic sol and STTP in a proportion of 34–39:60–65:1 wt.% were blended by a mixer (CKL, Multimix) at 120 rpm for 20 min to obtain a biocomposite slurry for bone scaffold fabrication. The viscosity of slurry, which was measured by a viscometer (DV-1 Prime, Brookfield) ranged from 11,000 to 15,000 cP. Slurry with appropriate viscosity possessed suspension effects. When fabricating an overhanging structure, support effects can be provided to facilitate the fabrication of bone scaffolds with complex shapes [15,16].

2.2. Experimental procedures

In this study, a layer additive technique was applied to a self-developed RP apparatus for forming biomedical composite bone scaffolds. The procedure involved the following steps. (1) A 3D simulation scaffold model of the external bone was created by a computer graphics software (e.g., Pro/Engineer); (2) the 3D model was converted into 2D image files with a slicing software (e.g., Magic RP); (3) the biomaterials were mixed in appropriate proportions to form a slurry as the raw material; (4) the slurry was paved onto the working platform, maintaining a specific layer thickness of 0.1 mm; (5) suitable processing parameters were adopted for selective laser sintering; (6) scanning of the 2D sliced image was performed using a CO_2 laser; and (7) steps 4 to 6 were repeated until a 3D green body of scaffold was finished [17]. Fig. 1 shows the flowchart that summarizes the process for fabricating a 3D bone scaffold.

2.3. In vitro cell culture

To ensure the biocompatibility of the biometric bone scaffolds, 12 specimens were produced using the method shown in Fig. 1. They were 7 mm × 7 mm × 2 mm in size and 0.1 mm thick. Four specimen sets, with each set comprising three specimens, were heat-treated at room temperature, 1200 °C, 1300 °C, and 1400 °C, and then placed in petri dishes to be sterilized in 75% alcohol for 10 min. Subsequently, osteoblast-like cells (MG63) were implanted onto the specimen surfaces, and the specimens were then placed in incubators, which were maintained at a constant temperature of 37 °C. Into the incubators, 5% CO_2 was injected. After the specimens were cultured for 4 h, 24 h, 4 days, and 7 days, the microculture tetrazolium test (MTT) reagents were added. Subsequently, the optical density (OD) values were measured to determine the cell survival rate. Finally, the state of cell adhesion and growth was observed using a scanning electron microscopy (SEM, JSM-6500F, JEOL) [18].

2.4. Characterization of materials

The morphology and microstructure of the bone scaffolds were analyzed using a SEM. The specimens were heat-treated in a high-temperature furnace (LHT04-17, Nabertherm). The bending and compression strengths were measured with a universal material testing machine (HT-9102, Hung Ta Instrument). The apparent porosity of the scaffolds was measured by the liquid displacement method. The density and porosity were evaluated according to the Archimedes principle. The surface roughness was measured by an α-step profiler (ET-4000A, Kosoka).

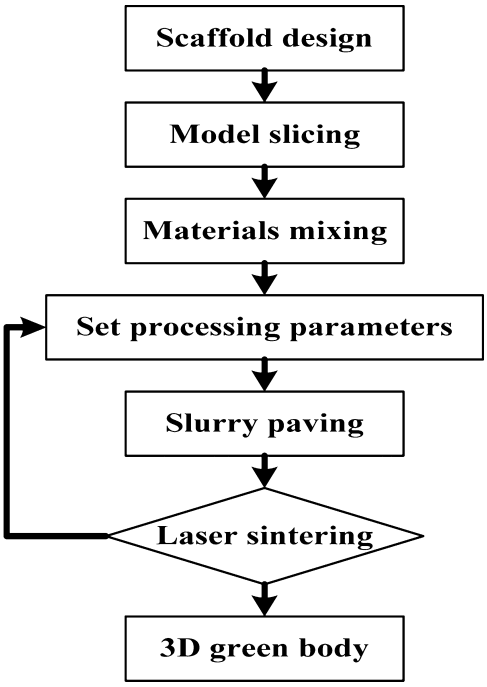

Fig. 1. Flowchart for fabricating 3D bone scaffold using selective laser sintering.

3. Results and discussion

3.1. Process parameters and biocomposite slurry

Whether biocomposite slurry solidifies depends on the absorbed laser energy density of the materials used. The relationship of energy density, energy, scan speed, and scan hatch of laser can be expressed as:

$$Pd = \frac{P}{Vs \times Hs}$$

where, *Pd*: laser energy density (J/mm^2), *P*: laser energy (W), *Vs*: laser scan speed (mm/s), *Hs*: laser scan hatch (mm).

Solidification of slurry begins after absorbing appropriate amounts of laser energy. In this study, the layer thickness and laser scan hatch were set to be 0.1 mm. Moreover, the laser frequency was set at 10 KHz. Subsequently, laser sintering technology was applied in the fabrication process with various laser irradiation energies and at difference scan speeds to observe how the slurry solidified. The suitable processing ranges of the laser parameters were tested using 7 mm × 7 mm × 2 mm specimens. After 30 trials, the process parameter window for specimen fabrication was obtained as shown in Fig. 2. Furthermore, the feasible ranges of the working parameters for the laser sintering process are listed in Table 1.

Fig. 3 shows biocomposite specimens obtained under different laser energies and scan speeds. As can be seen, the specimen shown in Fig. 3(b), fabricated under laser energy 12 W and scan speed 300 mm/s, is a complete green body formed upon solidification of slurry after absorbing appropriate laser energy density. In