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# Fabrication of porous titanium scaffolds by stack sintering of microporous titanium spheres produced with centrifugal granulation technology

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ABSTRACT

Microporosity plays a key role in bioactivity and osteoinductivity of a biomaterial scaffold. A simple new approach to fabricating load-bearing porous titanium (Ti) scaffolds with uniform porous structure, highly controllable pore size and excellent biocompatibility was developed in the present study. This method was based on stack sintering of microporous Ti spheres produced with centrifugal granulation of commercial Ti powders. Macropores (180.0–341.8 μm) and micropores (6.1–11.8 μm) of the scaffolds were dependent on the sizes of the Ti spheres and the Ti powders, respectively. The compressive strength of the scaffolds (83.4–108.9 MPa) was high enough for the repair of load-bearing bone defects. Besides, the abundant micropores occurred on the rough and convex surface of the Ti spheres in the scaffolds were more favorable for adsorption of serum proteins, and thus promoted the growth of mesenchymal stem cells (MSCs).



## 1. Introduction

With superior mechanical property, corrosion resistance and good biocompatibility, Ti and its alloys have been widely used for bone grafts or substitutes in load-bearing sites [1–3]. Nevertheless, stress shielding, due to the elastic modulus mismatch between Ti implant and bone, would lead to bone resorption and loosening of the implant [4,5]. In recent years, as a promising load-bearing bone grafts, porous Ti scaffolds have attracted much attention of researchers. First of all, by controlling its porosity and pore size distribution, the porous Ti scaffolds could be biomechanically compatible. Meanwhile, macropores of porous Ti scaffold could allow ingrowth of bone tissue, promoting fixation of the implant [6–9].

Porous structure parameters, such as open porosity, size distribution and interconnectivity of pores, play important roles in biological performance of a biomaterial scaffold. Typically, macropores above 100 μm were usually required to facilitate the rapid bone ingrowth and vascularization in the scaffold [10]. Interconnected macropores were more favorable for transportation and exchange of nutrient substances, as was necessary for growth of cells in the scaffold [10,11]. Besides, people also found that micropores on the walls of macropores played an active role in enhancing bioactivity and osteoinductivity of the scaffold. They could promote adsorption of osteogenic proteins, and thus enhance adhesion, proliferation and differentiation of cells [12–14]. Also, they could be utilized to carry drugs or growth factors such as bone morphogenetic protein-2 (BMP-2) [15,16].

Thus far, a variety of methods have been developed to fabricate porous Ti scaffolds, such as replication of polymer sponge [17], $H_2O_2$ foaming [18,19], space holder method [20,21], selective laser melting [22], plasma spraying [23], compacted Ti fiber sintering [24] and so forth. However, they either have relatively complex processing technique or cannot control pore size accurately. Stack sintering of spherical Ti beads could be a convenient method for fabricating a scaffold with homogeneous pore distribution, open porosity and highly controlled pore size. However, this scaffold had limited porosity and lacked micropores [25].

In the present study, a simple new approach to fabricating load-bearing porous Ti scaffolds with uniform porous structure, highly controllable pore size and excellent biocompatibility was developed. This scaffold was made by stack sintering of microporous Ti spheres produced with centrifuge granulation of commercial Ti powders. The porous structure and mechanical property of the fabricated porous Ti scaffolds were characterized. Protein adsorption and cell culture were carried out to evaluate their biocompatibility.

## 2. Materials and methods

Fig. 1 illustrates fabrication of porous Ti scaffold by stacking sintering of microporous Ti spheres produced with centrifugal granulation technology. Commercial Ti powders (Baoji Quanxing Titanium Industry Co. Ltd., China) were sieved to the required size range (38–45 μm, 54–63 μm and 88–97 μm). The sieved Ti powders were added into a self-made centrifugal granulator, whose rotate speed was adjusted to 200 rpm so as to keep vorticity of the powders. Then, 5% poly(vinyl alcohol) (PVA) aqueous solution was sprayed to cohere the powders into spheres, while the new powders were continuously added until

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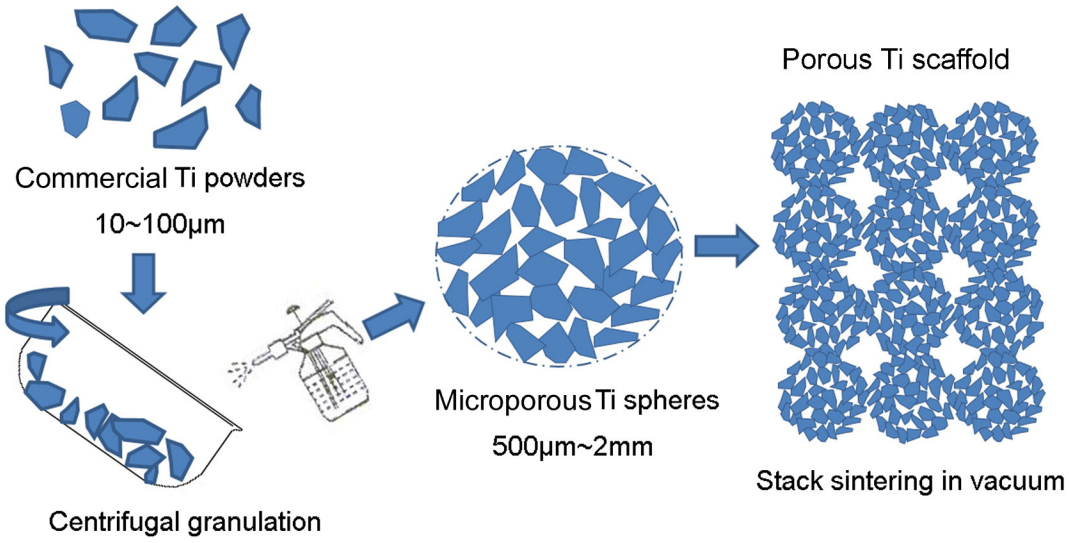


**Fig. 1.** Schematic diagram illustrating fabrication of the porous Ti scaffolds.

the Ti spheres grew up to the certain size. The Ti spheres were collected and sintered at 1100 °C for 1 h in a vacuum sintering furnace (ZR-50-21, Shanghai Cheng Hua Electric Furnace Co. Ltd., China) to make them have certain strength. Finally, the sieved Ti spheres (500–600 μm, 800–900 μm and 1000–1250 μm) were filled into a cylindrical mold, compacted and sintered in a vacuum of $1 \times 10^{-3}$ Pa at 1300 °C for 2 h. For comparison, commercially available Ti beads (Baoji Quanxing Titanium Industry Co. Ltd., China) were also used to fabricate the scaffold by the same method.

Table 1 presents the different porous Ti scaffolds fabricated in the present study. Morphology of them was observed using a field emission scanning electron microscopy (FE-SEM, S4800, Hitachi, Japan). Mercury intrusion porosimetry (Auto Pore IV9500, Micromeritics, USA) was used to measure pore size distribution and porosity of the samples ($\Phi 14 \times 3$ mm), and three parallel samples for each scaffold were analyzed. Uniaxial compression tests were performed on the cylindrical samples ($\Phi 4 \times 8$ mm) to evaluate mechanical strength of the scaffolds using a materials testing machine (Instron 8874, Norwood, MA, USA). A compressive load was applied to each sample at a crosshead speed of 1.0 mm/min until the evident failure. Before testing specific surface area (SSA), a commonly used acid-alkali treatment was used to activate the porous Ti scaffolds according to the previous method [26]. Simply, the samples were immersed in a mixture solution consisting of 48% $H_2SO_4$ and 18% HCl for 1 h at 70 °C, and subsequently in 6 M NaOH solution at 70 °C for 5 h. Nitrogen adsorption tests were then performed on the treated samples using a surface area and pore size analyzer (GeminiVII 2390 t, Micromeritics, USA). SSAs of the samples were determined by the Brunauer–Emmett–Teller (BET) method and calculated using the data over the relative pressure range of $0.05 \leq p/p_0 \leq 0.25$.

To evaluate biocompatibility of the porous Ti scaffolds, protein adsorption and cell culture experiments were performed on the samples, respectively. Protein adsorption was carried out in 24-well plates. The samples after acid-alkali treatment were placed into the wells, and then 1 mL of human serum (Beijing Minhai Biotechnology, China) per well was added. The plates were incubated for 4 h at 37 °C in an incubator, and then the samples were removed and washed with 0.01 M phosphate buffer solution (PBS) so as to remove those loose-binding proteins. The adsorbed proteins on the samples were eluted by 2% sodium dodecyl sulfonate (SDS) solution, and then the desorbed fractions were collected for protein quantitative analysis (BCA™ protein assay kit, Pierce, USA). MSCs were isolated from bone marrow of newly born rabbits' femora and tibiae according to the previous method [13], and the passage 3 cells were used for cell culture. MSCs ($1 \times 10^5$) were seeded onto the samples and cultured for 3 days. The attached cells were stained with 0.1% fluorescein diacetate (FDA, Topbio Science, China) for 15 min, and visualized as green fluorescence. Morphology of MSCs was observed using an inverted confocal laser scanning microscope (CLSM) (TCS SP 5, Leica Microsystems, Germany).

**Table 1**
Designation for the fabricated porous Ti scaffolds.

| Designation | | The Ti powders (μm) | The Ti spheres (μm) |
|---|---|---|---|
| Group 1 | Ti-600/54 | 54–63 | 500–600 |
| | Ti-900/54 | 54–63 | 800–900 |
| | Ti-1250/54 | 54–63 | 1000–1250 |
| Group 2 | Ti-900 | – | 900 (Ti beads) |
| | Ti-900/38 | 38–45 | 800–900 |
| | Ti-900/54 | 54–63 | 800–900 |
| | Ti-900/88 | 88–97 | 800–900 |

## 3. Results and discussion

By stack sintering of microporous Ti spheres produced with centrifugal granulation technology, the porous Ti scaffolds with uniform porous structure and highly controllable pore size were fabricated successfully. The macroscopic appearance and microstructure of the Ti spheres and the fabricated porous Ti scaffolds are shown in Figs. 2 and 3, respectively. The Ti spheres produced with centrifugal granulation of commercial Ti powders had nearly spherical shape and rather rough surface (Fig. 2a and b), on which a large number of micropores occurred (Fig. 2c). However, the commercial Ti beads had also spherical shape but smooth surface, on which no micropores could be observed (Fig. 3d). By using different size ranges of the Ti spheres, the scaffolds with different porous structure could be easily obtained (Fig. 3a). According to the fabricating method, macropores in the scaffolds resulted from the gaps among the Ti spheres (beads) and thus had the convex arc and channel-like shape (Fig. 3c and d). For bone repair scaffold, its porous structure plays an important role in determining its ability to induce bone ingrowth, and much work has been done to explore the relationship between pore characteristic and osteogenesis of the scaffold [10,27–29]. Size and shape of macropores in the scaffold have been confirmed to affect its osteogenic outcome notably. According to the report of Hubert et al., the minimum macropore size about 100 μm was necessary to regenerate mineralized bone [28]. Wang et al. fabricated two types of hydroxyapatite (HA) scaffolds with different shapes of