An improved polymeric sponge replication method for biomedical porous titanium scaffolds

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Abstract

Biomedical porous titanium (Ti) scaffolds were fabricated by an improved polymeric sponge replication method. The unique formulations and distinct processing techniques, i.e. a mixture of water and ethanol as solvent, multiple coatings with different viscosities of the Ti slurries and centrifugation for removing the extra slurries were used in the present study. The optimized porous Ti scaffolds had uniform porous structure and completely interconnected macropores ($\sim 365.1\text{ }\mu\text{m}$). In addition, two different sizes of micropores (~ 45.4 and $\sim 6.2\text{ }\mu\text{m}$) were also formed in the skeleton of the scaffold. The addition of ethanol to the Ti slurry increased the compressive strength of the scaffold by improving the compactness of the skeleton. A compressive strength of $83.6\pm 4.0\text{ MPa}$ was achieved for a porous Ti scaffold with a porosity of $66.4\pm 1.8\%$. Our cellular study also revealed that the scaffolds could support the growth and proliferation of mesenchymal stem cells (MSCs).

Keywords: titanium; porous scaffold; sponge replication; ethanol; multiple coatings

1. Introduction

Titanium (Ti) and its alloys have been extensively applied as load-bearing orthopedic devices in clinic due to their superior mechanical property, high corrosion resistance and good biocompatibility [1, 2]. Artificial joints and dental implants are their two representative applications. However, mismatch of Young's modulus between human bone and Ti implant may lead to the occurrence of stress-shielding, resorption of surrounding bone, and the loosening and failure of Ti implants [3-5]. In order to overcome the problem mentioned above, porous structures on Ti implants were developed. On one hand, the porous structures can reduce the mechanical properties of the implant. As a result, the mechanical strength and elastic modulus of Ti implant could be adjusted to a comparable level with native bone tissue by controlling the porosity and pore size distribution of the porous implant. On the other hand, the pores, especially macropores allow the transportation of body fluids and further promote bone ingrowth [6, 7]. As a result, a variety of processing techniques have been developed to fabricate biomedical porous Ti, such as powder or fiber sintering [8-10], plasma spraying [11], gas foaming [12], freeze casting [13, 14], space-holder [15, 16], sponge replication [17, 18], and 3D rapid prototyping [19, 20].

Recently, polymeric sponge replication method has been proved to be a promising and effective method because of its potential in fabricating Ti scaffolds with high porosity, excellent interconnections between the macropores, and similar pore shape with that of cancellous bone [17, 18, 21-23]. In the sponge replication technique, controlling the rapid drying of the coated slurry plays a crucial role. Aqueous solutions containing binders to prepare Ti slurries typically resulted in a slow drying rate. Thus the relatively heavy Ti slurry would flow downward before solidification, leading to the uneven porous structure of the final Ti scaffold. In order to increase

the amount of the coated Ti slurry, a multiple coatings technique is often adopted. However, the uniform slurry concentration at each coating process may have an adverse effect on the amount of the coated Ti slurry and the mechanical strength of the Ti scaffolds. Ahmad et al. produced Ti scaffolds with porosity up to 73 % by using the sponge replication process with repeatedly dipping and drying processes. However, the compressive strength of as-prepared porous Ti was only 14.85 MPa [24]. Using the same method, Lee et al. fabricated Ti scaffolds with a porosity of ~70 % and a compressive strength of 18 ± 0.3 MPa [21]. The above porous Ti scaffolds have much lower mechanical strengths than that of bone. Therefore, in order to well satisfy the clinical needs for high strength porous Ti, it is necessary to modify the traditional sponge replication method.

In this study, an improved sponge replication method to prepare porous Ti scaffolds was developed. Firstly, in order to reach a fast drying rate and maintain appropriate viscosity of the Ti slurry, a novel solvent, i.e. a mixture of water and ethanol was introduced to the preparation of the Ti slurry. The addition of ethanol can also remove the bubbles in the slurry, and thus densify the skeleton of the scaffold. Secondly, in order to obtain a high amount of coated Ti slurries during multiple coatings, change of viscosity of the Ti slurry with the increase of the coating times was used in this work. A mixture of ethanol and deionized water as solvent was used to make Ti slurry. The green body was prepared by a centrifuging process and multiple coatings technique to eliminate the extra Ti slurry. After optimization of processing parameters, porous Ti scaffolds with a uniform porous structure, high mechanical strength and good biocompatibility were prepared and characterized.

2. Materials and methods

Commercially pure Ti powders that comply with the Chinese standard YS/T 654-2007 was

purchased from Chengdu HuaRui Group Ltd., China. Polyurethane (PU) foams (40 pores per inch, Guangan City Xuhui Co. Ltd., China) were used as the replicated templates. The dimension of PU foams was about 15×20×37 mm. Polyvinyl Alcohol (PVA, Chengdu Kelon Chemical Reagent Factory, China) solution was used as binder. A mixture of deionized water and ethanol was used as the solvent of PVA.

The process to prepare porous Ti scaffolds with the improved polymeric sponge replication method is following. The Ti slurry was prepared by dispersing the Ti powders into a PVA solution. The PU foam was immersed into the Ti slurry until it was fully impregnated. Next, the foam was taken out and centrifuged to remove the extra slurry, leaving a thin layer of Ti slurry on the struts of the foam. The foam coated with the Ti slurry was then dried for 2 hours at 60 °C in an oven. The procedure of coating, centrifugation and drying was repeated for several times until the appropriate amount of the Ti slurry on the struts was obtained. Finally, the dried green bodies were heated to 600 °C at a heating rate of 5 °C/min and calcined for 1 hour to burn out the PU foam. The scaffolds were then sintered at 1350 °C for 2 hours under a vacuum of 10^{-3} Pa in a vacuum sintering furnace (ZR-50-21, Shanghai Cheng Hua Electric Furnace Co., China).

The viscosity of the Ti slurry was measured by a viscometer (DV2T, Brookfield Engineering Labs, USA) at a speed of 100 rpm with a spindle SC4-21 and at room temperature (~20 °C). Morphologies and microstructures of the sintered porous Ti samples were observed by a field emission scanning electron microscopy (FE-SEM, S4800, Hitachi, Japan). The pore size distribution and porosity of the samples were analyzed using a mercury intrusion porosimetry (Auto Pore IV9500, Micromeritics, USA). The compressive strength of cylindrical samples ($\Phi 6 \times 10$ mm) was evaluated by a universal material testing machine (AGS-X, SHIMADZU, Japan).

at a crosshead speed of 1.0 mm/min.

The cellular compatibility of the samples was evaluated by in vitro cell culture experiments. The used MSCs were isolated from bone marrow of newly-born rabbits' femur and tibia according to a previously established protocol [25]. The cells were seeded on the porous Ti samples ($\Phi 9 \times 2$ mm) and at a density of 5×10^4 cells/ml for confocal laser scanning microscopy (CLSM) observation and MTT assay. Tissue culture plates were used as the control. The cells on the samples were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10 % fetal bovine serum (FBS, Beijing Minhai Biotechnology, China) and 1 % penicillin/streptomycin at 37 °C in an atmosphere with 100 % humidity and 5 % CO₂. After culturing for 4 days, the cells attached to the porous Ti scaffolds were stained with fluorescein diacetate (FDA, Topbio Science, China) and propidium iodide (PI, Topbio Science, China) according to the manufacturer's instructions. The viability and morphology of the attached cells were observed using an inverted CLSM (TCS SP 5, Leica, Germany). In FDA/PI staining, live cells can react with FDA in green color, while dead cells can react with PI in red color. After culturing for 1, 4 and 7 days, the cell were incubated with 0.5 mg/ml MTT for 4h at 37 °C. Then the supernatants were removed, and dimethyl sulphoxide (DMSO) was added to dissolve the purple formazan salts. The cell proliferation and viability were examined by a multifunctional full wavelength microplate reader (Varioskan Flash, Thermo Scientific, USA) at the wavelength of 490 nm.

3. Results and discussion

Figure 1a and 1b illustrates the Ti powder and PU foam used in the present study. The Ti particles have irregular shape, and the particle sizes are between 38-45 μm . The PU foam has

interconnected open porous structure, with pore size of $\sim 600\text{ }\mu\text{m}$ and strut thickness of $\sim 100\text{ }\mu\text{m}$.

Figure 2 gives the process flowchart of the improved polymeric sponge replication method. The

used Ti slurries and technological parameters in this modified replication method are shown in

Table 1 and 2, respectively. Polymeric sponge replication method has been widely used for

fabrication of porous ceramics and metals [26-29]. The current replication method is quite

different from the traditional methods in terms of several critical processing parameters. Firstly,

the choice of liquid medium is different. Traditional methods generally use water as the liquid

phase of the slurry, but this improved method chose a mixture of ethanol and water as the liquid

phase. Secondly, the method of removing extra slurry is different. Traditional methods often

removed the extra slurry by squeezing after the foam impregnation, but the improved method

removed the extra slurry by centrifugation in order to prevent the formation of the closed pores.

Thirdly, the varied slurry viscosity was used in the multiple coatings technique. Traditional

methods usually chose the same viscosity of the slurry for every coating process, but slurries with

three different viscosities were used at different stage of the coating process in the improved

method. In order to insure more Ti slurries coated on the struts of the PU foam and avoid blocking

of pores, the viscosity of the used Ti slurry decreased gradually with the increasing of coating

times.

The property of the slurry for polymeric sponge replication process plays an important role in

quality of the final products. Deionized or distilled water was used as a traditional liquid phase for

the preparation of slurries. Such slurries have slower drying rates and different flow rates that lead

to uneven porous structure of the obtained scaffold. In addition, air bubbles can be easily formed

in the slurry, which would not only influence the coating quality of slurry on the struts, but also

produce microstructure defects such as cracks, pores on the wall and other large flaws in the scaffolds [22, 30]. For the preparation of ceramic slurries, ethanol was often used as an anti-foaming agent [31]. Based on the unique properties of ethanol as a solvent, such as rapid volatility and anti-foaming effects, a mixture of water and ethanol was chosen as the liquid phase of the Ti slurry in the present study.

To achieve good attachment of the Ti slurry on the struts of the PU foam, appropriate viscosity of the slurry is required [22, 30, 32, 33]. Figure 3a shows the effect of the ethanol content on the viscosity of the Ti slurry. It could be seen that the viscosity of the Ti slurry increased with the increasing of ethanol content. Usually, slurry with a higher viscosity could adhere to the struts of the PU foam easier than that with a lower viscosity. Thus a thick coating could be formed on the struts. Besides, in the preparation of the Ti slurries, we found that higher ethanol content in the slurry exhibited better effect on eliminating air bubbles than lower ethanol content. However, if the viscosity of the slurry is too high, the extra slurry couldn't be removed easily from the PU foam by centrifugation, which could lead to the blockage of the pores in the scaffold. According to the data shown in Figure 3a, the viscosity of the Ti slurry (slurry I) had a rapid rise when the ethanol content was above 25 vol. %. Therefore, we chose the ethanol content of 25 vol. % in the liquid phase for the preparation of the Ti slurry in the subsequent experiments.

Figure 3b presents the influence of the PVA content on the viscosity of the Ti slurry. The viscosity of the Ti slurry had a slight increase when the PVA content increased from 1 to 5 wt. %. The viscosity of the Ti slurry then increased sharply after the PVA content reached 5 wt. %. A high content of the PVA binder was advantageous for the attachment of the slurry on the struts of the PU foam. However, a high content of PVA binder could also result in a surge of the slurry's

viscosity. Therefore, the PVA content of 5 wt. % was chosen for the preparation of the Ti slurry.

Figure 3c shows the effect of the ratio of solid to liquid on the viscosity of the slurry. When the ratio of solid to liquid was below 3 g/10 ml, the viscosity of the slurry only had a slight increase. However, when the ratio of solid to liquid was above 8 g/10 ml, the viscosity of the slurry exhibited a sharp increase. In the present study, we chose a varied ratio of solid to liquid to both match the multiple coatings technique and achieve better coating on the struts of the PU foam. Three Ti slurries with different ratios of solid to liquid were used, as shown in Table 1.

Except for the viscosity of the slurry, both the method of removing extra slurry and times of coating are important factors to prepare porous Ti scaffolds with uniform porous structure and high mechanical strength. In the present study, we removed the extra slurry by centrifugation method instead of traditional squeezing method, because the traditional method was unsuitable for our multiple coatings technique. It is well known that mechanical strength of a porous scaffold increases with the decreasing of the porosity of the scaffold. The porosity of a Ti scaffold can be affected by the thickness of the Ti coating on the struts of the PU foam. Therefore, the process of multiple coatings could be used to prepare porous Ti scaffold with high mechanical strength [6, 21]. After each coating, the extra slurry would be removed by centrifugation of the PU foam coated with the Ti slurry. During the course of the centrifugation, a higher rotational speed would produce a higher centrifugal force, which may result in the deformation of the PU foam. However, if the rotational speed is too low, the extra slurry could not be removed to leave the porous structure open. Moreover, with the increase of coating times, the Ti coating on the struts of the PU foam was thickened. For the same rotational speed and slurry concentration with previous coatings, a partially closed porous structure could be developed. Therefore, variation of rotational speed and

slurry concentration is necessary with the increase of coating times.

Table 2 reports the centrifugal rotational speed, the slurry concentration and the responding coating times during the preparation of porous Ti scaffolds. At the first three coating times, Slurry I with higher viscosity was applied for the foam impregnation to insure more Ti slurries coated on the struts of the PU foam. Although the rotational speed increased with the coating times, the foam was blocked when coated up to the fourth time of coating. Therefore, from the fourth time of coating, Slurry II with middle viscosity was applied for the subsequent impregnation. However, the foam was blocked again when coated up to the seventh time. Therefore, Slurry III with lower viscosity was applied for the final two times of impregnation.

Figure 4 shows the SEM images of the microstructure of porous Ti scaffolds by the sponge replication method without or with addition of ethanol. With the addition of ethanol in the slurry, the Ti coated skeleton became denser than without the addition of ethanol, which is due to the anti-foaming effect of ethanol. Using the improved replication method, the change of skeleton thickness of the porous Ti scaffold with the coating times is shown in Figure 5. Obviously, the skeleton of the Ti scaffold became thicker and thicker when the coating times increased from four to seven. Furthermore, the total porosities of the porous Ti scaffolds are calculated by the formula:

$p=1-\rho/\rho_0$, where p , ρ and ρ_0 represent the porosity, actual density and theoretical density of the scaffold, respectively. The compressive strengths of the scaffolds were tested as well. The porosity and mechanical properties are summarized in Table 3. The porosity of the scaffold decreased from 84.2 ± 1.2 to 66.4 ± 1.8 % with the increase of the coating times from 5 to 9. The compressive strength of the resultant porous Ti scaffolds increased from 8.9 ± 0.6 to 83.6 ± 4.0 MPa. Therefore, the porosity and mechanical strength of the porous Ti scaffold prepared by the method could be

easily adjusted by controlling the coating times.

The ultimate goal of this work is to develop porous Ti scaffolds with an improved sponge replication method for the repair of bone defect under load-bearing conditions. It has been reported that human cancellous bone has a porosity of approximately 75 %, and its macropore size ranges from 100 to 600 μm [30]. Moreover, researchers have done much work to investigate the effects of macropore size on bone ingrowth into the scaffold. The results suggested that the macropore size ranging from 100 to 500 μm could be the optimal pore size for bony ingrowth [18, 34, 35]. Recently, some researchers reported that the micropores distributed on the wall of the macropores may have positive effects on new bone formation [36]. The load-bearing function of human bone is mainly provided by the cortical bone, whose compressive strength is about 80-120 MPa [37]. Therefore, the ideal porous Ti scaffold not only has high porosity and appropriate pore size for tissue ingrowth, but also has high mechanical strength to bear loads. By using the current method and optimizing the processing parameters, porous Ti scaffolds with both appropriate porosity and pore size for bony ingrowth, and strong mechanical properties to support loads were successfully developed.

Figure 6 shows the typical SEM images of the optimized porous Ti scaffolds. The scaffolds replicated the porous structure of the PU foams very well. A uniform porous structure and completely interconnected macropores were also observed clearly. Although the skeleton of the scaffold was relatively dense, some micropores could be found. The pore size distribution of the scaffold was evaluated by a mercury intrusion porosimetry, and the result is shown in Figure 7. Three characteristic peaks were observed in the curve, which correspond to the pore sizes of 6.2, 45.4 and 365.1 μm , respectively. The peaks of 6.2 and 45.4 μm indicated that the micropores

occurred in the scaffold, which could be attributed to the generated gas and formation of sintering necks during the sintering process of Ti powders and the burning off of the struts of the PU foam, respectively. The macropore peak suggested a nice replication of the porous structure of the PU foam. The result of mercury intrusion porosimetry agrees well with the SEM observation, and confirmed the expected porous structure of the as-prepared Ti scaffold.

Figure 8 shows a typical compressive stress-strain curve of the optimized porous Ti scaffold. The average compressive strength and elastic modulus of the scaffold were 83.6 ± 4.0 MPa and 2.0 ± 0.5 GPa, respectively. The mechanical strength of the scaffold developed by the current improved sponge replication method is higher than that of those porous Ti prepared by the traditional methods [18, 21, 22, 24, 30, 32, 38]. During the preparation process of the porous Ti scaffold using this improved sponge replication method, the addition of ethanol may play a key role in the enhanced mechanical strength of the scaffold. As shown in Figure 4, the addition of ethanol increased the skeleton density of the scaffold notably, and thus may improve its mechanical strength.

In vitro cell experiments using the primary MSCs were performed to evaluate the cellular compatibility of porous Ti scaffolds. Figure 9 shows the morphology of the attached cells on the scaffold surface after culturing for 4 days. The green and red colors represented live and dead cells, respectively. It could be seen that many live cells attached to the surface of pore walls and spread well, while few dead cells existed. Meanwhile, Figure 10 presents the proliferation and viability of MSCs on the scaffolds after culturing for 1, 4 and 7 days by MTT assay. Compared to the control group, the porous Ti scaffolds held back the proliferation of MSCs to a certain degree, as could be ascribed to the influence of the porous surface of the scaffolds. However, it is worth noting that

the number of live MSCs increased notably with the culture time, suggesting that the porous Ti scaffolds could support the cells growth well. The results confirmed that the porous Ti scaffolds made by our improved sponge replication method has good biocompatibility with MSCs and can support the attachment, growth and proliferation of the cells. The detailed biological evaluation for the Ti scaffolds will be carried out in the next work.

4. Conclusions

This work developed an improved polymeric sponge replication method to prepare biomedical porous Ti scaffolds. Porous Ti scaffolds with open and interconnected porous structure were obtained after optimizing processing parameters including liquid medium, centrifuging speed and viscosities of Ti slurries. The as-prepared Ti scaffolds have both macropores ($\sim 365.1\ \mu\text{m}$) and micropores (two sizes of ~ 45.4 and $\sim 6.2\ \mu\text{m}$). The addition of ethanol in the liquid medium of Ti slurry greatly improved the mechanical strength of the scaffold. The compressive strength of an optimized porous Ti scaffold with a porosity of $66.4 \pm 1.8\%$ achieved $83.6 \pm 4.0\ \text{MPa}$. Furthermore, the porous Ti scaffolds supported the growth and proliferation of MSCs. Taken together, the porous Ti scaffolds prepared by this modified replication method may have a great potential to repair bone defects in load-bearing sites.

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Figure Legends

Figure 1 SEM images of (a) titanium powder and (b) PU foam.

Figure 2 Flowchart of the improved polymeric sponge replication method for porous Ti scaffolds.

Figure 3 Effects of (a) ethanol content, (b) PVA content and (c) ratio of solid to liquid on the viscosity of the Ti slurry.

Figure 4 SEM images of the fabricated porous Ti scaffolds by the sponge replication method without (a, b) and with (c, d) addition of ethanol in the solvent.

Figure 5 SEM images of the skeleton thickness of the fabricated porous Ti scaffolds with different coating times. (a) 4 times, (b) 5 times, (c) 6 times and (d) 7 times.

Figure 6 Typical SEM images of the optimized porous Ti scaffolds. (a) $\times 30$, (b) $\times 100$.

Figure 7 Pore size distribution of the porous Ti scaffold analyzed by mercury intrusion porosimetry.

Figure 8 A typical compressive stress-strain curve of the optimized porous Ti scaffold.

Figure 9 (a) CLSM observation of MSCs attached on the scaffold surface, (b) Partial enlarged view of the red box.

Figure 10 MTT assay for the proliferation of MSCs on the Ti scaffolds. * $p < 0.05$

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Figure 10

Table 1 Compositions of Ti slurries for the improved polymeric sponge replication method.

Composition	Slurry I	Slurry II	Slurry III
Ethanol content in solvent (vol. %)	25	25	25
PVA content in PVA solution (wt. %)	5	5	5
Ti powder (g)/10 mL PVA solution	8	6	3
Viscosity (cP)	262.3±6.4	191.8±1.7	111.7±9.4

Table 2 The technological parameters (Ti slurries, rotational speed of centrifugation and coating times) of the multiple coatings technique in the improved polymeric sponge replication method.

Slurries	Rotational speed (rpm)	Coating times							
		1	2	3	4	5	6	7	8
Slurry I (8g/10mL)	850	✓	✓	xx					
	900	×	✓	✓					
	1000		×	✓	xx				
Slurry II (6g/10mL)	1100			×	✓	xx			
	1200				×	✓	xx		
	1300					✓	✓	xx	
Slurry III (3g/10mL)	1400					×	×	✓	xx
	1500								✓

Deformation (×) Blocking (xx)

Table 3 Porosities and compressive strengths of the porous Ti scaffolds prepared with multiple coating technique.

Coating times	5	6	7	8	9
Porosity (%)	84.2±1.2	77.9±0.5	74.0±1.8	69.8±0.6	66.4±1.8
Compressive strength (MPa)	8.9±0.6	31.1±3.7	38.0±3.8	59.5±3.8	83.6±4.0