Preparation of mesoporous bioglass coated zirconia scaffold for bone tissue engineering

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Abstract—Porous yttria-stabilized zirconia (YSZ) has been regarded as a potential candidate for bone substitute due to its high mechanical strength. However, porous YSZ is biologically inert to bone tissue. It is therefore necessary to introduce bioactive coatings onto the walls of the porous structures to enhance its bioactivity. In this study, porous YSZ scaffolds were prepared using a replication technique and then coated with mesoporous bioglass due to its excellent bioactivity. The microstructures were examined using scanning electron microscopy and the mechanical strength was evaluated via compression test. The biocompatibility and bioactivity were also evaluated using bone marrow stromal cell (BMSC) proliferation test and simulated body fluid test.

Keywords: scaffold, porous structure, mesoporous bioglass, zirconia(YSZ), Compressive strength

I. INTRODUCTION

For the filling and reconstruction of non-healing bone defects, the application of porous ceramic scaffold as bone substitutes is considered as a reasonable choice. The porous scaffold structure can aid cell migration and cell/gene delivery and provides a mechanical support to the newly formed tissue [1]. However, the mechanical properties of porous bioactive simplex ceramics are undesirable. Many studies to date have indicated that the compressive and bending strength of porous bioactive simplex ceramics are limited [2-5]. The porous ceramics such as the hydroxyapatite scaffold and the pure mesoporous bioglass scaffold have compressive strength of 1.75 MPa [6] and 60 KPa [7], respectively. On the other hand, porous yttria-stabilized zirconia (YSZ) is relatively strong and tough compared to other porous bio ceramics, but has the problem of biological inertness to bone tissues. Therefore, many studies of zirconia based ceramics focused on combining the mechanical properties of zirconia with the bioactivity of other materials, such as hydroxyapatite coating. Up to now, however, there are few studies on mesoporous bioglass coating on porous zirconia. In this study, porous YSZ was prepared using a replication method and mesoporous bioglass (MBGs) was adopted as the coating material to improve its bioactivity. The structure-property relationship and the bioactivity were investigated.

II. EXPERIMENT PROCEDURE

A. Materials

The materials used to prepare the porous bioceramics were 3 mol% yttria stabilized zirconia powder (Aldrich Chemical Company, Inc.) with an average particle size of 0.265 μm and a specific surface area of 4.01 m²/g. Polyvinyl alcohol (PVA, SIGMA-ALDRICH, Inc.) solution containing 97.5 wt.% water and 2.5 wt.% active substance was used as a binder for ceramic slurries. Sodium polymethacrylate (Richard E. Mistler, Inc.) solution was used as dispersant and HCl (0.1 M) and NaOH (0.1 M) were used to modify the pH of the slurries. The scaffold template was made from acrylonitrile butadiene styrene (ABS).

B. Preparation of ABS scaffold templates

ABS scaffold templates were firstly designed using Solidworks software and constructed using a 3D Rapid Prototyper, as shown in Fig. 1. The diameter of the rod in the template is 0.5 mm to obtain small channels in the final scaffold. These regular mesopore-channels are expected to provide osteoinductivity and guide bone tissue growing well into the pores. As shown in Fig. 1, the template samples were designed in cubic and cylindrical shapes with different porosities and sample sizes. The detail of the template design is summarized in Tab. 1.

![Figure 1: Template design (A and C) and rapidly prototyped samples using a 3D printer](image-url)
C. Preparation of porous scaffolds

3 mol% yttria stabilized zirconia powders were mixed with 1 wt.% PVA, 2 wt.% sodium polymethacrylate, and appropriate distilled water to form zirconia slurry. The ABS templates were coated with wax to reduce possible thermal expansion during the sintering process. Zirconia slurry was infiltrated into the templates to obtain the porous structures. After drying in air for two days for pyrolysis of the organic phases, the sintering was carried out at 1400°C for 2 hours [3, 7-8]. A slow heating rate of 1 °C/min was used to burn out the ABS and minimize the cracking due to thermal expansion. Then, 5°C/min heating rate was used to 1400°C [8] to form the porous scaffolds.

D. Preparation of mesoporous bioglass (MBGs) coating

The MBGs (SiO2–CaO–P2O5) sols were prepared following the method reported by Pereira et al. [9] and Zhu et al. [10]. In this study, 6g of Pluronic® F-127 (Sigma-Aldrich) and 8.9g of tetraethyl orthosilicate (TEOS, 98%, Sigma-Aldrich) were added to 65g of 98% ethanol and stirred till the solution became clear. Then, 5g of 1mol/L hydrochloric acid (HCl) was added with mixing for 15 minutes. Finally, 1.89g of calcium nitrate (Ca(NO3)2)2H2O, >99.0%, Sigma-Aldrich) and 0.73g triethyl phosphate (TEP, 99.8%, Sigma–Aldrich) were added and mixed for 45 min. The obtained mixture was stirred at 30°C for 24 hours. The molar ratio of SiO2: CaO: P2O5 was 80:16:4.

A dip-coating process was used for coating the MBGs. The porous zirconia samples were immersed into the MBGs sol-gel solution for 2 min. The samples were then centrifuged under 500 rpm for 30 seconds to remove the extra sols. After drying for one day in air at room temperature, the coated porous zirconia was sintered at 1200°C for 2 hours. Then, the process was repeated for the second coating but the temperature was controlled at 700°C for 3 hours. The heating rate was 5°C/min for sintering the two coatings.

E. Characterization

1) Porosity of the porous zirconia ceramic.

The porosity of a scaffold was determined by measuring the dimensions and the mass of the scaffold and calculated using the following formula:

\[ P = \left( 1 - \frac{m}{\rho \cdot V} \right) \times 100\% \]  

where \( P \) is the porosity, \( m \) is the mass of the scaffold, \( \rho \) is the true density of the zirconia and \( V \) is the volume of the scaffold.

2) Mechanical testing

Compression test was conducted to evaluate the mechanical strength and its dependence with sample size, sample shape and porosity. The test was conducted using a Hounsfield testing machine at a loading rate of 0.5mm/min.

3) In-vitro biocompatibility test

To evaluate bone marrow stromal cell (BMSC) proliferation, BMSCs were seeded on biomaterial disks in 24-well plate at a density of 5x103 cells/well and incubated for 4 h. 20 mg of zirconia scaffold specimens were added to the culture plate. Cells were then incubated at 37°C in 5% CO2 for 7 days. Then, 40 μL of 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution (Sigma, Aldrich) was added in each well and incubated for 4 h at 37°C. The reaction was terminated by the addition of 100 μL dimethyl sulfoxide. The absorbance of the formazan was read at 495 nm using an Enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad Laboratories, Pty., Ltd. Gladesville, New South Wales, Australia). The MTT assay is to assess cell viability and grow based upon the conversion of MTT to formazan. Results were expressed as absorbance reading from each well minus the optical density (OD) value of blank wells. For comparison, BMSC proliferation without the addition of specimens and zirconia scaffold without adding cells were also evaluated by the same procedure.

4) In-vitro bioactivity test

It is believed that simulated body fluid (SBF) is useful for prediction of the in-vivo bone bioactivity of a given material [11-12]. To this end, the scaffolds prepared were separated into three groups. The uncoated scaffolds (Group 1) were immersed into the simulated body fluid (SBF) for 7 days at 37°C. The scaffolds coated with the bioglass were immersed into the SBF for 4 days (Group 2) and 7 days (Group 3) at 37°C.

5) Scanning electron Microscopy (SEM)

The scaffolds were coated with gold using a sputter coater (BioRad SC5000). Then, the microstructure of the mesoporous bioglass coating and the scaffolds were examined using a scanning electron microscope (PEI QUANTA 200) with the acceleration voltage of 15 kV and 25 kV.
III. RESULTS AND DISCUSSION

A. Porosity and shrinkage of uncoated zirconia scaffolds

Fig. 2 shows the sectional view of uncoated zirconia scaffold. The size of the big channel is about 0.5–0.8mm. Small pores can be observed in the scaffolds, Fig. 2 (b). However, there still have some small cracks between pores. Tiny cracks can be also observed. It is due to the difference in the thermal expansion coefficient between the ABS template and zirconia.

Based on Equation 1, the dimensional change after the sintering can be measured to evaluate the shrinkage rate. The shrinkage rate and porosity estimated are 19.14–29.59%, and 63–68±2.5%, respectively.

B. Mechanical properties of uncoated and coated scaffolds

1) Effect of scaffold size and shape on compressive strength

Compressive strength and its dependence with sample size, sample shape and porosity were evaluated. Fig. 3 shows the variation of compressive strength with the sample end surface area for the scaffolds with cubic and cylindrical shapes. It is clear that the compressive strength decreases with the end surface area. This is because the material volume is proportional to the end surface area. The higher the volume, the higher the density of defects the scaffold has. In addition, the scaffolds with a cylinder shape have a higher strength than those with a cubic shape. The reason may be attributed to a reduced surface area and less possible stress concentration in the cylindrical samples. As expected, a higher strength can be observed in the coated samples, indicating the healing effects of coating on small defects such as tiny cracks.

2) Effect of porosity on compressive strength

The variation of compressive strengths with porosity in the scaffolds with different template design (strut spacing) is shown in Fig. 4. The compressive strength decreases with the porosity. Although there is significant data scatter, the general trend is in agreement with the investigation by others [7, 13].

3) Effect of MBGs coating on compressive strength

It has been observed that the strength increase in the one-layer coated scaffold is very limited. On the other hand, Fig. 5 shows the compressive strength in the scaffolds with two-layer coatings. It is clear that a higher compressive strength is associated with all coated samples tested. In other words, two-layer MBGs coating is more efficient than one layer coating in increasing mechanical strength. The possible reason is further reduction of tiny defects such as cracks after repeated coatings.

C. BMSC Proliferation test and MTT assay

Fig. 6 shows the SEM images of BMSC proliferation test. It seems that both uncoated and coated zirconia scaffolds have good biocompatibility. The bone marrow stromal cells were migrating, attaching and proliferating well on the pore walls but not on other areas because the pore curvature could
provides optimum compression and tension on the cell’s mechanoreceptors [14].

Fig. 7 shows the average optical density (OD) value of uncoated and MBGs coated zirconia scaffolds at day 1, day 3, and day 6. The OD value slightly increases after 6 days, and there was a slight increase of the OD value in the coated scaffold. Therefore, both uncoated and coated scaffolds have good cell viability without obvious cytotoxicity.

**FIGURE 6:** SEM images of uncoated (A) and MBGs coated (B) scaffolds

**FIGURE 7:** The average OD value of uncoated and MBGs coated zirconia scaffold at day 1, 3, and 6.

**D. Simulated Body Fluid (SBF) Test**

The bioactivity of MBGs coated zirconia scaffolds were evaluated in SBF. There was nothing changed on the surface of the uncoated zirconia scaffolds (group 1) after immersing into the SBF for 7 days at 37°C. The scaffolds in group 2 and group 3 were coated with mesoporous bioglass and immersed into the SBF for 4 days and 7 days, respectively.

In Fig. 8 shows the SEM images of the mesoporous bioglass coating after the SBF test. The coating layer has average thickness of 7 μm. The formation of apatite was not observed on the wall surfaces in group 2. On the other hand, some hoarfrost was formed on the coating layers in the samples of the group 3. Compared to the work of Kokubo et al [11], the apatite is believed to form on the coatings. As shown in Fig. 9, the compositional analysis indicates a high concentration of Si and Ca on the coating in the group 3 (7days). The high Ca is believed to be caused by the precipitation of Ca from SBF solution, indicating a high possibility of forming apatite. However, not much Ca could be observed in the group 2. It means the significant bioactive can be observed after 7 days SBF testing. Therefore, it can be concluded that the coated porous zirconia has high bioactivity.

**FIGURE 8:** SEM images of MBGs coated scaffolds in SBF after 4 days (A) and 7 days (B) at 37°C.

**FIGURE 9:** Compositional analysis of coating surface in group 3

**IV. CONCLUSION**

Porous zirconia scaffold structures had high porosities of 63±2.8% to 68±2.5% with shrinkage rates of 19.14% – 29.59%. Most macropores were interconnected with pore sizes of 0.5–0.8mm. The compressive strength of the porous zirconia decreased with the increase of specimen size and the porosity. The uncoated cube and cylinder porous zirconia scaffolds respectively showed the compressive strength of the uncoated scaffolds with cubic and cylindrical shapes was in the range of 18.95–46.76 MPa and 38.24–110.51 MPa. A higher compressive strength was observed in the bioglass coated scaffolds, i.e., 34.22–55.01 MPa and 44.35–123.32 MPa, respectively in the ones with cubic and cylindrical shapes. Repeated coatings could further increase the mechanical strength.
The BMSC proliferation test and MTT assay results demonstrated that both uncoated and MBGs coated zirconia scaffolds have good biocompatibility and cell viability without obvious cytotoxicity. After the simulated body fluid (SBF) testing for 7 days, apatite was formed on the coating surface, indicating good bioactivity.

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