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# POROUS ZIRCONIA SCAFFOLD MODIFIED WITH MESOPOROUS BIOGLASS COATING

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**Abstract:** Porous yttria-stabilized zirconia (YSZ) has been regarded as a potential candidate for bone substitute as its high mechanical strength. However, porous YSZ bodies are biologically inert to bone tissue. It is therefore necessary to introduce bioactive coatings onto the walls of the porous structures to enhance the bioactivity. In this study, the porous zirconia scaffolds were prepared by infiltration of Acrylonitrile Butadiene Styrene (ABS) scaffolds with 3 mol% yttria stabilized zirconia slurry. After sintering, a method of sol-gel dip coating was involved to make coating layer of mesoporous bioglass (MBGs). The porous zirconia without the coating had high porosities of 60.1% to 63.8%, and most macropores were interconnected with pore sizes of 0.5-0.8mm. The porous zirconia had compressive strengths of 9.07-9.90MPa. Moreover, the average coating thickness was about 7μm. There is no significant change of compressive strength for the porous zirconia with mesoporous biogalss coating. The bone marrow stromal cell (BMSC) proliferation test showed both uncoated and coated zirconia scaffolds have good biocompatibility. The scanning electron microscope (SEM) micrographs and the compositional analysis graphs demonstrated that after testing in the simulated body fluid (SBF) for 7 days, the apatite formation occurred on the coating surface. Thus, porous zirconia-based ceramics were modified with bioactive coating of mesoporous bioglass for potential biomedical applications.

**Key words:** scaffold, porous structure, mesoporous bioglass, yttria-stabilized zirconia (YSZ), Compressive strength.

## 1 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 (Lin, Kikuchi, & Hollister, 2004). However, the mechanical properties of porous bioactive simplex ceramics are undesirable. Many studies to date have indicated that the compressive strength and bending strength of porous bioactive simplex ceramics are limited (Wei et al., 2010; Kim et al., 2003; Miao, Hu, Liu, & Huang, 2007; Roohani Esfahani, Tavangarian, & Emadi, 2008). Porous ceramics such as hydroxyapatite scaffold and pure mesoporous bioglass scaffold have compressive strength of 1.75MPa (Jun, Koh, Lee, & Kim, 2007) and 60KPa (Wu, Zhang, Zhu, Friis, & Xiao, 2010) respectively. On the other hand, porous yttria-stabilized zirconia (YSZ) is relatively strong and tough compared to other porous bioceramics, 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 bioactive materials. However, there are few studies on mesoporous bioglass coating on porous zirconia. The purpose of this study was to apply a bioactive coating onto the pore walls of the porous zirconia. After this process, the surface of the porous zirconia will become bioactive, and this bioactive coating can provide additional bonding to surrounding bones and promote healing rates.

This article reports the preparation of porous zirconia ceramic by infiltration of Acrylonitrile Butadiene Styrene (ABS) scaffolds (by 3D prototyper) with 3 mol% yttria stabilized zirconia slurry, and followed by firing. After preparation of mesoporous bioglass (MBG), the dip coating process was applied to the pore wall surface of the porous zirconia ceramic. Then, the mechanical property was tested by a Hounsfield testing machine. The biocompatibility was evaluated by using Human bone marrow stromal cell (BMSC) proliferation test. After the simulated body fluid (SBF) testing for 7 days, the porous structure and biological activity were examined by scanning electron microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analysis.

## 2 EXPERIMENTAL PROCEDURE

### 2.1 Preparation of Mesoporous Bioglass (MBG)

The MBGs ($SiO_2$–$CaO$–$P_2O_5$) sols were prepared following a previously reported method by Pereira et al (1994) and Zhao (2007). In this project, 6g of Pluronic® F-127 (Sigma-Aldrich), 8.9g of tetraethyl orthosilicate (TEOS, 98%, Acros), 65g of 98% ethanol, 5g of 1mol/L hydrochloric acid (HCL), 1.89g of calcium nitrate($Ca(NO_3)_24H_2O$, >99.0%, Sigma-Aldrich) and 0.73g triethyl phosphate (TEP, 99.8%, Sigma-Aldrich) were used during the synthesize process. The molar ratio of $SiO_2$:$CaO$:$P_2O_5$ in this project was 80:16:4.

### 2.2 Preparation of simulated body fliud (SBF)

The simulated body fluid (SBF) was used as an incubation solution for bone-like apatite formation on the pore wall of porous zirconia ceramics. The method for preparing 1L SBF was according to a journal paper reported by Kokubo and Takadama (2006). Ion-exchanged and distilled water was needed to prepare the SBF. Tab. 01 lists the 9 reagents and their purity and amounts for preparing 1000mL SBF solution in this study.

**TABLE 01: Purity and amounts of various reagents for preparing 1000mL SBF solution**

| Reagents | Purity (%) | Amounts |
|---|---|---|
| NaCl | >99.5 | 8.0349 g |
| $NaHCO_3$ | >99.5 | 0.3549 g |
| KCl | >99.5 | 0.2254 g |
| $K_2HPO_4 3H_2O$ | >99.0 | 0.2314 g |
| $MgCl_2.6H_2O$ | >98.0 | 0.3112 g |
| 1M-HCl | - | 39ml |
| $CaCl_2 2H_2O$ | >96.0 | 0.3867 g |
| $Na_2SO_4$ | >99.0 | 0.0725 g |
| Tris | >99.0 | 6.1182 g |
| 1M-HCl | - | 0-5 ml |

### 2.3 Preparation of porous zirconia ceramics

The struts of the scaffolds should be designed to be less than 1 mm thick so that the pores in the final zirconia would roughly be controlled to less than 1 mm. The gap in the scaffolds should also be about 0.5 to 1 mm. The scaffold samples were designed by

Solidworks software and built by 3D printer. The material of the scaffold samples was Acrylonitrile Butadiene Styrene (ABS). Then, 3 mol% yttria stabilized zirconia (Aldrich Chemical Company, Inc.) slurry was embedded into the scaffold samples to obtain the porous structure. After drying the green sample, a pyrolysis of the organic phase was carried out and calcination process was done to get the porous zirconia in air atmosphere at 1300°C for 2 hours (Barreiro, Rey, Souto, & Guitián, 2009). This process was carried out in order to burn out the ABS and secure an initial form of the porous zirconia.

After dip coating process, the dried coated porous zirconia was sintered at 1280°C for 2 hours. The heating rate was controlled at 5°C/min. The main purpose for this process was making the coating film firmly bond to the pore walls of porous zirconia. Afterwards, the coating process was repeated and the second coated porous zirconia was sintered at 700°C for 2 hours. The heating rate still remained at 5°C/min. The purpose for this process was removing the organic composition and forcing the second coating film to form bioactive glass.

### 2.4 **Sample characterization**

#### *2.4.1 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 (1):

$$P=(1-m/(\rho\_Zirconia\times V))\times 100\% \quad (1)$$

where P is the porosity, m is the mass of the scaffold, ρ_Zirconia is the true density of the zirconia and V is the volume of the scaffold.

#### *2.4.2 Mechanical Testing of the Porous Zirconia Ceramics*

A compressive testing was involved in mechanical testing. It was performed on cubic shaped specimens of coated and uncoated scaffolds with different porosities. The compressive force-deformation curve of a scaffold was measured at room temperature with a cross-head speed of 0.5mm/min on a Hounsfield testing machine. Then, the compressive strength was calculated by the force at the failure point dividing the contact area. The effect of porosity on compressive strength was discussed.

#### *2.4.3 Bone Marrow Stromal Cell (BMSC) Proliferation Test*

To evaluate BMSC proliferation with the existence of microspheres, BMSCs were seeded on biomaterial disks in 24-well plate at a density of 5×103 cells/well and incubated for 4 h. 20mg of materials was added to the culture plate. Cells were then incubated at 37ºC 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 37ºC. 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 assesses 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 value of blank wells. For the control, BMSC proliferation without the addition of materials and zirconia scaffold without adding cells were evaluated by the same procedure.

#### *2.4.4 Scanning Electron Microscope (SEM)*

The microstructures of the scaffolds were observed by scanning electron microscope (SEM). For SEM observation, the scaffolds were cut with a razor blade and the scaffold samples were mounted onto aluminium stubs with a carbine tape. The scaffold samples were coated with a gold film in a sputter coater (BioRad SC500). The mesoporous bioglass coating structure and microstructure of the scaffolds were then examined using a scanning electron microscope (FEI QUANTA 200) with the acceleration voltage of 15 kV and 25kV.

## 3 RESULTS AND DISCUSSION

### 3.1 **Porosity Calculation**

From the section 2.4.1, the porosity is related to the density of the 3 mol% yttria stabilized zirconia (3Y-TZP), mass of the scaffold and volume of the scaffold. The theoretical density of 3Y-TZP was taken as 6.08 g/cm3 in the calculation (Yin et al., 2000; Xue, Lu, & Ma, 2009). The mass and volume of the scaffolds were measured from 8.41g to 8.44g and 3.528cm$^3$ to 3.838cm$^3$. Hence, the porosity of these scaffolds was calculated between 60.1% - 63.8%.

### 3.2 **Mechanical Testing**

Fig. 01 shows the compressive force-deformation curves of uncoated scaffold and coated scaffold under different porosities. From curve 1 and curve 2, there is no obvious difference between these two curves. The yield forces for uncoated and coated scaffolds are around 4365N and 4396N. The contact area of the uncoated scaffold was 21mm×21mm = 0.000441m$^2$, and the contact area of the coated scaffold was 21mm×21.5mm = 0.0004515m$^2$.

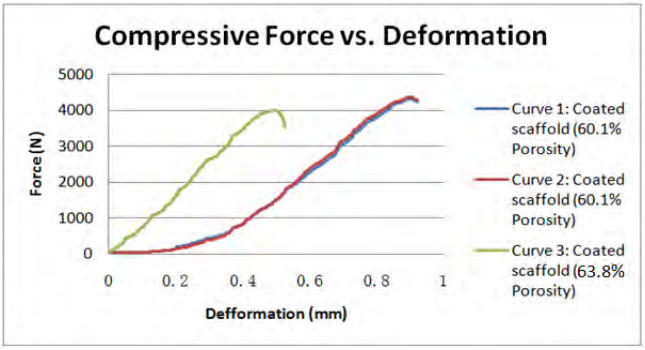


**FIGURE 01: Compressive Force-Deformation curves of uncoated and coated scaffolds**

By using Stress=Force/Area, the compressive strength of the uncoated and coated scaffolds are about 9.90MPa and 9.74MPa. These results also correspond to the paper reported by Miao et al. (2007). The compressive strength error between these two scaffolds is less than 1.7%. Any technical error or human error would affect the error. Therefore, the error is small enough and it can be considered that the mesoporous bioglass coating would not change the scaffold‘s compressive strength during this project. From curve1 and curve 3, it can be seen that the higher porosity scaffold has lower yield force. The yield force of the 63.8% porosity scaffold is about 4006N. The contact area between these two scaffolds is the same, which means the compressive strength of the 63.8% porosity scaffold is about 9.07MPa. Hence, it can be considered that the compressive strength decreased with the increase of porosity (Wei et al., 2010).

### 3.3 **BMSC Proliferation Test**

The SEM images of BMSC proliferation test results (Fig. 02 overleaf) presented in this study demonstrated that both uncoated and coated zirconia scaffold have good biocompatibility. The bone marrow stromal cells were migrating, attaching and proliferating well on the pore walls rather than other area, because the pore curvature could provides optimum compression and tension on the cell‘s mechanoreceptors (Boyan et al., 1996).

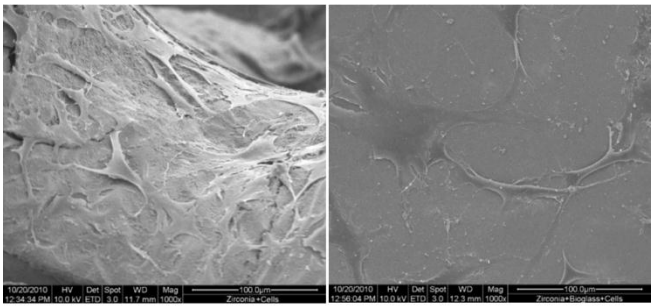

**FIGURE 02: SEM micrographs of uncoated (Left) and MBGs coated (Right) scaffolds**

## 3.4 SEM and EDS Analysis

The scaffolds were separated into three groups. The uncoated scaffolds were in group 1 immersed into the simulated body fluid (SBF) for 7 days at 37°C. The group 2 and group 3 scaffolds were coated mesoporous bioglass and immersed into the SBF for 4 days and 7 days at 37°C.

**Group 1:**

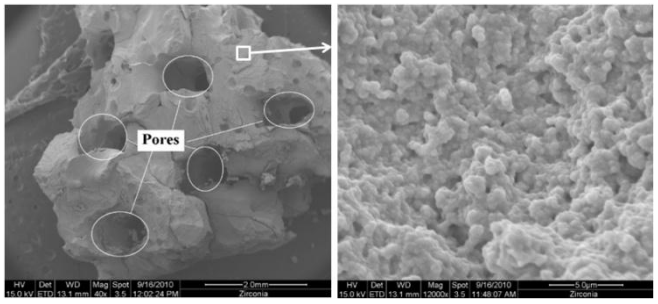


**FIGURE 03: SEM micrographs of the uncoated scaffolds with low magnified (×40, left) and high magnified (×12000, right)**

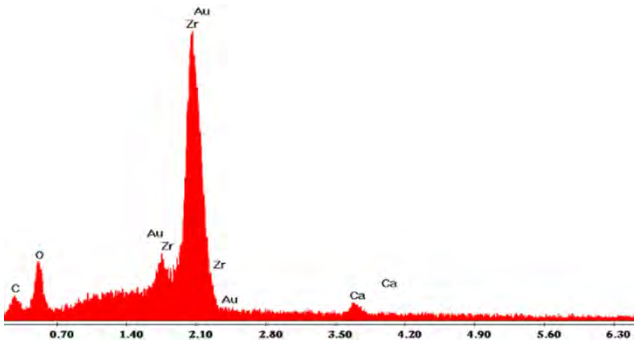


**FIGURE 04: Compositional analysis of the uncoated scaffolds**

The SEM images showed the diameter of pores is about 0.5mm to 0.8mm. (Fig. 03) It clearly can be seen that there is no coating or formation on the pore wall surface. Besides, the microstructure of the pore wall surface shows the zirconia particles are arranged compactly. It indicates the porous zirconia has finely sintered. However, there still have some small cracks between pores. It is caused by thermal expansion of the ABS props and zirconia itself. A research to minimize the scaffold cracks can be one of the future works after this report.

The EDS results provided the composition of an uncoated scaffold pore wall surface. (Fig. 04) Each peak waveband means the different element on the pore wall surface. The largest amounts of elements are Zr and Au. The reason for largest amount of Au is the scaffolds are gold coated before doing SEM. The small amount of Ca was probably the residue calcium ion from simulated body fluid. Therefore, the largest amount of Zr supports the main composing of scaffold is Zirconia.

**Group 2:**

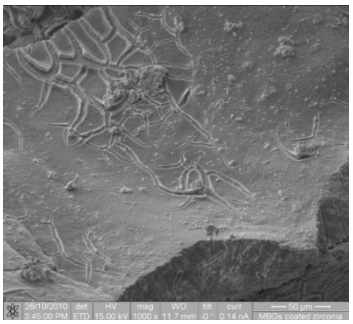

**FIGURE 05: SEM micrograph of group 2 scaffolds.**

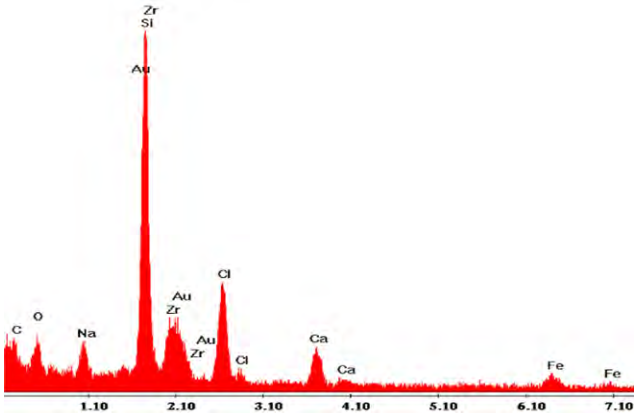


**FIGURE 06: Compositional analysis of the group 2 scaffolds coating surface**

The group 2 scaffolds were coated mesoporous bioglass and immersed into the SBF for 4 days. It can see the two mesoporous bioglass coating layers on the pore wall surface. (Fig. 05) The coating layer has average thickness of 7µm. In addition, there still have some cracks on the coating layers surface. These cracks should be caused by the shrinkage of mesoporous bioglass after sintering. Besides, the micrograph of high magnified coating layer displays the mesoporous bioglass has crystallized at 700°C. Whereas, so far it is hard to see the apatite formation on the pore wall surface of group 2 scaffolds. The EDS (Fig. 06) also indicates that there is no apatite formation on the coating surface. The most amounts of elements are Zr, Si, and Au. The element of Si is the main component of bioglass. The other elements should be the residue ions from simulated body fluid.

**Group 3**:

The coating layers and microstructure of the group 3 scaffolds are similar with the group 2 scaffolds. Furthermore, there are some hoarfrost formations on the group 3 scaffold coating layers (Fig. 07). Comparing these two micrographs with Kokubo and Takadama's paper (2006), these formations would be the apatite formation.

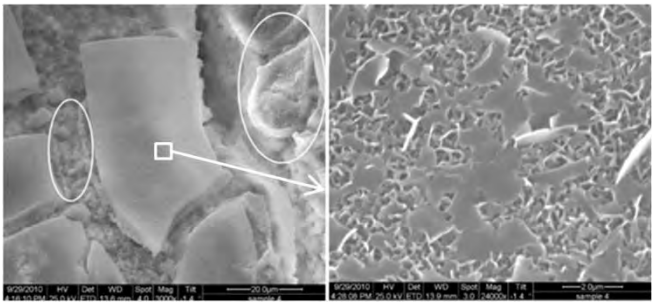

**FIGURE 07: SEM micrograph of group 3 scaffold coating layer with low magnified (×3000, left) and high magnified (×24000, right)**

The compositional analysis lists that the most amounts of elements are the Si and Ca (Fig. 08). As mentioned before, these two elements indicate the coating surface contains lots of bioglass and apatite. It means the pore wall surface of the group 3 scaffolds is bioactive. Therefore, it can be considered that the porous zirconia has successfully modified with bioactivity coating.

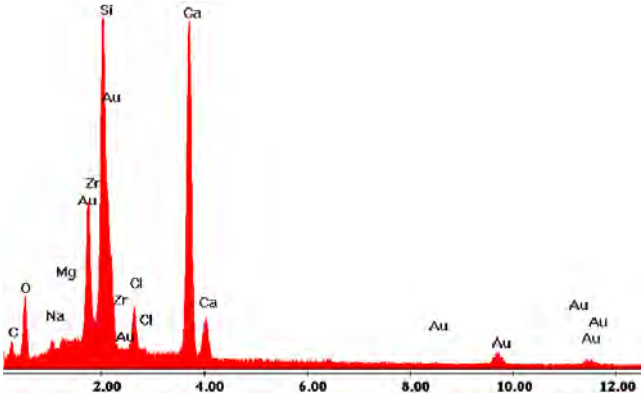


**FIGURE 08: Compositional analysis of the group 3 scaffolds coating surface**

## 4 CONCLUSIONS

The uncoated porous zironia had high porosities of 60.1% to 63.8%. Most macropores were interconnected with pore sizes of 0.5-0.8mm. Depending on the porosity, the uncoated porous zirconia showed compressive strengths of 9.07-9.90MPa.

The porous zirconia scaffolds were further coated with mesoporous bioglass (MBGs). The average coating thickness was about 7μm. During the compressive testing, there was no obvious evidence showing the mesoporous bioglass coating changed the compressive strength of the porous zirconia. The BMSC proliferation test results demonstrated that both uncoated and coated zirconia scaffold have good biocompatibility. After the simulated body fluid (SBF) testing for 7 days, mesoporous bioglass coated porous zirconia showed the apatite formation on the coating surface. Therefore, the porous zirconia has been successfully modified with the mesoporous bioglass coating.

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