칼슘 포스페이트 결정화 유리를 이용한 스캐폴드의 구조와 기계적 물성의 관계

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Relation between Structure and Mechanical Properties of Porous Scaffold Using Calcium Phosphate Glass-ceramics

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ABSTRACT

본 연구의 목적은 칼슘 포스페이트 결정화 유리를 이용하여 다공성 스캐폴드를 제조하고, 다공성 구조와 기계적 물성 간의 관계를 규명하는 것이다. 칼슘 포스페이트 결정화 유리와 증류수의 비율이 1.5이고, 바인더로 사용한 폴리비닐 알콜의 함량이 5 wt%일 때 폴리우레탄 스펀지에 슬러리가 가장 잘 코팅되었다. 코팅한 스펀지를 건조한 후, 스펀지 및 유기첨가제를 연소시키기 위해 600 ℃까지 3 ℃/min의 속도로 승온한 후, 800 ℃에서 2시간 동안 칼슘 포스페이트 유리를 결정화 열처리하였다. 300~600 μm의 기공이 잘 연결된 다공성 스캐폴드를 제조할 수 있었으며, 결정화 열처리에 따른 결정과 유리의 분율에 따라 스캐폴드의 압축강도를 제어할 수 있었으며, 스캐폴드의 다공성 구조는 micro CT를 이용하여 분석하였다.

KEY WORDS: Calcium phosphate, Scaffold, Micro CT

INTRODUCTION

The shortcomings of autografting and allografting, with respect to the limited donor site (Goulet et al, 1997) tissue rejection (Mizutani et al, 1990; Moore et al, 1984) and disease transfer (Barriga et al, 2004; McCann et al, 2004), have inspired the use of synthetic bone graft substitute materials. Recently, tissue engineering of bone regeneration concept and not simple bone replacement presents an alternative approach to repair the defected site by encouraging new bone ingrowth into the damaged site. Porous biomaterials, so-called scaffolds, for bone tissue engineering require adequate mechanical and biological properties for tissue regeneration. The 3-dimensional interconnected porous scaffold with sufficient mechanical strength provides the structural support for the cells to proliferate and maintain their differentiated function, and its architecture defines the ultimate shape of regenerated bone (Langer와 Vacanti, 1993; Hollinger와 Chaudhari, 1996). In addition, the scaffold should ideally replace the new tissue structure through sequential remodeling cycles, enabling the repair site to maintain an optimal balance between form and function (Hing et al, 2007). Therefore, osteoblast stem cells that are obtained from the patient's hard tissues can be expanded in culture and seeded onto a scaffold. The scaffold will slowly be degraded and resorbed as the new tissue structure grows *in vitro* and *in vivo* (Langer와 Vacanti, 1993).

Bioceramics such as hydroxyapatite (HA), Bioglass®, apatite-based glass or glass ceramics have been developed for a variety of different hard and soft tissue implants. These ceramics have excellent biocompatibility

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and bone bonding or regeneration properties. Over the past 30 years, there has been great interest in use of calcium phosphates, a principal inorganic constituent of natural bone, as scaffolding materials for bone tissue engineering (LeGeros, 1991). LeGeros and Lee have reported on calcium phosphate glass in the system $CaO-CaF_2-P_2O_5-MgO-ZnO$, which has a similar composition to natural bone and is characterized as having a very low Ca/P ratio of 0.6. It was noted that calcium phosphate glass showed a greater dissolution rate in buffer solutions and an increased bioactivity after exposure to either simulated body fluid or fetal bovine serum (LeGeros와 Lee, 2004). Calcium phosphate glass was also observed to promote of bone-like tissue forma and have an enhanced alkaline phosphatase activity in vitro (Lee et al, 2004). In addition, calcium phosphate glass promoted new bone formation in the critical-sized calvarial defect of Sprague-Dawley rats (Moon et al, 2005).

Different phases of calcium phosphates were employed to fabricate porous scaffolds to accommodate bone tissue regeneration *in vitro* or *in vivo*. The polymeric sponge method produces open cell porous ceramic scaffolds through replication of a porous polymer template. The scaffolds prepared by the polymeric sponge method have a controllable pore size, interconnected pores, and desired geometry.

The utilization of computer aided technologies in tissue engineering has evolved over time and was termed by Sun *et al.* as 'computer-aided tissue engineering (CATE)'. The X-ray micro-computed tomography (micro-CT) is a computer-aided 3D reconstruction of the structure of a material that has had considerable development over the last decade. Data collected through in vitro computed tomographic assessment of scaffolds as cancellous bone has proven valuable in clinical and preclinical studies of structural properties. Micro-CT starts from a set of 2D images taken in a fine pitch along the rotational axis, which is then fed into a computer program to produce 3D images of the sample. This methodology is similar to the CT scanning technique, routinely used for medical imaging, but the 3D reconstructions may be developed within a little micron resolution. Micro-image analysis is gaining popularity in the biomechanical field due to its ability to accurately measure the hard tissues mineral content. The unique feature of the X-ray micro-CT is that the 3D computed reconstruction can be sliced along any direction to gain accurate information on the internal geometric properties and structural parameters of the sample.

The purpose of this study was not only to prepare porous scaffolds by polymeric sponge method using calcium phosphate glass and crystallized glass, but also to investigate mechanical properties. Besides, we assessed scaffold architecture using micro-computed tomography (CT).

MATERIALS AND METHODS

1. Materials

Calcium phosphate glass in the system $CaO-CaF_2-P_2O_5-Na_2O$ was prepared with Ca/P ratio of 0.55 using raw materials such as $CaCO_3$, CaF_2, H_3PO_4, and Na_2CO_3. Mixed batches were dried for 24 hrs at 100° C and they were melted in a platinum crucible at 1200° C. After the glass was melted in a Kanthal super furnace, it was poured onto a copper plate at room temperature. The crystallized glasses were prepared by crystallizing as-quenched glasses for 2 hrs at 600° C. As-quenched and crystallized glasses were crushed with an alumina mortar, and sieved to produce particles with less than 45 μ m.

Two types of reticulated polyurethane ester sponge (Regicell, Jehil Urethane Co., Korea) were used in this experiment. One has 45 three-dimensionally interconnected open pores per inch (ppi) and another has 60 ppi.

2. Fabrication of porous scaffold

Calcium phosphate slurry was prepared by dispersing the prepared calcium phosphate glass, crystallized glass or various contents of mixed powders (Table 1) into distilled water with organic additives. Polyvinyl alcohol, polyethylene glycol and dimethyl formamide

Table 1. The code of scaffold prepared by the mixed powder with calcium phosphate glass and crystallized glass

Code	Content of glass (wt%)	Content of Crystalline (wt%)
G100	100	0
G75C25	75	25

Table 2 Porosity values as obtained by micro CT scanning

Code	Polymeric sponge (ppi)	Porosity (%)	Specific surface (mm^2/mm^3)	Thickness of strut (μm)	Spacing of strut (μm)
G100	45	84.6±1.4	31.4±0.8	120.3±4.1	529.0±2.6
	60	83.6±1.0	40.4±2.3	102.8±2.4	355.9±6.5
G75C25	45	82.9±1.2	33.4±1.6	94.6±1.1	463.1±20.6
	60	80.8±1.4	44.2±0.9	79.3±0.7	335.5±6.3
G50C50	45	83.0±1.3	33.9±0.5	94.8±2.1	467.9±18.2
	60	79.6±0.9	45.9±0.6	81.8±1.1	299.6±10.0
G25C75	45	81.4±1.7	33.0±0.4	95.0±0.5	481.1±17.9
	60	79.6±1.5	45.9±3.9	80.9±2.8	341.9±8.6
C100	45	86.6±0.8	31.4±1.4	112.8±4.3	563.9±10.7
	60	84.8±2.0	41.5±4.5	93.0±1.5	381.5±11.1

were selected as binder, dispersant and drying chemical control additive, respectively. First, polyvinyl alcohol (PVA-1500 Duksan Pure Chemical Co., Korea) was hydrolyzed and stirred in distilled water at a temperature of 50℃ at 5 and 10 wt%. After cooling to the room temperature, polyethylene glycol (PEG-400, Duksan Pure Chemical Co., Korea) was added at 5 wt%, and followed by addition of dimethyl formamide (DMF; Aldrich, USA) at 10 wt%. Preparation of the calcium phosphate slurry was completed by dispersing the prepared powders into distilled water containing the organic additives from 0.5 to 1.5 of powder/water ratio (g/mL).

Prior to the sponge coating process with sponges, the surface layer of the sponge was treated in a 2% NaOH solution for 20 min ultrasonically to improve the surface layer' s hydrophilicity (Park et al, 2006). After cleaning and drying, the porous sponge was subjected to a coating process. It was immersed into the slurry and taken back several times. This was done in order to remove the excess residual slurry from the sponge. Compressed air was blown into the pores of the sponge to perforate the clogged pores. After sponge coating it was then dried at room temperature and heat-treated in a Kanthal furnace. The condition of the heat-treatment was based upon a thermal analysis. First, the temperature was raised up to 600℃ at 3℃/min in order to burn out the sponge entirely, and the temperature was held there constant for 2 hrs to volatize the organic additives such as binder, dispersant and drying chemical control additive. Then the remaining calcium phosphate glass was sintered for 2 hrs at 800℃.

3. Characterization

In order to set up the heat-treatment condition of the sponge coated with the calcium phosphate slurry, the polymeric sponge was thermally analyzed by TG/DSC(STA 1500, Netsch Co., Ltd., Germany). An optical microscope and a scanning electron microscope (S4200, Hitachi, Japan) examined the microstructure of the coated, dried and sintered scaffolds. The compressive strength was determined by a universal testing machine (3366, Instron®, USA) at 0.5 mm/min of the crosshead speed. Parafilm of 0.2 mm in thickness were placed between each surface of the block and the compression punches in order to eliminate any unexpected effects due to an uneven horizontal surface level. The compressive strength was calculated from the determined maximum compressive load by the following equation.

$$S = F/A$$

where F is a maximum compressive load (N), A is the cross sectional area perpendicular to the load axis (mm^2), and S is the compressive strength (MPa). The significant differences between the determined compressive strengths were analyzed using one-way ANOVA with a post hoc test. A p-value <0.05 was considered statistically significant.

The scanner used to examine the structure of the scaffolds was a Skyscan 1076 *in vivo* micro CT. The scaffolds were placed with the height and width parallel to the scanning plane. The resolution was set at 9 μm and an averaging of three was employed together with a filter of 0.5 mm aluminum, a rotation step of 0.6°, and a rotation angle of 180°. A cubical region of interest (ROI) with 5 mm as the length and the width, and a height (approximate 5 mm) was chosen by selecting 101 slices of scanned data for thescaffolds. After estimating the volume of the scaffold within the ROI, the empty space was obtained by subtracting the scaffold volume within the ROI from the volume of the ROI. Dividing the volume of the empty space by the volume of the ROI gave porosity.

RESULTS AND DISCUSSION

1. Porous calcium phosphate scaffold

Porous scaffold was prepared by using a polyurethane ester sponge. In previous study (Park et al, 2006), the sponge was chemically treated in NaOH solution to improve wetting of aqueous slurry on it. With the treatment in NaOH solution, much more slurry could be homogeneously coated on the surface of sponge, due to increased surface roughness and specific surface area. The slurry was prepared in an aqueous system including water, and defects like cracks on the slurry coating layer may occurred on drying due to abrupt and large shrinkage caused from the high surface tension of water. In order to prevent these cracks, various organic additives can be added as a drying chemical control additive. Of these additives, DMF is a very good candidate because of its lower surface tension and higher evaporation temperature when compared to those of water. Therefore, though the water evaporates on drying, DMF can be still remained between the particles and moderate local surface tension of the coating layer to prevent abrupt shrinkage, and so the microcracks can be eliminated.

Fig. 1 shows photographs of the polymeric sponges after coating of the slurry prepared with various contents of the calcium phosphate glass powder and PVA. When powder/water ratio was 0.5, the content of powder was insufficient to coating fully the polymeric sponge. The pore of the sponge was clogged because of the slurry's viscosity. The sponge with powder/water ratio of 1.0 does not have the clogged pore, but was not also coated perfectly. The slurry with powder/water ratio of 1.5 and 5 wt% PVA showed the best homogeneous coating(Fig. 2). PVA is used as a binder and the more contents of PVA as a binder, the better coating efficacy was. This is because an increased thixotropy are available as the PVA content is increased. However, when powder/water ratio of 1.5 and the binder content of 10 wt%, the slurry coated onto sponge's surface seemed to be agglomerated. This is thought to happen that the powder concentration was much higher to coating uniformly.

The final step for scaffold fabrication is the heat-treatment. The role of heat-treatment is to eliminate the polymeric sponge and organic additives at a temperature of around 600°C. After that, the remaining calcium phosphate glass was sintered at 800°C. The microstructure of the heat-treated glasses was observed using a SEM, as is shown Fig. 3.

2. The mechanical and structural properties of porous calcium phosphate scaffold

The compressive strength was determined by a universal testing machine and showed in represented Fig. 4. G100 and C100 not mixed only glass and crystallized glass were the lower strength values than other groups. The compressive strength values of 45 and 60 ppi in G100 were 0.49 and 0.61 MPa, respectively, and were each 0.51 and 0.62 MPa in C100. The compressive

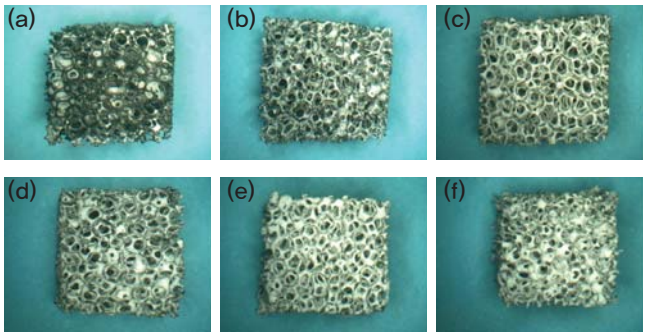

Figure 1. *Photographs of the sponges coated by slurry with various powder/water ratio and PVA content [(a) powder/water ratio 0.5, PVA 5 wt%, (b) powder/water ratio 0.5, PVA 10 wt%, (c) powder/water ratio 1.0, PVA 5 wt%, (d) powder/water ratio 1.0, PVA 10 wt%, (e) powder/water ratio 1.5, PVA 5 wt%, (f) powder/water ratio 1.5, PVA 10 wt%)].*

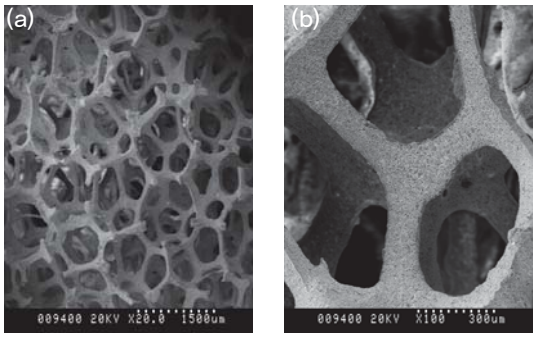

Figure 2. *SEM photographs of porous scaffold (45ppi) coated by slurry with powder/water ratio 1.5 and PVA 5 wt% (a) ×20, (b) ×100.*

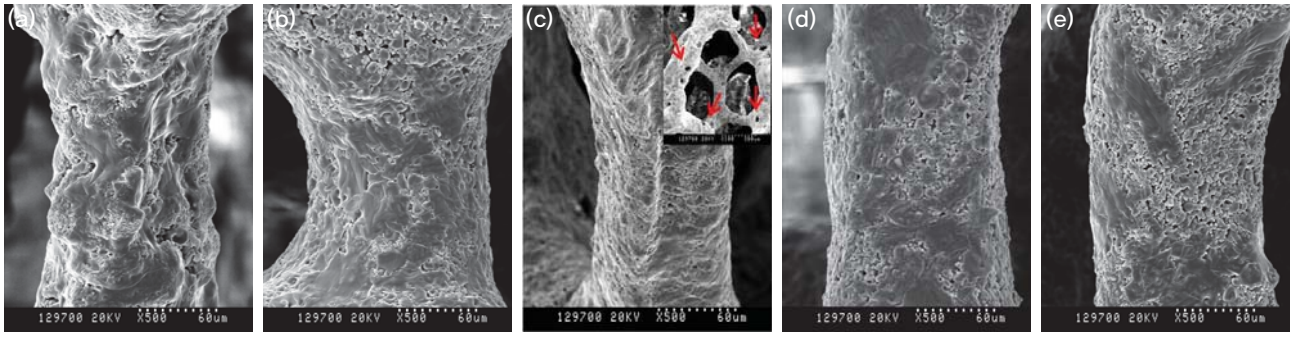

Figure 3. *SEM photographs of microstructure of sintered scaffold with 45ppi [(×500 (a) G100, (b) G75C25, (c) G50C50, (d) G25C75, (e) C100, arrows in (c) are holes in struts)].*

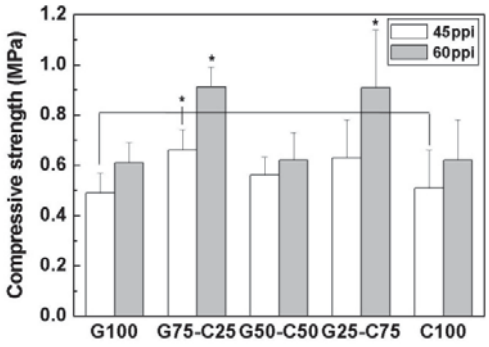

Figure 4. *Compressive strength of the sintered scaffold prepared from various contents of mixed powders * p 〈 0.05.*

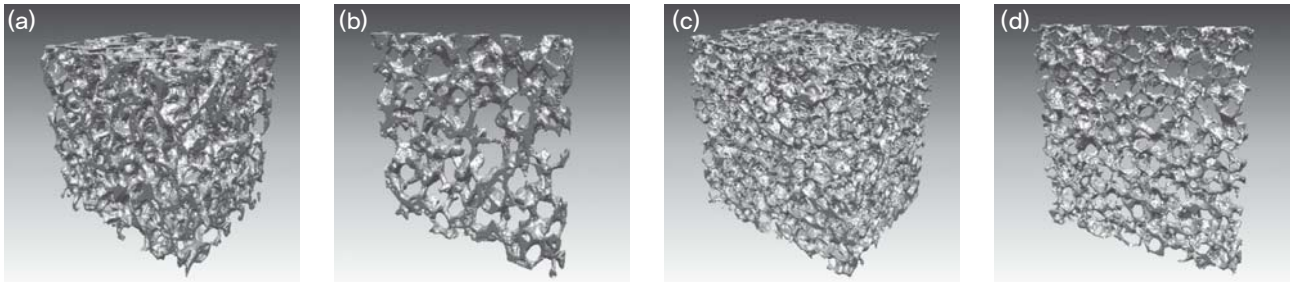

Figure 5. *Images of 3D reconstruction (a, c) and cross-section of 30 slices (b, d) of a scaffold with 45 ppi (a, b) and 60 ppi (c, d) of G25C75.*

strength of G75C25 both 45 and 60 ppi was the highest value of each 0.66 and 0.91 MPa. This is thought to that glass and crystallized glass played a role as a filler to increase the strength in G75C25 and G25C75, respectively. However, in case of G50C50 mixed with the same proportion of glass and crystallized glass, the compressive strength was lower than other mixed group (G25C75 and G75C75). This could be due to pores existed in struts of scaffolds, as is shown Fig. 4. These pores may be formed on the heat-treatment due to different thermal properties of glass and crystallized glass. In our study, simple mixture of glass and crystallized glass could improve the compressive strength of the calcium phosphate scaffold. Also, a repeated slurry coating/sintering process is able to increase the compressive strength of sintered porous body (Park et al, 2006).

Previous studies have shown that the compressive strength of porous hydroxyapatite scaffolds (porosity 69-86%) and 45S5 Bioglass® scaffolds (porosity 84~89%) prepared via a polymer sponge template method were about 0.03~0.29 and 0.42~0.6 MPa, respectively (Kim et al, 2005; Miao et al, 2004; Chen et al, 2006; Gibson과 Ashby, 1999). The strength of the calcium phosphate scaffolds prepared in this study was higher than that of hydroxyapatite and Bioglass® scaffolds and this falls in the range of 0.2~4.0 MPa of the compressive strength of spongy bone (Gibson과 Ashby, 1999; Wu et al, 2008).

X-ray micro CT has been proposed to analyze the scaffold architecture. Fig. 5 shows the 3D model and cross-sectional image of 30 slices of scanned G25C75. The 3D models and cross-sectional images in all groups are very similar. Table 2 shows the sample code, calculated porosity values, specific surface, mean thickness of strut, and mean spacing of strut (pore size) from the 3D modeling program (Ctan).

The porosity, mean thickness of strut and mean pore size of 45 ppi was higher than that of 60 ppi. But, the specific surface of 60 ppi was higher than that of 45 ppi; this was due to more struts of polymeric sponge with 60 ppi. The G100 and C100 of only glass or crystallized glass were the higher porosity and pore size than other groups with mixed powder. The structural properties of the mixed groups (G75C25, G50C50, and G25C50) were similar. This is because the shrinkage of crystallized glass was smaller than that of glass in sintering process. Namely, the more reaction of glass in G100 in sintering process made thicker struts, while the pore size of C100 exhibited the larger value by the smaller shrinkage of crystallized glass.

The structural properties of the scaffold have been studied usually with SEM for the examination of the structure of scaffolds and by quantifying deposition geometries for actual porosity measurement. Because of lack of accuracy of the above mentioned methods, micro CT has been proposed to analyze the scaffold architecture. Scaffold visualization coupled with calculation of actual porosity and strut thickness was possible depending on the software that processes the scan data (Lin et al, 2003).

There are several advantages to investigate the physical properties of the scaffold by micro CT scanning. Firstly, due to non-destruction of sample in using micro CT the sample can be also used for other test. Powerful 3D software such as Ctan and Ctvol enables the assay and visualization of the 3D image of the scaffold. Moreover they allow the user to examine and analysis in detail any part of the scaffold in regard of internal architecture and interconnectivity, as digital manipulation can be easily achieved with 3D models (Tuan et al, 2005).

CONCLUSIONS

The highly porous scaffolds with interconnecting pores with 300~600 μm were successfully fabricated using calcium phosphate glass and crystallized glass by polymeric sponge method. The various contents of mixed powders of glass and crystallized glass could improve the compressive strength of the sintered scaffold. We have demonstrated successfully that systematic variation of macroporous scaffold architecture can be studied by computer imaging technology using micro CT.

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