# NOVEL MACROPOROUS TISSUE ENGINEERING SCAFFOLD USING AMORPHOUS CALCIUM PHOSPHATE GLASS

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**Abstract: In this study, we investigated the fabrication method of a 3D reticulated scaffold with interconnected pores of several hundred micrometers using calcium phosphate glass in the system of $CaO$-$CaF_2$-$P_2O_5$-$MgO$-$ZnO$ and a polyurethane sponge as a template. Calcium phosphates glass slurry was homogenously thick coated when the weight percentage of the calcium phosphate glass powder was 40% with 8 wt% of polyvinyl alcohol as a binder. Addition of 10 wt% dimethyl formamide as a drying control chemical additive into a slurry almost prevented the crack formation during drying. Sintering of the dried porous block at 850°C exhibited the densest microstructure as well as the entire elimination of the organic additives. Repeating the process significantly increased compressive strength of sintered porous body due to the thickening of the struts. To summarize, macroporous calcium phosphate glass can be fabricated with 200~800 μm of pore size and 3D interconnected open pore system. It is thought that this kind of biodegradable glass scaffold combined with osteogenic cells has potential to be studied further as a tissue-engineered bone substitute.**

## Introduction

Tissue engineering presents an alternative approach to the repair of a damaged tissue; it avoids the need for a permanent implant made of an engineered material. The underlying principle involved is the regeneration of living tissue where a loss or damage has occurred as a result of injury or disease. A suitable temporary scaffold material exhibiting adequate mechanical and biological properties is required to enable tissue regeneration by exploiting the body's inherent repair mechanism, i.e. a regenerative allograft [1]. 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* [2]. The 3D-constructed scaffold provides the necessary support for the cells to proliferate and maintain their differentiated function, and its architecture defines the ultimate shape of the regenerated bone [3].

The design of bioresorbable scaffolding materials and the manufacturing technology of porous scaffolds are at the heart of bone tissue engineering approaches. The scaffolds serve as 3D templates for initial cell attachment and subsequent tissue regeneration [4]. Synthetic bioresorbable polymers have been attracting attention as tissue engineering scaffolds, in particular PLA, PGA, and PLGA. However, a number of problems have been encountered regarding the use of these polymers in tissue engineering applications. These problems are due to the release of acidic degradation products that lead to inflammatory responses [5-6]. Another limitation of biodegradable polymer is that they lack of bioactive function, i.e., in particular for bone tissue applications where they do not allow for bone apposition or bonding on the polymer surface [7].

Another good candidate for a tissue engineering scaffold is calcium phosphates because of their good biocompatibility and osteintegrative properties [8-9]. However, their slow biodegradation is still remains a problem, especially for the filling of large bony defects. But β-TCP is known to be completely resorbable at skeletal sites [8]. Recently, LeGeros and Lee have reported on calcium phosphate glass(CPG) 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 [10]. CPG was also observed to promote of bone-like tissue formation and have an enhanced alkaline phosphatase activity in vitro [11]. In addition, CPG promoted new bone formation in the critical-sized calvarial defect of Sprague-Dawley rats [12].

A number of fabrication technologies have been applied to process biodegradable and bioresorbable materials into 3D polymeric scaffolds of high porosity and surface area. The conventional techniques for


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scaffold fabrication include freeze drying, fiber bonding, foaming, solvent casting, particulate leaching, polymeric sponge method, membrane lamination and melt molding [13-14].

In this study, we investigated the optimal manufacturing process of CPG scaffold in a $CaO$-$CaF_2$-$P_2O_5$-$MgO$ system by the polymeric sponge methods.

## Materials and Methods

CPG in the system $CaO$-$CaF_2$-$P_2O_5$-$MgO$-$ZnO$ was prepared with Ca/P ratio of 0.6. A molar ratio of $CaO/CaF_2$ was fixed to at 9. MgO and ZnO were added 1% in weight percentage, respectively. Mixed batches were dried for 12 hr at 80°C and calcined for 1 hr at 450°C. Then they were melted in a platinum crucible at 1250°C. After the glass was melted in a kanthal super furnace, it was poured onto a graphite plate at room temperature. As-quenched glasses were crushed with an alumina pestle and attrition milled to less than 20 μm for the average size. Reticulated polyurethane sponge having 45 ppi(pores per linear inch) was used as template in this experiment.

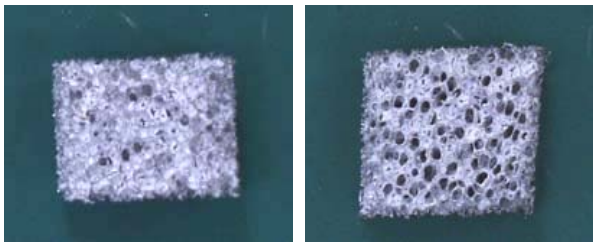
CPG slurry was prepared by dispersing the prepared CPG powder into distilled water with organic additives such as binder, dispersant and a drying chemical control additive(DCCA). Polyvinyl alcohol(PVA), polyethylene glycol(PEG) and dimethyl formamide(DMF) were selected as binder, dispersant and drying chemical control additive, respectively. First, PVA was hydrolyzed and stirred in distilled water at a temperature of 50°C at various amounts from 2 to 8 wt%. After cooling to the room temperature, PEG was added at 5 wt%, and followed by addition of DMF up to 10 wt%. Preparation of CPG slurry was completed by dispersing the CPG powder into distilled water containing the organic additives from 10 to 40 wt%.

The porous sponge was subjected to an infiltration process. It was immersed into CPG slurry and taken back several times, followed by rolling it through the teflon twin rollers whose spacing was controlled to compress and shrink the sponge up to 75% in thickness. 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 infiltration 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°C very slowly at 1°C/min in order to burn out the sponge entirely, and the temperature was held there constant for 2 hr to volatize the organic additives such as binder, dispersant and drying chemical control additive. Then the remaining CPG was sintered for 2 hr at various temperatures from 650 to 850°C. The full procedure listed above was repeated twice to thicken the framework of the porous block.

In order to set up the heat-treatment condition of the sponge infiltrated with CPG, the polymeric sponge was thermally analyzed by TG/DTA (STA 1500, Netsch, Germany). The glass transition temperature of the prepared CPG was determined by a TMA (2940, TA Instrument, USA) in order to predict the sintering temperature. The viscosity of the CPG slurry was measured by a cone and plate type viscometer (RH, Brookfield, USA). An optical microscope and a scanning electron microscope (S 4200, Hitachi, Japan) examined the microstructure of the infiltrated, dried and sintered scaffolds. Each block was 5 mm in diameter and 8 mm in height. The maximum compressive load of each of the 10 porous blocks was determined by a universal testing machine (4501, Instron, USA) at 1.0 mm/min of the crosshead speed.

## Results

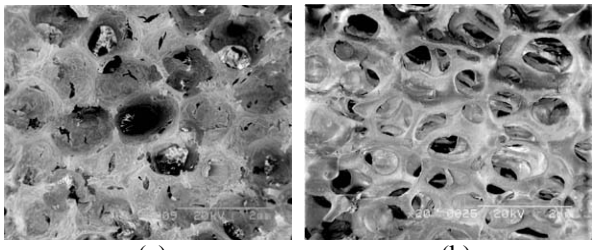
Photograph of the polymeric sponges after infiltration of CPG slurry with PVA is represented in Figure 1. When the content of the CPG powder was lowest as 10 wt%, a thin film of the slurry clogged the pores. This was due to its low surface tension. With increasing the content of the glass powder up to 25 wt%, the thin film formation was eliminated, however, the viscosity of the slurry was still low. When the content of the glass powder was fixed, coating efficacy was improved with increasing the binder content. The best condition for homogenous-thick coating of the slurry in this study was 40 wt% glass powder and 8 wt% binder.



(a) (b)

Figure 1: Polymeric sponge infiltrated by CPG slurry containing (a) 10 wt% CPG with 2 wt% PVA, and (b) 67 wt% CPG with 8 wt% PVA.

This clogged pore phenomena could be also observed using a scanning electron microscope, as is shown in Figure 2.



(a) (b)

Figure 2: SEM photographs of polymeric sponges infiltrated by CPG slurry containing (a) 10 wt% CPG with 2 wt% PVA, and (b) 67 wt% CPG with 8 wt% PVA.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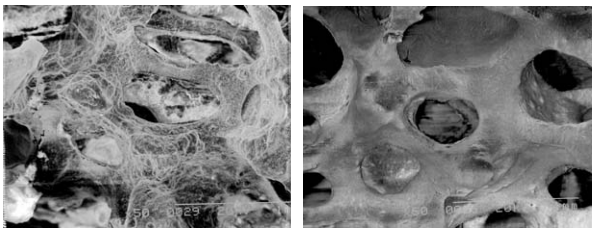
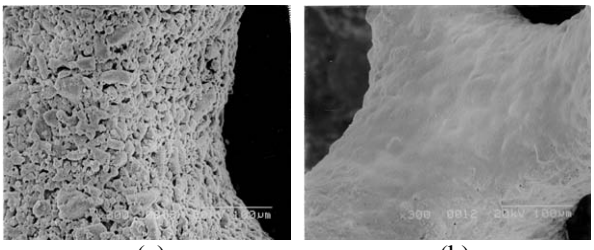
The next step for the porous scaffold fabrication is drying. Without DCCA, lots of cracks were formed and the surface of the coated film was very rough and heterogeneous, as is exhibited in Figure 3. The surface was much smoother and more homogeneous with 5 wt% DMF as the DCCA. However, the cracks were still present. When the addition of DMF increased up to 10 wt%, you can see the absolutely prevention of crack formation during the drying process.



(a) (b)

Figure 3: SEM photographs show the crack formation in the strut of the macroporous scaffolds after drying at 60°C (a) without and (b) with DMF (10 wt%).

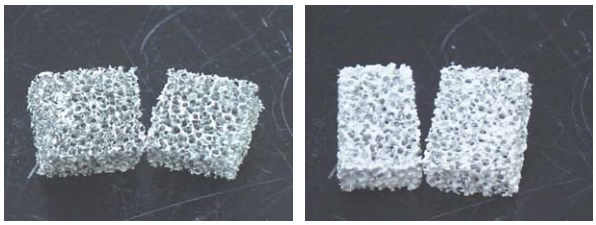
The final step for scaffold fabrication is the heat-treatment. The role of heat-treatment is to eliminate the polymeric sponge and organic additives at a temperature of around 600°C. After that, the remaining CPG was sintered at various temperatures from 650 to 850°C, as is shown in Figure 4.



(a) (b)

Figure 4: SEM photograph shows the strut of the porous skeletals after sintering at (a) 650°C and (b) 850°C.

When sintering at 850°C, the glass exhibited a white color, while sintering at a lower temperature presented dark colored glass, which means that there are some remains of the sponge or additives. The microstructure of the heat-treated glasses was observed using a SEM. The glass powders sintered at 650°C just contacted with surrounding powders. With increasing the sintering temperature, the voids between the powders were decreased. You can see that the dense microstructure after sintering at 850°C is without voids and cracks.



(a) (b)

Figure 5: Photographs of the sintered scaffolds at 850°C (a) once and (b) twice.

We repeated this process to thicken the frames of the scaffold. After sintering at 850°C, slurry was infiltrated again and dried and sintered at 850°C. With this repeating process, as is represented in Figure 5, we can obtain much thicker scaffolds.

As a result, the compressive strength was increased by almost two twice from 0.7 MPa to 1.4 MPa, however, there was no significant difference ($p>0.05$).

**Discussion**

In this study, macroporous CPG scaffold was prepared by using a polyurethane sponge. PVA is used as a binder and a large amount of slurry could be coated onto the sponge as the PVA content was increased for the same powder concentration. This is because of the relatively higher powder concentration in the slurry and an increased thixotrophy are available as the PVA content is increased. For example, in case of powder concentration of 40 wt% with 2 wt% PVA (a relatively low binder content), the slurry coated onto sponge's surface seemed to be slightly separated into agglomerated particles and water. This is thought to happen because the added amount of binder is not enough to surround each particle effectively and also, a much higher thixotropy is required. When increasing the binder content up to 8 wt%, this enabled an effective envelopment of particles and a homogeneous slurry coating behavior.

The heating rate of as-dried porous samples turned out to be one of the important factors for obtaining successful results. The samples that were heat-treated faster than a rate of 3°C/min were collapsed locally or the samples were very weak. This resulted from a softening and elimination of the sponge before the formation of necking particles and the lack of sufficient binding strength of the particles. In case of samples heat-treated as slowly as 1°C/min, the original configuration with a three-dimensionally interconnected open pore system was maintained well after firing and the struts of porous skeletal consisted of homogeneous and dense grains.

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 a process using a polymeric sponge for porous ceramics, it is known that these microcracks on the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coating layer may appear from a non-uniform coating thickness, the presence of a locally un-coated layer, the difference in coefficient of thermal expansion between polymeric sponge and the coated layer, the vapor pressure generated by sponge evaporation on firing or the residual internal stress due to the drying [15-16].

These are the reasons microcracks need to be controlled; otherwise, the mechanical strength will be considerably decrease due to them [17-18]. In order to control these cracks, various organic additives can be added as a DCCA. 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. It was shown that microcracks did not appear with 10 wt% of DMF.

As-dried porous CPG specimens are crystallized and sintered by heat-treatment. The determined glass transition temperature of the glass powder was 595$^{o}$C for setting up at an optimum heat-treatment temperature. The prepared porous samples were heat-treated at different temperatures ranging from 650 to 850$^{o}$C. The specimen fired at 650$^{o}$C showed gray color due to the residual hydrocarbon component of sponge. In contrast, sintering at 850$^{o}$C enabled the specimens to be white porous glass with a dense microstructure of struts; this was thought to be the optimum firing temperature.

Also, a repeated slurry coating/sintering process affected the increased compressive strength of sintered porous body; this was due to thickening of struts. The compressive fracture behavior was similar to that in other reports, where elastic deformation was predominant at initial stage of compressive load, followed by subsequent fracture of the struts of the reticulated structure [19-20].

From this polymeric sponge method, macroporous CPG can be fabricated with 200~800 μm of pore size and a 3D interconnected open pore system. It is thought that this kind of biodegradable scaffold combined with osteogenic cells has potential to be studied further as a tissue-engineered bone substitute.

## Conclusions

In this study, we investigated the fabrication method of a 3D reticulated scaffold with interconnected pores of several hundred micrometers using CPG in the system of CaO-$CaF_2$-$P_2O_5$-MgO-ZnO and a polymeric sponge as a template. Macroporous CPG can be fabricated with 200~800 μm of pore size and 3D interconnected open pore system. It is thought that this kind of biodegradable glass scaffold combined with osteogenic cells has potential to be studied further as a tissue-engineered bone substitute.

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