J MATER SCI 41 (2006) 4357–4364

Feasibility of three-dimensional macroporous scaffold using calcium phosphate glass and polyurethane sponge

YOUNG-SANG PARK
Research Center for Orofacial Hard Tissue Regeneration, Yonsei University College of Dentistry, Seoul 120-752, Korea; Department and Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, Seoul 120-752, Korea

KYOUNG-NAM KIM, KWANG-MAHN KIM
Research Center for Orofacial Hard Tissue Regeneration, Yonsei University College of Dentistry, Seoul 120-752, Korea; Brain Korea 21 Project for Medical Science, Yonsei University, Seoul 120-752, Korea; Department and Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, Seoul 120-752, Korea

SEONG-HO CHOI, CHONG-KWAN KIM
Research Center for Orofacial Hard Tissue Regeneration, Yonsei University College of Dentistry, Seoul 120-752, Korea; Brain Korea 21 Project for Medical Science, Yonsei University, Seoul 120-752, Korea; Department of Periodontics, Yonsei University College of Dentistry, Seoul 120-752, Korea

RACQUEL Z. LEGEROS
Department of Biomaterials and Biomimetics, New York University College of Dentistry, New York NY 10010, USA

YONG-KEUN LEE*
Research Center for Orofacial Hard Tissue Regeneration, Yonsei University College of Dentistry, Seoul 120-752, Korea; Brain Korea 21 Project for Medical Science, Yonsei University, Seoul 120-752, Korea; Department and Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, Seoul 120-752, Korea
E-mail: leeyk@yumc.yonsei.ac.kr

Tissue engineering presents an alternative approach to the repair of a damaged tissue by avoiding the need for a permanent implant made of an engineered artificial material. A suitable temporary scaffold material that exhibits adequate mechanical and biological properties is required to enable tissue regeneration by exploiting the body's inherent repair mechanism, i.e. a regenerative allograft. Synthetic bioresorbable polymers have been attracting attention as tissue engineering scaffolds. However, a number of problems have been encountered such as inflammatory responses and lack of bioactivity. Another good candidate for a tissue engineering scaffold is the calcium phosphates because of their good biocompatibility and osteointegrative properties. Their slow biodegradation is still remains problem, especially for the filling of large bony defects. In this study, we investigated the fabrication method of a three-dimensional 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 phosphate 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

---

*Author to whom all correspondence should be addressed.

0022-2461 © 2006 Springer Science + Business Media, Inc.
DOI: 10.1007/s10853-006-6261-0

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 500∼800 μm of pore size and a three-dimensionally 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.

---

1. Introduction

Bone regeneration by autografting tissue transplantation is one of the most promising techniques in periodontal and maxillofacial surgeries, and in orthopedic surgery as well. Treatment concepts based on those techniques would eliminate problems of immune rejection and pathogen transfer [1], however, the inherent donor site is somewhat limited [2]. The shortcomings of allografting, with respect to tissue rejection [3, 4] and disease transfer [5], have inspired the development of tissue engineering in which the material properties of synthetic compounds are dissociated by cells into the host in a manner that will result in the formation of new tissue [6, 7].

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 [8]. 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* [7]. The 3-dimensional 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 [9].

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 three-dimensional templates for initial cell attachment and subsequent tissue regeneration [10]. Matrix materials must satisfy a number of requirements. The solid phase must be biocompatible and promote cell adhesion and growth. Over time, as the cells produce their own natural extracellular matrix, the synthetic matrix should degrade into nontoxic components that can be eliminated from the body. Synthetic bioresorbable polymers have been attracting attention as tissue engineering scaffolds for the last 10 years, 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 [11, 12]. 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 [13].

Another good candidate for a tissue engineering scaffold is calcium phosphates because of their good biocompatibility and osteointegrative properties [14, 15]. 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 [14]. Recently, 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 [16]. Calcium phosphate glass was also observed to promote of bone-like tissue formation and have an enhanced alkaline phosphatase activity in vitro [17]. In addition, calcium phosphate glass promoted new bone formation in the critical-sized calvarial defect of Sprague-Dawley rats [18].

A number of fabrication technologies have been applied to process biodegradable and bioresorbable materials into three-dimensional polymeric scaffolds of high porosity and surface area. The conventional techniques for scaffold fabrication include freeze drying, fiber bonding, foaming, solvent casting, particulate leaching, polymeric sponge method, membrane lamination and melt molding [19, 20].

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

2. Materials and methods

2.1. Preparation of calcium phosphate glass

Calcium phosphate glass in the system CaO-CaF_2-P_2O_5-MgO-ZnO was prepared with Ca/P ratio of 0.6 using raw materials such as $CaCO_3$, CaF_2, H_3PO_4, MgO, and ZnO. 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 h at 80°C and calcined for 1 h 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 40 μm for the average size.

2.2. Polymeric sponge

Reticulated polyurethane ester sponge (Regicell, Jehil Urethane Co., Korea) was used in this experiment. This sponge has 500 three-dimensionally interconnected open pores per each linear mm.

2.3. Preparation of calcium phosphate glass slurry

Calcium phosphate glass slurry was prepared by dispersing the prepared calcium phosphate glass powders into distilled water with organic additives such as binder, dispersant and a drying chemical control additive (DCCA). Polyvinyl alcohol, polyethylene glycol and dimethyl formamide were selected as binder, dispersant and drying chemical control additive, respectively. First, polyvinyl alcohol (PVA; Du Pont, USA) 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, polyethylene glycol (PEG-400, Duksan Pure Chemical Co., Korea) was added at 5 wt%, and followed by addition of dimethyl formamide (DMF; Aldrich, USA) up to 10 wt%. Preparation of the calcium phosphate glass slurry was completed by dispersing the glass powder into distilled water containing the organic additives from 10 to 40 wt%.

2.4. Infiltration and heat-treatment

Prior to the infiltration process, the surface layer of the sponge was treated in a 2% NaOH solution for 20 min ultrasonically to improve the surface layer's hydrophilicity. After cleaning and drying, the porous sponge was

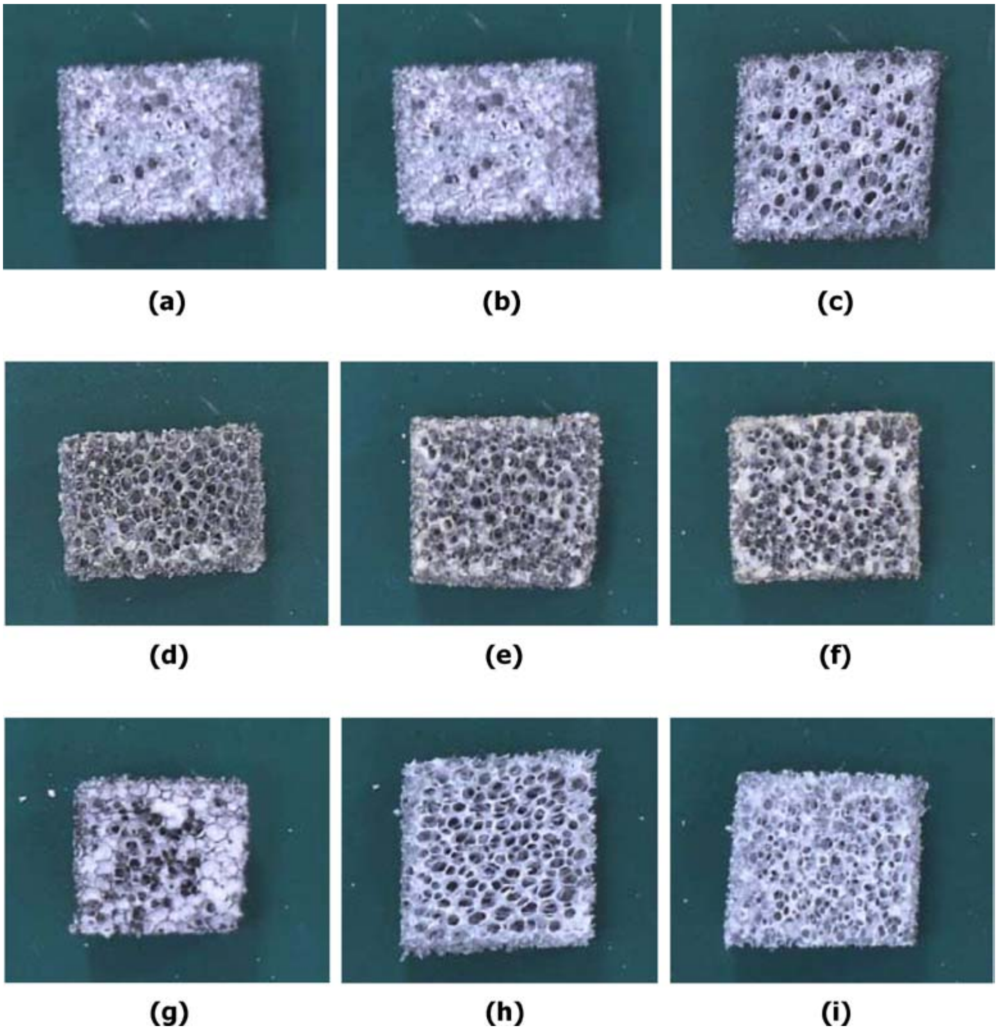


Figure 1 Photographs of polyurethane ester sponge foams infiltrated by the calcium phosphate glass slurry with various contents of the calcium phosphate glass powder and PVA ((a) powder 10 wt%, PVA 2 wt%, (b) powder 10 wt%, PVA 4 wt%, (c) powder 10 wt%, PVA 8 wt%, (d) powder 33 wt%, PVA 2 wt%, (e) powder 33 wt%, PVA 4 wt%, (f) powder 33 wt%, PVA 8 wt%, (g) powder 67 wt%, PVA 2 wt%, (h) powder 67 wt%, PVA 4 wt%, (i) powder 67 wt%, PVA 8 wt%).

subjected to an infiltration process. It was immersed into the glass 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 h to volatize the organic additives such as binder, dispersant and drying chemical control additive. Then the remaining calcium phosphate glass was sintered for 2 h at various temperatures from 650 to 850°C. The full procedure listed above was repeated twice to thicken the framework of the porous block.

2.5. Characterization

In order to set up the heat-treatment condition of the sponge infiltrated with the calcium phosphate glass, the polymeric sponge was thermally analyzed by TG/DTA (STA 1500, Netsch Co., Ltd., Germany). The glass transition temperature of the prepared calcium phosphate glass was determined by a TMA (2940, TA Instrument, USA) in order to predict the sintering temperature. The viscosity of the calcium phosphate glass slurry was measured by a cone and plate type viscometer (RH, Brookfield, USA). An optical microscope and a scanning electron microscope (S4200, Hitachi, Japan) examined the microstructure of the infiltrated, dried and sintered scaffolds. Each block was 5 mm in diameter and 8 mm in height and they were heat treated two times. 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. Rubber plates of 0.2 mm in thickness were placed between each surface of

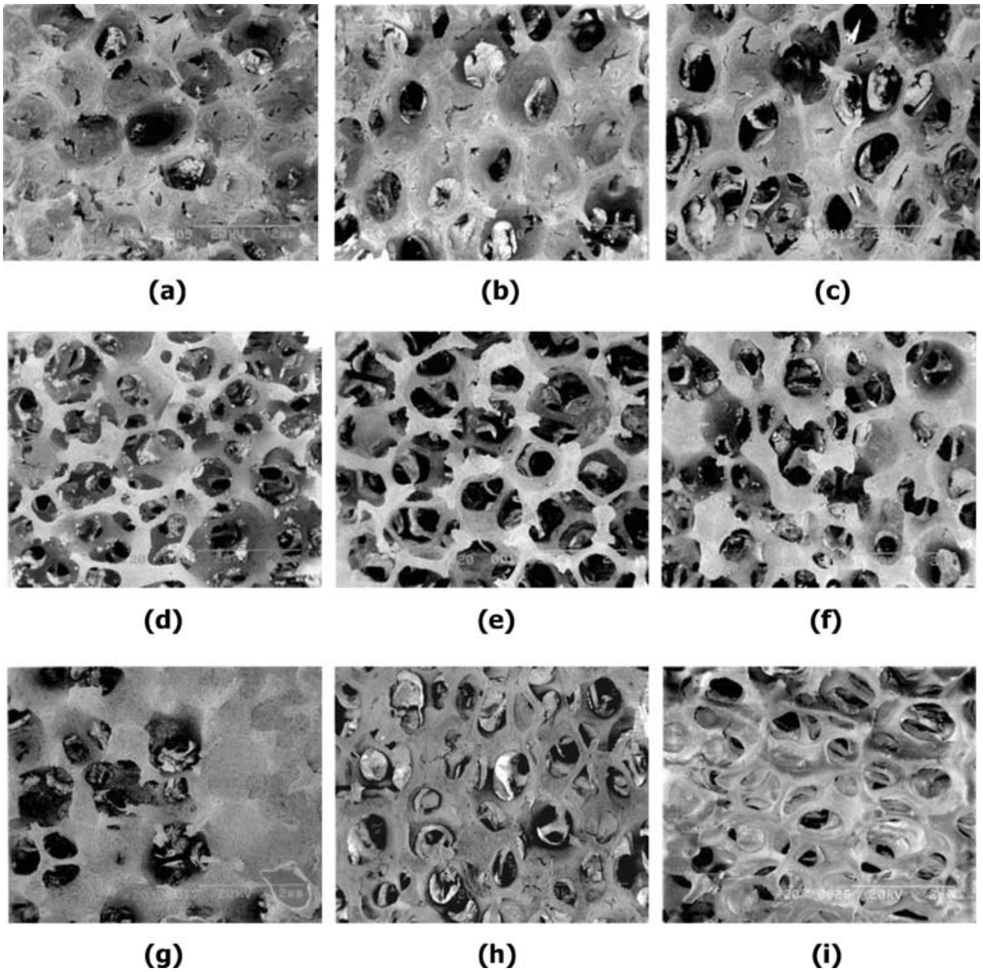


Figure 2 SEM photographs of polyurethane ester sponge foams infiltrated by the calcium phosphate glass slurry with various contents of the calcium phosphate glass powder and PVA. ((a powder 10 wt%, PVA 2 wt%, (b) powder 10 wt%, PVA 4 wt%, (c) powder 10 wt%, PVA 8 wt%, (d) powder 33 wt%, PVA 2 wt%, (e) powder 33 wt%, PVA 4 wt%, (f) powder 33 wt%, PVA 8 wt%, (g) powder 67 wt%, PVA 2 wt%, (h) powder 67 wt%, PVA 4 wt%, (i) powder 67 wt%, PVA 8 wt%).

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 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 difference between the determined compressive strengths corresponding to the heat-treatment times were analyzed by a Kruskal-Wallis test.

3. Results

Photographs of the polymeric sponges after infiltration of the calcium phosphate glass slurry with various contents of the calcium phosphate glass powder and PVA are represented in Fig. 1. When the content of the calcium phosphate glass 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. This clogged pore phenomena could be also observed using a scanning electron microscope, as is shown in Fig. 2.

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 Fig. 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 absolute prevention of crack formation during the drying process.

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 various temperatures from 650 to 850°C, as is shown in Fig. 4. 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.

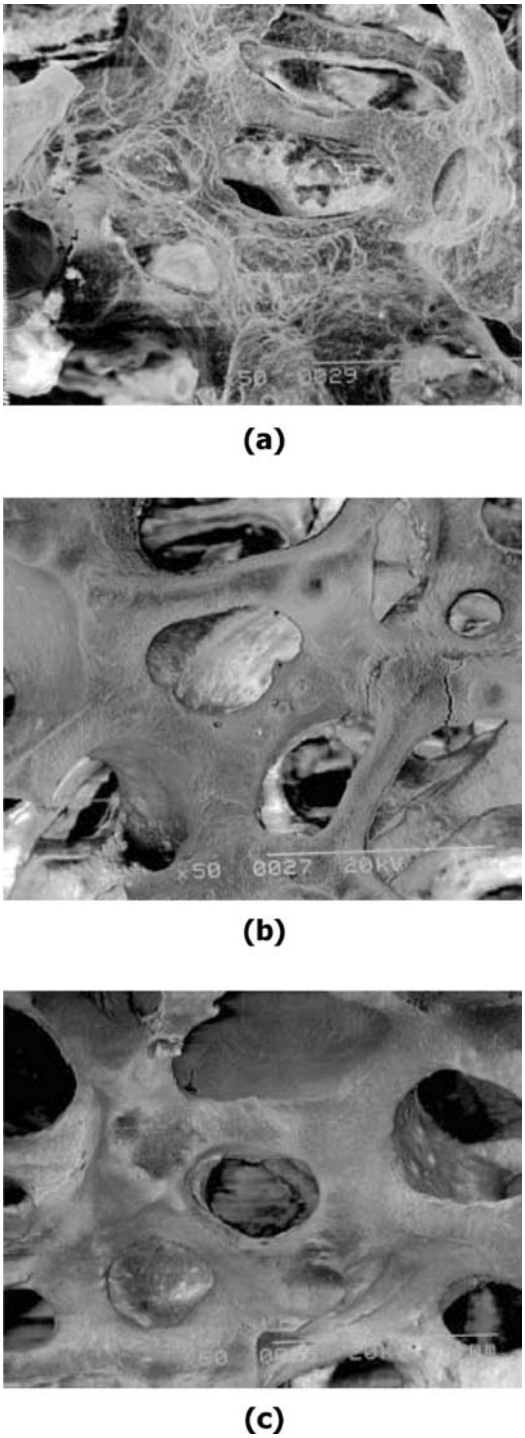
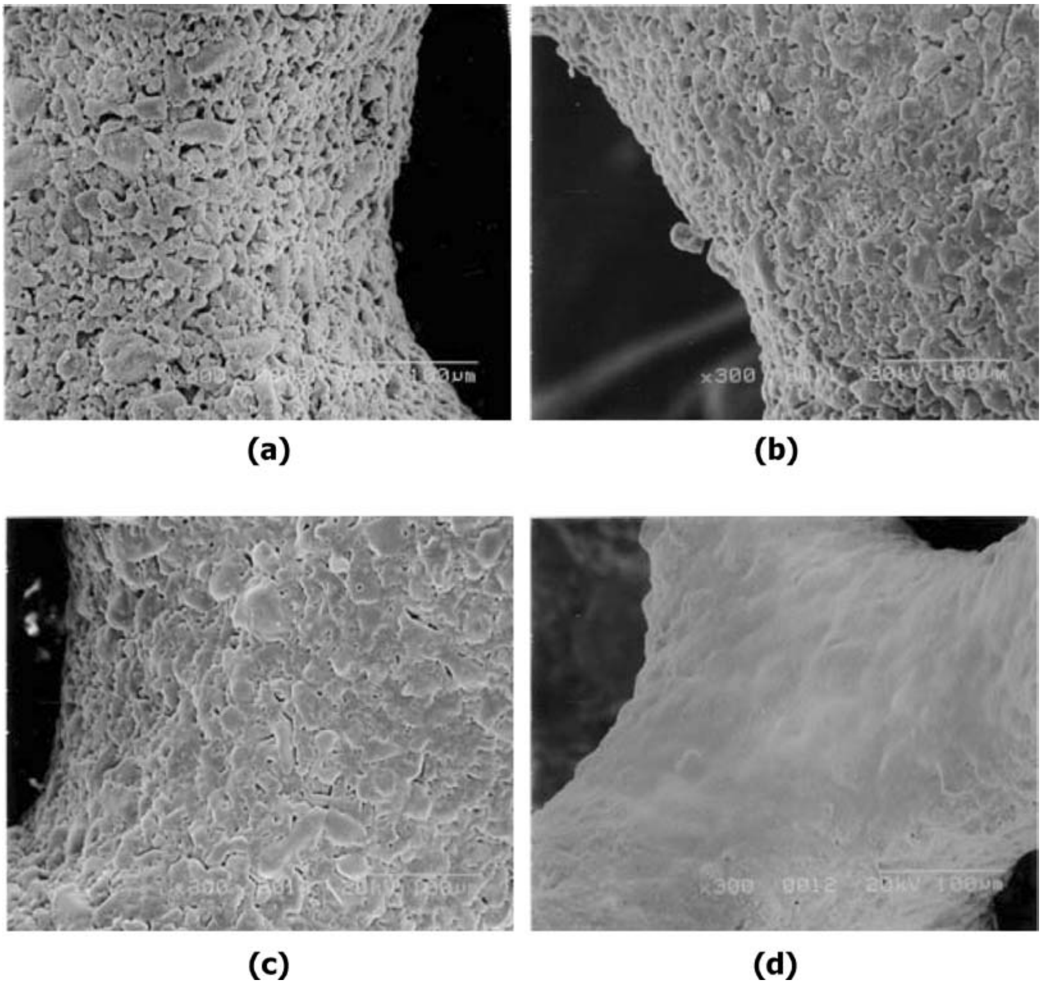


Figure 3 SEM photographs show the polyurethane ester sponge foams after infiltration and subsequent drying at 60°C with various contents of dimethyl formamide. (a: 0 wt%, b: 5 wt%, c: 10 wt%).

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 Fig. 5, we can obtain much thicker scaffolds. As a result, the compressive strength was increased by almost two twice, as is shown in Fig. 6. However, there was no significant difference ($p > 0.05$).



(a) (b) (c) (d)

Figure 4 SEM photographs show the polyurethan ester sponge foams after sintering at various temperatures. (a: 650°C, b: 700°C, c: 800°C, d: 850°C).

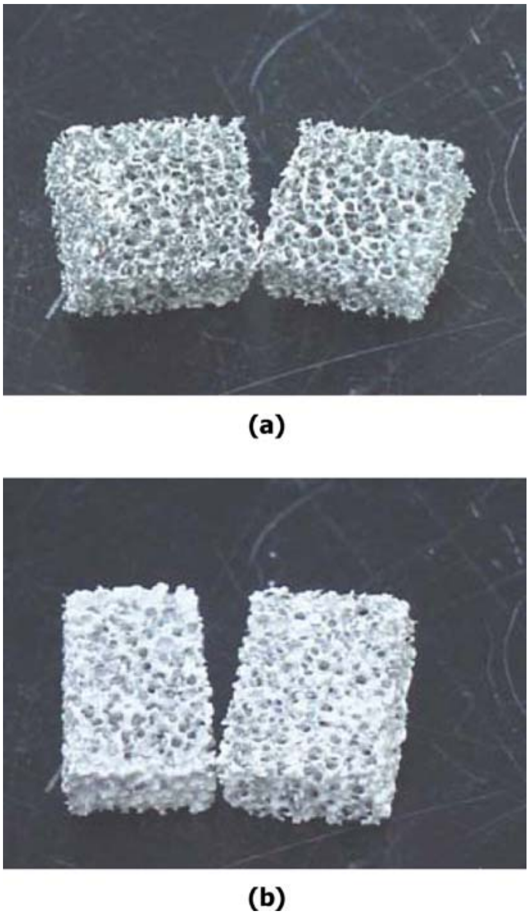
4. Discussion

In this study, macroporous calcium phosphate glass was prepared by using a polyurethane ester sponge. 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; this resulted in an elevated surface energy state of the sponge. There appeared to be many impurities on the surface of sponge before NaOH treatment, which may have caused non-uniformity of the slurry coating layer and given rise to defects such as abnormal grain growth during sintering. For these reasons, these impurities need to be eliminated before the slurry coating. In this current experiment, chemical treatment of sponge was best with a 2% NaOH solution for 20 min, and beyond that time, the chemical degradation and spring-back properties of sponges were considerably decreased.

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 coat-



(a)

(b)

Figure 5 Photographs of polyurethan ester sponge foams after sintering at 850°C (a) once and (b) twice.

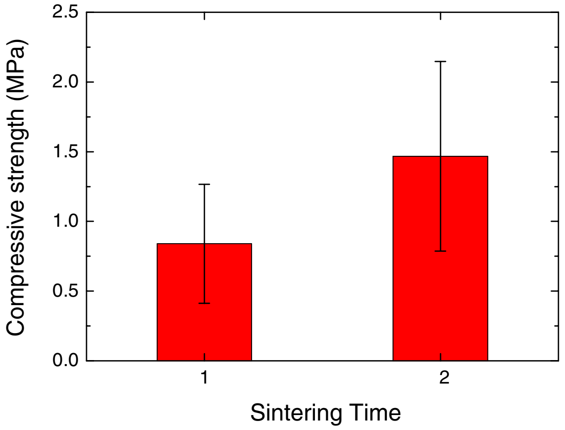


Figure 6 Compressive strength of polyurethan ester sponge foams according to the sintering time at 850°C.

ing layer may occurred on drying due to abrupt and large shrinkage caused from the high surface tension of water. In a process using a polyurethane sponge for porous ceramics, it is known that these microcracks on the coating layer may appear from a non-uniform coating thickness, the presence of a locally uncoated 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 [21, 22].

These are the reasons microcracks need to be controlled; otherwise, the mechanical strength will be considerably decrease due to them [23, 24]. 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 calcium phosphate porous glass specimens are crystallized and sintered by heat-treatment. The determined glass transition temperature of the glass powder was 595°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°C. The specimen fired at 650°C showed gray color due to the residual hydrocarbon component of sponge. In contrast, sintering at 850°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 [25, 26].

From this polymeric sponge method, macroporous calcium phosphate glass can be fabricated with 500∼800 μm of pore size and a three-dimensionally 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.

Acknowledgments

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ1-PG1-CH8-0001).

References

1. G. J. NAUGHTON, W. R. TOLBERT and T. M. GRILLOT, *Tissue Eng.* **1** (1995) 211.
2. N. F. BISSADA and U. HANGORSKY, *Dent Clin North Am.* **24** (1980) 739.
3. A. MIZUTANI, T. FUJITA, S. WATANABE, K. SAKAKIDA and Y. OKADA, *Int Orthop.* **14** (1990) 243.
4. J. R. MOORE, T. W. PHILLIPS, A. J. WEILAND and M. A. RANDOLPH, *J. Orthop. Res.* **1** (1984) 352.
5. J. T. MELLONIG, A. B. PREWETT and M. P. MOYER, *J. Periodontol.* **63** (1992) 979.

6. M. E. AICHELMANN-REIDY and R. A. YUKNA, *Dent. Clin. North Am.* **42** (1998) 491.
7. R. LANGER and J. VACANTI, *Science* **260** (1993) 920.
8. M. KELLOMAKI, H. NIIRANEN, K. PUUMANEN, N. ASHAMMAKHI, T. WARIS and P. TORMALA, *Biomaterials* **21** (2000) 2495.
9. J. O. HOLLINGER and A. CHAUDHARI, *Cells Mater.* **2** (1992) 143.
10. D. W. HUTMACHER, T. SCHANTZ, I. ZEIN, K. W. NG, S. H. TEOH and K. C. TAN, *J Biomed. Mater. Res.* **55** (2001) 203.
11. V. MAQUET and R. JEROME, *Mater. Sci. Forum.* **250** (1997) 115.
12. C. M. AGARWAL and R. B. RAY, *J. Biomed. Mater. Res.* **55** (2001) 141.
13. H. SCHLIEPHAKE, F. W. NEUKAM, D. HUTMACHER and J. BECKER, *J. Oral. Maxillofac. Surg.* **52** (1994) 57.
14. K. KURASHINA, H. KURITA, Q. WU, A. OHTSUKA and H. KOBAYASHI, *Biomaterials* **23** (2002) 407.
15. J. DONG, T. UEMURA, Y. SHIRASAKI and T. TATEISHI, *ibid* **23** (2002) 4493.
16. R. Z. LEGEROS and Y. K. LEE, *J. Mater. Sci.* **39** (2004) 5577.
17. Y. K. LEE, J. SONG, S. B. LEE, K. M. KIM, S. H. CHOI, C. K. KIM, R. Z. LEGEROS and K. N. KIM, *ibid.* **69A** (2004) 188.
18. H. J. MOON, K. N. KIM, K. M. KIM, S. H. CHOI, C. K. KIM, R. Z. LEGEROS and Y. K. LEE, *J. Biomed. Mater. Res..* **74A** (2005) 497.
19. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
20. T. M. FREYMAN, I. V. YANNAS and L. J. GIBSON, *Prog. Mater. Sci.* **46** (2001) 273.
21. D. D. BROWN and D. J. GREEN, *J. Am. Ceram Soc.* **77** (1994) 1467.
22. F. F. LANGE and K. T. MILLER, *Adv. Ceram Mater.* **2** (1987) 827.
23. R. BRENZY and D. J. GREEN, *J. Am. Ceram Soc.* **74** (1991) 1061.
24. R. BRENZY and D. J. GREEN, *ibid.* **72** (1989) 1145.
25. L. J. GIBSON and M. F. ASHBY, Structure and Properties (Pergamon Press, Oxford, U.K., 1988).
26. C. Q. DAM, R. BRENZY and D. J. GREEN, *J. Mater. Sci.* **5** (1990) 163.

Received 16 November 2004
and accepted 17 August 2005