

Preparation of a PLGA/Calcium Silicate Composite with Gradient Pore Structure

Bong-Kyu Choi¹, Suk Young Kim² and Sang-Hoon Rhee³

¹*Department of Oral Microbiology and Immunology, Dental Research Institute and BK21 HLS, School of Dentistry, Seoul National University, Seoul, Korea*

²*School of Materials Science & Engineering, Yeungnam University, Gyeongsan, Korea*

³*Department of Dental Biomaterials Science, Dental Research Institute and BK21 HLS, School of Dentistry, Seoul National University, Seoul, Korea*

Keywords: Composite, PLGA, Calcium Silicate, Pore, Bioactivity.

Abstract: The PLGA/SiO₂-CaO composite, which have a gradient pore structure, was newly prepared by the expansion of carbon dioxide gas in the PLGA matrix. The bioactive SiO₂-CaO particles were made by a sol-gel method from tetraethyl orthosilicate and calcium nitrate tetrahydrate under acidic condition followed by the heat treatment at 600°C for 2 h. The PLGA/SiO₂-CaO composite was then prepared by a solvent casting using chloroform as solvent. The composite was loaded into the high pressure chamber and then carbon dioxide gas was introduced achieving a final pressure of 10 MPa. After 3 days, the gas was released quickly and the gradient pore structure was developed. The samples were observed by FE-SEM and its bioactivity was tested in simulated body fluid.

1 INTRODUCTION

The representative bone bonding materials are calcium phosphates, bioactive-glasses, and –glass ceramics. They are known to bond to bone directly without intervening fibrous tissues. However, their applications are only limited to non-load bearing sites because their fracture toughness are very low, which results in low mechanical reliability.

Composites have been extensively studied as alternations of a bioactive ceramic because a polymer can give a ductility and flexibility while a ceramic can give hardness, strength, and bioactivity. One method to make a bioactive composite is just mixing the ceramic particles and polymers mechanically. Typical ones are thermal blending and solvent casting methods. However, the drawback of thermal blending method is that it is hard to increase the ceramic portion in the composites as well as the occurrence of phase separations due to the different wettabilities between two materials. Ceramics show hydrophilicity while most synthetic polymers have hydrophobicity so they cannot mix together easily. Solvent casting method is very simple way to make ceramic/polymer composite but it also shows the disadvantage when mixing two phases. After casting

the ceramic/polymer mixture, the sedimentation of ceramic particles must occur due to the density differences between solvent and ceramic particles. Thus, thick polymer top layer generally forms. In addition, phase separation between ceramic particles and polymer matrix occurs after drying. Thus, ceramic/polymer nano-composites are developed, where the polymer is linked to ceramic precursor at the molecular level.

However, the reported process to make the composite is hard to get porous structure. The bone grafting materials must have porous structure because it induces the growth of blood vessels into the material.

The salt leaching method is generally used to make porous structure when using the solvent casting method to make ceramic/polymer composites. However, this method is hard to make the connections among the pores. In addition, it cannot be applicable to the process for making nano-composite because the calcium salt, which is inevitable component to produce bioactivity in vivo, is also leached out when washing out the salt after the casting.

Mooney et al. (1996) reported the new method to produce porous structure in PLGA scaffold by the

expansion of carbon dioxide gas in high pressure chamber. The carbon dioxide has some solubility in the PLGA. Thus, when the high pressure of carbon dioxide is applied to the PLGA scaffold and then released quickly, it becomes to expand in the PLGA matrix. Resultantly, the pores are developed at the places where the gas existed. However, the disadvantage of this method is that it is also hard to connect between the pores. Thus, the salt leaching method is combined together to make porous structure. However, there has been no report to apply this method to make a porous bioactive composite material, yet.

In this study, we prepare the PLGA/calcium silicate composite by the solvent casting method and then a gradient pore structure was introduced using the expansion of carbon dioxide gas in a high pressure chamber.

2 MATERIALS AND METHODS

2.1 Preparation of Calcium Silicate Powders

The calcium silicate ($\text{SiO}_2\text{-CaO}$) particles were prepared with the starting composition of $70\text{SiO}_2\text{:}30\text{CaO}$ in molar ratio. The calcium silicate particles were prepared by hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS, Nacalai Tesque) in calcium nitrate tetrahydrate (Nacalai Tesque) and polyethylene glycol (PEG, Aldrich) in aqueous solution. The molecular weight of PEG used in this experiment was about 10000.

PEG and calcium nitrate tetrahydrate were dissolved in distilled water and then concentrated nitric acid (60 wt%, Nacalai Tesque) was added. TEOS was added to the above solution under stirring. After 20 min, the solution was transferred to a polystyrene box with its top sealed tightly, and kept at 40°C in a convection oven for gelation and aging for 1 day. The obtained wet gel was immersed in distilled water for 3 h with the distilled water renewed every hour. After the wet gel was dried at 40°C for 7 days, it was heated at 600°C for 2 h and then pulverized using a planetary ball mill.

2.2 Preparation of a PLGA/Calcium Silicate Composite

The $90\text{PLGA}/10\text{SiO}_2\text{-CaO}$ composite (in wt%) was made by the solvent casting. PLGA powder ($90\text{PLA}10\text{PGA}$, IV 0.55 ~ 0.75, Medisorb,

Alkemes) was dissolved in chloroform (4%) after which $\text{SiO}_2\text{-CaO}$ particles were added to the solution. After stirring vigorously for 1 h, the mixture was poured into a Teflon mould and dried under ambient conditions.

2.3 Preparation of a PLGA/Calcium Silicate Composite with a Gradient Pore Structure

The $90\text{PLGA}/10\text{SiO}_2\text{-CaO}$ composite was loaded into the high carbon dioxide pressure chamber and then carbon dioxide gas was introduced into the chamber achieving final pressure of 10 MPa. The specimens were allowed to equilibrate and saturate with the carbon dioxide gas for 3 days, forming a single phase $\text{PLGA}/\text{CaO-SiO}_2/\text{CO}_2$ gas solution at room temperature. Subsequently, the carbon dioxide gas was quickly released bringing the chamber to ambient pressure. (Mooney et al., 1996)

2.4 Bioactivity Test

The bioactivity of the $\text{PLGA}/\text{CaO-SiO}_2$ composite was assessed by evaluating its capability to form low crystalline hydroxyl carbonate apatite on its surface in simulated body fluid (SBF). (Kokubo et al., 1990) The SBF was prepared by dissolving reagent grade NaCl, NaHCO_3 , KCl, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , and Na_2SO_4 in ion exchanged distilled water. Their ionic concentrations were Na^+ 142, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 (in mM). The solution was buffered at pH 7.4 with tris(hydroxymethyl) aminomethane ($(\text{CH}_2\text{OH})_3\text{CNH}_2$) and 1 M hydrochloric acid (HCl) at 36.5°C . Specimen disks 12 mm in diameter by 4 mm in thickness were cut, sterilized under UV lamp for 30 minutes, and then dried on a clean bench. Subsequently, the specimens were incubated in 30 mL of the SBF at 36.5°C for 7 days. After incubation, the $\text{PLGA}/\text{CaO-SiO}_2$ composite was removed, gently rinsed with ion-exchanged distilled water several times, and dried at room temperature.

2.5 Characterization

Microstructure was observed by a field emission scanning electron microscopy (FE-SEM; S-4700, Hitachi). The crystal phase of the specimens before and after soaking in the SBF was evaluated by a thin film X-ray diffractometry (TF-XRD; D8 Discover, Bruker).

3 RESULTS AND DISCUSSION

Figure 1 shows the FE-SEM photograph of the PLGA/CaO-SiO₂ composite after the solvent casting. A thick surface layer was the PLGA while the CaO-SiO₂ particles were not observed at the surface. However, the hemispherical convex surfaces denoted as white arrows in Figure 1 must be the CaO-SiO₂ particles, which placed under the thick PLGA film.

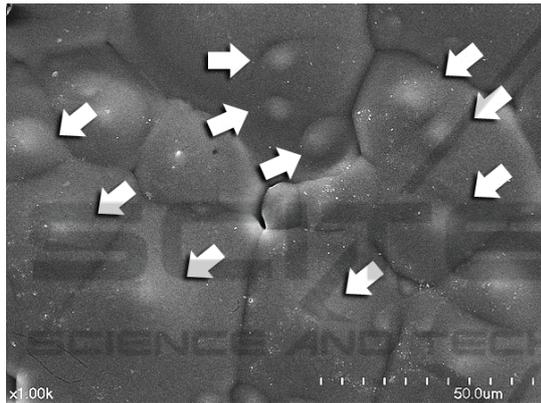


Figure 1: FE-SEM photograph of the PLGA/CaO-SiO₂ composite after the solvent casting.

Figure 2 shows the XRD diffraction pattern of the PLGA/CaO-SiO₂ composite after the solvent casting. Only broad hallow peak was observed to occur. It means there is no crystalline phase.

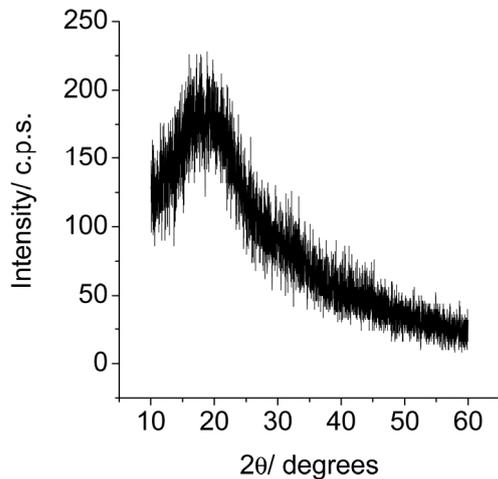


Figure 2: The XRD diffraction pattern of the PLGA/CaO-SiO₂ composite after the solvent casting.

Figure 3 shows the (a) low and (b) high magnification FE-SEM photographs of the fractured PLGA/CaO-SiO₂ composite after the carbon dioxide

gas treatment. The thick PLGA surface layer was inflated in large degree after the quick release of carbon dioxide gas and resultantly it made large pores at the surface of the composite (Figure 1(a)). However, the PLGA/CaO-SiO₂ composite region, which was previously placed under the thick PLGA layer, was not inflated as much as the PLGA. Thus, only small pores were produced (about 10 ~ 30 μm in size) (Figure 3(b)). The pore sizes were gradually become larger from the bottom to top surface of the specimen. The bottom layer of the composite showed open pore structure (white arrow in Figure 3(a)).

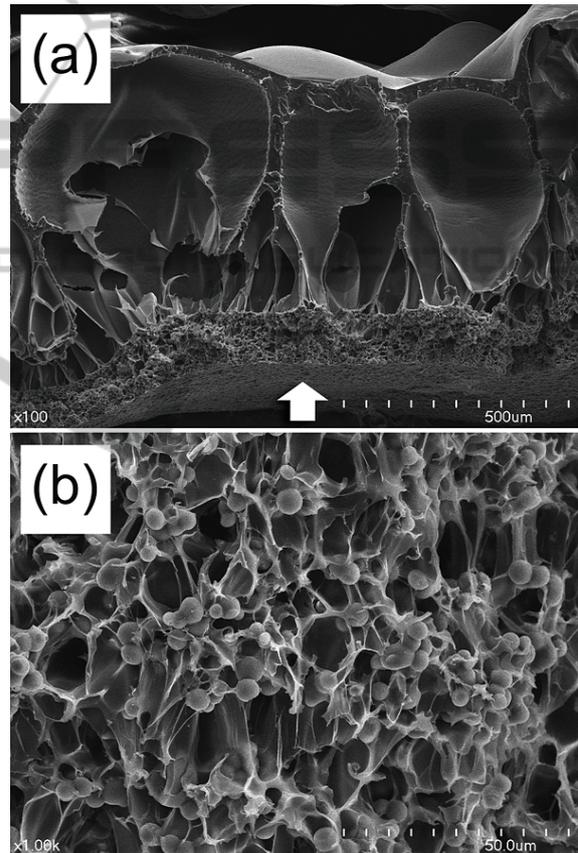


Figure 3: (a) Low and (b) high magnification FE-SEM photographs of the fractured PLGA/CaO-SiO₂ composite after the carbon dioxide gas treatment.

Figure 4 shows the (a) low and (b) high magnification FE-SEM photographs of the PLGA/CaO-SiO₂ composite after soaking in the SBF for 1 week at 36.5°C. Small flake-like low crystalline hydroxyl carbonate apatite crystals were observed to occur on the surface of the PLGA/CaO-SiO₂ composite. They started to occur from the surface of the CaO-SiO₂ particles and then spread

out all the surface of the composite even on the PLGA surface. It means this PLGA/CaO-SiO₂ composite has bioactivity and it also has a potential to have a good osteoconductivity.

From the results, it can be summarized that the PLGA-calcium silicate composite which had the gradient pore structure was successfully made using the expansion of carbon dioxide gas in the high pressure carbon dioxide gas chamber. The practical implication of this result is that this material can be used as a bone grafting material or a scaffold material for bone tissue engineering due to its high porosity and apatite forming capacity in the SBF.

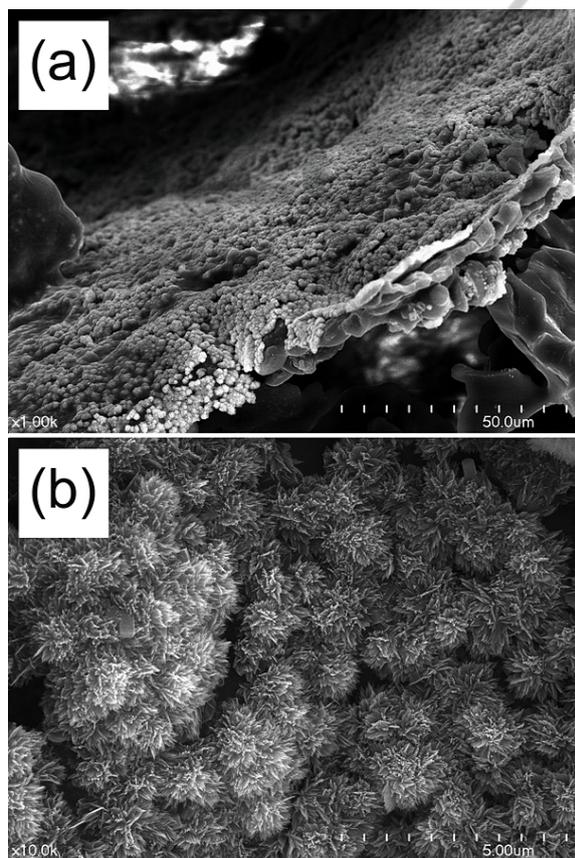


Figure 4: (a) Low and (b) high magnification FE-SEM photographs of the PLGA/CaO-SiO₂ composite after soaking in the SBF for 1 week at 36.5°C.

4 CONCLUSIONS

The PLGA/calcium silicate composite which had gradient pore structure was newly developed. The calcium silicate particles could be made by sol-gel method while the PLGA/calcium silicate composite could be made by solvent casting method. The thick

PLGA layer was formed during the casting process due to the sedimentation of the calcium silicate particles by the different densities between PLGA and calcium silicate particles. After the treatment of carbon dioxide gas, the gradient pore structure was developed in the composite. This composite showed the bioactivity in the SBF and it means it has a high potential to be used as a bone grafting material or the scaffold materials for bone tissue engineering.

ACKNOWLEDGEMENTS

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government (MEST) (20110007746).

REFERENCES

- Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T., 1990. *J. Biomed. Mater. Res.*, 24, 721-734.
- Mooney, D. J., Baldwin, D. F., Suh, N. P., Vacanti, J. P., Langer, R., 1996. *Biomaterials*, 17, 1417-1422.