Biodegradable Polymer-Nanoceramic Composite for Bone Regeneration

Sang-Soo Kim, [1] *Min Sun Park,* [2] *Byung-Soo Kim* [1,*]

[1]Department of Bioengineering, Hanyang University, Seoul, Korea
[2]Department of Chemical Engineering, Hanyang University, Seoul, Korea
[*]bskim@hanyang.ac.kr

Introduction

Biodegradable polymer/ceramic scaffolds can overcome the limitations of conventional ceramic bone substitutes.[1] However, the conventional methods (e.g., solvent casting and particulate leaching (SC/PL)) of polymer/ceramic composite scaffold fabrication often use organic solvents, which might be harmful to cells or tissues.[2] Moreover, scaffolds fabricated with the conventional methods have limited ceramic exposure on the scaffold surface since the polymer solution envelopes the ceramic particles during the fabrication process. In this study, we developed a novel fabrication method, gas foaming and particulate leaching (GF/PL), for the efficient exposure of ceramic onto the scaffold surface, which would enhance the bone regeneration potential of the scaffold.

Experimental

Preparation of PLGA/HA Scaffolds. Porous poly(D,L-lactide-co-glycolide)/hydroxyapatite (PLGA/HA) composite scaffolds were fabricated by the modification of a previously described GF/PL method.[3,4] Porous PLGA/HA scaffolds were also fabricated by the modification of a previously described solvent casting and particulate leaching (SC/PL) method[5] and used as a control.

Cell Seeding and In Vitro Culture. Aliquots of 50 μl of the cell suspension of rat calvarial osteoblasts (2.0×10^6 cells/scaffold) were seeded onto the tops of the pre-wetted scaffolds and cell/scaffold constructs were cultured in a humidified incubator at 37 °C with 5% (v/v) CO_2.

In Vivo Implantation. Fabricated scaffolds were implanted without cell seeding into the critical size defects (diameter = 8 mm) in rat skulls for eight weeks *in vivo* and bone regeneration was evaluated using micro CT and histological analyses.

Results and discussion

Scaffold Characterization. Gas foaming and subsequent salt leaching of scaffolds containing a high percentage of NaCl particles led to the formation of highly porous structures. The average porosities of the GF/PL and SC/PL scaffolds were 91 ± 3% and 86 ± 3%, respectively. The GF/PL scaffolds exhibited enhanced mechanical properties as compared to the SC/PL scaffolds. The average compression modulus was 2.3 ± 0.4 and 4.5 ± 0.3 MPa for the SC/PL and GF/PL scaffolds, and the average tensile modulus was 2.0 ± 0.1 and 26.9 ± 0.2 MPa for the SC/PL and GF/PL scaffolds, respectively. Selective staining of ceramic particles with von Kossa's silver nitrate indicated that HA nanoparticles were exposed to the scaffold surface more abundantly in the GF/PL scaffold than in the conventional SC/PL scaffold.

In Vitro Osteoblast Culture on PLGA/HA Scaffolds. Both types of the PLGA/HA composite scaffolds allowed for the adhesion and proliferation of the seeded rat calvarial osteoblasts over the in vitro culture period. Osteoblasts grew more rapidly in the GF/PL scaffolds than in the SC/PL scaffolds. The average cell density of the GF/PL scaffolds was 2.48×10^6 cells/scaffold after four weeks in culture, while that of the SC/PL scaffolds was 2.19×10^6 cells/scaffold, corresponding to 86.5% and 69.7% increases in cell density for the GF/PL and SC/PL scaffolds, respectively.

The ALP activity of the osteoblasts cultured on PLGA/HA composite scaffolds increased during the four-week culture period and decreased at eight weeks. In contrast, the ALP activity of the osteoblasts grown on the PLGA scaffolds without HA was low and did not show significant changes during the culture period.

The calcium deposition by cultured osteoblasts was significantly higher on the GF/PL scaffolds than on the SC/PL scaffolds. The deposition on both types of the PLGA/HA scaffolds gradually increased during the culture period. On the PLGA scaffolds without HA, calcium deposition was significantly lower than on both types of the PLGA/HA scaffolds. The calcium deposition on the PLGA scaffolds remained constant at low levels for the first four weeks, and increased slightly at eight weeks.

In Vivo Implantation Study. The implantation of both types of the PLGA/HA composite scaffolds into critical size defects in rat skulls without cell seeding resulted in enhanced bone formation *in vivo* compared with the PLGA scaffold. Eight weeks after implantation, new bone with lamellar structures and osteoid formation was appreciated in the SC/PL and GF/PL scaffolds at the defect edges and mid-sites of the grafts. The GF/PL scaffolds exhibited significantly enhanced bone regeneration when compared with the SC/PL scaffolds. Micro-computed tomography of the regenerated tissues showed that bone formation was more extensive on the GF/PL scaffolds than on the SC/PL scaffolds.

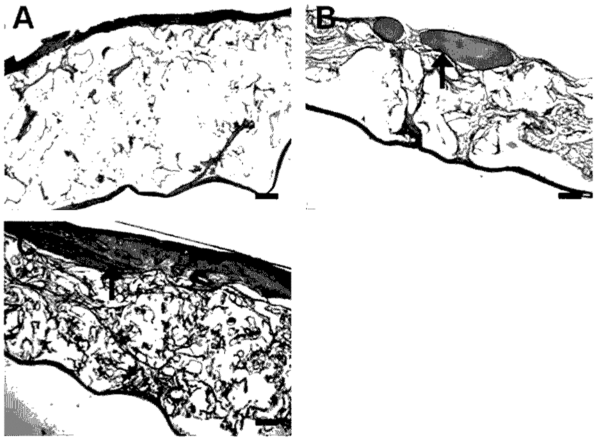

Figure 1. Histological evaluation of specimens retrieved at 8 weeks after implantation. Hematoxylin and eosin staining. (A) GF/PL-no HA, (B) SC/PL, and (C) GF/PL scaffold.

Implantation of the osteoblast-seeded PLGA/HA composite scaffolds into subcutaneous site of athymic mice resulted in new bone formation *in vivo* in ectopic sites at five and eight weeks after implantation. Five weeks after implantation, a small amount of woven bone was detected in both the SC/PL and the GF/PL scaffolds. Eight weeks after implantation, osteogenesis had progressed, and more bone with lamellar structures appeared. Histomorphometric analyses of the mid-portion sections of the regenerated tissues showed enhanced bone formation in the GF/PL scaffolds, compared with the SC/PL scaffolds and the PLGA scaffolds with no HA, at five and eight weeks after implantation. In contrast, the cell-seeded PLGA scaffolds with no HA had produced nearly no new bone *in vivo* for eight weeks. Most of the pores of the PLGA scaffolds with no HA were filled with loose fibrous connective tissues without evidence of bone formation at five and eight weeks after implantation.

Conclusions

PLGA/HA composite scaffold fabricated by GF/PL method showed enhanced mechanical property, hydrophilicity and osteoconductivity compared with the SC/PL scaffolds, and this enhancement was most likely due to a higher extent of exposure of HA particles to the scaffold surface. The biodegradable polymer/bioceramic composite scaffolds fabricated by the GF/PL method could enhance bone regeneration efficacy for the treatment of bone defects compared with conventional composite scaffolds.

References

[1] Peter, S.J., Lu, L., Kim, D.J. and Mikos, A.G. *Biomaterials* **2000**, *21*, 1207.
[2] Jung, Y., Kim, S.S., Kim, Y.H., Kim, S.H., Kim, B.S., Kim, S., Choi, C.Y. and Kim, S.H. *Biomaterials* **2005**, *26*, 6314.
[3] Harris, L.D, Kim, B.S. and Mooney, D.J. *J. Biomed. Mater. Res.* **1998**, *42*, 396.
[4] Kim, S.S., Park M.S., Jeon, O., Choi, C.Y. and Kim, B.S. *Biomaterials* **2006**, *27*, 1399.
[5] Lu, H.H., El-Amin, S.F., Scott, K.D. and Laurencin, C.T. *J. Biomed. Mater. Res. A* **2003**, *64*, 465.
[6] Kokubo, T. *Thermochim. Acta.* **1996**, *479*, 280.