Fabrication of a poly(D,L-lactide-co-glycolide)/hydroxyapatite composite scaffold with enhanced osteoconductivity

Sang-Soo Kim[1,2], So-Jung Gwak[3], Kang-Min Ahn[4], Min Sun Park[3], Jong-Ho Lee[4], Cha Yong Choi[2,5], Byung-Soo Kim[1,3].
[1]Department of Bioengineering, Hanyang University, Seoul 133-791, Korea.
[2]Interdisciplinary Program for Biochemical Engineering and Biotechnology, Seoul National University, Seoul 151-742, Korea.
[3]Department of Chemical Engineering, Hanyang University, Seoul 133-791, Korea.
[4]Department of Oral and Maxillofacial Surgery, College of Dentistry, Seoul National University, Seoul 110-744, Korea.
[5]School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Korea.

Statement of Purpose: Biodegradable polymer/ceramic scaffolds can overcome the limitations of conventional ceramic bone substitutes.[1] However, the conventional methods 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 for the efficient exposure of ceramic onto the scaffold surface, which would enhance the osteoconductivity of the scaffold.

Methods: Porous poly(D,L-lactide-co-glycolide)/hydroxy-apatite (PLGA/HA) composite scaffolds were fabricated by the modification of a previously described gas foaming and particulate leaching (GF/PL) method.[3] In brief, PLGA, HA, and NaCl particles were mixed at a mass ratio of 1:1:9, and the mixture was loaded into a disk mold and compressed at 2000 psi for 1 min. The solid disks were exposed to high pressure CO_2 gas (800 psi) for 48h and then decreased the gas pressure to ambient pressure. NaCl particles were subsequently removed by leaching the scaffolds in distilled water for 48 h. PLGA scaffolds without HA were also fabricated by the GF/PL method (GF/PL-no HA). Porous PLGA/HA scaffolds were also fabricated by the modification of a previously described solvent casting and particulate leaching (SC/PL) method[4] and used as a control.

Porosity, morphology, and mechanical properties of fabricated scaffolds were examined. To examine the distribution and extent of surface exposure of HA in the scaffolds, HA was visualized with a hydrophilic dye (trypan blue) staining or von Kossa's silver staining. The scaffolds were then tested for their wettability. For this, trypan blue solution was dropped on the top of scaffold and the time required for complete absorption of the solution into the scaffold was measured.

Fabricated scaffolds were implanted to critical size defects in rat skulls for eight weeks *in vivo* and bone regeneration was evaluated using micro CT and histological analyses.

Results / Discussion: Gas foaming and subsequent salt leaching of scaffolds containing a high percentage of NaCl particles led to the formation of highly porous structures with no evidence of an external, nonporous skin layer. 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 and a hydrophilic trypan blue dye indicated that HA nanoparticles were exposed to the scaffold surface more abundantly in the GF/PL scaffold than in the conventional SC/PL scaffold.

When the wettability of GF/PL, SC/PL, and GF/PL-no HA scaffolds was examined by dropping trypan blue dye solution on the scaffolds, the faster wetting of the GF/PL scaffold compared with SC/PL and GF/PL-no HA scaffold was observed.

The implantation of both types of the PLGA/HA composite scaffolds into critical size defects in rat skulls 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.

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:

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