Acellularized Scaffold for Osteogenic Differentiation of Rabbit Bone Marrow Stromal Cells

Yu-Jin Hong[1,2], Ik Hwan Kim[2], Dong Keun Han[1], Kwideok Park[1,*]
[1]Biomaterials Research Center, Korea Institute of Science and Technology, Seoul, Republic of Korea (kpark@kist.re.kr)
[2]College of Life Science and Biotechnology, Korea University, Seoul, Republic of Korea

Statement of Purpose: A major hurdle in bone tissue engineering is the lack of osteoinductive biomaterials and the difficulty of blood vessel formation within a tissue-engineered bone. While many parameters are often interconnected, a fundamental aspect is how to realize a biomimetic three-dimensional (3D) scaffold that can perfectly mimic the unique properties of natural extracellular matrix (ECM). The hierarchical network of ECM provides a natural environment in which cells can survive and function properly. In this study, a tissue cell-derived ECM is utilized as a substrate of stem cells seeded in porous scaffold. Osteogenic potential of ECM is of interest in this work. We hypothesize that a naturally self-assembled ECM-based structure can provide stem cells with a favorable environment for cell adhesion and differentiation. **Methods: Fabrication of porous PLGA/HA/β-TCP Scaffolds:** PLGA solution (13%, w/v) was mixed with HA and β-TCP, maintaining the weight ratio at 1:0.2:0.8, respectively. Sodium bicarbonate (200-300μm) was blended with the mixture of PLGA solution and the inorganics. Once packed in a disc-shaped silicon mold, they were briefly dipped in liquid nitrogen and subjected to lyophilization for 2 d. They were then subjected to a gas foaming process in a 20% citric acid, producing a porous composite scaffold. **Decellularization and characterization:** To make a decellularized scaffold, NIH3T3 fibroblasts or MC3T3-E1 preosteoblasts were seeded at $1x10^6$ cells per scaffold, respectively and cultured for 1 week. The cell/scaffold constructs were then decellularized using a cocktail solution, where the components include 10mM Tris-HCl (pH 8.0), 1.5M NaCl, 1% EDTA, 1% Triton X-100, RNase A (20 μg/ml), and DNase I (0.20 mg/ml). The constructs were placed in a shaker bath at 37 ^oC, with a continuous shaking at 100 rpm for 48 hr and then subsequent washing in PBS for 24 hr. The decellularized scaffolds from fibroblasts were assigned FDS and those from preosteoblasts were assigned PDS. After the decellularized scaffolds were cryo-sectioned, immunofluorescent staining of type I collagen was carried out with FDS and the immunostained samples were subjected to DAPI staining for nucleic debris detection in the FDS. **In vitro osteogenic differentiation of MSCs:** Bone marrow was harvested from the tibia and the femur of 4-month old New Zealand white rabbits weighing 500-600 g. Animal experiments were conducted under the ethical approval from our institute. The passaged MSCs (P3) were seeded at $5x10^5$ cells in three different scaffolds: control, FDS, and PDS. MSC-seeded constructs were then put in the osteogenic DMEM medium, containing 100nM dexamethasone, 50μM ascorbate-2-phosphate, 10mM β-glycerophosphate, 10% FBS, and 1% P/S. Each group was cultured in vitro for up to 4 weeks without the addition of BMP-2. Alizarin red S and von Kossa staining were used to identify the mineralized areas from the constructs. **Nude mouse implantation:** For ectopic bone formation, the constructs were implanted in the nude mouse subcutaneously. Tested groups were control, FDS, and PDS. When these samples were recovered after 3 weeks post-implantation, histological analysis and ALP activity test were carried out. **Results:** While DAPI staining revealed blue spots in the scaffold, the blue stains mostly disappeared after the decellularization, suggesting the removal of the nucleic components of the tissue cells (Fig. 1L). In addition, green stains were observed in the FDS upon the immunofluorescent staining before as well as after the decellularization, demonstrating that type 1 collagen, representative of the cell-derived ECM, remained after the treatment. Cultured in osteogenic medium in vitro, Alizarin red stains, an indicator of calcium deposition, were found in a greater area of FDS and PDS than control (Fig. 1R). H&E staining of in vivo samples also showed that FDS and PDS could induce osteogenesis of MSCs and thus develop a tight network of bone tissue matrix (Fig. 2).

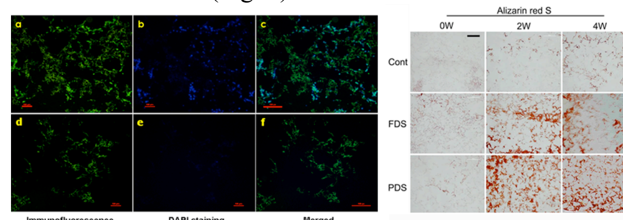

Fig. 1. FDS stained with DAPI and immunofluorescenced with type I collagen (L) and Alizarin red staining of FDS and PDS cultured with MSCs for 4 weeks in vitro (R). Scale bar: 100μm.

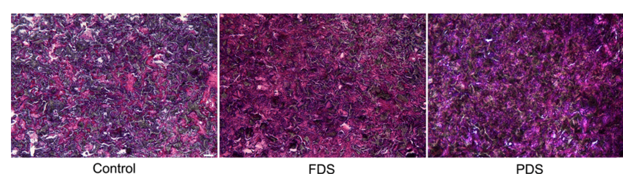

Fig. 2. H&E staining of the retrieved constructs from the nude mouse implantation for 3 weeks. Scale bar: 100μm.

Conclusions: The present results exhibited that introduction of naturally derived ECM into 3D scaffold is feasible and FDS and PDS were effective in prompting osteogenic differentiation of MSCs. This study hints that tissue-cell derived ECM may provide a suitable microenvironment for stem cells.

References: Zhang et al. Biomaterials. 2009;30:4021-4028; Rivron et al. Biomaterials. 2009;30:4851-4858.

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