Enhanced Osteogenesis of Mesenchymal Stem Cells in Calcium Phosphate/Collagen/Nanotube Scaffolds

Lijie Zhang[1,2] and Hicham Fenniri[3]
[1] Department of Bioengineering, Rice University, Houston TX, 77005, USA
[2] Department of Biomedical Engineering, University of California, Davis CA 95616, USA
[3] National Institute for Nanotechnology and Department of Chemistry, University of Alberta, Edmonton, AB, T6G 2M9, CANADA

Statement of Purpose:
Each year numerous joint or bone injuries caused by trauma, wear or tear and diseases, happened frequently in the world. Traditional autograft and allograft treatments still have many limitations and can not satisfy current high expectations of patients. Therefore, this study aimed to create novel mesenchymal stem cell (MSCs) encapsulated bone or osteochondral constructs based on hydroxyapatite (HA), β-tricalcium phosphate (TCP), type I collagen, and nanostructured rosette nanotubes (RNTs). Specifically, the calcium phosphates and collagens are main components of the extracellular matrix in natural bone or cartilage, thus serve as popular bone or cartilage substitutes. RNTs are a class of biologically inspired nanomaterials obtained through the self-assembly of DNA motifs in water. Previous studies have reported that RNTs functionalized with various peptide side chains [1] have excellent cytocompatibility properties and can remarkably improve osteoblast (bone-forming cell) functions when embedded in hydrogels, thus hold great potentials for bone repairs. In addition, as a promising cell source for regenerative medicine, MSCs have the self-renewal capability and multiple differentiation potency suitable to regenerate different tissues. Consequently, in this study, different compositions of TCP, HA, collagen, and RNT scaffolds were fabricated and the osteogenic differentiation of MSCs was investigated on these scaffolds for improving bone and osteochondral tissue engineering applications.

Methods: 0.005 mg/mL RNTs with RGDSK (RNT-RGDSK) were prepared by dissolving twin based Guanine∧Cytosine motifs with RGDSK peptides in water. Then the TCP/HA/collagen/RNT and TCP/HA/RNT nanocomposites were prepared by soaking porous TCP/HA/collagen (85:15 of TCP:HA, Medtronic) and TCP/HA (40:60, Medtronic) composites in 0.005 mg/mL RNT solution for 45 min at room temperature and were dried overnight. Uncoated collagen sponges, TCP/HA and TCP/HA/collagen served as controls. All scaffolds were sterilized using ethylene oxide. Furthermore, human bone marrow-derived MSCs were expanded up to passage #3 under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air). MSCs were then seeded at 10^5 cells per scaffold and cultured in a standard osteogenic medium for 7, 14 and 21 days. Cell culture medium was changed every other day. At each prescribed time point, cells were lysed and the left scaffolds were immersed in a 0.6 N HCl. Total DNA contents were assessed with the PicoGreen dsDNA assay kit (Invitrogen). The alkaline phosphatase activity was evaluated by an alkaline/acid phosphatase assay kit (Upstate). The calcium deposition was evaluated by a calcium reagent kit (Pointe Scientific).

Results: This study demonstrated that TCP/HA/collagen/RNT nanocomposites improved osteogenic differentiation of MSCs when compared to scaffolds without RNTs or collagen controls (Figure 1). Specifically, results showed that TCP/HA/collagen/RNT scaffolds had greater alkaline phosphatase activities than TCP/HA/collagen after 21 days of culture. Furthermore, when compared to collagen controls, increasing amounts of calcium depositions were observed on biomimetic TCP/HA/collagen/RNT, TCP/HA/RNT nano scaffolds and TCP/HA/collagen as well as TCP/HA. Figure 2 revealed the SEM image of TCP/HA/collagen scaffolds.

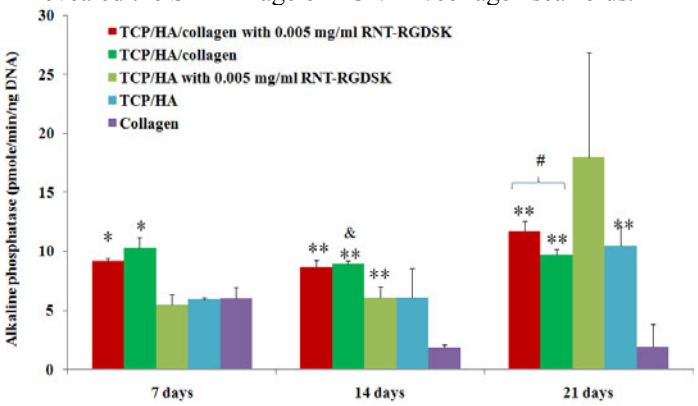

Figure 1. Enhanced alkaline phosphatase activity of MSCs on calcium phosphate/collagen/nanotube scaffolds. Data are mean values ±SEM, n=4. #p<0.01 vs. TCP/HA/collagen at 21 days; *p<0.05 vs. all other scaffolds at 7 days; **p<0.05 vs. collagen at 14 and 21 days; &p<0.05 vs. TCP/HA/RNT at 14 days.

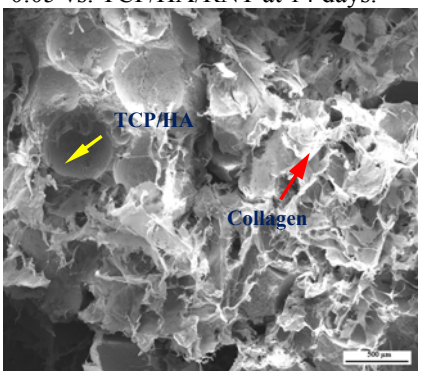

Figure 2. SEM image of TCP/HA/collagen scaffolds.

Conclusions: In summary, this study suggested that the biomimetic calcium phosphate/collagen/nanotube scaffolds were cytocompatible for mesenchymal stem cells and enhanced osteogenic differentiation of MSCs. The chondrogenic differentiation of MSCs on the scaffolds will be investigated next.

References:

1. Zhang L., et al. Biomaterials. 2009;30(7):1309-1320.