Cell-laden nanofibrous membrane design for osteochondral repair

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Statement of Purpose: Biphasic scaffolds have gained increasing attention for the regeneration of osteochondral interfacial tissue because they are expected to effectively define the interfacial structure of tissue that comprises stratified cartilage with a degree of calcification. Here, we propose a biphasic nanofiber (NF) construct made of polymer nanofiber (NF) and its mineralized form (mNF), which were used as the matrix for the culture of articular chondrocytes (ACs) and mesencymal stem cells (MSCs), respectively. The efficacy of the biphasic scaffold-cell constructs was investigated in vitro and in vivo after implantation in nude mice.

Methods: The PLCL solution was subjected to electrospinning to prepare nanofibers, and the prepared nanofibers were mineralized through solution-mediated reactions. ACs and MSCs were seeded on each sheet of NF and mNF, respectively. Thereafter, each medium was replaced with the chondrogenic or osteogenic defined medium. ACs-NF layered constructs were placed together with MSCs-mNF layered constructs to produce the multi-layers of cell-laden NF constructs. The morphology and characteristics of NFs were analyzed by SEM, FT-IR, XRD and EDS. The biological behaviors of the cell/NF constructs were analyzed by CCK-8, real time PCR, Alcian blue, ALP stain, H&E, safranin-o, and ARS staining.

Results: Bare NFs exhibited smooth surfaces, while the mNFs showed the surface coverage of nanocrystallites. Fiber diameters of bare NFs ranged from 400 to 600 nm, but mNFs were approximately 2-fold thicker than the bare NFs due to homogeneously deposited mineral crystals. The FT-IR spectra of mNFs revealed that the vibration bands relating phosphate groups were shown at wave numbers of 1029 and 563 cm^{-1}, and the XRD pattern showed peaks at 26^{o} and 32^{o}. The EDS spectrum in mNFs, contrasted to that in bare NFs, showed additional peaks of calcium and phosphorus elements. Cells highly elongated along the NFs and mNFs, favoring the underlying substrate condition. Cells grew actively on NFs, exhibiting an ongoing increase in growth kinetics. Although the initial level was lower on NFs than on culture plate, the cell growth on NFs at 7 days became almost comparable to that on culture dish. Much stronger gene expression of SOX9, type 2 collagen, aggrecan, and ALP was found in the differentiated group than in the undifferentiated control group at the 7, 14 day culture, strongly indicating that the upregulated mRNA of these genes responded to each defined medium environment in which the cells grew. The image of cells grown on NFs at the interfacial region showed a well integration of the layered structure and a number of cells migrated and populated within the NFs during the 4 weeks of implantation. ACs-NFs and MSCs-mNFs showed favorable tissue reaction through the implantation period and that each layer was effective in developing specific tissue ECMs.

Conclusion: ACs and MSCs cultured on NFs successfully exhibited the phenotypes of chondrocytes and osteoblasts, respectively. Furthermore, new cartilage and bone tissues were formed in the implanted area of cell-laded biphasic constructs at 4 weeks in nude mice.