Neuronal differentiation of mesenchymal stem cells on aligned nanofibers immobilized with nerve growth factor

Young Il CHO[1], Shin Young PARK[2], Hye Sung KIM[2], Seo Young JEONG[1], Hyuk Sang YOO[2,*].
[1]Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Republic of Korea
[2]Department of Biomaterials Engineering, School of Bioscience and Bioengineering, Kangwon National University, Chuncheon, Republic of Korea
*corresponding author: hsyoo@kangwonac.kr

Statement of Purpose: With respect to neural engineering applications, patterned adhesive areas contact guidance cues, delivery of nerve growth factors (NGF) and electrically conductive substrates have been particularly used with the goal of improving axon regeneration after trauma. However, whether these stimuli influence axon formation, axon elongation or both has not been extensively studied. Immobilized NGF was used as the model chemical ligand, whereas surface microtopography in the form of microchannels was investigated as the physical stimulus. These studies were performed presenting the cues as surface properties of a designed material, which could be adapted for neural engineering applications to better design biomaterials that modulate neuron responses. In the present study, NGF was chemically conjugated to the surface of electrospun nanofibers for nerve repair.

Methods: PCL–PEG block copolymer was synthesized by conjugating PEG-diamine to activated PCL. PCL–PEG/PCL mixtures in a mixture of methanol and chloroform at 15% (w/v) were injected through 27G needles at a speed of 1 ml/h. The amount of PCL–PEG block copolymer in the polymers was 10% (w/w). For alignments of electrospun nanofibers, fibers were collected at a rotating speed of 1800 rpm (drum diameter=100mm). The amine groups exposed on the surfaces of the nanofibers were measured by a fluorescamine assay. NGF, 1-hydroxybenzole (HOBt), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were added to soaked nanofibers in 1 ml of PBS (pH 8.0). The reaction was performed at room temperature with gentle stirring for 24 h. The NGF-conjugated nanofiber was extensively washed with distilled water to remove unreacted NGF. NGF-conjugation to the nanofiber was confirmed with a commercially available sandwich ELISA kit. The effects of nanofibers immobilized NGF on neural cell differentiation compared quantitatively by qRT-PCR

Results: As shown in Figure 1, morphology was examined by FE-SEM. Diameter and aligned angle of nanofibers were 390nm and 3.6°, respectively. Fluorescamine assay confirmed that amino groups exposed on the surface of the nanofibers was 2.4nmol/mg when the blend ratio of PCL-PEG was 10% (w/w) in the nanofibers. The amount of NGF was added at 50 ng/cm^2. All amine groups on the nanofibers were employed to chemically conjugated NGF. In order to determine the amount of NGF on the nanofibers after the conjugation reaction, ELISA was employed to measure NGF in the NGF-nanofiber. Sample volumes were collected from NGF-nanofibers incubated in PBS at 37℃ for 24h. The average concentration of NGF in solution was 1.077±0.23 ng/ml (n=3). (Figure 2) Soluble and immobilized concentrations cannot be directly compared, the soluble NGF concentration was probably much smaller than the immobilized NGF concentration. qRT-PCR (Figure 3)

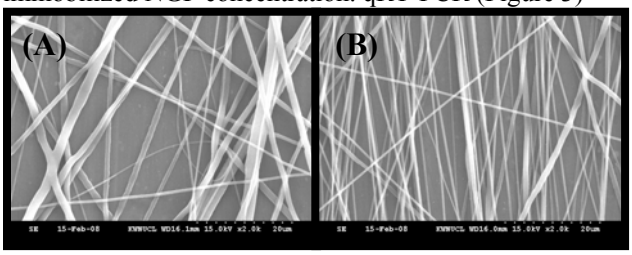

Figure 1. Scanning electron microscopy of PCL-PEG/PCL nanofibers when needle to collector distances were 5cm (A) and 10cm (B).

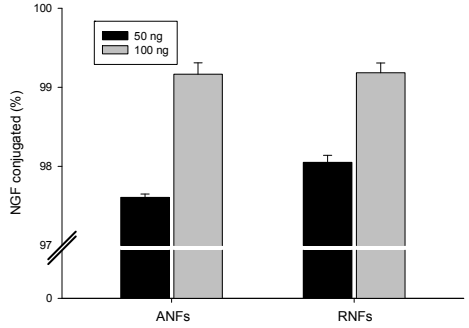

Figure 2. The conjugation percentage of NGF on aligned nanofibers (ANF) and random nanofibers (RNF). (n=3) NGF concentrations in conjugation medium were 50 and 100 ng per cm^2

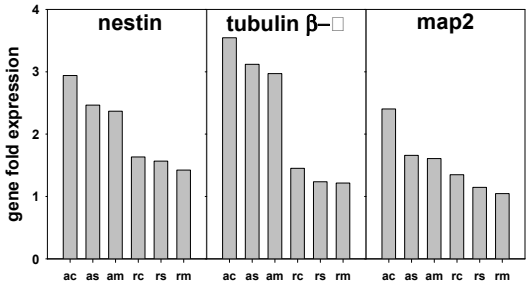

Figure 3. Real-time PCR (qRT-PCR) results showing neuronal expressions of mesenchymal stem cells cultivated in aligned nanofibers with conjugated NGF (ac), with NGF solution (as), in culture medium, random nanofibers with conjugated NGF (rc), with NGF solution (rs), and in culture medium (rm)

Conclusions: Aligned nanofibers with surface-immobilized NGF showed superior effects on neuronal differentiation of mesenchymal stem cells.

References: Chen PR. Biomaterials. 2005;26:6579-6587.
Choi JS. Biomaterials. 2008;29:587-596.
Gomez N. Biomaterials. 2007;28:271-284.