Human Annulus Fibrosus Tissue Engineering: Cell Attachment and Extracellular Matrix Production in Polyamide Nanofiber 3D Culture

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**Introduction:** Tissue engineering offers the potential to correct a number of conditions associated with disc degeneration: low cell numbers, altered cell-cell communication, and production of inappropriate or inadequate extracellular matrix (ECM). Nanofibrillar matrices have the potential to promote microenvironments which may mimic in vivo conditions and resemble connective tissue. Our objective here was to investigate cell attachment and ECM production of human disc cells seeded onto randomly oriented electrospun polyamide nanofibers.

**Materials and Methods:** Studies were carried out following approval of the authors' Human Subjects Institutional Review Board. Annulus cells were isolated from four cervical spine surgical disc specimens, expanded in culture as previously described (1), and seeded into either a) routine plastic culture (control), b) nanofiber surface, or c) nanofiber surface with a net positive charge. Nanofiber surfaces tested contained randomly oriented electrospun polyamide nanofibers (UltraWeb™-coated culture dish, Corning). Cells were cultured for 9 days, harvested, embedded in paraffin, and stained with toluidine blue. Immunolocalization studies were carried out for type II collagen (col II) (rabbit anti-human col II, Biodesign International, 1:100) and chondroitin sulfate (CS) (mouse monoclonal anti-proteoglycan deltaDI-4S, ICN Biomedicals, 1:100). The secondary antibody was Dako LSAB2 biotinylated Link for HRP/AP followed by peroxidase-conjugated streptavidin and DAB. Negative controls did not contain primary antibodies.

**Results:** The nanofiber surfaces tested here have fibers with a random orientation and average fiber diameter of 280 nm (Figure 1A; NP, nanoparticles). As expected in monolayer annulus cell culture, there was little matrix production by control plastic-grown cells (Figure 1B; NP, nanoparticles). When cultured on the nanofiber surface, annulus cells formed long cell processes coursing through the substrate (Figure 1C). When cultured on charged nanofiber surfaces, cells again formed long processes (Figure 2B and C). Unique to the charged surface was deposition of large localized regions of ECM with proteoglycan content (pink regions, Figure 2A, C, D).

**Discussion:** In conclusion, these novel studies have shown that human annulus cells attached well to the nanofibers, extended long processes into the nanofiber 3D structure, and grew well over 9 days with improved ECM formation on the nanofiber charged substrate. Our results show that culture of annulus cells on nanofibers is permissive for secretion and assembly of type II collagen and chondroitin sulfate. Nerurkar et al. have used bovine caudal annulus cells in studies of oriented electrospun nanofibrous scaffolds (2), and also showed collagen and proteoglycan deposition.

Results shown here have future application in cell-based tissue engineering strategies for biologic therapies for disc degeneration. Work is on-going in our lab to investigate cell signaling induced by nanofibrillar 3D culture of annulus cells.

**References:**

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