Effects of polycaprolactone nanowire surfaces on adipogenic and chondrogenic differentiation of adipose-derived stem cells

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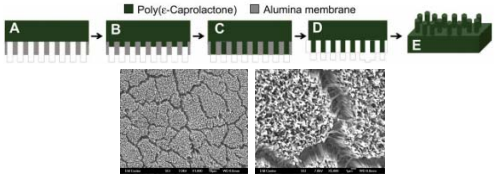
Statement of Purpose:

Despite many advances in tissue engineering, there are still significant challenges associated with restructuring, repairing, or replacing damaged tissue in the body. Currently, a major obstacle has been trying to develop a scaffold for cartilage tissue engineering that provides the correct mechanical properties to endure the loads associated with articular joints as well as promote cell-scaffold interactions to aid in extracellular matrix deposition[1]. In addition, adipogenic tissue engineering is widely growing due to an increased need for more innovative reconstruction therapies following adipose tissue traumas and cosmetic surgeries. Recently, lipoaspirate tissue has been identified as a viable alternative source for mesenchymal stem cells because it contains a supportive stroma that can easily be isolated. Adipose-derived stem cells (ADSC) can differentiate into a variety of mesodermal lineages including the adipogenic and chondrogenic phenotypes. Biodegradable polymeric scaffolds have been shown to be a promising alternative and stem cells have been widely used to evaluate the compatibility, viability, and bioactivity of these materials. Polycaprolactone (PCL) is a bioresorbable polymer which has been shown to promote cell proliferation[2]. The fundamental concept behind successful synthetic tissue-engineered scaffolds is to promote progenitor cell migration on to the scaffold, induce, and finally integrate with host tissue[1]. In this study, we investigate the nanoscale adhesion, proliferation, and chondrogenic differentiation of adipose derived stem cells on smooth PCL and PCL nanowire surfaces.

Methods:

PCL nanowire (NW) surfaces were fabricated using a template synthesis method. Smooth PCL pucks were placed flat against alumina oxide membranes and heated slightly above the melting temperature while the PCL was extruded into the pores of the membranes. Following extrusion, the alumina was dissolved in NaOH, rinsed profusely, dried, and stored.



a.

b.

Figure 1: *a). Schematic of NW fabrication b). SEM images of NWs at 1000x and 5000x*

Human adipose derived stem cells were cultured, expanded, and seeded in 48 well plates on both PCL and NW surfaces at a density of 5,000 cells/cm^2 in pre-adipocyte medium. Cultures were maintained at 37°C and 5% CO2 for the duration of the study. ADSC proliferation was investigated on both surfaces with cell responses observed on days 1, 4, and 7. During the proliferation phase, cell viability was determined using an MTT based toxicology assay and cell morphology was observed using scanning electron microscopy. In addition, cells live were viewed under fluorescence microscopy using CMFDA (cytoplasm), DAPI (nucleus), and rhodamine phalloidin (actin) stains to determine cell survival, adherence, morphology, and spatial organization across both scaffolds. The long-term differentiation phase consisting of weeks 1, 2, and 3 after the proliferation phase was observed using either chondrogenic or adipogenic differentiation media. ECM production was observed via Alcian Blue and Oil Red O for chondrogenic and adipogenic differentiation, respectively. Immunofluorescent labeling for sox-9, collegen-2, PPARγ and adiponectin was conducted on all 3 weeks. As with the proliferation phase, morphology was observed under scanning electron microscopy.

Results:

MTT results showed a general increase in cell viability on both surfaces during proliferation and ADSCs on NW surfaces displayed more elongated morphologies in comparison with smooth PCL. It was found that after 3 weeks of differentiation, ADSCs under adipogenic conditions on NW surfaces had increased lipid formation. In addition, both surfaces displayed higher expression of adipogenic proteins (PPARY and adiponectin) during immunofluorescence. However, it was found that after 3 weeks of differentiation, ADSCs under chondrogenic conditions on NW surfaces exhibited decreased proteoglycan formation as well as lower expression of chondrogenic proteins (sox-9 and collagen-2) during immunofluorescence.

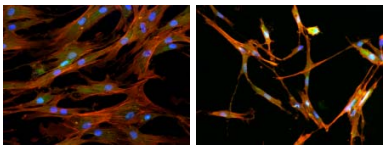


Figure 2: *CMFDA/DAPI/Rhodamine phalloidin stains of ADSCs on smooth PCL (left) and NW surfaces (right)*

Conclusions:

Biodegradable NW surfaces hold promise for future treatments of adipose tissue related damages and defects. However, although NW surfaces provide a high cell-surface interaction, the surfaces to not adequately promote ADSCs to differentiate into the chondrocyte phenotype as well as smooth PCL.

References:

[1]. Awad, H. A. (2004). "Chondrogenic differentiation of adipose derived stem cells in agarose, alginate, and gelatin scaffolds." Biomaterals 25(16): 3211-3222.

[2]. Woodruff, M. A. and D. W. Hutmacher (2010). "The return of a forgotten polymer—Polycaprolactone in the 21st century." Progress in Polymer Science **35**(10): 1217-1256.