Roles of Nanofiber Structure and Chemistry in Directing Stem Cell Morphology, Osteogenic Differentiation and Gene Expression

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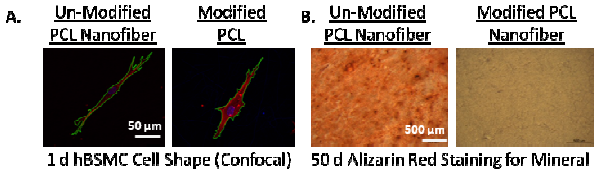
Statement of Purpose: Nanofiber technology has emerged as a promising tool to recapitulate the native extracellular matrix (ECM) environment for tissue engineering and regenerative medicine strategies. Cell-material interactions in the nanofiber system are largely dependent on nanofiber properties such as fiber diameter, alignment and biological functionality as well as scaffold pore size and porosity. Therefore, in order to understand the mechanisms underlying cell-material interactions in the nanofiber environment, systematic studies with controlled material properties are required. In previous studies we determined that nanofiber scaffold structures induced osteogenic differentiation without osteogenic supplements (1). In the current study, we have systematically investigated the roles of nanofiber structure and chemistry in directing the response of human bone marrow stromal cells (hBMSCs). In our material system, scaffold structure is kept constant while chemistry is varied by hydrolysis. Stem cell morphology, differentiation and gene expression are then investigated.

Methods: Poly(ε-caprolactone) (PCL) nanofibers (NF) were fabricated via electrospinning and PCL films via spin-coating (SC) [1]. PCL NFs and SC films were chemically modified by hydrolysis in 1 mol/L NaOH (7 h, 37 °C). hBMSCs were cultured on un-modified and NaOH-modified scaffolds and cultures were investigated for cell shape (1 d) using immunostaining and confocal imaging with 10 cell shape metrics determined by implementing a modified snake outline algorithm and custom MATLAB program [2]. Osteogenic differentiation (14 d) (alkaline phosphatase assay), calcification (50 d) (alizarin red staining) and microarray gene expression profiles (14 d) (BRB Array Tools and DAVID Bioinformatics Resources for gene ontology (GO) analysis) were also measured. Statistical significance was determined using 1-way ANOVA and Tukey's post test.

Results: Modified NF scaffolds (MOD PCL NF) and modified SC films (MOD PCL SC) were more hydrophilic with an increase in surface carboxyl concentration compared to un-modified scaffolds. (water contact angle 145° ± 6° to 112° ± 4° on NF to MOD NF and 75° ± 7° to 48° ± 7° on SC to MOD SC) There was no significant difference in fiber diameter (average NF diameter 611 nm +/- 87 nm) or SC film topology after modification. Cell shape metrics on NF vs SC scaffolds were significantly different (10/10 metrics with $p < 0.05$) while for NF vs. MOD PCL NF fewer significantly different metrics were identified (only 4/10 metrics with ($p < 0.05$)) (Fig 1A). Results suggest that cell shape is largely determined by NF scaffold structure and not chemistry. Investigating osteogenic differentiation, hBMSCs cultured on NF scaffolds for 50 d showed positive alizarin red staining for mineral (Fig 1B). This was not the case for MOD PCL NF scaffolds indicating



C.

Effect of Structure: ALL NF vs. ALL SC (Regardless of Chemistry)

GOTERM: Cellular Component

Annotation Cluster 1 Enrichment Score 2.0	Gene Count	P-Value
extracellular region part	5	0.0017
extracellular region	6	0.0035
proteinaceous extracellular matrix	3	0.016
extracellular matrix	3	0.018

Effect of Chemistry: ALL MOD vs. ALL UN-MOD (Regardless of Structure)

KEGG PATHWAY

	Gene Count	P-Value
Glycolysis / Gluconeogenesis	9	1.50×10^{-9}
Fructose and mannose metabolism	7	3.70×10^{-8}
Pentose phosphate pathway	4	4.30×10^{-4}

Figure 1: hBMSC morphology (A), mineralization (B), and gene clustering (C) in NFs and SC films of varying chemistry.

that both the structure and chemistry of the NF scaffolds contribute to NF mediated osteogenic differentiation (Fig 1B). For microarray gene expression analysis scaffolds were grouped into classes of ALL NF, ALL SC, ALL MOD, and ALL UN-MOD to investigate the effects of structure and chemistry on gene expression. Annotation cluster analysis of GO terms enriched from ALL NF vs ALL SC genes (identified by significance analysis of microarrays) resulted in GO terms related to extracellular regions/parts/matrix (Fig 1C). ECM-related genes were down-regulated on SC films but were up-regulated on NFs, regardless of chemical modification. Annotation cluster analysis of pathways and GO terms enriched from ALL MOD vs ALL UN-MOD genes resulted in pathways primarily related to glycolysis and metabolism (Fig 1C). Genes related to glycolysis were generally up/minimally regulated in UN-MOD substrates while down regulated in MOD substrates.

Conclusions: We have demonstrated a synergistic effect of scaffold structure and chemistry in promoting an osteogenic response from hBMSCs. Scaffold structure had a more profound effect cell morphology as well as on gene expression related to extracellular matrix proteins, while changes in scaffold chemistry resulted in changes to glycolysis pathways, indicating possible mechanisms of action in NF-stem cell interactions.

References: [1] Kumar G, et al. Biomaterials 32, 9188, 2011; [2] Driscoll MK, et al. Aging 4, 119, 2012

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