Effect of Nanofiber Scaffolds on 3D Shape of Stem Cells

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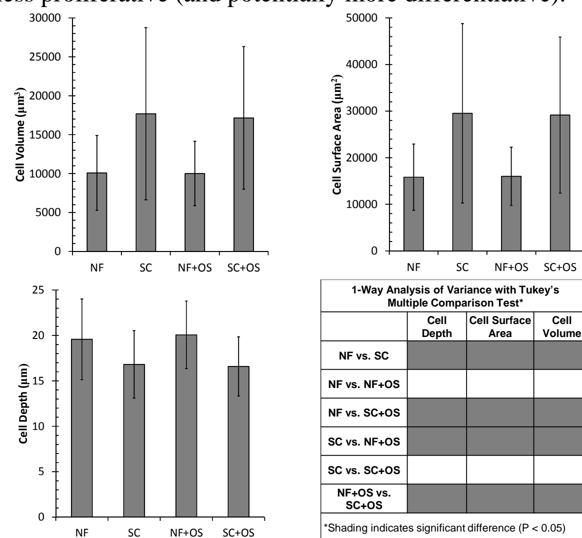
Statement of Purpose: Previous work has demonstrated that culture of osteoprogenitor cells on nanofiber scaffolds can potentiate osteogenic differentiation [1-4]. The nanofibrous structure causes cells to adopt elongated shapes which may drive their differentiation. However, the effect of scaffold structure on 3D cell shape has not been measured in scaffolds. Herein, primary human bone marrow stromal cells (hBMSCs) were cultured in nanofiber scaffolds, imaged in 3D using confocal microscopy and 3D cell shape was analyzed by computational approaches. A large number of cells were imaged, 115 per treatment, to provide statistical confidence in the results.

Methods: Electrospun nanofibers (NF) were made from PCL solution [(poly(ε-caprolactone), relative molecular mass 80,000 g/mol; 15% mass fraction in 5:1 volume ratio chloroform:methanol, 0.5 mL/h pump rate, positive lead on 18 gauge needle, grounded aluminum foil target, 18 cm needle to target, 16 kV, 6 h spinning]. NFs were electrospun onto TCPS disks (12 mm, hot-punched from tissue culture polystyrene dishes) that were placed on the target. Scanning electron microscopy was used to determine the mean diameter of the NFs (589 nm, S.D. 116, n = 151 fibers). For planar controls, PCL was spuncoat (SC) onto TCPS dishes (10% by mass in glacial acetic acid, 0.8 mL, 1000 rpm, 30 sec), annealed at 60°C for 30 s and hot-punched into 12 mm dia. SC disks. All samples were affixed to the bottom of 48-well plates with silicon grease, sterilized with ethylene oxide, degassed 2 d in a desiccator under house vacuum, incubated 2 d in medium with serum (α-minimum essential media, 16.5% by vol. fetal bovine serum, 4 mmol/L L-glutamine, with penicillin-streptomycin) and seeded with hBMSCs (Texas A&M, female, 29 years, pass 5, 2500 cells/well, 0.5 mL medium, 37 °C, 5% by vol. CO_2). Some samples were incubated with osteogenic supplements (OS) as indicated to induce hBMSC osteogenic differentiation (10 nM dexamethasone, 20 mM beta-glycerophosphate, 0.05 mM L-ascorbic acid). After 24 h culture, samples were fixed in 3.7% by vol. formaldehyde, washed and permeabilized with 0.1% by mass Triton X-100. The samples were stained with Alexa Fluor 546-phalloidin (F-actin stain, 330 nmol/L) and DAPI (4',6-diamindo-2-phenylindole, dihydrochloride, 0.03 mmol/L). Samples were imaged wet using water immersion. Z-stacks of fluorescence confocal images were collected (Leica TCS SP5 laser-scanning confocal microscope, water-immersion 63x objective, 50 z-slices/cell, voxel dimensions 240 nm × 240 nm × 710 nm. Actin and nuclei were imaged in 115 randomly selected cells for each of the 4 treatments (460 total cells imaged). Cell Depth, Surface Area, and Volume were computed from the z-stacks using Matlab. Voxels containing cellular material were distinguished from empty voxels using adaptive thresholding. Each z-slice was preprocessed using morphological operations to remove noise, and all contiguous regions of cellular material except the largest were removed from the z-stack. Cell Volume and Surface Area were computed by counting cell voxels and cell-surface voxels, respectively. Cell Depth was computed by finding the distance between the highest and lowest points on the cell along the shortest principal axis.

Results & Conclusions: A statistically robust analysis of 3D stem cell shape in nanofiber scaffolds has been performed where 115 hBMSCs were imaged in 3D for each of 4 treatments considered (460 total z-stacks). 3D shape metrics, Cell Depth, Surface Area and Volume, were significantly different for hBMSCs cultured on NF and SC substrates (Fig. 1). Addition of OS did not influence 3D cell shape. The results demonstrate that hBMSCs on nanofibers have a smaller size but take on a more 3D morphology (higher Cell Depth) than during culture on planar SC substrates. Large cells tend to have higher growth rates than smaller cells [5-7], suggesting that nanofibers may enhance osteogenic differentiation by driving cells into shapes with smaller volumes that are less proliferative (and potentially more differentiative).



1-Way Analysis of Variance with Tukey's Multiple Comparison Test*

	Cell Depth	Cell Surface Area	Cell Volume
NF vs. SC			
NF vs. NF+OS			
NF vs. SC+OS			
SC vs. NF+OS			
SC vs. SC+OS			
NF+OS vs. SC+OS			

*Shading indicates significant difference (P < 0.05)

Figure 1. hBMSC 3D shape metrics were determined by confocal imaging following 1 d culture on NF or SC samples both with and without OS. Data are means with S.D. (n =115).

References: [1] Kumar G, et al. Biomaterials 32, 9188, 2011. [2] Liu et al., ACS Nano 7, 6928, 2013. [3] Smith LA, et al. Biomaterials 30, 2516, 2009. [4] Ruckh et al. Acta Biomaterialia 6, 2949, 2010. [5] Cavalier-Smith. J Cell Sci 34, 247, 1978. [6] Folkman J, Moscona A. Nature 273, 345, 1978. [7] Chan YM, Marshall WF. Organogenesis 6, 88, 2010.

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