Measuring Organelle Shape in 3D in Stem Cells Cultured on Nanofiber Scaffolds

Wojtek Tutak[1,2], Giri Jyotsnendu[1,2], Peter Bajcsy[3], Carl G. Simon, Jr.[1]
[1]Biosystems & Biomaterials Division, [2]Dr. Anthony Volpe Research Center, [3]Software & Systems Division,
National Institute of Standards & Technology, Gaithersburg, MD, USA

Statement of Purpose: Previous work has demonstrated that culture of osteoprogenitor cells on nanofiber scaffolds can potentiate osteogenic differentiation [1-4]. Culture in nanofiber scaffolds causes changes to cell morphology, and these morphological changes may cause cell functional changes. Herein, it was hypothesized that nanofiber effects on cell morphology could influence cell behavior by altering organelle structure. Primary human bone marrow stromal cells (hBMSCs) were cultured on nanofiber scaffolds and the 3D shape of their organelles was measured using confocal microscopy.

Methods: Electrospun nanofibers (NF) were made from PCL solution [(poly(ε-caprolactone), relative molecular mass 80,000 g/mol; 10% mass fraction in 3:1 volume ratio chloroform:methanol, 2 mL/h pump rate, positive 18 gauge needle, ground aluminum foil target, 15 cm from needle to target, voltage 16.5 kV, 1.5 h spin time]. NFs were electrospun onto TCPS disks (16 mm dia., hot-punched from tissue culture polystyrene dishes) that were placed on the target. Scanning electron microscopy was used to measure mean NF dia. (325 nm, S.D. = 256 nm, n = 60). 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 16 mm dia. SC disks. All samples were affixed to the bottom of 24-well plates with silicon grease, sterilized with ethylene oxide, degassed 3 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, 24 years, pass 4, 15000 cells/well, 1 mL medium, 37 °C, 5% by vol. CO_2). After 24 h culture, medium was removed and replaced with serum-free medium containing 350 nmol/L Mitotracker Red CMXRos (1H,5H,11H,15H-Xantheno[2,3,4-ij:5,6,7-i'j']diquinolizin-18-ium, 9-[4-(chloromethyl)phenyl]-2,3,6,7,12,13,16,17-octahydro-, chloride, stains mitochondria). Plates were incubated for 1.5 h in the cell culture incubator, washed with PBS (phosphate buffered saline) and replenished with the fresh serum-free medium. CellLight Peroxisome-GFP was added to each well (6 μL/well, baculovirus transfects with green fluorescent protein coupled to a peptide that targets peroxisomes) and plates were incubated for 16 h in the cell incubator. Samples were fixed in 3.7% by vol. formaldehyde and permeabilized with 0.2% by mass Triton X-100. The samples were stained with Alexa Fluor 546-phalloidin (F-actin stain, 20 nmol/L in PBS) and DAPI (4',6-diamindo-2-phenylindole, dihydrochloride, 300 nmol/L in PBS). Samples were washed in PBS, washed in water and air dried. Z-stacks of fluorescence confocal images were collected (Leica TCS SP5 laser-scanning confocal microscope, oil-immersion 63x objective, 50 z-slices/cell, voxel dimensions 123 nm × 123 nm × 126 nm) for nuclei (435 nm to 480 nm), mitochondria (590 nm to 619 nm), peroxisomes (515 nm to 545 nm) and F-actin (558 nm to 585 nm). Organelles were imaged in 15 randomly selected cells for both NF and SC. Using ImageJ software, images were thresholded and organelle volumes were determined.

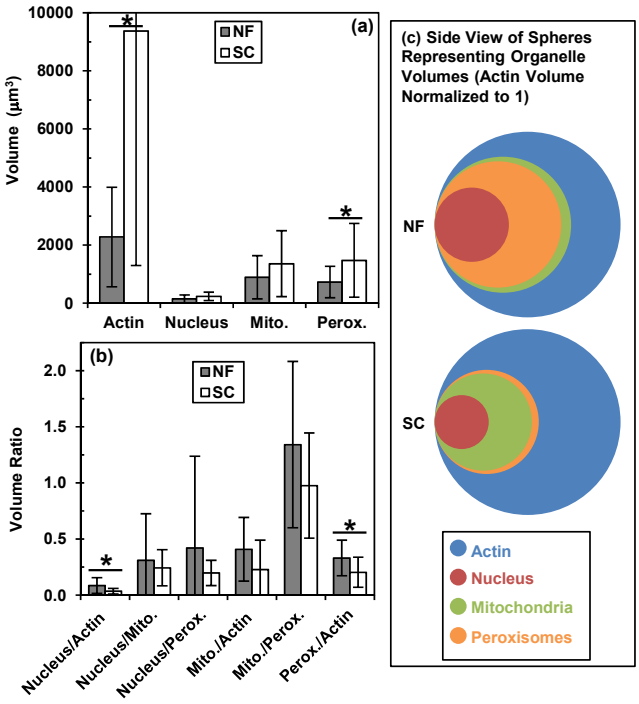

Figure 1. (a) hBMSC organelle volumes were determined by confocal imaging following 1 d culture on NF or SC samples. (b) Volume ratios for all 6 possible organelle pairings were calculated . (c) The colored circles represent side views of spheres that have the volumes of the indicated organelles. The diagram illustrates how the volume fractions of the organelles change during culture on NF (c). All data are for 15 cells (error bars = standard deviation) and asterisks are significant differences (t-test, $P<0.05$).

Results & Conclusions: hBMSCs and their organelles had a smaller volume during culture on NF scaffolds as compared to SC planar substrates (Fig. 1a). However, the nuclei and peroxisomes occupied a significantly ($P < 0.05$) larger fraction of the hBMSC cell volume during culture on NFs (Fig. 1b,c). Peroxisomes generate energy from catabolism of fatty acids and the increase in their cell volume fraction during NF culture could enable increased energy production [5]. Likewise, the increased volume fraction of the nuclei for NFs may enhance output of mRNA available for translation and protein synthesis [6].

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] Chan YM, Marshall WF. Organogenesis 6, 88, 2010. [6] Cavalier-Smith. J Cell Sci 34, 247, 1978.