Nanofiber Scaffolds Induce Morphological Changes in hBMSCs Critical for Osteogenic Differentiation

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Statement of Purpose: Utilizing tissue scaffold architecture to induce stem cell differentiation is of tremendous importance to the regenerative medicine community. To this end, we have investigated the effect of nanofiber scaffold structure on primary human bone marrow stromal cell (hBMSC) differentiation. hBMSC function was tested on biocompatible poly(ε-caprolactone) (PCL) and poly(DL-lactic acid) (PDLLA) nanofibers. Flat spun-coat surfaces of the same materials were used as controls. Since cell differentiation is tightly linked to cell morphology, we used high resolution confocal microscopy to measure and compare cell morphology on PCL nanofibers and films. Further, we have compared cellular morphologies during osteogenesis driven by nanofibers to that driven by osteogenic supplements (OS). We find that OS induce hBMSCs to adopt morphologies similar to that found on nanofibers. These results indicate that nanofiber scaffold architecture induces hBMSCs to adopt morphologies associated with osteogenic differentiation.

Methods: PCL films (80000 g/mol, 10% mass/vol. in acetic acid) were prepared by spin-coating (1200 RPM, 30 sec) onto 100 mm TCPS discs, then punched into 10 mm discs. PCL nanofibers were electrospun (16.5 kV, 2.0 mL/h) onto TCPS discs from solution (10% mass/vol. in 3:1 chloroform:methanol). PDLLA films (100000 g/mol, 15% mass/vol. in acetic acid) were prepared by spin-coating (1500 RPM, 30 sec) onto TCPS discs. PDLLA nanofibers were electrospun (18 kV, 1.5 mL/h) onto TCPS discs from solution (15% mass/vol. in hexafluoroispropanol) and fluorescent PDLLA fibers included 5 μg/mL Rhodamine 123. Discs were loaded into 48-well plates, sterilized (ethylene oxide), degassed, and washed in culture media to prepare for cell plating. hBMSCs (29 yr. female, Tulane University NIH hBMSC Center) were plated at 5000 cells per disc (α-MEM, 16% fetal bovine serum) with or without OS (dexamethasone, ascorbic acid, β-glycerophosphate). Cells on scaffolds were fixed (3.7% mass/vol. formaldehyde) and permeabilized (0.2% mass/vol. Triton X-100). Cells were stained with AlexaFluor 546 phalloidin (33 nM, red actin) and Sytox green (1 μM, green nuclei). To measure osteogenesis, calcium deposition was assessed by Alizarin red staining (1 h, 1% mass/vol. Alizarin red). Confocal imaging was performed on a Zeiss LSM-510 microscope.

Results: In the absence of OS, hBMSCs underwent osteogenic differentiation on PCL or PDLLA nanofibers but not on PCL or PDLLA spun-coat films (Fig. 1a-d). hBMSCs cultured on nanofiber scaffolds exhibited elongated, highly branched morphologies (Fig. 1e-g). Those cultured on spun-coat films were highly spread with significantly decreased branching and increased roundness. On the spun-coat scaffolds in the presence of OS, hBMSCs adopted morphologies statistically similar to those cultured on nanofibers in the absence of OS. These results indicate that decreased area, elongation, and branching of hBMSCs at early time points may be required for osteogenic differentiation to occur. Fluorescent PDLLA nanofibers revealed that hBMSCs adhere to and project along nanofibers (Fig. 1h-i).

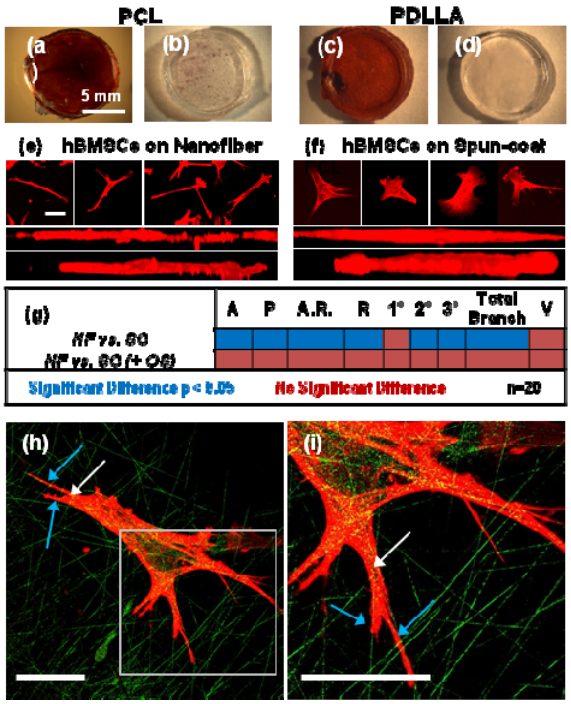

Figure 1: Alizarin red staining of hBMSCs cultured 50 d without OS on PCL or PDLLA nanofibers (a,c) or spun-coat films (b,d). Confocal z-stack projections of hBMSCs in the x-y and y-z planes (20 μm scan shown) for nanofiber (e) and spun-coat (f) scaffolds. (g) Statistical analysis (analysis of variance with Tukey's) of nine hBMSC morphological quantifiers (area, perimeter, aspect ratio, roundness, primary, secondary and tertiary branching, total branches, volume) for PCL nanofibers versus PCL spun-coat with and without OS. (h,i) Dual-fluorescence confocal micrographs of actin-stained hBMSCs cultured on fluorescent PDLLA nanofibers in the absence of OS. White arrows point to branch points with blue arrows showing projections aligning with fibers. Scale bars are 50 μm for e, f, h and i.

Conclusions: We have identified critical morphological characteristics required for osteogenesis and determined that nanofiber scaffolds induce similar cell morphologies as do OS.

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