Effect of Polymer Scaffold Structure on Human Bone Marrow Stromal Cell Differentiation

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Statement of Purpose: Many protocols for fabricating polymer scaffolds have been tested for tissue engineering applications. These approaches afford control over scaffold topology, structure and pore geometry. In addition, much work has demonstrated that cell differentiation is sensitive to topology at sizes ranging from nano- to micro- to macroscale.[2] Cells also exist in vivo in a 3D environment and cells cultured in a 3D environment in vitro can behave more physiologically than those cultured on a 2D flat surface.[2] For these reasons, we have investigated the effect of different scaffold topologies on differentiation of primary human bone marrow stromal cells (hBMSCs).

Methods: All scaffolds were made from PCL (poly(ε-caprolactone), relative molecular mass 80000) and were designed to fit in a 48-well plate (12 mm dia.). "Salt-Leached": 30 % by mass PCL solutions in chloroform were mixed with sieved NaCl (0.25 mm to 0.425 mm) to make a paste that was put into Teflon molds, air dried and salt-leached in water. "Gas-Foamed": 30 % by mass PCL solutions in chloroform were mixed with sieved ammonium bicarbonate (0.25 mm to 0.425 mm) to make a paste that was put into Teflon molds, air dried and foamed in warm water (40°C) for 2 h. "Phase-Separated": 30 % by mass PCL solutions in 7:3 chloroform:butanol (by volume) were mixed with sieved ammonium bicarbonate (0.25 mm to 0.425 mm) to make a paste that was put into Teflon molds, frozen at -80°C for 2 h, immersed in methanol at -20°C for 18 h and foamed in warm water (40°C) for 2 h. "Nanofibers": 15 % by mass PCL solutions in 9:1 chloroform:methanol was pumped at 0.5 mL/h into an electrospinning apparatus running at 15 kV. Polystyrene disks (12 mm) were placed on the aluminum foil target to collect fibers. "Spincoated": 10% by mass PCL solutions in acetic acid were spuncoat onto polystyrene disks (12 mm dia.) and air dried. "TCPS": This is control 2D tissue culture polystyrene. hBMSCs (29 yr. old female, Tulane University) were cultured according to supplier protocols. hBMSCs were seeded on scaffolds (10,000 cells/well) and cultured in medium without differentiation supplements. Cells on scaffolds were fixed (formaldehyde) and permeabilized (Triton X-100) for staining and imaged by fluorescence microscopy.

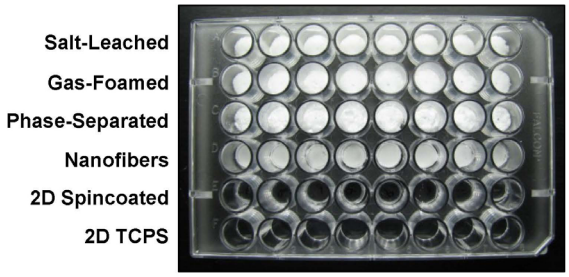

Fig. 1: Picture of 48-well platform for screening cell function on scaffolds with different topologies.

Results: A 48-well plate was used as a platform for screening hBMSC response to scaffolds (Fig. 1) with different topologies (Fig. 2). hBMSCs displayed different morphologies on the different scaffold topologies (Fig. 3). hBMSCs were the most spread and had the most numerous and well-defined actin fibers on TCPS. hBMSCs and their cytoskeleton attained more stellate 3D configurations in the scaffolds compared to 2D systems (spincoated and TCPS).

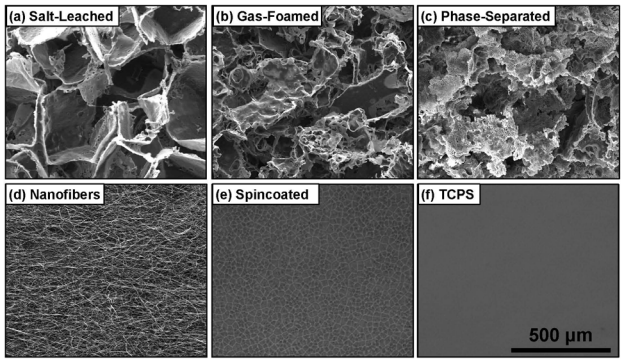

Fig. 2: Scanning electron (a-d) and phase contrast micrographs (e-f) of scaffolds with different topologies.

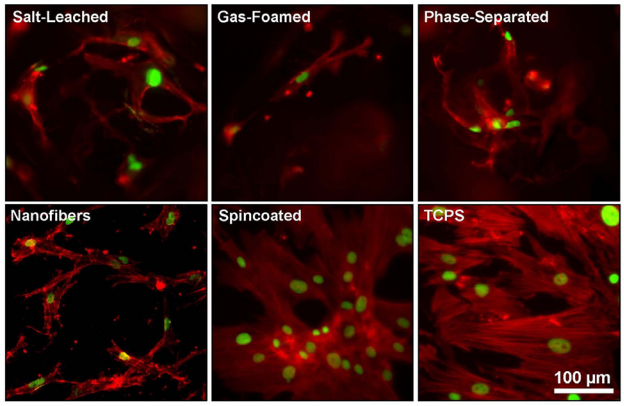

Fig. 3: Fluorescence micrographs of hBMSCs cultured 7 d on scaffolds with different topologies. Green is nuclei stained with Sytox green. Red is actin stained with Alexa fluor 546 phalloidin.

Conclusions: Cell morphology and the actin cytoskeleton show widely different morphologies on the different topologies presented by the different types of scaffolds. Ongoing work is assessing hBMSC proliferation and differentiation in the different scaffolds and will provide insight into which scaffolds can best promote hBMSC differentiation down different lineages.

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Reference:

[1]Oh S, et al., PNAS 2009; 106: 2130-5.
[2]Yamada KM, Cell 2007; 130: 601-10.