Effect of 3D Scaffold Structure on Osteogenic Differentiation of Human Bone Marrow Stromal Cells

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Introduction: Structural properties of polymeric scaffolds for tissue engineering play a key role in directing osteogenesis. These properties depend on the chemical nature and the fabrication process of biomaterial. There are many protocols for fabricating scaffolds for bone tissue engineering applications which afford control over scaffold topography. In addition, much work has demonstrated that cell differentiation is sensitive to topography at sizes ranging from nano- to micro- to macroscale. Thus, we have investigated the effect of different scaffold topographies on differentiation of human bone marrow stromal cells (hBMSCs).

Materials and Methods: All scaffolds were made from PCL [poly (ε-caprolactone), relative molecular mass 80000 g/mol] and were designed to fit in a 48-well plate (12 mm dia.). "Salt-Leached" (SL): 30 % by mass PCL solutions in chloroform were mixed with sieved NaCl (0.25 mm - 0.425 mm) to make a paste that was put into Teflon molds, air dried and salt-leached in water. "Gas-Foamed" (GF): 30 % by mass PCL solutions in chloroform were mixed with sieved ammonium bicarbonate (0.25 mm - 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" (PS): 30 % by mass PCL solutions in 7:3 chloroform:butanol (by volume) were mixed with sieved ammonium bicarbonate (0.25 mm - 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" (NF): 15 % by mass PCL solution in 9:1 by volume chloroform:methanol was pumped at 0.5 mL/h into an electrospinning apparatus running at 15 kV. Polystyrene disks (12 mm) were placed on an aluminum foil target to collect nanofibers. "Freeform Fabricated" (FFF): Scaffolds were purchased in 96-well plates (3DBiotek; disc-shaped 5 mm dia., 2 mm height; 0.3 mm strut dia.). "Spin-Coated" (SC): 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 Gene Therapy Center) were cultured according to supplier protocols. hBMSCs were seeded on scaffolds (10,000 cells/well) and cultured in medium with and without osteogenic supplements (OS) (dexamethasone, ascorbic acid, β-glycerophosphate). Cells on scaffolds were fixed (formaldehyde), permeabilized (Triton X-100), stained and imaged by fluorescence or stereomicroscopy.

Results: PCL was used as the polymer in all 3D scaffolds so that contributions from material chemistry could be isolated from topological effects. PCL spun-coat films were used as a 2D control as well as TCPS. Only NF scaffolds were able to induce osteogenesis by hBMSCs in the absence of OS (Fig. 1). Cell morphology was assessed since it is linked to cell function. hBMSCs were well spread with well-defined actin filaments on 2D substrates (SC, TCPS) and FFF. hBMSCs assumed a stellate morphology with poorly defined actin filaments on the 3D scaffolds (SL, GF, PS, NF).

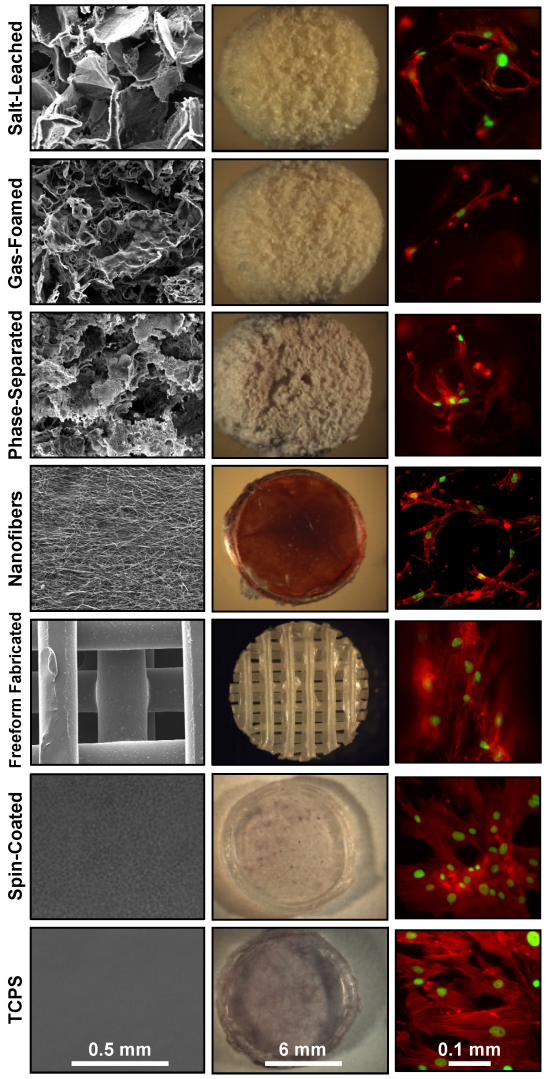

Fig. 1: Left column is scanning electron (SL, GF, PS, NF, FFF) and phase contrast (SC, TCPS) micrographs of scaffolds. Middle column is stereomicrographs of calcium staining (Alizarin red) for osteogenesis by hBMSCs cultured 50 d on scaffolds without OS. Right column is fluorescence micrographs of hBMSCs cultured 7 d on scaffolds without OS (green = nuclei = Sytox green; red = actin = Alexa fluor 546 phalloidin).

Conclusion: All scaffold morphologies supported hBMSC osteogenic differentiation in the presence of OS, but only NF scaffolds induced hBMSC osteogenesis in the absence of OS. Cell imaging suggests that nanofibers may drive hBMSCs to adopt a morphology that drives osteogenic differentiation.

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