

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

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Introduction: Structural properties of polymeric scaffolds for bone 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 topology. In addition, much work has demonstrated that cell differentiation is sensitive to topology at sizes ranging from nano- to micro- to macroscale¹. Thus, we have investigated the effect of different scaffold topologies on differentiation of human bone marrow stromal cells (hBMSCs).

Materials and Methods: All scaffolds were made from PCL [poly (ϵ -caprolactone), mass-averaged 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 to 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 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” (PS): 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” (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. “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 year 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: Libraries of 4 different 3D scaffold architectures were assembled in 48-well plates: SL, GF, PS, NF. PCL was used as the polymer in all 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. hBMSCs produced a calcium-containing matrix on all scaffolds and substrates by 21 d culture with OS (not shown). However, 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). hBMSCs assumed a stellate morphology with

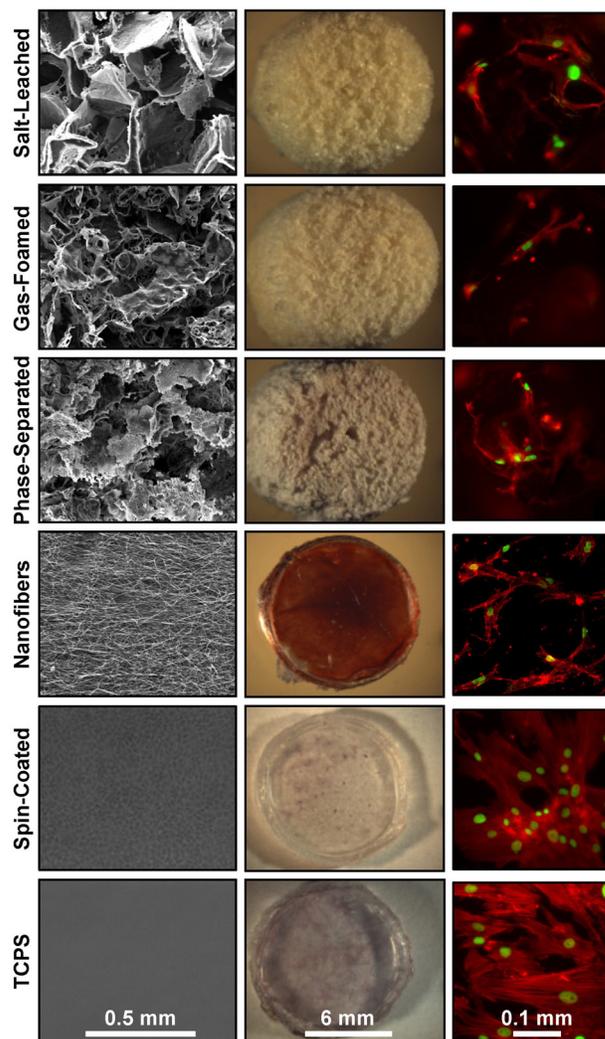


Fig. 1: Left column is scanning electron (SL, GF, PS, NF) and phase contrast (SC, TCPS) micrographs of the structure of the different scaffolds. Middle column is stereomicrographs of calcium staining (Alizarin red) for osteogenic differentiation of hBMSCs cultured on scaffolds without OS for 50 d. Right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

poorly defined actin filaments on the 3D scaffolds (SL, GF, PS, NF).

Conclusion: These results demonstrate that many scaffold morphologies can support hBMSC osteogenic differentiation in the presence of OS, but that only NF scaffolds can induce hBMSC osteogenesis in the absence of OS.

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Reference: ¹Yamada KM, Cell 2007; 130: 601-10.