Hydrogel Nanofiber Stiffness Influences Mesenchymal Stem Cell Spreading and Vascular Differentiation in 3D Matrix

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Statement of Purpose: Vascular diseases are the leading causes of death in the western world. Treatment of a diseased or dysfunctional blood vessel often involves surgical replacement of it with a vascular graft. Synthetic vascular grafts always cannot match the efficacy of natural vessels, leading to short-term or long-term graft failure. Recent developments in vascular tissue engineering have shown exciting potentials for promoting cells to regenerate blood vessels. However, there is a limited availability of vascular cells. Stem cells are thus increasingly explored for regenerative medicine. Particularly important is the microenvironment niche that regulates cell responses such as migration, proliferation and differentiation. Recent studies have shown that changes in the local stiffness of a 2-dimensional (2D) polyacrlymide hydrogel or polydimethylsiloxane polymer influence cell response. But few have studied cell responses to changes in the matrix stiffness of mimetic 3D microenvironments. The natural vessel exhibits three layers composed of hydrogel nanofibers with varied stiffness: the basement membrane around endothelium in the intima layer is the most elastic, connective tissue in the adventitia layer is the stiffest and the stiffness of smooth muscle in the media layer is in between. Our overall hypothesis is that the stiffness of 3D hydrogel nanofibers within a mimetic range regulates morphology, spreading and vascular differentiation of mesenchymal stem cells (MSCs) for regeneration. Herein, electrospun nanofiber grafts composed of photopolymerizable polyethelene glycol (PEG) hydrogel are utilized as matrix for 3D culture. The percentage and molecular weight of PEG as well as the amount of photopolymerization are varied to achieve a range of nanofiber elastic moduli.

Methods: Dimethylacrilite groups are synthesized onto PEG-3000 (Sigma-Aldrich), forming PEGdma-3000. PEGdma-3000 is placed in solution with PEO, DI-H_2O, and Ingacure 2959 and electrospun onto a rotating grounded collector at a flow rate of 1ml/hr, a voltage of 25 kV, and a collector distance of 18 cm. The resultant nanofiber graft (NFG) is composed of 49.98% wt PEGdma 3000, 49.98% wt PEO, and 0.04% wt initiator NFGs are photo-polymerized in a vacuum under 365 nm UV light for a period of 5, 15, 30 or 60 minutes. NFGs are then soaked in DI-H_2O for 24 hrs to remove PEO and residues. Mechanical testing is then performed on hydrated grafts and FTIR performed on dry grafts. Grafts are sterilized and coated with type I collagen before MSCs are seeded. After 16-hour culture, Live/Dead assay is performed to evaluate cell viability and spreading. Image-J is used to measure cell perimeter and area. Immunostaining cells with VE-cadherin (endothelial cell marker) and α-actin (smooth muscle cell marker) are used to characterize vascular differentiation.

Results: Images of the NFGs show a 3D fibrous matrix that swells when exposed to water, mimicking the in-vivo extracellular matrix environments (Fig 1). Tensile test

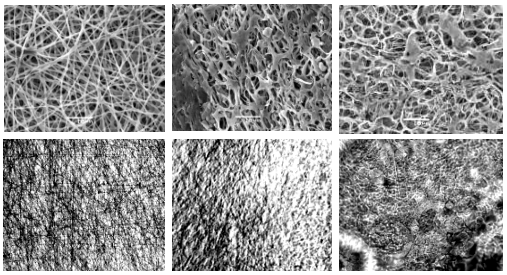

Fig 1: SEM (top left) and optical (bottom left) images of dry PEGdma-3000 NFG. SEM (top: middle and right) images of freeze-dry hydrated NFGs. Optical (bottom: middle and left) images of hydrated NFGs. NFGs are polymerized for 5 min (middle) or 30 min (right).

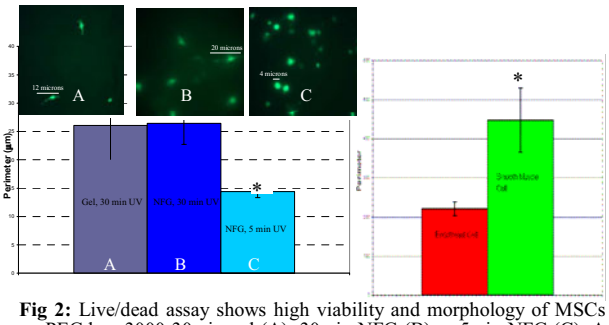

Fig 2: Live/dead assay shows high viability and morphology of MSCs on PEGdma-3000 30min-gel (A), 30min-NFG (B) or 5min-NFG (C). A comparison of average cell perimeter of MSC on each graft (left) and that of endothelial and smooth muscle cells (right) are performed.

show that 30-min NFGs have an elastic modulus ranging from 20 to 50 kPa. FTIR shows a decrease in the carbon carbon double bond peak (1640 cm^{-1}) as photopolymerization time increases. Measurements of cell perimeter and cell area show that MSCs on 5min-NFG have a statistically smaller cell area and perimeter then MSCs on 30min-NFG (Fig 2). MSCs on the NFGs readily migrated into the 3D matrix while those on the gel stay on the surface. Measurements of the cell perimeter and area of endothelial cells and smooth muscle cells also show that endothelial cells have a statistically smaller area and perimeter than SMCs. Roughly 70% of the MSCs on the 30min-NFG demonstrated VE-cad markers, while the remaining 30% had SMA markers. Fewer MSCs showed vascular differentiation on the 5min-NFG.

Conclusions:

PEGdma 3000 NFGs have been fabricated in the mimetic stiffness range with modulus varying from 20 to 50 kPa. The amount of crosslinking in the grafts and thus the graft stiffness can be controlled by photopolymerization time. MSCs readily migrated into the 3D NFG and showed increased cell perimeter and area with increasing NFG stiffness. The MSC morphology change with the NFG stiffness is also correlated to the vascular differentiation of MSC on the NFG. It is likely that NFG with a shorter UV exposure results in lower crosslinking and stiffness, providing a biomechanical nanofiber environment for endothelial differentiation of MSC; while NFG with a higher UV exposure provides a biomechanical nanofiber environment for smooth muscle differentiation.