**Modular poly(ethylene glycol) scaffolds with multiple levels of porosity for cell transplantation and vascularization**

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**Statement of Purpose:** Rapid host blood perfusion is essential for the survival of implanted cardiac tissues. Pre-vascularization strategies seek to accelerate host perfusion of scaffolds by pre-forming tubules of endothelial cells and support cells.[1,2] Previously, we demonstrated new methods for producing scaffolds from poly(ethylene glycol) (PEG) modules that self-assemble in the presence of living cells.[3] Here, we introduce a new method that allows for the formation of channel-like pores lined with endothelial cells. Degradable PEG 'strings' containing endothelial cells (EC) are crosslinked with non-degradable poly(ethylene glycol) (PEG) microspheres. Cells and modules are assembled into scaffolds in the presence of 20-40% dextran, which causes the PEG modules to de-swell. This enhances mechanical strength and leads to the formation of a network of pores within the scaffolds, within which another cell type may reside (e.g. HL-1 cardiomyocyte cell line). Hydrolytic degradation of the EC-containing strings results in the formation of an EC-lined macropore network. The ability to introduce multiple cell types and multiple levels of porosity in one step may enhance the fabrication of rapidly vascularizing constructs for cardiac tissue engineering.

**Methods:** *Microsphere fabrication:* RGD-functionalized non-degradable and degradable microspheres were fabricated as previously described.[3]
*Scaffold fabrication*: Non-degradable microspheres and HL-1 cells were suspended in DMEM and rotated for 1.5 h to promote cell adhesion. Microspheres were then resuspended in a dextran solution and centrifuged. The resulting scaffolds were incubated for 1 h, after which they were placed into Claycomb Complete Media (CCM, SAFC Biosciences). A Live/Dead Cell Viability Assay (Invitrogen) and scanning confocal microscopy were used to determine cell survival.

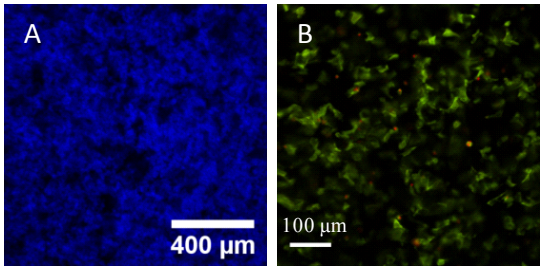


**Figure 1:** (A) Porous scaffold produced by crosslinking PEG microspheres in the phase separated state in 40% dextran. (B) Live (green) and dead (red) HL-1s in a non-degradable microsphere scaffold.

*String fabrication:* Degradable, RGD-containing PEG microspheres were suspended in a dextran solution and rotated to form strings due to flow-induced aggregation. A separate dextran suspension containing human umbilical vein ECs (Lonza) was then added to the string suspension and rotated for 1 h to promote cell adhesion to the strings.

**Results:** *Effect of dextran on scaffold porosity:* PEG and dextran phase separate, but neither solution is particularly toxic to cells. Non-degradable PEG microspheres (about 5 microns in diameter) are formed[3] and then crosslinked in a dextran solution. After buffer exchange, the scaffolds swell to produce connected pores (Fig. 1).
*HL-1s in non-degradable scaffolds:* HL-1 viability 24 h after scaffold formation was 81.1 +/- 4.7% (n=3) (Fig. 1). Cells were spread, indicating adhesion to the RGD peptides on the microspheres. After two weeks, cell numbers had increased eight-fold over day 0.
*String and scaffold formation:* End-over-end rotation in the presence of 10-20% dextran led to the formation of crosslinked strings of microspheres with length-to-diameter ratios of about 5-10 due to flow-induced aggregation.

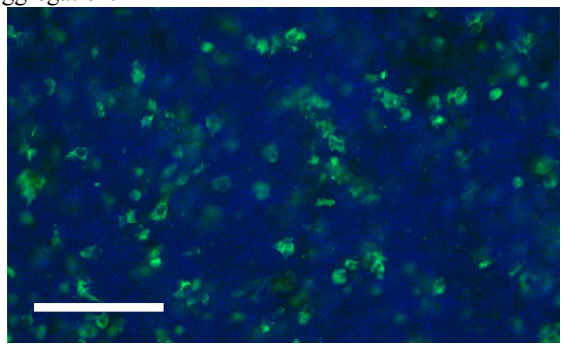

**Figure 2:** ECs (green) attached to fast-degrading PEG strings in a non-degradable microsphere (blue) scaffold. Scale bar is 300 µm.

*ECs on degradable strings:* Rotating ECs with degradable microsphere strings allowed the cells to adhere to RGD peptide in the microspheres, after which the strings were mixed with non-degradable microspheres to form scaffolds. EC-lined macropores were observed after dissolution of the strings (Fig. 2).

**Conclusions:** The ability to produce a scaffold containing a network of EC-lined macropores represents a major step towards the production of pre-vascularized cardiac tissue constructs.

**References:**

1. Chen X, et al., Tissue Eng A. 15, 1363, 2009.
2. Leung BM, Sefton MV. Tissue Eng A (in press), 2010.
3. Scott EA, Nichols MD, Kuntz-Willits R, Elbert DL. Acta Biomater 6, 29, 2009.