Heparin Releasing Hydrogels Promote the Contractile Phenotype of Cultured Vascular Smooth Muscle Cells

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Statement of Purpose: One of the challenges in vascular tissue engineering is selective promotion of smooth muscle cell (SMC) growth (such as to populate a new scaffold) while also facilitating the eventual re-differentiation of these cells back to a contractile, quiescent phenotype. The purpose of this study was to explore a heparin releasing hydrogel scaffold as a strategy to promote the re-differentiation of cultured, synthetic human SMCs toward a contractile phenotype.

Methods: Poly(ethylene glycol) diacrylate (PEGDA) was synthesized by conjugation of PEG diol (MW 3000) with acryloyl chloride in the presence of triethylamine (1:3:2.2 molar ratio) in dichloromethane under argon overnight then precipitated in diethyl ether and collected by filtration. Human coronary artery SMCs (HCASMCs, Lonza) were expanded in SmGM-2 (Lonza). To determine the response to heparin, HCASMCs were cultured on fibronectin (FN) in low serum medium (LSM, DMEM + 2% FBS), which facilitates differentiation toward a contractile phenotype, supplemented with 0-400 ug/ml of heparin (from porcine intestinal mucosa, Sigma). After 6 d, smooth muscle α-actin protein levels were quantified by cell based-ELISA (cELISA) and normalized to DNA content determined using Picogreen (Invitrogen). Heparin releasing depots were formed by polymerizing PEGDA discs (10-30% w/w, D=10 mm, H=1.2 mm) in the presence of heparin (1 mg/ml, 100 µg/gel) using Irgacure 2959 (Ciba) and UV irradiation (365 nm, 0.4 mW/cm^2, 10 min). Resulting hydrogel depots were bathed in phosphate buffered saline (PBS, pH 7.4) at 37°C and 80 rpm agitation. At various time points, PBS was removed and replaced. The heparin content in the buffer samples was determined using the dimethylmethylene blue (DMMB) assay. Heparin releasing scaffolds for SMC culture were formed by encapsulating heparin (1 mg/ml) in PEGDA discs (30% w/w, D=25 mm, H=1.6 mm) then polymerizing a thin film of PEGDA containing GRGDSP-PEG-acrylate (5 mM, MW 3400) on the top surface. Chondroitin sulfate (CS, 1 mg/ml) was loaded in analogous gels as a negative control. HCASMCs were seeded on these scaffolds and cultured in LSM. After 3 d, mRNA expression of the contractile markers α-actin, calponin, SM22α, and smooth muscle myosin heavy chain (SM-MHC) were determined using quantitative, real-time RT-PCR.

Results: The expression of α-actin protein by HCASMCs cultured on FN increased in a dose-dependent fashion as the concentration of heparin increased from 0 to 400 ug/ml with a maximal effect between 100-400 ug/ml (Fig. 1A), indicating that heparin is a potent inducer of α-actin expression. Heparin releasing depots formed from PEGDA hydrogels provided sustained release of heparin ranging from 1-2 d for 10% gels to >10 d for 30% gels (Fig 1B). This range would be adequate to induce changes in SMC differentiation marker expression. HCASMCs cultured on both RGD-modified scaffolds increased expression of contractile marker genes after 3 d relative to a post seeding (t = 4h) baseline. However, heparin releasing scaffolds induced greater up-regulation of marker genes compared with CS releasing control scaffolds by about 1.5 fold (range: 1.35-1.62 fold) for all marker genes examined (Fig. 1C).

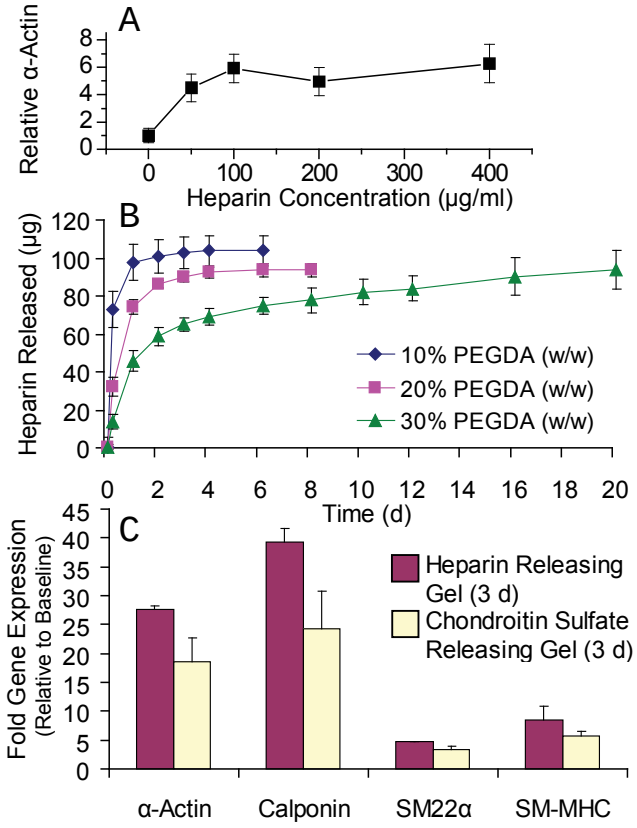

Fig. 1. A. Relative α-actin protein expression with increasing heparin concentration after 6 d, determined by cELISA **B.** Cumulative release of heparin from PEGDA hydrogels with increasing PEGDA concentrations **C.** Relative mRNA expression of SMCs seeded on heparin or CS releasing PEGDA scaffolds. Baseline mRNA expression was determined after a 4 h seeding period on FN.

Conclusions: Heparin potently induced expression of markers of differentiated, contractile SMC cell phenotype on FN and synthetic RGD-bearing PEGDA hydrogels. PEGDA scaffolds which release heparin over a range of time frames have been engineered. HCASMCs cultured on these heparin releasing scaffolds had higher expression of contractile phenotype markers than control scaffolds which released CS. These results suggest that RGD-bearing, PEGDA-based heparin scaffold systems can be used to promote contractile SMC phenotype.

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