RGD-Containing Hydrogels Support Re-expression of Markers of Contractile Vascular Smooth Cell Phenotype

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Statement of Purpose: Poly(ethylene glycol) diacrylate (PEGDA) hydrogels modified with pendant adhesive peptides have attracted interest as scaffolding materials for vascular tissue engineering. The purpose of this study was to assess quantitatively the role of specific peptide-cell interactions in re-expression of markers of contractile vascular smooth muscle cell (SMC) phenotype from cultured synthetic human SMCs.

Methods: Thin hydrogel film substrates were formed on γ-methacryloxypropyl silane coated glass coverslips from PEGDA (MW 6000, 20% w/w) copolymerized with peptide-PEG-acrylate conjugates (MW 3400, 5-10 mM) using Irgacure 2959 (Ciba) and UV irradiation (365 nm, 0.4 mW/cm^2, 10 min). Human coronary artery SMCs (HCASMCs, Lonza) were expanded in culture using SmGM-2 growth media (Lonza) which promotes cell proliferation and suppresses SMC contractile marker expression. For attachment studies, SMCs were seeded on substrates bearing the extracellular matrix protein derived peptides GRGDSP, VAPG, YIGSR, GKDGEA, KQAGDV, and GSWSGSPPRRARVT (10 mM) overnight in serum-free media, rinsed, and attachment was determined by quantifying DNA content with Picogreen (Invitrogen). To assess differentiation, hydrogels with GRGDSP (5 or 10 mM) and combination GRGDSP/YIGSR (5/5 mM) were seeded as above, then cultured in low serum medium (2% v/v FBS in DMEM) containing 400 μg/ml heparin (LSM+H), which promotes SMC differentiation, for 6 d. RNA was isolated after seeding, 2 d, and 6 d. The relative expression of the contractile markers smooth muscle α-actin and calponin was determined using real time RT-PCR. Protein expression and appropriate cytoskeletal organization of α-actin and calponin were confirmed by immunofluorescent (IF) staining. Focal adhesion formation on RGD bearing hydrogels (5 mM) was also confirmed by IF staining for vinculin.

Results: HCASMCs showed specific attachment and spreading in serum free conditions only on RGD containing hydrogels. Cell interactions with non-RGD peptide gels were indistinguishable from peptide-free control gels, with negligible cell attachment and no spreading. To assess the ability of these peptides to affect SMC phenotype subsequent to attachment, YIGSR was copolymerized with the adhesive GRGDSP peptide. Real-time RT-PCR studies showed a steady increase in the levels of α-actin (Fig. 1A) and calponin (Fig. 1B) mRNA on all of the substrates studied with only minor differences between gel substrates and fibronectin (FN) or laminin-1 (LN) coated tissue culture polystyrene. The substantial (> 10-fold) difference in marker expression between cells cultured in SmGM-2 and those cultured in LSM+H, regardless of the substrate, suggests that soluble factors in the media are more potent regulators of SMC phenotype than cell substrate. IF studies on hydrogels further confirm re-expression of both α-actin and calponin and appropriate co-localization of these proteins along α-actin filaments (Fig. 1C). Focal adhesion formation was similar on RGD hydrogels (Fig. 1D), FN, and LN after 6 d suggesting that one potential explanation for the lack of substrate dependent differences in marker expression may be the rapid deposition of the peri-cellular matrix that provides similar cell-matrix interactions regardless of initial substrate.

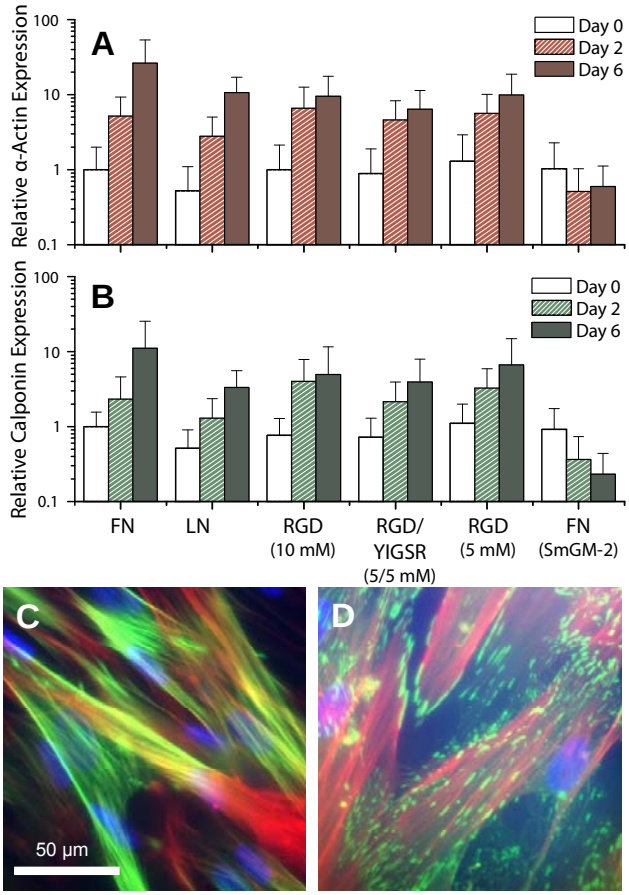

Fig. 1. A-B. Expression of smooth muscle α-actin (A) and calponin (B) mRNA on FN, LN, and various RGD modified hydrogel films determined by real time RT-PCR. **C.** IF staining for calponin (green) and α-actin (red) on RGD hydrogels after 6 d in LSM+H. **D.** IF staining for vinculin (green) and α-actin (red) on RGD hydrogels after 6 d. (Blue: DAPI nuclei)

Conclusions: HCASMCs showed specific attachment and spreading only on RGD containing hydrogels. These gels supported re-expression of α-actin and calponin that was quantitatively similar to FN and LN. These data suggest that RGD-bearing hydrogel scaffolds can promote contractile SMC phenotype for vascular tissue engineering applications given appropriate stimuli.

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