Endothelial Tube Stabilization and SMC Differentiation by TGF-β1 Loaded PEGylated Fibrin Gels

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Statement of Purpose: We have previously demonstrated that hMSC embedded within PEGylated fibrin gels without loaded or exogenous growth factors (GFs) demonstrate capillary tube morphology and endothelial cell (ECs) phenotype.[1] However, capillary tube formation was not correlated to functional output in an acute MI model.[2] Smooth muscle cells (SMCs) stabilize neovascularization and are required for mature vascular formation. Thus, a system that provides signals for SMC differentiation and maturation is desirable. Transforming growth factor-β1 (TGF) induces upregulation of late SMC markers from hMSCS, and stabilizes capillary tube formation.[3] Thus, a system that can maintain TGF presentation should provide signals for SMC differentiation and maturation. We have loaded TGF into a fibrin gel PEGylated with a homobifunctional PEG via conjugation and physical affinity. TGF maintained bioactivity and was sequestered within gels for at least 14 days.[4] The aim of the current study is to embed hMSCs into a TGF loaded PEGylated fibrin gel to induce EC and SMC differentiation. Further, multiple GFs can be loaded within the PEGylated fibrin gels via entrapment, conjugation, or physical affinity and released with varying kinetics if additional differentiation signals are required.[4]

Materials and Methods: Differentiation of hMSCs towards SMC has demonstrated a dependency on TGF concentration.[3] HMSCs (Lonza) were seeded at 2 k-cells/cm^2 on tissue culture treated poly(styrene) (TCP) and exposed to TGF concentrations of 0, 0.1, 1, and 10 ng/ml in maintenance media (Lonza) for 7d. Media was changed at day 4. HMSCs were lifted at days 1, 4, and 7 and RNA was isolated using an RNeasy® kit (Qaigen). Reverse transcriptase was performed using a high capacity cDNA kit (ABI). QRT-PCR was performed with primers (ABI) against EC (VE-CAD, CD31, VEGF, vWF, CD13) and SMC markers (ANGPT1, ANGPT2, CAL, DES, SMA, PDGF-Rβ). β-actin was utilized as an endogenous control and RNA production was normalized to control hMSCs.

Porcine Fib (Sigma) was PEGylated with PEG-(SCOP)$_2$, 3400 MW (NOF America) at 1:10 molar ratio over 1 hour. Human TGF (R&D Systems) was reacted with the PEGylated Fib for 30 min in PBS, pH 7.8 at 37ºC. HMSCs in maintenance media are mixed with GF loaded Fib solution. Human thrombin in 40 mM $CaCl_2$ (Sigma) was then added to the PEGylated Fib to begin crosslinking. The final concentration of TGF was 25 ng/ml; Fib, 10 mg/ml; hMSCs, 50 k-cells/ml; and thrombin, 12.5 U/ml. PBS was used in lieu of PEG or TGF as controls. Reverse transcriptase qRT-PCR was performed with the same primers listed above at days 7 and 14. Immunohistochemistry against select markers was utilized to validate EC and SMC differentiation.

Results and Discussion: RNA production of hMSCS cultured on a monolayer was measured over 7 days. Figure 1 demonstrates that at day 7, hMSCs demonstrated upregulation of SMA and calponin compared to hMSCs at day 1. Further, 10 ng/ml TGF induced greater upregulation of calponin compared to unexposed cells. EC markers were downregulated at 10 ng/ml TGF concentration, which matches current literature.[3] Nevertheless, hMSCs embedded within TGF loaded PEGylated fibrin gels demonstrate similar EC tube morphology compared to unloaded PEGylated fibrin gels.

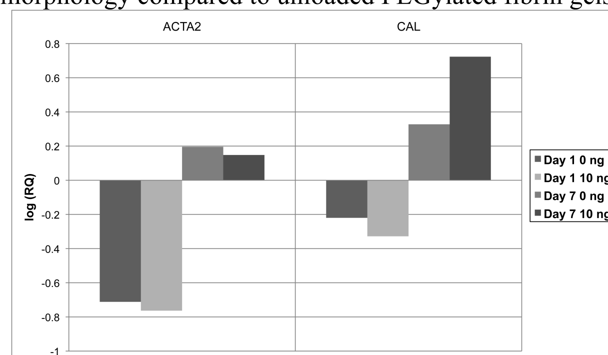

Figure 1: TGF induced SMC differentiation on TCP

HMSCs when embedded within unloaded PEGylated fibrin gels have demonstrated upregulation of EC and SMC markers (Figure 2) up to 14 days. SMC phenotype was not as well maintained as EC phenotype, and IHC demonstrated that CD31 and vWF were highly upregulated but SMA upregulation was limited. Thus, PEGylated fibrin gels loaded with TGF should promote SMC differentiation while maintaining EC differentiation.

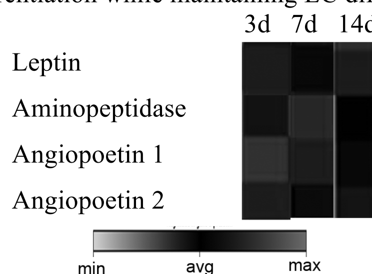

Figure 2: SMC differentiation of hMSCs in fibrin gels

Conclusions/Summary: HMSCs when exposed to TGF on TCP have demonstrated upregulation of SMC markers as quantified via reverse transcriptase qRT-PCR. Further, hMSCs embedded within unloaded PEGylated fibrin gels demonstrate upregulation of EC and SMC markers. Nevertheless, TGF is required to maintain SMC differentiation.

References:

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