Growth Factor Release from a Chemically Modified Elastomeric Poly(diol citrate) Scaffold Promotes Angiogenesis in vivo

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Statement of Purpose: Attempts to regenerate functional urinary bladder tissue have been hampered by several obstacles including appropriate scaffold choice and poor vascularization of developing tissue. The elastomeric properties of poly(1,8-octanediol-co-citrate) (POC) scaffolds have recently been shown to aid in urinary bladder regeneration when seeded with epitope defined, human mesenchymal stem cells in the context of a nude rat urinary bladder augmentation model.[1] Data from these studies indicate superior muscle formation with regard to morphologic, histologic, and protein expression profiles as compared to similarly seeded bladder smooth muscle cell control samples. In order to further promote increased tissue growth and development, we sought to create heparan sulfate binding POC (POC-HS) scaffolds that would allow for the binding and extended release of growth factors conducive to localized angiogenesis.

Methods: POC scaffolds were prepared as previously described.[1] POC-HS were created by activating the carboxylic acid groups found on POC in MES buffer [2-(N-morpholino)ethanesulfonic acid] pH 6.0, containing 0.15g 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 0.09g N-Hydroxysuccinimide. Scaffolds were subsequently rinsed in water and incubated in MES buffer, containing 75mg PEG-diamine (MW 2000) for 12 hours at room temperature with agitation. Heparan sulfate was activated by adding 0.4g EDC and 0.097g NHS to a 1% solution of heparan sulfate in 50mL of MES buffer pH 6.0 for 1 hour at room temperature (RT). POC scaffolds were incubated in the activated heparan sulfate solution for 4 hours at RT under agitation. Scaffolds were rinsed three times in water and stored in PBS. Subsequently, human growth factors (GF) vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF-1) (100ng/ml each, R and D Systems, Minneapolis, MN) were individually mixed into a PBS solution containing 0.1% BSA by manual pipetting and added to POC-HS scaffolds (0.5cm diameter, 0.5mm thickness) for 3 hours at RT. Scaffolds were removed, rinsed in PBS and underwent GF release and quantification with the appropriate enzyme-linked immunosorbent assay (ELISA) Assays (R and D Systems). Control samples consisted of POC scaffolds without heparan sulfate that were loaded with GFs under identical incubation conditions and processed accordingly. Athymic nude rats (NCI Animal Production Program, Frederick, MD) underwent subcutaneous implantation with either Condition A)POC scaffold alone, B)POC-VEGF, or C)POC-HS-VEGF (300ng VEGF/scaffold). Conditions A-C were repeated twice/animal (n=5 animals). Animals were sacrificed at 4 weeks post-implantation. Vessel density in scaffold implanted areas was quantified utilizing Adobe Photoshop Software (Adobe Systems, San Jose, CA) based upon Trichrome staining of 5μm thick specimens.

Figure 1

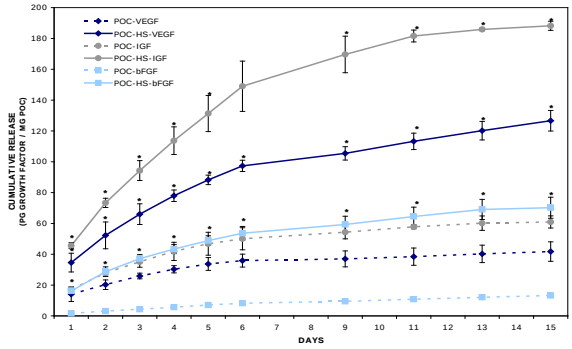
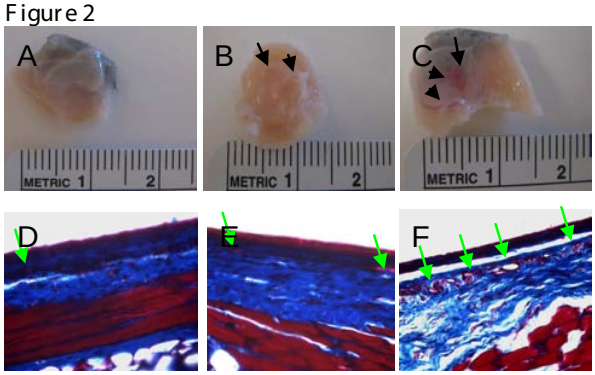


Figure 2



Results: In vitro cumulative GF release from POC-HS based scaffolds demonstrated an extended GF release profile which was statistically significant when compared to POC-GF control samples at nearly all time points ($p<0.05$, utilizing a Student's t-test) over a 15 day period (Figure 1). Gross subcutaneous specimens of Conditions A-C (Figure 2A-C) demonstrated increased vascularity with POC-HS-VEGF. Vessel quantification revealed that Condition A contained 32.6±3.3 vessels/implant region, while Conditions B and C contained 60.6±7.5 and 131.4±12.3 vessels/ implant region, respectively (n=10 implants/Condition; mean ±95% CI, Figure 2D-F). Arrows indicate areas of vessels/vascularization.

Conclusions: Data demonstrate that POC can be chemically modified to release pro-angiogenic GFs over time and promote localized angiogenesis.

References: 1. Sharma AK. Biomaterials 2010;31(24):6207-6217.