Characterization of Hybrid Poloxamine/Collagen/Hyaluronan Hydrogel for Bladder Tissue Engineering

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Statement of Purpose: Recent clinical success in bladder tissue engineering by Atala el al (1) demonstrated the feasibility of this technology to combine synthetic polymer scaffolds with host cells for bladder reconstruction. Yet, there remain several unresolved issues. For example, use of PLGA for scaffold does not permit proper distension and contraction of the tissue. Culturing of smooth muscle cells (SMCs) on scaffolds with random pore architecture does not allow organizing cells into the highly oriented architecture of the native bladder. Moreover, prolonged cultures of SMCs often result in loss of the contractile phenotype of these cells. Our group previously demonstrated that 3D cultures of bladder SMCs in collagen gel subjected to sustained tension exhibited cell orientation along the direction of the applied stimulus and significantly greater levels of contractile phenotype markers compared to the no-tension control (2). We hypothesize that exposure of 3D culture of bladder SMCs to appropriate mechanical stimuli leads to guided directional cell growth and retention of contractile phenotype. The long-term objective of the present study is to develop both a biomaterial that matches the native tissue mechanical properties and an approach to guide SMC growth into organized bundle structures for the bladder tissue engineering applications.

Methods:

Preparation of Tetronic T1107-acrylate/collagen/hyaluronan hydrogel To make hydrogel with a four arm PEO-PPO block copolymer (Tetronic T1107, BASF, Evans City, PA), it was first acrylated to allow photopolymerization. Briefly, T1107 dissolved in toluene was azeotropically distilled, redissolved in dichloromethane, and mixed with triethylamine on ice. Acryloyl chloride was added dropwise over 2 hrs and the reaction was run for 24 hrs. The product was precipitated in ethyl ether, neutralized, and dehydrated. The final product was dried under vacuum overnight.

3D culture of bladder smooth muscle cells (SMC) T1107-acrylate (5.0wt%) and type-I collagen (0.5wt%, MP Biomedicals, Solon, OH) were dissolved in 0.02N acetic acid and a photoinitiator, Irgacure 2959 (0.05wt%), was added. This solution was mixed with varying concentrations of HA (0.10, 0.15, 0.20wt%) and neutralized with NaOH in custom-made Teflon molds (3x1x0.3cm wells). Rat bladder SMCs (4, 8, or $16x10^6$ cells/ml suspended in 3X DMEM) or equal volume of PBS (for acellular gels) were subsequently added to molds to make the final volumes of 1.5ml and 0.75ml. A thin layer of mineral oil was laid on top to block oxygen and gels were cured under 365nm UV light for 10 min.

Mass swelling ratio quantification Acellular hydrogel were equilibrated in 10% PBS solution for 24 hr and lyophilized. Dry weights (w_d) were recorded, the specimens were allowed to swell for 24 hr in distilled water and wet weights (w_s) were recorded. Mass swelling ratio, q, was calculated by ratio of wet to dry weight.

Tensile Testing Acellular hydrogels were subjected to uniaxial tensile loading in the ambient air using MTS Synergie 100 (MTS Systems Corporation) at a rate of 5mm/min until breaking point. Force and elongation data were collected to determine maximum strain and tensile strength. The stiffness was determined from the slopes of the stress-strain curves.

Cell Viability and Morphology Hydrogel SMC constructs were cultured for up to 7 days and live/dead stained and fluorescence microscopy were used for qualitative viability testing.

Results: Swelling tests showed a slight increase in mass swelling ratio with an increase in HA content. Stress-strain behavior from tensile testing showed a general trend of increased stiffness with increased concentration of HA. Analysis of peak stress showed that gels with 0.10% and 0.15% HA content exhibited a lower peak stress than 0.20% gels. Analysis of peak strain showed that 0.10% gels exhibited a higher peak strain than 0.15% and 0.20% gels. Viability tests revealed that thick constructs (1.5ml) contained viable cells mainly near surfaces and dead cells on the interior. However, thin constructs (0.75 ml) contained nearly 100% viable cells throughout the gel thickness.

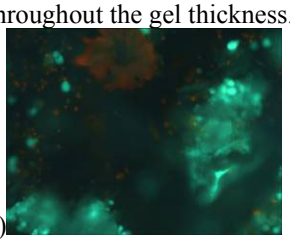
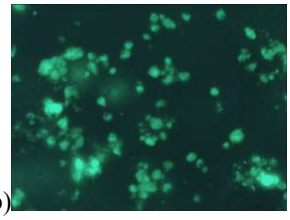
a)  b) 

Figure 1: Viability of SMCs in (a) high (1.5ml) and (b) low (0.75ml) volume construct after 7 days of culture.

Conclusions: The increase in mass swelling ratio with higher HA concentrations observed in the present study suggests that HA expands hydrogel pore size to allow for increased water uptake. Peak stress and strain trends show that an increase in HA content provides strength for the gels while compromising ductility. Together, these results suggest that mechanical behaviors of the hybrid Tetronic-collagen-HA hydrogels are dependent on the hydration level. The results of cell viability testing indicates that high volume gels do not support sufficient nutrient diffusion to the innermost cells of cultures as do low volume gels. Future research includes cell morphology analyses with confocal microscopy, phenotype characterization using RT-PCR and Western analyses, and exposure of 3D SMC culture constructs to varying levels of static and cyclic tension with custom bioreactor for guided tissue growth in vitro.

References: (1) Atala A et al., Lancet 367:1241-6.
(2) Roby TS, et al., Ann Biomed Eng. 2008; 36:1744-51.