Sustained gene delivery from micro-fibrous, elastomeric polymer scaffolds

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Statement of Purpose: The encapsulation of viral particles in polymeric scaffolds can protect these vectors from degradation and immune responses, enabling their sustained release in an active format.[1,2] While various polymeric matrices have been utilized to deliver viral vectors in a sustained and localized manner, such matrices have not included biodegradable thermoplastic elastomers. Such elastomers, however, are attractive for application in a variety of soft tissue repair scenarios and as tissue engineering scaffolds for mechanically active tissues. The objective of this study was to develop micro-fibrous elastomeric scaffolds incorporating viral vectors and to evaluate the effect of several processing strategies on the infection profiles of these materials. Specifically, recombinant adeno-associated virus (rAAV), known for its persistent transgene expression and low pathogenicity, was incorporated into biodegradable, elastomeric poly(ester urethane)urea (PEUU) and processed by electrospinning into three formats designed to influence the controlled release behavior.

Methods: rAAV serotype II encoding for green fluorescent protein (GFP) was manufactured using CsCl gradient ultracentrifugation.[3] PEUU was synthesized from polycaprolactone diol, butyl diisocyanate and putrescine using a 2-step one-pot synthetic strategy.[4] Virus and PEUU were combined and electrospun into micro-fibrous mats with 1 of 3 architectures: solid fibrous scaffolds, core-sheath fibrous scaffolds, and porous core-sheath fibrous scaffolds (**Fig. 1**). Solid fibers were made by dispersing rAAV in PEUU solution (PEUU in HFIP:DCM) and electrospinning onto a rotating mandrel. Core-sheath fibers were made by co-axial electrospinning where rAAV was mixed in polyethylene glycol (PEG) in PBS as the core solution and PEUU solution created the sheath. Porous core-sheath fibers were fabricated by adding PEG into the PEUU sheath solution. Release kinetics of AAV from all 3 scaffolds were evaluated by assessing transduction efficiencies of HEK 293 cells, which were cultured in 12-well plates with scaffolds added. At predetermined time points, cells were trypsinized and the transduction efficiency was quantified by flow cytometry. The scaffolds were then transferred to new wells with a cell monolayer pre-cultured at the bottom.

Results: Core-shell structures were successfully achieved via co-axial electrospinning (**Fig. 1b**). Pores of ~100 nm were formed on the sheath using PEO as a porogen (**Fig. 1c**). All 3 scaffolds demonstrated typical elastomeric behavior with Young's moduli of 1.1-1.6 MPa, tensile strengths of 1.3-7.7 MPa and breaking strains of 194-284%. After 3-days incubation, close to 50% of cells were transduced by the two core-sheath scaffolds, followed by a drop to ~30% in two weeks (**Fig. 2**). The infection efficiencies of the two core-shell fibrous mats remained almost constant at 20-30% from weeks 9-15, and a significant decrease was observed at week 18, where ~10% cells were transfected. The solid fibrous scaffolds exhibited a low level of cell transduction (~5%) throughout the culture period.

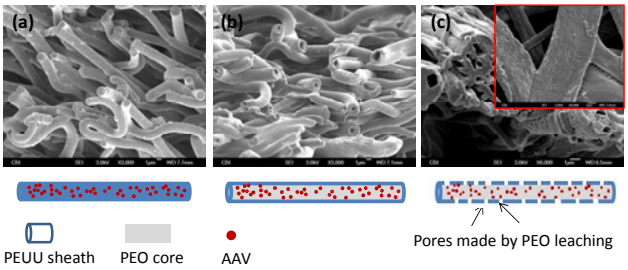

Fig. 1. Electron micrographs of the cross sections of (a) solid fibers; (b) core-sheath fibers; and (c) porous core-sheath fibers. The inset shows the nano-pores on the fiber surface in (c). All scaffolds were incubated in PBS for 3 days and freeze-dried before SEM observation. The schematic images illustrate AAV encapsulation strategies for a typical fiber in each corresponding scaffold.

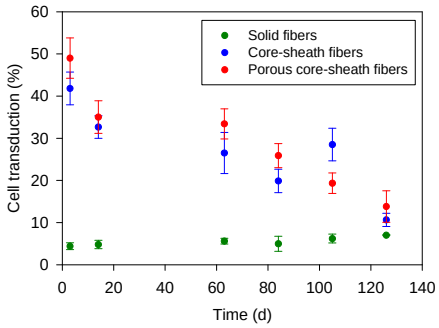

Fig. 2. HEK 293 cells incubated with AAV-GFP fibrous scaffolds remain positive over 18 weeks.

Conclusions: Sustained transgene expression over 4 months was observed for cells exposed to AAV-loaded fibrous scaffolds fabricated by coaxial electrospinning. The extended release behavior, prolonged transgene expression, and the elastomeric mechanical properties make this scaffold an attractive option for soft tissue engineering where both gene delivery and appropriate mechanical support are desired.

References: [1]. Jang, J-H. et al. *Mol Ther*, 2011; 19, 1407-1415. [2]. Liao I. et al. *J Control Release*, 2009; 139, 48-55. [3] Tang, Y et al. *Gene Ther*, 2010; 17, 1476-1483. [4]. Guan J. et al. *J Biomed Mater Res*, 2002; 61, 493-503.