Isolation and Characterization of Inner Wall Endothelial Cells of the Schlemm's Canal using a Citrate-based Polymer

Shwetha Ramachandra[1,2], Amir Vahabikashi[1], Mark Johnson[1], Guillermo Ameer[1,2].
[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA
[2]Center for Advanced Regenerative Engineering, Northwestern University, Evanston, IL, USA

Statement of Purpose: Glaucoma is defined as an optic neuropathy caused by characteristic damage to the optic nerve subsequently leading to vision loss. It is a significant global health problem and the second leading cause of blindness in both the United Stated and the world, affecting nearly 67 million people worldwide[1]. The Schlemm's Canal (SC) is a unique lymphatic like endothelium lined vessel that encircles the cornea through which the aqueous humor is drained to maintain optimum intraocular pressure of 15.5 ± 2.6 mm Hg[2,3]. Understanding the functional contribution of the endothelia in the SC still remains a very important goal in glaucoma research. The objective of this research is to isolate the SC endothelial cells (SCECs) using a novel laminin derived peptide conjugated to a thermoresponsive hydrogel. The structural and mechanical properties as well as the potential of the hydrogel to isolate the SCECs was evaluated *in vitro.*

Methods: PPCN was synthesized using a polycondensation reaction of PEG, Citric acid and GDD followed by a free radical polymerization with N-isopropylacrylamide. Laminin derived A5G81 peptide was conjugated to PPCN through maleimide chemistry. Briefly, PPCN was conjugated to BMPH at a 0.1 molar ratio, and the peptide was reacted to PPCN-BMPH at 0.1 molar ratio. Conjugation was verified through MALDI-TOF, NMR, and TNBSA assay. Rheological characterization was carried out on a TA instruments DHR rheometer with a 20 mm 2° cone peltier plate geometry. Gelation temperatures for different concentrations of PPCN and PPCN-A5G81 were studied in a temperature ramp from 15°C to 45°C. A gel injection system was optimized by inserting several catheters and syringe needles into the SC of cadaveric eyes and assessing the flow of the gel and damage to the SC through H&E. After a system was optimized, gels were injected into the SC under sterile conditions. Cells were cultured in 6 well standard plates and MTT as well Live/Dead was performed to assess compatibility of cells with the hydrogels. Immunocytochemistry was performed for markers specific to the SCECs such as PECAM-1, VE Cadherin, vWF, Fib-2 and Integrin α6.

Results: PPCN and PPCN-A5G81 were successfully synthesized and characterized using NMR and MALDI-TOF. The amount of peptide tethered to PPCN was quantified and found to be 0.4mM. Both PPCN and PPCN-A5G81 gelled at suitable working temperatures and demonstrated shear thinning behavior (**Fig 1).** A gel injection system was optimized and PPCN and PPCN-A5G81 was injected into the SC **(Fig 2).** The cells demonstrated viability in both PPCN and PPCN-A5G81 **(Fig 3)** and expressed markers specific to SCECs found *in vivo* in both normal and glaucomatous tissues **(Fig 4).**

References:

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3.(Stamer W.D., Invest. Opthalmol. Vis. Sci.; 1998; 39;1804-1812)

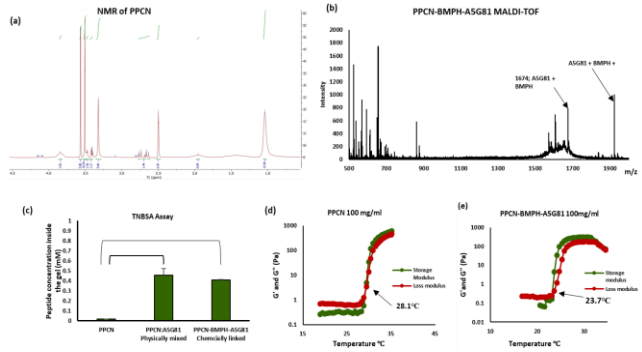

Figure 1: (a) NMR of PPCN;(b) MALDI-TOF characterization of PPCN-A5G81;(c) TNBSA assay of primary amines; Gelling temperatures of (d) PPCN and (e) PPCN-A5G81

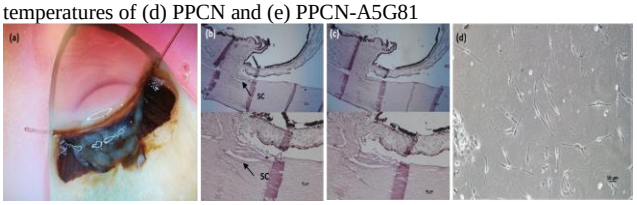

Figure 2: (a) Insertion of catheter into the SC; (b) H&E of SC without catheter inserted; (c) H&E of SC with catheter inserted; (d) SCEC culture at week 2, note the spindle like structure.

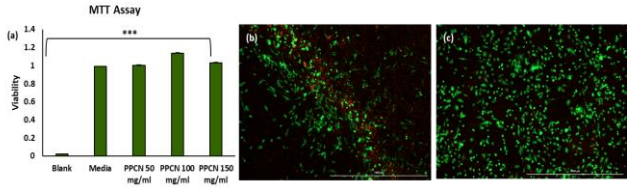

Figure 3: (a) MTT assay of SCECs in different concentrations of PPCN; (b) Live/dead of SCECs in PPCN; (c) and PPCN-A5G81.

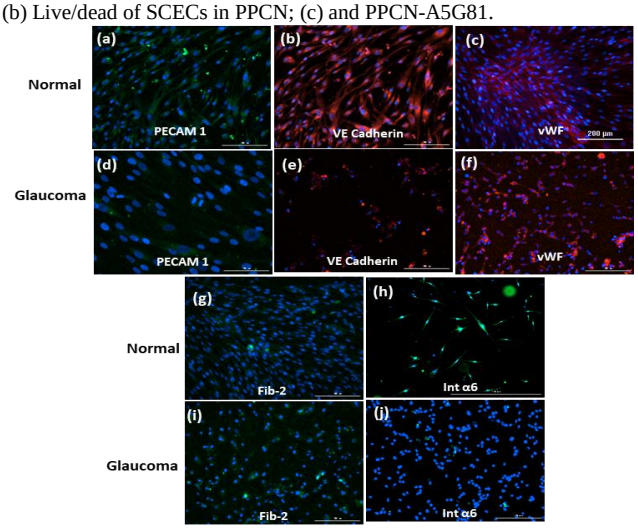

Figure 4: Immunocytochemistry for SCEC markers.

Conclusions: PPCN was successfully conjugated to A5G81 peptide and this conjugation minimally affected the LCST of the gel. It demonstrated injectability through a microcatheter system without damage to the SC. The cells isolated by this technique showed good viability in both PCN and PPCN-A5G81 and expressed markers native to SCECs in both normal and glaucomatous eye specimens. Thus, this peptide-linked hydrogel could potentially be used to effectively isolate SCECs.