Electrochemical Compaction Yields Transparent and Stable Collagen Matrices for Corneal Applications

Ranjani Iyer[1], Vipuil Kishore[1,2]
[1]Dept of Biomedical Engineering, [2]Dept of Chemical Engineering, Florida Institute of Technology.

Introduction: Corneal disease due to trauma, ulceration or infection has rendered more than 10 million people blind across the world[1]. Transplantation of donor corneas is the current gold standard for the treatment of corneal disease. However, short supply and immune related complications are major concerns. Over the past two decades, corneal tissue engineering has gained significant interest as an alternative treatment method for corneal disease. In this study, we employed an electrochemical compaction methodology to synthesize highly dense collagen matrices that mimic the transparency of native cornea. We hypothesize that optimal crosslinking will significantly improve the stability and mechanical properties of electrochemically compacted collagen (ECC) matrices. Further, we also hypothesize that crosslinked ECC matrices will support the viability and proliferation of human corneal keratocytes. The objective of the study is to develop a collagen-based functional biomaterial that mimics the transparency, density, stability, and mechanical properties of native cornea. Realization of a tissue mimicking collagen matrix will provide compositional, topographical and mechanical cues to the cells populating it and thereby promote the formation of *de novo* cornea-like matrix for corneal tissue engineering applications.

Materials and Methods: Dialyzed collagen solution (Advanced Biomatrix, CA) was loaded between two planar electrodes and an electric field was applied (3V, 45 min) to form a highly dense ECC sheet. The ECC sheets were then physically crosslinked using UV-Riboflavin or chemically crosslinked using EDC-NHS or Genipin. Uncrosslinked sheets served as controls. Transparency was assessed using an UV-vis spectrophotometer. Stability was determined by measuring the resistance of ECC sheets to collagenase treatment. Tensile tests were performed to assess the strength and stiffness of ECC matrices. Cell viability (live-dead assay) and proliferation (Alamar blue assay) was assessed by culturing primary keratocytes (20,000 cells/cm^2) on ECC sheets for 7 days.

Results and Discussion: The transparency of the ECC sheet is clearly evident in Fig. 1A. Light transmission results indicated that the transparency of EDC-NHS crosslinked ECC sheets was superior to native cornea (Fig. 1B). Uncrosslinked ECC sheets degraded within 6 hours upon collagenase treatment (Fig. 1C). However, chemical crosslinking of ECC sheets significantly improved the stability to 195 hours (EDC-NHS) and 256 hours (genipin) compared to 110 hours for human cornea[2]. Results from mechanical tests showed that the strength and stiffness of uncrosslinked ECC sheets were 5 kPa and 17 kPa, respectively. Upon crosslinking, the mechanical properties of the sheets improved significantly. Specifically, crosslinking with EDC-NHS resulted in ECC sheets with strength of 0.2 MPa and stiffness of 2 MPa. Cell viability was maintained on crosslinked ECC sheets. Higher rates of proliferation were observed on crosslinked ECC sheets compared to uncrosslinked sheets.

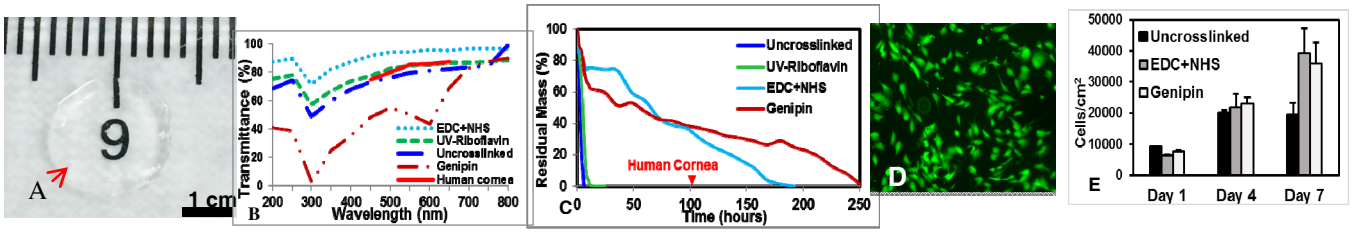

Figure 1: (A) Transparent ECC sheet (red arrow). (B) Transparency of ECC matrices and human cornea[2]. (C) Stability of ECC sheets. (D) The green staining shows the live cells on EDC-NHS crosslinked ECC sheets. (E) Cell proliferation on uncrosslinked and chemically crosslinked ECC sheets.

Conclusions: In summary, EDC-NHS crosslinking improves the transparency, stability and mechanical properties of ECC sheets. Future studies will focus on optimizing the EDC-NHS crosslinking conditions to further improve the mechanical properties to converge upon the strength (3.8MPa)[2] and stiffness (15MPa)[2] of native cornea. The most optimally crosslinked ECC sheets will be used for the development of hemi-corneas (stroma+epithelium). Together, ECC sheets have considerable promise for use in corneal repair applications.

References: 1.Whitcher et al., Bull WHO, 2001; 2. Ahn et al, Acta Biomaterialia,2013