Highly Transparent Dense Collagen Sheets for Corneal Tissue Engineering Applications

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Statement of Purpose: Corneal disease is the second major cause for blindness, second only to cataracts[1]. In the United States, over 72,000 corneal transplants are performed each year costing an estimated 6 billion dollars[2]. Transplantation of donor cornea is currently the gold standard for the treatment of corneal disease. However, limited supply and immune related complications are major limitations. Over the past two decades, tissue engineering is being pursued with a goal to develop a functional biomaterial for corneal repair and regneration. Although much work has been done on this front, a fully functional tissue engineered cornea as an alternative to donor cornea is yet to be realized. In this study, highly dense and transparent collagen sheets were synthesized using electrochemical compaction. The transparency, stability, water retention capacity, mechanical properties and cellular compatibility of the electrochemically compacted collagen (ECC) sheets were determined.

Methods: ECC sheets were synthesized as described earlier[3]. Briefly, dialyzed collagen solution was loaded between two parallel graphite electrodes and an electric field was applied (3V, 45 min). The electric field drives the collagen molecules to form a dense ECC sheet that is recovered from the cathode at the end of the process (Fig. 1A). The ECC sheets were crosslinked using two different strategies: 1) physical crosslinking using UV-riboflavin and 2) chemical crosslinking using EDC-NHS. Uncrosslinked ECC sheets were used as controls. The transparency of the ECC sheets was quantified by measuring light transmission in the UV-vis range using a spectrophotometer (200–800 nm). The stability of the ECC sheets was determined by evaluating their resistance to collagenase treatment. The water retention capacity of the ECC sheets was determined by first drying the samples under vacuum and measuring the dry weight. Following this, the sheets were hydrated for 24 hours and weighed again and the equilibrated water content in the sheets was computed. Mechanical assessment of the EDC-NHS crosslinked ECC sheets was carried by loading the sheets monotonically under tension until failure (0.5N/min; Q800 TA Instruments). Stress-strain curve was plotted and ultimate tensile strength, elongation at break and Young's modulus was calculated. A preliminary cell study was performed by seeding NIH/3T3 fibroblasts (ATCC) on EDC-NHS crosslinked ECC sheets and assessing cell viability using Live-dead assay and cell proliferation using Alamar Blue assay.

Results: The transparency of EDC-NHS crosslinked ECC sheets was over 90% in the visible range and significantly higher than that of native cornea[4] (Fig. 1B) The stability results showed that upon EDC-NHS crosslinking, the ECC sheets were stable in collagenase until 192 hours compared to 110 hours for the native cornea as reported in the literature[4] (Fig. 1C) Crosslinking reduced the water retention capacity of the ECC sheets (Fig. 1D). Mechanical testing revealed that ECC sheets have a strength of 80±20 KPa, elongation at break of 22±11% and a stiffness of 950±400 KPa demonstrating that ECC sheets are significantly weaker than native cornea (3.8 MPa)[4]. Results from cell studies showed that cell viability on ECC sheets is maintained until day 7 (Fig. 2A). Further, higher rates of proliferation were observed on ECC sheets compared to tissue culture plastic (Fig. 2B).

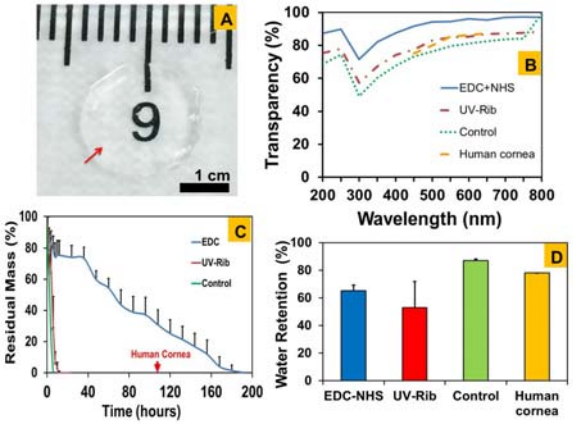

Figure 1: (A) Picture of ECC sheet (red arrow). Transparency (B), Stability (C) and Water retention (D) of ECC sheets.

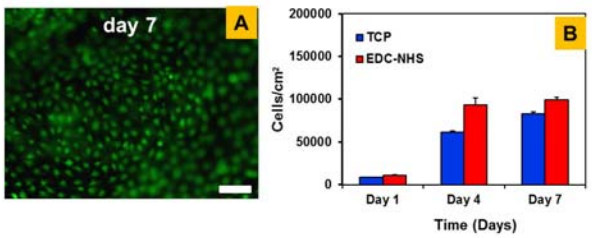

Figure 2: (A) Cell viability and (B) cell proliferation on ECC sheets compared to tissue culture plastic.

Conclusions: The transparency and stability of ECC sheets are on par with native cornea. However, the water retention capability and mechanical properties of ECC sheets are suboptimal. To address these limitations, future studies will focus on incorporating glycosaminoglycans such as chondroitin sulfate within the ECC sheets to improve the water retention capability of the sheets. To improve the mechanical properties of the ECC sheets, studies that focus on optimizing the electrochemical compaction process variables (voltage, time) and EDC-NHS crosslinking conditions are ongoing. The feasibility of incorporating other polymers such as chitosan to form hybrid materials is also being explored. Cell culture studies with corneal epithelial cells and keratocytes are imminent. In conclusion, the results indicate that ECC sheets have considerable promise to be developed as a functional biomaterial for corneal applications.

References: [1] Whitcher, Bull World Health Organ, 2001; [2] Eye Bank Association of America, Annual Report, 2013; [3] Kishore et al., ASME SBC, 2013; [4] Ahn et al;Acta Biomaterialia, 2013.