Development of Biomimetic Materials for Sutureless Ocular Surface Repair

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Statement of Purpose: To address the challenges associated with existing methods of ocular surface repair, Luna Innovations is developing a unique ocular biomaterial capable of protecting the injured surface and stimulating ocular healing. In this biomaterial, the healing properties of the amniotic membrane are mimicked in an improved hydrogel material, and the mechanical properties are enhanced using nanofiber reinforcements that effectively mimic the natural structure of the ocular tissue. The dressing production technique for a proprietary composition has been developed, and standard techniques have been used to establish appropriate handling properties, cytocompatibility with primary human corneal epithelial cells, and a refractive index of 1.335 with 85% transmission at 550 nm. Photochemical tissue bonding has been implemented, and a sutureless process has been demonstrated *in vitro* using excised albino rabbit eyeballs.

Methods: Luna Innovations designed and assembled an electrospraying/spinning system for fabrication of the dressings. The system uses dual power sources (Gamma High Voltage), multiple syringe pumps, and a custom-built high-speed rotating mandrel for capture. Primary human corneal epithelial cells (HCECs) were cultured for both direct and indirect cytocompatibility testing using the lactate dehydrogenase (LDH) assay to report on toxicity as compared to controls. UV-Vis spectroscopy was used to quantify transparency of the samples by quantifying percent transmission through the constructs at 550 nm. A Vee Gee Model C10 Abbe Refractometer with bromonapthalene as a wetting agent was used to characterize dressing refractive index. Degradation of the construct was monitored both visually and by weight loss utilizing simulated tear solution (2.68 mg/mL lysozyme, 6.50 mg/mL D-glucose, 1.34 mg/mL gamma globulin, 6.50 mg/mL sodium chloride, 2.68 mg/mL bovine serum albumin, and 0.08 mg/mL calcium chloride). Nanofiber alignment was determined using the FibrilTool ImageJ (NIH) plugin and SEM images of the dressings. Photochemical tissue bonding was studied using excised rabbit eyeballs, dressings loaded with 0.01 - 0.1% rose bengal, and an Iridix OcuLight GL laser (532 nm) at 0.25 W with repeated 1 min administrations.

Results: The goal of this study was to demonstrate the feasibility of a nanofiber-reinforced hydrogel for use as a dressing for traumatic injuries to the exposed ocular surface. Luna first assembled a setup for bench-scale production of the dressing. Through careful production design and solvent selection, an identical applied voltage (25 kV) and separation distance (10 cm) was selected for both the electrospraying and electrospinning process. Cytocompatibility testing with HCECs indicated the dressings had no additional toxicity as compared to low density poly(ethylene) positive controls.

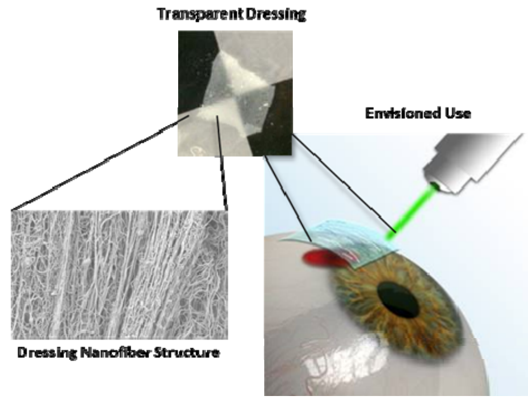

Figure 1. Luna's BIOcular™ is designed for sutureless application to promote regeneration of the ocular surface.

UV-Vis spectroscopy indicated over 85% transmission of 550 nm light for a hydrated dressing, and the refractive index of the final dressing was found to be 1.335. Using image processing of SEM micrographs, Luna confirmed alignment of the nanofiber-reinforcing layers and demonstrated control over the degree of alignment during production, with final dressings having an anisotropy value of 0.2. Luna also utilized 532 nm light (green) to demonstrate photochemical tissue bonding of the dressing to the surface of an excised albino rabbit eyeball. The concentration of the photoactive dye rose bengal was systematically altered in the hydrogel component of the dressing and was determined capable of adhesion to the surface with no cytotoxic effect to HCECs at 0.05% (wt/vol). Finally, Luna was able to demonstrate controlled degradation (5 min to 4 wk) in simulated ocular fluids by controlling the crosslinking of the hydrogel component.

Conclusions: Luna has succeeded in demonstrating *in vitro* feasibility of its dressing for use as an ocular biomaterial to aid in healing of the ocular surface. Luna designed and built an electrospinning/spraying system for production of the dressings and subsequently investigated dozens of iterations of production. After determination of each component's optimal fabrication conditions, the dressing was characterized for its optical, mechanical, and biological properties, and each was deemed appropriate for use in ocular repairs. The dressing was found to have a refractive index of 1.335, a transmission of 85% for 550 nm light, and to be cytocompatible with primary human corneal epithelial cells.

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