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Corneal endothelial tissue engineering using multi-layered polymer membranes

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Introduction: Corneal endothelial damage and diseases are two of the major contributors to blindness or severe visual impairment worldwide[1]. The corneal endothelium is the innermost cell layer of the cornea and consists of a monolayer of hexagonal cells that maintain the stroma in a state of relative dehydration through a “pump-and-leak” mechanism. A critical loss of cells due to damage, disease or aging leads to corneal edema which in turn results in opacification of the cornea [2]. Currently, the only treatment consists of a (partial) corneal transplantation from healthy cadaveric donor tissue. Unfortunately, only 1 donor is available for every 70 cases [3]. To tackle this donor shortage, the present work focusses on the development of transparent (>90%), thin (≤5µm), multilayered sheets constituting a poly(D,L-lactide) (PDLLA) layer for structural rigidity and a crosslinkable gelatin-based hydrogel as an extracellular matrix (ECM) mimic. These sheets provide a supporting function for corneal endothelial cells to enable subsequent ocular implantation thereby restoring the damaged endothelium and patient’s vision.

Methods: Multi-layered sheets (ø = 12mm) were developed through successive spincoating steps. A sacrificial gelatin layer (H_2O, 10w/v%) was spincoated on a glass plate followed by a PDLLA (Corbion, PURASORB PDL20) layer for structural integrity (THF, 4w/w%). Next, a layer of one of the crosslinkable gelatin derivatives, crosslinkable gelatin derivatives, gelatin-methacrylamide (Gel-MA), gelatin-methacrylamide- amino-ethylmethacrylate (Gel-MA-AEMA) [4] and gelatin-norbornene (Gel-NB) [5], is spincoated (H_2O, 10w/v%) as final layer, after applying an argon plasma treatment (0.8mbar, 30s) to the PDLLA. Finally, crosslinkable gelatin was crosslinked using UV-A (6 mW/cm^2, 30 min) irradiation. Isolation of the sheets occurred by immersing in warm water (40°C), thereby dissolving the sacrificial gelatin layer. The sheets were characterized for their transparency (UV-VIS/NIR, 390-700 nm), thickness (white light interferometry) and glucose permeability (side-by side diffusion setup, glucose assay kit) as well as for their surface composition (XRD) and compatibility with corneal endothelial cells using both primary and B4G12 cells.

Results: Multi-layered sheets were successfully produced. The sheet thicknesses ranged between 0.8 and 1.5µm which is thinner than the natural Descemet’s membrane (i.e. 10-20µm). All produced sheets showed a transparency of over >95% in dry state and > 98% in the wet state throughout the visual spectrum with no significant differences observed between the applied gelatin derivatives. The sheets were sufficiently permeable (>$2.36*10^{-3}$ cm/s) towards glucose, both in the presence and absence of the gelatin-derived coating. Upon seeding of the sheets with B4G12 cells and primary corneal endothelial cells, the cells developed their characteristic hexagonal shape. Immunocytochemical staining confirmed the presence of Na^+/K^+ ATPase pumps and tight junctions (ZO-1), indicating good cellular proliferation.

Conclusion: Transparent (>90%), thin (<10 µm), multi-layered sheets were successfully produced. These sheets were sufficiently permeable for glucose. Additionally, the sheets were able to support the proliferation of B4G12 and primary corneal endothelial cells. The produced sheets are thus promising candidates to function as tissue engineered alternatives for donor corneas.

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