Biomimetic Collagen-Phosphorylcholine Hydrogels as Corneal Implants for High Risk Patients

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Introduction: The cornea is the transparent covering in the front of the eye. It is responsible for over 75% of transmission of light to the retina for vision. The cornea is composed of three major cellular layers, an outermost epithelium, a middle stroma containing keratocytes and an innermost, single-layered endothelium. Corneal diseases are a leading cause of blindness throughout the world, leading to loss of corneal transparency and deteriorating vision. There are a wide variety of infectious and inflammatory eye diseases that cause corneal scarring and may lead to total blindness. Transplantation with human donor corneas is the still the main treatment and is accepted worldwide for corneal blindness. However, the availability of high quality human donor corneas is lower than the demand, leading to the development of artificial substitutes. However, even if readily available, some clinical conditions are not amenable to donor human cornea transplantation and carry high risk of graft rejection and failure. These include inflammation caused by chemical burns, infections and autoimmune problems.

To address high-risk transplantation problems, we developed biomimetic implants comprising recombinant human collagen (RHC) and 2-methacryloyloxyethyl phosphorylcholine polymer (MPC).[1] We previously showed that these RHC-MPC materials are able to stably integrate within rabbit models of alkali burned corneas, repelling neovascaularization.[2] Here, we show the route for advancing RHC-MPC implants into the clinic and early results where they were tested in patients for whom human donor cornea grafting is contraindicated due to the high risk of rejection or transplant failure.

Methods: Under Class 100 conditions and following GMP, interpenetrating networks of RHC and MPC were fabricated. The first network comprised RHC crosslinked with EDC/ NHS; and the second network comprised MPC polymerized with poly(ethylene glycol) diacrylate (PEGDA). MPC and PEGDA polymerization was initiated by Ammonium persulphate (APS) and N,N,N,N-tetramethylethylenediamine (TEMED). The hydrogels, moulded into corneal shaped implants underwent *in vitro* and *in vivo* animal testing as part of the quality control criteria. In accordance to the Declaration of Helsinki, with ethics approval according to the laws in the Ukraine, trial registration (ClinicalTrials.gov Identifier:NCT02277054), and after written informed consent, 3 patients requiring transplantation to treat the severe corneal pathologies presenting as persistent ulceration and scarring were grafted with RHC-MPC implants at the Filatov Institute of Eye Diseases and Tissue Therapy (Odessa, Ukraine).

Results: Implants made under GMP were sterile and endotoxin levels were below the acceptance limit. Implants were more transparent then normal human cornea and were stable in high concentrations of collagenase. FTIR confirmed the presence of both RHC and MPC within the implants. The water content of the implants was higher and denaturation temperature was lower than human cornea, but the implants were nevertheless sufficiently robust for clinical grafting. Ultrastructure analysis confirmed that implants contained uniaxial aligned collagen filament. Biocompatibility testing showed that the RHC-MPC constructs were cell friendly and supported corneal epithelial cell proliferation and stratification. Subcutaneous implantations in rats showed that the implants were well tolerated and did not elicit immune reactions. A 12-month grafting study into corneas of mini-pigs showed regeneration of epithelium, sub-epithelial nerves and stroma that was promoted by the initially cell-free implants. Histopathological examination of implants after harvesting showed neo-corneas that resembled normal unoperated contralateral control eyes in morphology. Early clinical results (2 weeks post-operation to 3 months) from 3 patients showed that implants remained transparent, free of edema and neovascularization in all patients. Complete epithelial coverage of the implant occurred in one patient after four weeks of the surgery, while the remaining two still had incomplete coverage and sutures at the time of examination. Visual acuity improved in two patients.

Conclusions: We have shown that RHC-MPC implants are biocompatible, non-immunogenic *in-vitro* and in animal studies and are able to promote regeneration. Early clinical data suggests that they may be useful in treating high-risk patients. However, a complete Phase 1 clinical study is needed to evaluate the true potential of the implant in corneal transplantation.

Reference:

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