expressing markers typical to HCECs. The donor age and isolation method significantly affected the HCECs culture results. Under all conditions tested, ECM protein coating promoted cell-cell interactions and proliferation of HCEC. The results indicate that the corneal endothelium can be regenerated by seeding the cultured HCECs on a proper carrier material. This study demonstrated that the bioengineered neocorneas could be a new potential source for transplantation.

21.P09
Bioengineering gelatin hydrogel for corneal endothelial cell repair

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Introduction: Corneal endothelium can be cultured in vitro, and together with appropriate scaffolding material, is transplanted inside of patient's eye to repair the dysfunctional endothelium. Gelatin is a hydrolysed product from collagen, and has been extensively used in medical field. The purpose of this study is to develop a bioengineering gelatin hydrogel for corneal endothelial cell repair.
Methods: Gelatin hydrogel was obtained by cross-linking with1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) / N-hydroxysuccinimide (NHS). Human corneal endothelial cells (hCECs) were seeded on the gelatin hydrogel. New Zealand rabbit was used for the implantation of gelatin hydrogel covered with/without hCEC.
Results: A flexible and transparent hydrogel was obtained after cross-linking gelatin film with EDC/NHS. hCECs were seeded directly on gelatin hydrogel, and tight confluent cell layer was formed on hydrogel surface after several days culture. The growth rate of hCECs on gelatin hydrogel was similar to that on the tissue culture plate. ZO^{-1} and K-Na-ATPase markers were expressed by hCEC seeded on gelatin hydrogel. Round shaped gelatin hydrogel with a 7 mm diameter was easily implanted inside of rabbit eyes with only 2–3 mm incision, and no obvious inflammation and rejection response were found.
Conclusion: The Gelatin hydrogels, seeded with hCECs can be used to create corneas for transplantation and overcome the shortage of donated corneas.

21.P10
Bioactive peptide amphiphile nanofibers for cornea regeneration

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Corneal opacification is the most frequent cause of blindness. Although keratoprosthesis and keratoplasty are the most common methods of treatment, there are some limitations in their use. Peptide amphiphile (PA) molecules offer a promising new approach for regenerative medicine studies. These molecules are biocompatible and biodegradable and can be engineered to include bioactive sequences. The bioactive groups are used for mimicking the active epitopes in the natural extracellular matrix. In addition, the nanofibers formed by self assembly of PA molecules form a network that resembles the morphological properties of the natural matrix. Here, we utilized various PA molecules in order to determine their effects on human keratocytes in in vitro conditions. The PAs were designed to contain laminin or/and fibronectin-derived epitopes. The biocompatability and bioactivity of the PAs were analyzed via cell viability and proliferation assays with Alamar Blue and BrdU assays, respectively. The adhesion and morphological properties of keratocytes on PA surface was characterized by phalloidin/To-Pro-3 staining. Our results showed that PAs enhance the viability of these cells. Moreover, PA molecules have ability to augment cell proliferation. Keratocytes can also spread on these PA networks similarly to collagen matrices. In conclusion, we established that PA nanofibers can be used as an efficient scaffold for keratocytes and thus offer a new platform for corneal tissue engineering.

21.P11
Fabrication of novel artificial corneal substitute using silk protein derived materials

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Corneal transplantation is most promising way for recovering from corneal blindness. However, donor shortage in all over the world is serious problem. Therefore, development of an artificial cornea is one of the solutions to overcome this complicated situation. Silk protein derived materials such as fibroin have been tested as an artificial cornea because of its high biocompatibility. However, perfectly reliable material has not been established yet. We focused on novel silk protein derived material such as sericin gel films (SF) and hornet cast films (HF) as a novel artificial cornea. These films have high transparency and enough mechanical strength for clinical use. Therefore, we evaluate SF and HF under various sterilized conditions. Using autoclave treated HFs, we could establish bio-safety artificial cornea. However, low permeability of film materials affected the homeostasis of host cornea adversely. To solve this problem, we tried to fabricate electrospun fiber based materials from silk derived protein. From aqueous fibroin solution, we could prepare nanofiber mat. Our biological tests revealed that corneal cells could attach not only the surface of fiber mat but also the inside of fiber mat with keeping its phenotype. This result suggests that the materials might be applicable for artificial cornea. Unfortunately, current fibrous mats have insufficient transparency. Now we are spending our efforts to increase transparency of silk proteins-based fibrous substrates.

21.P12
Biological cornea glue - in vitro evaluation

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Introduction: An efficient biological method of corneal tissue repair that replaces surgical sutures is still unmet. Transglutaminase-2 (TG2) stabilizes connective tissue by covalent isopeptide crosslinks. We hypothesize that TG2 based tissue glue will covalently bond the corneal tissue layers to replace the need of sutures for wound closure.
Materials and methods: Human cornea was tested for the presence of TG2 and FXIIIa substrate sites using histochemical assay and imaged with fluorescent microscopy, electron microscopy and second harmonic generation imaging. The ability of endogenous and exogenous TG2 in crosslinking collagen was studied on fibroblast cultured matrix using enzyme inhibitors and commercial TG2 followed by gel electrophoresis. The enzymatic adhesion in corneal tissue was studied in cadaveric porcine corneal flap wounds by delivering concentrated enzyme to the tissue interface with or without substrate additives and then mechanical testing using instron tester.
Results: Human cornea showed abundant substrate sites for TG2 and a good colocalization of its substrate sites on the matrix proteins. Both endogenous and exogenous TG2 produced collagen crosslinking in the matrix. In mechanical testing, the breaking shear stress of the corneal flap wounds closed after the delivery of the active enzyme with the substrate additives was greater than the strength of fibrin glue.