Novel Extracellular Matrix Biomembrane for Corneal Repair
Ju Won Kim¹, Jung Soo Lee², Hyo Soon Park³, So Ra Park⁴, Byoung-Hyun Min⁵,⁶
Corresponding Author: bhmin@ajou.ac.kr
¹Cell Therapy Center, School of Medicine, Ajou University, Suwon, Korea, Republic of,
²REGENPRIME Co., Ltd, Suwon, Korea, Republic of,
³Shin & Park Kim’s eye clinic, Pusan, Korea, Republic of,
⁴Department of Physiology, College of Medicine, Inha University, Incheon, Korea, Republic of,
⁵Department of Orthopedic Surgery, School of Medicine, Ajou University, Suwon, Korea, Republic of,
⁶Department of Molecular Science and Technology, Ajou University, Suwon, Korea, Republic of

Introduction
As damage to the corneal epithelium due to limbal stem cells deficiency (LSCD) will cause serious eye disease, such as conjunctivalization, various studies were performed to cure this defect. Cultivated limbal stem cell transplantation is being used as a current treatment for LSCD. However, use of allogenic biological material as limbal stem cell (LSC) carrier is associated with risks of transmission of certain diseases and allograft rejection. Therefore development of non-toxic biological material is important. Recently, we have developed novel extracellular matrix (ECM) membrane product. The purpose of this study is to investigate whether an ECM membrane has adequate mechanical and biological properties to be used as LSC carrier for the regeneration of the corneal epithelium.

Materials and Methods
A novel ECM membrane was manufactured using cultured porcine chondrocytes and molded to have a convexity analogous to that of the natural cornea. The mechanical properties were measured and compared to those of commercially available denuded human amniotic membrane (HAM). Rabbit LSCs were labeled with PKH26 dye, and cultivated on the ECM membrane or HAM. The cell attachment and proliferation rates were evaluated, and then the LSC-ECM membrane construct was attached to a denuded rabbit corneal button and implanted onto the back of an athymic mouse. At 3 weeks after implantation, the grafts were analyzed by transmission electron microscopy (TEM), histology, and immunohistochemical staining of AE-5 and mucin-5AC.

Results
The ECM membrane had a base curve that corresponded to the surface curvature of the natural cornea, and it was 1.5-times (15 μm) thicker and exhibited higher light transmittance than HAM; moreover, there were no significant differences in cytocompatibility. On ex vivo cultivation, a new epithelium with PKH-labeled cells was formed on the denuded corneal button attached to the LSC-ECM membrane construct. TEM images of the graft showed a continuous cell layer covering the stroma with good cell-to-cell contact. Histological examination showed that the newly formed epithelium was similar to the normal cornea and expressed AE-5 but mucin-5A, which showed corneal epithelial specific phenotype.

Discussion and Conclusions
The ECM membrane was fabricated in the form of a contact lens to ensure that it fit to the corneal surface and possesses the mechanical properties and cytocompatibility required for LSC transplantation. The results of ex vivo implantation demonstrated that the ECM membrane can be used as an LSC cell carrier for repairing the corneal epithelium.

References

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