

## **Amniotic Membrane Suspension Stimulates Human Corneal Epithelialization In Vitro**

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**Purpose:** To investigate the biochemical effect of amniotic membrane suspension (AM) on corneal epithelial proliferation and migration.

**Methods:** Human corneal epithelial cells (HCECs) were cultured in a medium with different concentration of AM suspension. After manual scraping of HCECs, migration assay and immunocytochemical staining of cell adhesion molecule (E-cadherin) was performed to assess corneal epithelial migration. Cornea epithelial cell proliferation was evaluated by 5-Bromo-2-deoxyuridine (BrdU) incorporation, western blot using proliferating cell nuclear antigen (PCNA) and Reverse transcription–polymerase chain reaction (RT-PCR) using epithelial cell marker (CX 43).

**Results:** On migration assay, increased migration rates were observed as the concentrations of AM were increased with statistical significance in AM 15%, 30% treated cells compared to negative control. ( $P < 0.05$ ) On immunocytochemistry, large amounts of E-cadherin were found near the wound edges after 24 hours of injury. On proliferation assay, BrdU positive cell count / total cell counts (labeling index) were augmented according to the AM concentration with statistical significance ( $P < 0.05$  in AM 30% and positive control) In western blot analysis, expression cell cycle-associated protein, PCNA was gradually increased according to the concentration of AM. RT-PCR showed that HCECs exposed to AM had increased connexin43 mRNA levels ( $P < 0.05$  in AM 30%)

**Conclusion:** These results indicate that suspension form of AM maintains its beneficial effect and that AM suspension has positive effect on corneal epithelial migration and proliferation according to its concentration.