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Human Conjuntival Epithelial Cells cultivated ex vivo on Amniotic Membrane

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Introduction: The conjunctiva plays an important role in the ocular surface physiology: it represents a physical barrier against microorganisms and prevents liquid loss. Besides, it has immune cells, special cicatricial mechanisms and produces mucins, an important components of the tear film. There are different ocular surface diseases that affect the conjunctiva, as pterygium, tumors and symblepharon. Classically, conjunctival auto or allografts have been performed to treat many of these diseases. However, there are some limitations regarding the availability of conjunctival donor tissue.
Purpose: To establish human conjunctival epithelial cell culture on amniotic membrane.
Methods: A conjunctival fragment of approximately 2x4mm was harvested from different living donors who underwent cataract or pterygium surgery. All donors signed a inform consent prior to the procedure. The conjunctival fragment was sent to the laboratory. Under sterile conditions, the tissue was divided into an anterior and a posterior portion. The anterior portion was divided into two fragments. One was cultivated on denuded human amniotic membrane, and the other was placed on a culture plate. The cultures were incubated with a modified HEM media at 37ºC and 5% CO2. The culture medium was changed 3 times a week for 3 weeks. After this period, the cultures were air-lifted for 3 days and fixed for immunocytochemical analysis for epithelial cytokeratins (K3, MUC5) and proliferation markers (Ki-67). We also performed impression cytology to verify morphologic features of the cultures.
Results: Conjunctival epithelial cells (n=3) expanded successfully either on culture plate or amniotic membrane. Impression cytology demonstrated the presence of compact conjunctival epithelium and goblet cells. Immunocytochemical analysis showed positivity to K3, MUC5 and Ki-67.
Conclusions: We establish a method to cultivate human conjunctival epithelial and goblet cells *ex vivo* on human amniotic membrane. This method may

represent an important step to be used in the treatment of many ocular surface diseases.