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Last Name - Cristovam
First Name - Priscila
Middle - Cardoso

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Importance of 3T3 feeder layer to establish epithelial cultures from cell suspension obtained from cornealscleral rims

Priscila C. Cristovam, Maria A. Glória , Gustavo B. Melo, Charles C. Faria, Myrna S. Santos e José Álvaro P. Gomes

Purpose: To evaluate the importance of the presence of 3T3 fibroblasts for establishing limbal epithelial cultures from cell suspension obtained from cornealscleral rims (CSL). **Methods:** CSL from different donors (n=6) had their posterior stroma and endothelium stripped away. Each CSL was divided in three equal segments that were set up in tissue culture in three different conditions: one of the segments was cut in three small pieces which were placed with the epithelial side up on the bottom of the culture plate (group A). The other two fragments were cut in small pieces that were incubated with Trypsin 1g/ml (EDTA/PBS 0,02%) for 30 min at 35oC. The CSL pieces were removed and the cell suspension was centrifuged at 1500rpm for 5 minutes. The procedure was repeated with the same CSL pieces and the cells obtained were suspended on a SHEM media. One-thousand epithelial cells were placed on 100mm culture plates with (group B) or without (group C) irradiaded 3T3 fibroblasts and cultured in SHEM media which was changed every 2 days. The epithelial migration in group A and clone formation in groups B and C were evaluated by phase contrast microscopy. After 20 days, the media was removed and the attached cells were stained with rodamine. **Results:** All the epithelial cell suspensions that were cultured with 3T3 fibroblasts (group B) formed clones. Epithelial cell growth was observed in 4/6 rims (group A). No adhesion or clone formation was observed at the cell suspensions that were cultivated without 3T3 fibroblasts (group C). **Conclusion:** Epithelial cell suspension obtained from CSL in this model need to be cultivated with 3T3 fibroblasts in order to form clones.