Optimization of Conjunctival Epithelial Retrieval and Growth

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Introduction

The conjunctiva is a membrane covering the surface of the eye which produces mucins which stabilize the tear film. Many diseases such as chemical injuries, immune disorders and severe infections may lead to conjunctival destruction. This inevitably causes a painful eye, and secondary tear film instability often leads to visual disability. Present treatments are very limited and the outlook is often bleak. There is no satisfactory means of replacing conjunctiva. We hypothesize that by isolating conjunctival stem cells they could be expanded into sheets of epithelium suitable for transplantation.

Our initial aims were to develop a method of retrieving whole conjunctival tissue and to optimize epithelial growth.

Materials and Methods

 Conjunctival tissue was obtained from human cadaveric donors. A surgical technique to excise whole conjunctival tissue was developed. Various methods were employed to remove and culture conjunctival epithelial cells. Trypsinisation was performed with and without cloning rings for time periods of 1-20 minute cycles. Explants or cell suspensions were plated onto tissue culture plates with and without an inactivated 3T3 feeder layer or Matrigel basement membrane matrix (BD Biosciences).

Results

Whole human conjunctival tissue (figure 1) was successfully obtained by splitting both eyelids anteriorly-posteriorly along the grey line, performing a 360° limbal peritomy and dividing the fatty tissue and Tenon’s capsule behind the conjunctiva.

Primary colonies of epithelial cells grew after trypsinisation of the tissue for four 20minute cycles when co-cultured with an inactivated J23T3 feeder layer (figure 2). Very few cells were obtained after trypsinisation with cloning rings. No colonies were seen on tissue culture plates or Matrigel matrix. A mixed population of cells grew from the tissue explants with and without a feeder layer.

Discussion and Conclusions

We have developed a technique to retrieve whole conjunctival epithelium for research purposes and optimized conjunctival epithelial growth. This enables future comparison of the stem cell and growth properties of different areas of the human conjunctiva.

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Disclosures

The authors have no conflict of interest to disclose.