Culture and characterization of oral mucosal epithelial cells on a fibrin gel for ocular surface reconstruction.

Authors: Sheth R., Neale M.H., Shortt A.J., Massie I., Vernon A.J., Daniels J.T.

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Abstract:

Aim of the study: To develop a clinical grade fibrin gel for the culture of oral mucosal epithelial cells (OMEC) intended for ocular surface reconstruction in the treatment of limbal stem cell deficiency (LSCD).Materials and methods: Transparent fibrin gels composed of fibrinogen and thrombin were developed for the culture of epithelial cells. Oral mucosa was harvested from the buccal region of healthy volunteers and cultured as explants on fibrin gels. Tranexamic acid (TA), a clinically approved anti-fibrinolytic agent was added to prevent the fibrin gel from digesting due to cellular activity. The gels were stained for p63alpha (as a marker of poorly differentiated epithelial cells), CK19, CK13 and CK3 (expressed by OMEC). Epithelial cell stratification was observed using hematoxylin-eosin staining.Results: Addition of TA prevented gels from dissolving during the culture period. OMEC proliferated on the fibrin gel and attained confluence over a 2-week period (+/-2 d) and exhibited a typical epithelial, cobblestone morphology. Basal OMEC exhibited positive staining for p63alpha while the superficial cells exhibited positive staining for CK3. The cells expressed a strong immunoreactivity for CK19 and CK13 suggesting that they retained a normal oral epithelial phenotype.Conclusion: Fibrin gels, maintained in the presence of TA, to control the rate of substrate degradation, provide a more robust yet transparent substrate for the culture and transplantation of cultured OMEC. The fibrin gels are easily standardized, the components commercially available, and produced from clinically approved materials. The resulting stratified OMEC-derived epithelium displays characteristics similar to that of a human cornea, e.g. CK3 expression. The conventional dependence on a murine feeder layer for support of epithelial cells is unnecessary with this

technique and hence, provides for an attractive alternative for treatment of LSCD.