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Developing an *ex vivo* human cornea model for discovering the cellular interactions in simple limbal epithelial transplantation

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Abstract

Purpose : Simple limbal epithelial transplantation (SLET) and allogenic SLET (alloSLET) are successful, yet not fully understood treatment options for total limbal stem cell deficiency (LSCD). The aim of our study was to establish an *ex vivo* model of the SLET technique.

Methods : Human corneas, not suitable for donation, were provided by the Pathology Department with approval of the Institutional Ethics Board. Corneas were cut into 6-8 equal segments and the limbal palisades were prepared from 1-2 segments (donors). The remaining segments (recipients) were digested with dispase II, then the epithelium was denuded. Some denuded segments were treated with 0.1M NaOH to further damage the niche. A 1x1mm piece of donor limbus was laid on the denuded recipient cornea segments with or without amniotic membrane (AM), using fibrin glue. The models were cultured in SHEM medium with 10% FCS for 2-3 weeks, using air lifting technique. After culturing, all samples were cryosectioned and Pappenheim staining was applied. Immunfluorescence labeling for pancytokeratin (CK), CK15, vimentin, CD90 and CD31 was performed. The extent of epithelial outgrowth was assessed and statistically analyzed.

Results : Re-epithelialization originating from the donor tissue onto the recipients' corneal surface occurred in both the allogenic and autologous transplantation settings. Nevertheless, re-epithelization was significantly less successful in alloSLET compared to SLET (59.26% vs 100%, p=0.017). AM without donor did not induce epithelial regrowth. Neither the lack of AM (p>0.1), nor NaOH treatment (p>0.1) resulted in a significantly different epithelial outgrowth. The basal layers of epithelial outgrowths were CK15+/vimentin+, indicating the presence of progenitor cells. In the recipient and donor limbal tissues, disintegrated CD31+ vessels and numerous CD90+ mesenchymal cells were visualized.

Conclusions : We successfully established and validated an *ex vivo* human SLET model. The relatively poor alloSLET success is comparable to that is seen in real-life settings and may be due to donor damage by limbal resident immune cells. Recipient niche damage and lack of AM may not influence initial short-term epithelization. This model is a valuable tool for further investigation of limbal epithelial stem and niche cell interactions occurring in SLET.

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