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Study into the Interactions of Primary Limbal Epithelial Stem Cells and HTCEPI Using Tissue Engineered Cornea

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Abstract : Introduction: Though knowledge of the compositional makeup and structure of the limbal niche has progressed exponentially during the past decade, much is yet to be understood. Identifying the precise profile and role of the stromal makeup which spans the ocular surface may inform researchers of the most optimum conditions needed to effectively expand LESCs in vitro, whilst preserving their differentiation status and phenotype. Limbal fibroblasts, as opposed to corneal fibroblasts are thought to form an important component of the microenvironment where LESCs reside. Methods: The corneal stroma was tissue engineered in vitro using both limbal and corneal fibroblasts embedded within a tissue engineered 3D collagen matrix. The effect of these two different fibroblasts on LESCs and hTCEpi corneal epithelial cell line were then subsequently determined using phase contrast microscopy, histolological analysis and PCR for specific stem cell markers. The study aimed to develop an in vitro model which could be used to determine whether limbal, as opposed to corneal fibroblasts, maintained the stem cell phenotype of LESCs and hTCEpi cell line. Results: Tissue culture analysis was inconclusive and required further quantitative analysis for remarks on cell proliferation within the varying stroma. Histological analysis of the tissue-engineered cornea showed a comparable structure to that of the human cornea, though with limited epithelial stratification. PCR results for epithelial cell markers of cells cultured on limbal fibroblasts showed reduced expression of CK3, a negative marker for LESC's, whilst also exhibiting a relatively low expression level of P63, a marker for undifferentiated LESCs. Conclusion: We have shown the potential for the construction of a tissue engineered human cornea using a 3D collagen matrix and described some preliminary results in the analysis of the effects of varying stroma consisting of limbal and corneal fibroblasts, respectively, on the proliferation of stem cell phenotype of primary LESCs and hTCEpi corneal epithelial cells. Although no definitive marker exists to conclusively illustrate the presence of LESCs, the combination of positive and negative stem cell markers in our study were inconclusive. Though it is less traslational to the human corneal model, the use of conditioned medium from that of limbal and corneal fibroblasts may provide a more simple avenue. Moreover, combinations of extracellular matrices could be used as a surrogate in these culture models.

Keywords : cornea, Limbal Stem Cells, tissue engineering, PCR
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