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Cornea and External Disease

Nº CEP

NOVEL ENZYMATIC ISOLATION OF AN ENTIRE VIABLE HUMAN LIMBAL EPITHELIAL SHEET FOR EX-VIVO EXPANSION OF CORNEAL STEM CELLS

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PURPOSE: To develop a reproducible method of isolating an intact viable human limbal epithelial sheet.
MATERIALS AND METHODS: Human pigmented limbus was incubated at 4 oC for 18 h in SHEM containing 50 mg/ml Dispase II and 100 mM sorbitol. A loose limbal epithelial sheet was separated by a spatula. The remaining stroma was digested and subcultured. Viability of isolated cells was assessed. Isolated epithelial sheets and remaining stroma were subjected to immunostaining. Sheets of 1.5 mm length were cultured in SHEM on plastic until confluency and cell extracts were subjected to Western blotting.

RESULTS: Intact limbal epithelial sheets were consistently isolated. Pigmented palisades of Vogt revealed large superficial squamous cells and small basal cuboidal cells. No epithelial cells grew from the remaining stroma. Mean viability was 80.7 ± 9.1%. The basal epithelium was negative to keratin 3 and connexin 43, but was scatter positive to p63. The epithelial sheet showed negative staining to laminin 5 and collagen VII, but interrupted linear basal staining to collagen IV. The remaining stroma showed negative staining to laminin 5, positive linear staining to collagen IV in the basement membrane, and diffuse staining to collagen VII in the superior stroma subjacent to the basement membrane. Western blotting revealed that cells originated from the limbal sheets expressed keratin 3 and p63.

CONCLUSION: An intact limbal epithelial sheet can be consistently and reproducibly isolated and contains stem cell characteristics in the basal epithelium by degrading laminin 5 and part of collagen IV, and disassembling collagen VII.