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UCP A Novel Technique in Human Corneal Cell Cultures

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Link to Corneal Culture in Serum Free Media

Abstract

Advances in fundamental and toxicological studies using human corneal cells are hindered, no scarce availability of donor material but also by the relatively harsh enzymatic and coars isolation techniques of corneal cell populations for culture (Kahn, C.R et al. IOVS 34, 3429-Better yields have been reported from explant corneal pieces (Samples et al. Exp. Eye Res. 1991). However, due to their greater proliferative capacity, fibroblast contamination of epithel common whichever technique is used. (Engelmann et al. IOVS 29, 1656-1662 1988).

The novel technique presented here is to minimise fibroblast contamination at the initia culture by precise selective sectioning of the epithelial cell layers from the stroma. This is achie by means of an autoclavable hand-held microtome conceived and developed by the author ir Eye Research Laboratory. A British Patent (No. 990721.6) has been obtained and a Europea been filed.

Ninety-five percent of our human corneal tissues are excised corneoscleral discs of 1-2mm t discs are held between the jaws of the microtome and sectioned with a thin razor blade. containing the epithelial layers are transferred to culture flasks. These explants are left undi week during which time small squamous cells grow from the edges onto the flask substrate. F are similarly derived from the stromal sections. The cultures are confluent within 2-3 weeks.

The epithelial nature of the cells is identified by their polyhedral morphology and immunofluorescent labelling of cytokeratin. Fibroblasts produced from sectioned stromal identified by their characteristic spindle shape and immunofluorescently-labelled vimen **cultures** have been successfully obtained from donor ages ranging from 7 to 99 years old.

Purpose

To establish a protocol for the routine culture of pure epithelial and stromal cells from human

Introduction

The cornea is the projecting transparent anterior surface of the outer coat of the eye. Its prin is to provide the bulk of the refractive power of the eye's dioptric system.

Clinically the corneal tissue barrier plays an important role in drug delivery by the ophthalmic

The schematic diagram below shows the five main layers of the cornea although B Descemet's membranes are really modifications of the stroma.



• **The epithelium** has an important barrier function. It prevents evaporation at the air/cornea and provides a screen against harmful pathogens.

• The stromal layer performs the major light focusing function of the cornea.

• The endothelium has an efficient ion and water regulatory activity, which maintains the cla cornea.

• If cell lines can be generated from the three major cell types, not only would they provide the for experiments in their own right but also serve as a basis for human corneal constructs.

• The corneal tissues obtained from the eye banks, are excised corneal-scleral discs, which a suitable for transplants. Most have been incubated for up to 30 days.

• **Endothelia** from such corneas have not survived beyond the primary culture. The technique here were developed for epithelial and stromal cultures.

Method

STEP 1 First Cut

With the aid of the hand-held microtome, the corneal disc is cut through the stroma to give sections. The thicker one bears the epithelial layers and the thinner one the endothelium.





Procedure for Corneal Sectioning

- Scleral tissue is trimmed off to leave a disc of <15mm diameter
- The corneo-scleral disc is placed on the blue sample holder surface

- The clamping cylinder is dialled to the "ZERO" starting position by means of the positioning c
- Markings on the knob and the blue marking on the white clamping cylinder main body are al achieve this.

• The sample holder with the corneal disc is retracted by >2mm to clear it completely from t gap

• The microtome is firmly closed by bringing the two halves of the body together

• The sample holder is dialled clockwise towards the cutting gap until the cornea is just touchir surface of the white clamping cylinder

• The clamping cylinder is retracted by 100mm

• The sample holder is **advanced by 100mm** to insert the cornea into the cavity of the clamp body

• The explant is ready for sectioning.



- The cutting implement is a snapped half of a double-edged razor blade.
- The half-blade is inserted in the top of the cutting gap with the aid of the spring blade guid
- The blade is passed through the cornea with a smooth downward vertical action
- The cut is ended when the blade hits the cutting ledge
- A final gentle sawing action on the ledge bisects the tissue completely

• The two halves of the microtome are opened to expose the cut corneal sections one on each blade

• The two slices of tissue are removed and stored for further fine selective sectioning



Two Sectioned Corneal Slices

Method

STEP 2 Second Cut

Separating the Epithelium from the Stroma

The epithelial/stromal slice is longitudinally sectioned as described in Step 1 to obtain an epit of <100mm and multiple stromal sections.



Serial Sections from One Cornea

Method

STEP 3 Setting up the Cultures

• The **epithelial explants** are transferred to collagen-coated flasks and **the stromal explant** standard flasks.

• The explants are left for 1-2 hours to adhere to the flasks, which are kept in an upright posithen laid down.

- Periodical moistening of the tissues prevents the cells from desiccation during this period.
- The culture medium is EMEM enriched with 30% FCS
- The cultures are incubated at 350C in a humidified atmosphere containing 5% CO2 and 95%

Results

• Epithelial Cultures

Growth in the epithelial explants is initiated within 1-2 days and confluency is achieved within

A collagen -coated substrate is advantageous for producing confluent epithelial cultures

178 corneal epithelial cultures produced 80% primary cultures, 40% secondary cultures (P=1) tertiary cultures (P=2)

<u>Stromal Cultures</u>

Stromal explants do not usually exhibit growth for the first week but once established the fibre proliferate very rapidly to become confluent within 3-4 days

Stromal fibroblasts proliferated abundantly on plastic substrates

Fibroblast cultures were established from all corneal explants

• The accompanying Phase Photomicrographs show typical primary corneal epithelial and stror

The epithelial cells exhibit their characteristic polyhedral morphology with distinctive cell mer prominent nuclei.

The stromal fibroblasts are characteristically elongated. They tend to align parallel to each o bundles but where the bundles touch, there is a profusion of crossover fibres.

In cultures of over two weeks, the fibroblasts become a mass of swirling bundles.

<u>Characterisation of Corneal Cells</u>

Fluorescently labelled marker proteins, cytokeratin and vimentin are only found in the epithe fibroblast cells respectively as displayed in the fluorescent photomicrographs.





Conclusion and Future Project

Pure cultures of human corneal epithelial and stromal fibroblast cells can be routinely obtained rejected corneal transplant tissues.

• These cells are used for in vitro human corneal constructs

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