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Ultrastructural and Immunohistological Characterization of Cultured Human Corneal Epithelium

Thesis

Submitted in Partial Fulfillment of M.D. Degree in Histology

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بسم الله الرحمن الرحيم

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LIST OF ABBREVIATIONS

Ad12-Sv40 adenovirus 12 and simian virus-40

AE-5 antiepithelial-5

AIDS acquired immune deficiency syndrome

BPE bovine pituitary extract

c-AMP cyclic-adenosine monophosphate

c-DNA cyclic-deoxyribonucleic acid

DMSO dimethyl-sulfoxide

EDTA ethylene diamine tetra-acetic acid

EGF epidermal growth factors

ELISA enzyme linked immunosorbent assay

FBS fetal bovine serum

FNC fibronectin and collagen
GFAP glial fibrillar acidic protein

IF intermediate filament

IFM immunofluorescence microscopy

K keratin

KCl potassium chloride

KGF keratinocyte growth factor

KGF-R keratinocyte growth factor receptors

KGM keratinocyte growth medium m-RNA messenger-ribonucleic acid

NaCl sodium chloride

NFP neurofilament proteins
NGSL neutral glycosphingolipid
pAb-55 polyclonal antiboy-55

PBS phosphate-buffered saline PhCM phase-contrast microscopy

RNA ribonucleic acid

RT-PCR reverse transcription polymerase chain reaction

SDS sodium dodecyl sulfate

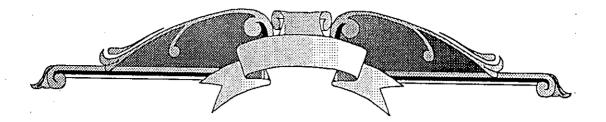
SDS-PAGE sodium dodecyl sulfate polyacrylamid gel electrophoresis

SIRC serial immortalized rabbit cells

T.E.M transmission electron microscopy

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Introduction

INTRODUCTION

The cornea is the circular clear tissue at the anterior part of the outer fibrous layer of the eyeball. It is both transparent and avascular. The cornea has two major functions; protection of the intraocular contents and refraction of light; to accomplish these two functions the cornea must maintain its strength and transparency.

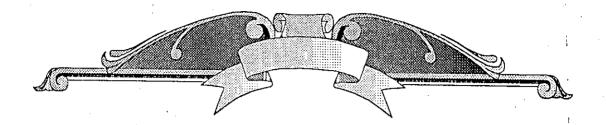
Microscopically, the cornea is composed of five layers; from front to back they are: (1) the epithelium, (2) Bowman's membrane, (3) the substantia propria, (4) the Descemet's membrane and (5) the Descemet's endothelium (Cormack, 1993).

The human corneal epithelium is nonkeratinized stratified squamous epithelium that consists of five to seven layers of cells. It has several unique characteristics among other stratified squamous epithelia of the body. It is extraordinarily regular in thickness over the entire cornea and it has absolutely smooth, wet, apical surface that serves as the major refractive surface of the eye. Its location on the surface of transparent cornea requires that it be transparent also. In addition to that, the epithelium carries out "routine" housekeeping functions of an epithelium that borders the external environment. These include provision of a barrier to fluid loss and pathogen entrance and resistance to abrasive pressure (Smolin and Thoft, 1994).

Because the cornea is exposed the corneal epithelium is vulnerable to various kinds of trauma such as chemical, radiation and irritation. Injury of the corneal epithelium may result in loss of sight (Bruner, 1992).

Previous and current studies had employed experimental animals such as rabbits, rats and animal-derived tissue cultures as alternative models, to test the ocular irritancy. These techniques are simple to perform, allow a quick economical results and use of laboratory animals that are easy to breed and maintain. Yet there are morphological and histochemical differences between the rabbit eye and the human eye that have led the animal model to be challenged almost since its inception (Kahn et al., 1993).

Alternatives to animal models have been proposed. Human corneal organ culture techniques have been developed (Doughman, 1980 and Richar et al., 1991) whereas tissue culture of human corneal epithelium has been used to model the ocular surface in vitro (Hainsowrth, 1991). Such a model may be useful as an adjunct in studying the mechanisms underlying human ocular irritancy by producing cells, living in a controlled environment, free from the disturbing influences of other parts of the body and species-specific (Kahn et al., 1993).



Aim of the Work