Ex Vivo Expansion of Limbal Epithelial Stem Cells: Amniotic Membrane Serving as a Stem Cell Niche

Thesis

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ABSTRACT

Stem cells of the corneal epithelium are located at the limbus and are ultimately responsible for renewal and regeneration of the corneal epithelium under normal circumstances and during wound healing. Diseases causing either a complete loss of limbal epithelial stem cells or severe destruction of the limbal stroma result in the pathologic state of total limbal stem cell deficiency.

A new technique termed ex vivo expansion of limbal epithelial stem cells has been developed to treat patients with limbal stem cell deficiency.

Key Words

- Cornea
- Limbus
- Stem cells
- Limbal stem cells

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LIST OF ABBREVIATIONS

AM	Amniotic membrane
AMT	Amniotic membrane transplantation
BM	Basement membrane
Cx	Connexin
DAB	Diaminobenzidine
dAM	Denuded AM
DMEM	Dulbecco's modified Eagle's medium
ECM	Extracellular matrix
EGF	Epidermal growth factor
FBS	Foetal bovine serum
H&E	Haematoxylin and eosin
iAM	Intact AM
K12	Keratin 12
K3	Keratin 3
KGF	Keratocyte growth factor
KGF-R	Keratinocyte growth factor receptor
LECs	Limbal epithelial cells
LESCD	Limbal epithelial stem cell deficiency
LESCs	Limbal epithelial stem cells
LSC	Limbal stem cell
LSCD	Limbal stem cell deficiency
LSCs	Limbal stem cells
NGF	Nerve growth factor
OCP	Ocular cicatricial pemphigoid
PBS	Phosphate buffered saline
PKP	Penetrating keratoplasty
PMC	Postmitotic cells
SC	Stem cell
SCs	Stem cells
SJS	Stevens- Johnson syndrome
TAC	Transient amplifying cell
TACs	Transient amplifying cells
TDC	Terminally differentiated cells
TGF-β	Transforming growth factor-beta

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INTRODUCTION

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INTRODUCTION

The cornea provides the eye with protection and the refractive properties essential for visual acuity ***(Daniels et al, 2001)***. There is continuous loss of cells from the surface of the corneal epithelium ***(Tseng, 1989; Kinoshita et al, 2001)***. The epithelium is renewed throughout life from a stem cell (SC) population thought to reside at the limbus, ***(Daniels et al, 2001)*** that is, the anatomical junction between the cornea and the sclero-conjunctiva ***(Schermer et al, 1986)***.

Limbal stem cells (LSCs) are supported by a unique stromal microenvironment called the *stem cell niche*. Destructive loss of LSCs and/or dysfunction of their stromal environment renders many corneas with a clinical entity called limbal stem cell deficiency (LSCD) ***(Grueterich et al, 2003a)***. In such patients, conjunctivalization of the cornea occurs as conjunctival epithelial cells and blood vessels peripheral to the limbus migrate onto the corneal surface ***(Huang and Tseng, 1991; Puangsricharern and Tseng, 1995)***. This process involves strong inflammation, neo-vascularization, opacification and visual loss ***(Huang and Tseng, 1991)***.

Several surgical procedures were developed using transplanted LSCs to restore vision in patients afflicted with LSCD ***(Chen and Tseng, 1990; Huang and Tseng, 1991)***. A new strategy of treating LSCD is to transplant a bio-engineered graft by expanding limbal epithelial stem cells (LESCs) ex vivo on amniotic membrane (AM) ***(Grueterich et al, 2003a)***.

Repopulation of the entire limbus with cultured stem cells (SCs) made it possible to repair the corneal epithelium and restore vision in these patients ***(Pellegrini et al, 1997; Rama et al, 2001)***. Cultured limbal stem cell (LSC) therapy is used in several clinical centres ***(Sangwan et al, 2003b)*** and has produced benefits for several types of LSC deficiencies ***(Schwab et al, 2000; Tsai et al, 2000; Koizumi et al, 2001a; Sangwan et al, 2003b)***.

AIM OF THE WORK

The aim of the present study is to attempt to expand LSCs from biopsies of limbal tissue taken from cadaveric corneo-scleral donor rims on AM.

Would it prove successful, this would encourage ophthalmologists to start exploring its potential application as an alternative to conventional limbal transplantation in patients with LSCD in Egypt.

REVIEW OF LITERATURE

CHAPTER (1)
LIMBAL STEM CELLS

Anatomy of the Ocular Surface

The normal ocular surface is covered by 2 distinct forms of non-keratinising stratified squamous epithelium: corneal epithelium and conjunctival epithelium ***(Daniels et al, 2001; Lavker and Sun, 2003)***.

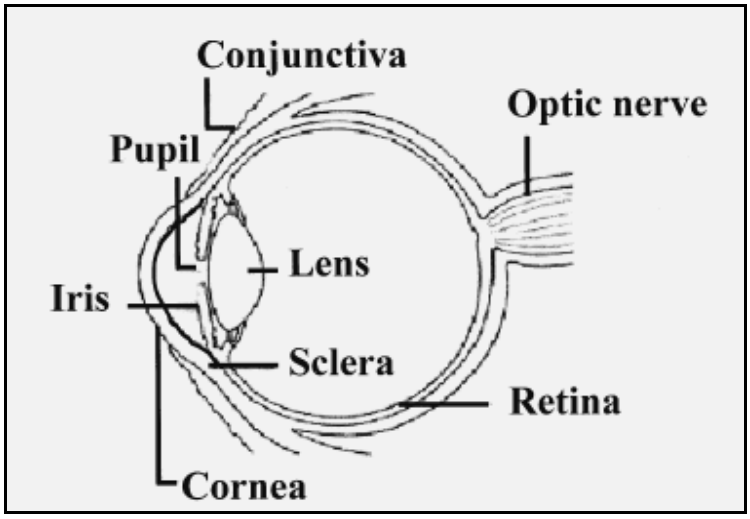

Figure (1) The human eye in cross-section ***(Daniels et al, 2001)***.

The conjunctival epithelium consists of loosely organized cell layers populated by mucin-secreting goblet cells. It lies on a well vascularized stroma ***(Schermer et al, 1986; Cotsarelis et al, 1989)***.

The corneal epithelium is composed of five to seven layers ***(Dua and Azuaro-Blanco, 2000a ; Dua and Azuaro-Blanco, 2000b)***. It is formed of basal cells, wing cells, and squames ***(Daniels et al, 2001)***. The superficial cells have microvilli. The remaining cells constituting the corneal epithelium interdigitate with and form desmosomal contacts with one another ***(Gartner and Hiatt, 2007)***.

The corneal epithelium is devoid of goblet cells. The cuboid basal layer lies on the avascular corneal stroma by Bowman's layer ***(Schermer et al, 1986; Cotsarelis et al, 1989; Kenyon and Tseng SC, 1989; Huang and Tseng, 1991; Pellegrini et al, 1997; Pellegrini et al, 1999)***.

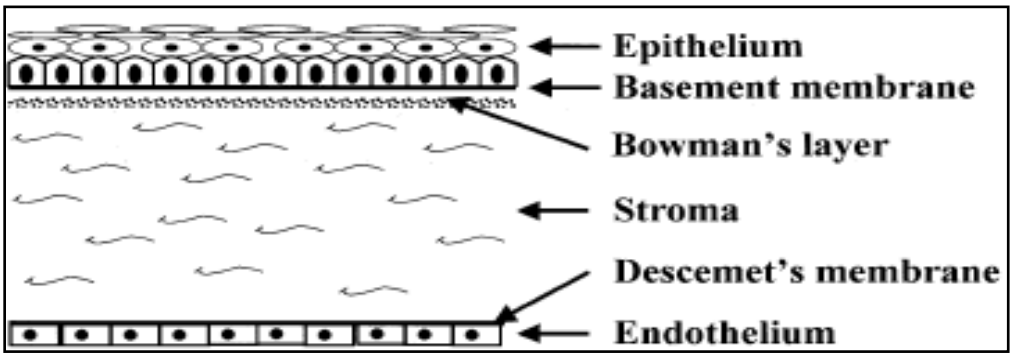

Figure (2) Diagrammatic representation of the human cornea in cross-section ***(Daniels et al, 2001)***.

The limbus is the transition zone between the cornea and sclero-conjunctiva. The limbal epithelium overlies a stroma containing a network of capillaries ***(Wei et al, 1993)***.

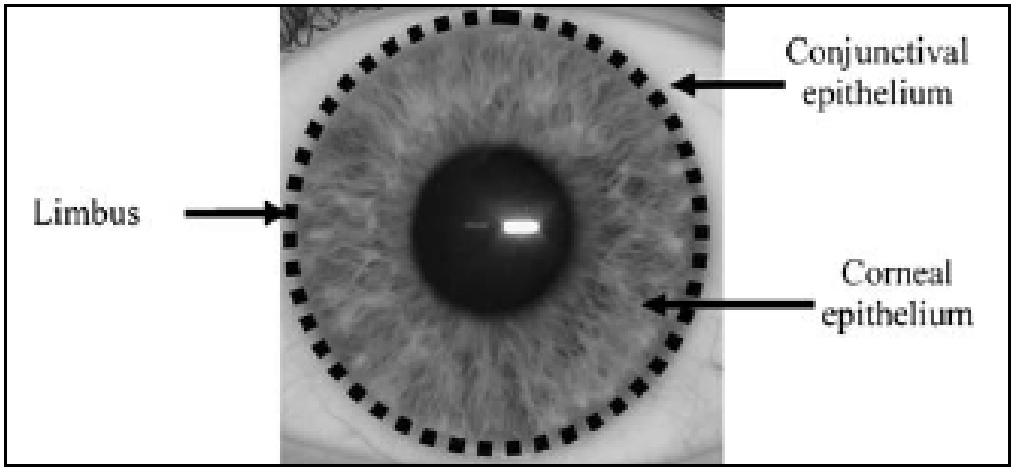

Figure (3) Location of the limbus. LESCs reside in the limbal region of the cornea, along the dashed line ***(Notara and Daniels, 2008)***.

The primary function of the corneal epithelium is to absorb nutrients and oxygen ***(Daniels et al, 2001)***, enable light to be transmitted to the interior of the eye ***(Wei et al, 1993)***, protect internal ocular structures from the external environment ***(Dua and Azuaro-Blanco, 2000a; Dua and Azuaro-Blanco, 2000b)*** and to serve as a support for the tear film ***(Wei et al, 1993)***.

The corneal epithelium superficial cells are constantly shed into the tear pool. Terminal differentiation of cells, coupled with cell death by apoptosis, prompts cell loss via desquamation, a process aided by eyelid blinking ***(Ren and Wilson, 1996)***.

A distinct population of SCs is located in the basal layer of the limbus ***(Davanger and Evensen, 1971; Schermer et al, 1986; Tseng, 1989)***. Limbal cells are the progenitors of corneal ***(Schermer et al, 1986; Cotsarelis et al, 1989)***, but not of conjunctival keratinocytes ***(Wei et al, 1996)***. SCs ensure that the corneal epithelium undergoes continual self-renewal and are responsible for epithelial tissue repair ***(Miller et al , 1993; Dua and Azuaro-Blanco, 2000a ; Dua and Azuaro-Blanco, 2000b; Daniels et al, 2001)***.