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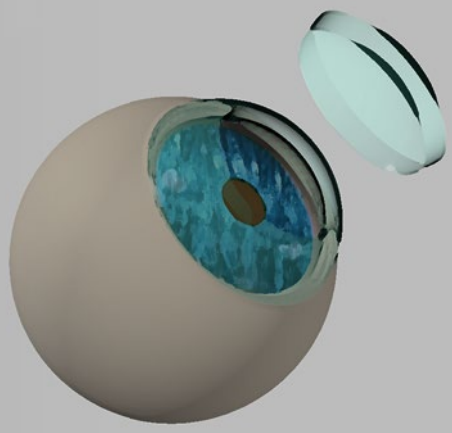

Mark Ahearne *Editor*

Corneal Regeneration

Methods and Protocols

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Contents

Chapter 4

Optimization of Human Limbal Stem Cell Culture by Replating a Single Limbal Explant

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Abstract

Cultured limbal epithelial stem cell transplantation is a clinical procedure used to regenerate the corneal epithelium in patients with limbal stem cell deficiency. The protocols used to expand limbal epithelial cells in vitro need to be optimized, since the scarcity of human ocular tissue donors is limiting the potential use of this procedure. Here, we describe a method to consecutively expand a single human limbal explant. With this method it is possible to obtain up to three limbal epithelial primary cultures from the same explant, thus increasing the efficiency of the in vitro cell culture.

Key words Cornea, Corneal epithelium, Corneoscleral samples, Limbus, Limbal explant, Limbal stem cells, Limbal primary cultures, In vitro expansion

1 Introduction

Limbal epithelial stem cells (LESCs) are responsible for corneal epithelium renewal [1, 2]. Corneal epithelium integrity is paramount for maintaining the corneal transparency required for optimal vision. However, several diseases, as well as chemical or physical insults, can compromise the integrity of the corneal epithelium, causing wounds and/or opacities. In many of these cases, corneal transplantation is a solution that may be used to restore sight. However, in the case of the destruction or dysfunction of LESCs or their niche, the outcome of corneal transplantation is usually poor [3]. In these cases, it is necessary to replace the missing stem cells to regenerate the corneal epithelium. To accomplish this, clinicians carry out cultured limbal epithelial transplantation (CLET). This therapy was first developed by Pellegrini et al. in 1997 [4] and has been reproduced in various clinical trials [5–11]. Since a limiting factor of this method is obtaining of enough

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