Functional reconstruction of corneal endothelium using nanotopography for tissue-engineering applications

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Abstract

Dysfunction in the corneal endothelium, which controls the hydration and transparency of the cornea, is one of the common reasons for transplantation. A tissue-engineered corneal endothelium is of interest for corneal regeneration and for in vitro testing of ocular drugs. In the native environment, corneal endothelial cells interact with the nanotopography of the underlying Descemet’s membrane. This study showed that nanotopography enhanced bovine corneal endothelial cell (BCEC) responses, creating a monolayer which resembled the healthy corneal endothelium. Topographies of different geometries were first tested to identify those that would elicit the most significant responses. A BCEC monolayer was then generated on both micro- and nanoscale pillars and wells. The BCEC monolayer cultured on topographies exhibited polygonal geometries with well-developed tight junction proteins. Scanning electron microscopy revealed that cells on pillars showed a higher density of microvilli, which was similar to native corneal endothelium. BCECs on nanopillars displayed a lower coefficient of variation of area (0.31) that was within the range of healthy corneal endothelium. More importantly, a BCEC monolayer cultured on nanopillars also had an enhanced Na+K+-ATPase immunofluorescence expression, mRNA upregulation and a higher Na+K+-ATPase activity. These results suggest that nanopillar substrate topography may provide relevant topographical cues, which could significantly enhance the formation and function of corneal endothelium.

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1. Introduction

The corneal endothelium is a monolayer of cells separating the corneal stroma from the anterior chamber. This layer controls corneal hydration, and thus the thickness and transparency of the cornea, by an ATP-dependent endothelial pump and a tight junction barrier [1]. Unlike corneal endothelial cells of other species, mature human corneal endothelial cells do not proliferate in vivo to replace dead or injured cells [2]. The area deficit caused by the death of corneal endothelial cells is instead compensated by cell enlargement and migration. When excessive endothelial cell loss occurs, the endothelium loses its function, causing corneal edema in the stroma and decreased corneal clarity, which eventually leads to loss of vision [3,4]. Corneal endothelial dysfunction is the most common reason for transplantation worldwide [5], and the existing available surgical treatments include penetrating keratoplasty (full-thickness transplantation) and endothelial keratoplasty [6].

The reconstruction of corneal endothelium has been of great interest due to its potential as a tissue-engineered replacement [7,8] or even as a tool for in vitro toxicology testing [9]. In vitro ocular toxicity tests offer a number of advantages over conventional animal tests: they are more economical, faster, avoid ethical issues with animal models, and reduce inconsistencies among data generated from different species [9,10]. Monolayers and stratified layers of corneal epithelial cells have been commonly used for testing drugs, contact lens or other irritants [11,12]. Recently, in vitro cultures of corneal endothelial cells have also been investigated as a means to test for toxic effects [13–15].

In the native environment, the basal layer of corneal endothelial cells is in contact with Descemet’s membrane. Descemet’s membrane consists of a densely packed interwoven mesh of nanosized fibers and pores [16]. Studies have also shown that cell–substratum and cell–cell interactions play important roles in vivo, and therefore the manipulation of these interactions in order to mimic the native environment can thus optimize conditions for cell