CHARACTERIZATION OF RETINAL PIGMENT EPITHELIAL CELLS 
AND ENDOTHELIAL CELLS WITHIN A MICROFLUIDIC DEVICE 
TOWARDS A RETINA ON A CHIP

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ABSTRACT

This paper reports a cell-based model of the ocular fundus within a microfluidic device. The characterization of human retinal pigment epithelial (ARPE) cells and human umbilical vein endothelial cells (HUVECs) cultured in the device was performed and found to exhibit properties found in native tissues. Also, in order to extend the culture model to an evaluation system for a drug delivery system (DDS) device, a microfluidic device was hybridized with native scleral tissues.

KEYWORDS: Ocular fundus, Organ on a chip, Retinal pigment epithelial cell

INTRODUCTION

Developing new three-dimensional (3D) in vitro cell culture models closely mimicking human tissues and organs (referred to as “organ on a chip”) has recently attracted great attention for disease modeling and drug discovery. In particular, there have been many attempts to mimic the digestive and respiratory systems such as the lung, liver and intestine. To date, however, few reported on on-chip culture models of sensory organs such as the eye exist [1]. Retinal diseases such as age-related macular degeneration (AMD) are one of the most common causes of visual impairment worldwide [2]. In the case of wet AMD, invasion of neovascularities from the choroid to the retina causes failure of the retinal function. However, the pathological mechanisms have not been completely elucidated. It is important to recapitulate certain aspects of human retinal functions, enabling the assessment of the cellular responses triggered by abnormal environment in the body.

EXPERIMENTAL

A microfluidic device having two parallel channels separated by a PDMS porous membrane containing an array of through-holes with a diameter of 10 microns was fabricated (Fig. 1). Human retinal pigment epithelial (ARPE) cells and human umbilical vein endothelial cells (HUVECs) were cultured in the device and morphological and functional characterizations were performed.

RESULTS AND DISCUSSION

When ARPE cells were cultured in the microfluidic device, a characteristic monolayer of hexagonal-shaped cells formed on the porous membrane (Fig. 2a). Also, as in native tissues, cells secreted vascular endothelial growth factor (VEGF) preferentially to the basolateral side (Fig. 2b). Moreover, the ratio of the VEGF secretion towards the basolateral side to that towards the apical side was higher when low glucose load was applied to the cells (Fig. 2c). Next, HUVECs were cultured in the microfluidic device...
and their response to VEGF was investigated (Fig. 3). When medium containing VEGF was allowed to flow through the upper channel while HUVECs existed on the lower side of the porous membrane (lower channel), cells migrated to the upper channel through the microholes in the membrane. The number of the migrating cells was four times larger compared to the case in which medium without VEGF was flowed (Fig. 3b). Finally, ARPE cells and HUVECs were co-cultured on either side of the membrane to mimic the ocular fundus tissue (Fig. 4).

Figure 2: (a) Immunostaining of the ARPE monolayer on the porous membrane (red: ZO-1, blue: nucleus, green: F-actin). (b, c) The concentration of VEGF secreted from cells to the apical and basolateral sides when (b) high or (c) low glucose medium was perfused.

Figure 3: (a) Sequential images of HUVECs on the porous membrane taken every hour. (b) Response of HUVECs to VEGF.

Figure 4: Co-cultured ARPE cells and HUVECs in the microfluidic device. ARPE cells and HUVECs were labeled with CellTracker Orange and Green, respectively.

The extension of the microfluidic culture model to an evaluation system for a drug delivery system (DDS) device was examined. We have been developing a transscleral DDS device that can deliver drugs to the retina by the transscleral route [3]. Primary evaluation of such devices by a culture model prior to animal experiments would accelerate the research. In order to modify the microfluidic cell culture model
to more closely mimic the structure of the ocular fundus, native sclera tissue from rabbits was integrated with microfluidic device (Fig. 5).

Figure 5: (a) Side and (b) top views of a microfluidic device hybridized with a native scleral tissue.

CONCLUSION
We have reported a cell-based model of the ocular fundus within a microfluidic device. The characterization of ARPE cells and HUVECs cultured in the device was performed and found to exhibit properties found in native tissues. Additional investigations of cultured ARPE cells in terms of trans-epithelial electrical resistance and permeability, and application of stimuli that induce pathological changes in ARPE cells would extend the system to a choroidal neovascularization model.

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