

A HUMAN BLINKING ‘EYE-ON-A-CHIP’

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ABSTRACT

This paper describes a human blinking eye-on-a-chip microdevice that reconstitutes the ocular surface in the human eye. To recapitulate the structural complexity of the ocular surface, we micro-patterned human cells derived from the cornea and conjunctiva on dome-shaped three-dimensional (3D) scaffolds in *in vivo*-like spatial arrangements. Moreover, this system was integrated with electromechanically actuated biomimetic eyelids created by 3D printing to simulate spontaneous eye blinking. Our eye-on-a-chip approach holds great potential to address critical limitations of existing eye models and may serve as an innovative platform to emulate human-relevant ocular physiology and pathology for biomedical, pharmaceutical, and environmental applications.

KEYWORDS: Microfluidics, Organ-on-a-chip, Biomimetics, Eye, Blinking, Cornea, Conjunctiva, Ocular surface, Cell culture, 3D printing

INTRODUCTION

The ocular surface is an anatomical and functional unit of the eye that protects the ocular system from external environments and provides a refractive surface for light transmission. As a principal protective barrier in this unit, the cornea consists of closely apposed epithelium and endothelium separated by a collagen-rich stromal tissue that contains keratocytes [1]. At the circumferential margin of the cornea, the corneal epithelium grades into the conjunctiva lined with goblet cells that are responsible for producing mucus component of the tear fluid [2]. The ocular surface is also under the constant influence of dynamic microenvironment created by spontaneous eye blinking-induced eyelid movements and concomitant spreading of the tear film that permits hydration and lubrication of the cornea and conjunctiva (Fig. 1A).

This structural, functional, and environmental complexity of the ocular surface, however, poses major technical challenges to *in vitro* investigation of its physiology and pathology using traditional cell culture models. As a result, research in this area has relied heavily on expensive and time-consuming *ex vivo* or *in vivo* animal studies that often fail to model biological responses in humans. These critical drawbacks of existing models have greatly limited our fundamental understanding and hampered the development of new therapeutic approaches to ocular surface diseases. To address this serious lack of physiological model systems, we have developed a microengineered organ-on-a-chip model of the human eye that replicates 3D architecture, physiological functionality, and dynamic mechanical microenvironment of the ocular surface.

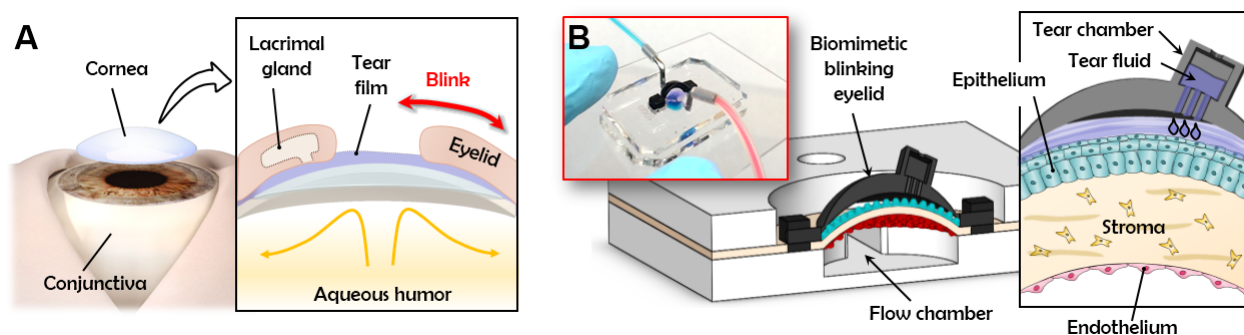


Figure 1. (A) The structure and microenvironment of the ocular surface in the human eye. (B) A microengineered organomimetic model that recapitulates the multi-layered 3D tissue structure, spontaneous blinking, and tear film dynamics of the human eye.

EXPERIMENTAL

The porous 3D shell scaffolds used in this model were generated by microengineering commercially available planar polystyrene cell culture scaffolds to generate 3D curvature that closely matched that of the cornea *in vivo*. These scaffolds were then incorporated into our eye-on-a-chip system by sandwiching them between upper and lower PDMS slabs that contained a circular chamber and a microfluidic channel, respectively. To recreate a stromal layer in the cornea, the sandwiched scaffold was impregnated with human primary keratocytes suspended in type I collagen gel. To demonstrate the feasibility of spatially patterning the scaffold surface to form the corneal and conjunctival epithelia, we first plated human corneal epithelial cells (HCECs) labeled with a green fluorescent dye at the center of the scaffold surface, and this step was followed by seeding of red-stained HCECs at the peripheral region. Simulation of eye blinking was accomplished by integrating a 3D-printed biomimetic eyelid into the upper chamber of the device. Blinking patterns and kinematics such as velocity, durations, and frequencies were precisely controlled by a computerized miniature DC motor.

RESULTS AND DISCUSSION

When hydrated during the seeding of keratocytes, the 3D shell scaffolds in our model retained the original curvature, and their structural stability was maintained throughout prolonged cell culture over 2 week (Fig. 2A). Our microengineered scaffolds were designed to have a radius of curvature of 5 mm to approximate the curvature of the human cornea [3] and contained complex networks of interconnected microscopic pores with an average diameter of 40 μm (Fig. 2B). When human keratocytes suspended in a collagen precursor solution were injected onto the scaffold surface, they penetrated deep into the scaffold and became lodged in the pores as gelation occurred. The primary keratocytes embedded in these porous scaffolds were maintained highly viable in culture over a period of 3 weeks.

To mimic the unique spatial distribution of different epithelial cell types on the ocular surface, we generated concentric patterns of epithelial tissues on our scaffolds using novel 3D cell patterning techniques. As shown in Fig. 2E, this approach enabled the selective deposition and growth of color-coded cell populations in different regions on the curved 3D scaffold surface to achieve *in vivo*-like epithelial patterning. These data show that our system provides new microengineering approaches to reproduce the 3D architecture and characteristic tissue organization of the human eye.

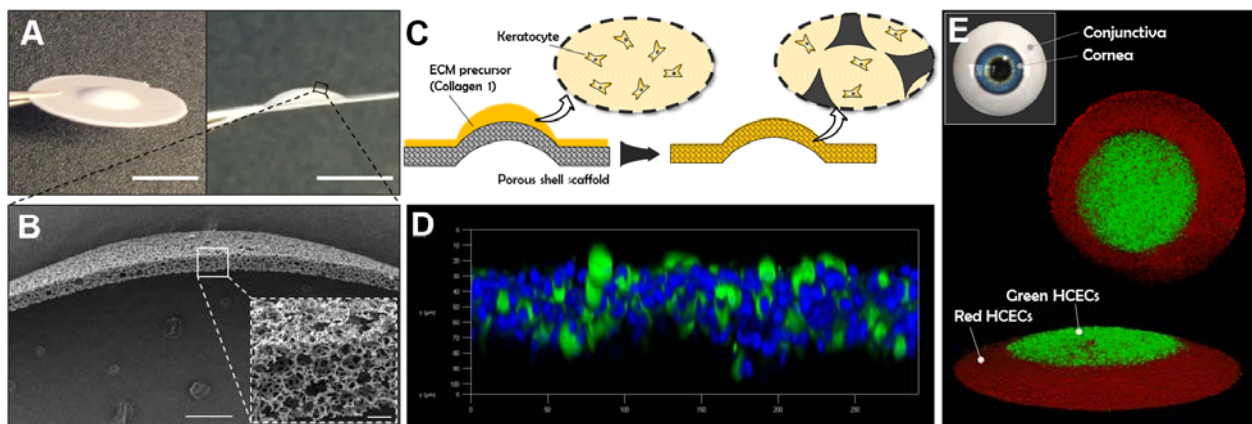


Figure 2. (A) Porous polystyrene shell scaffolds used in the eye-on-a-chip model. Scale bars, 5mm. (B) Scanning electron microscope (SEM) images of the scaffolds. Scale bars, 500 μm and 50 μm (inset). (C) Formation of corneal stroma through filling of scaffold pores with keratocytes and type I collagen gel. (D) Confocal image of fluorescently labeled keratocytes within the scaffold immediately after seeding. Green and blue represent cytoplasmic and nuclear staining, respectively. (E) 3D patterning of green and red HCECs on the curved scaffold surface to recapitulate human corneal and conjunctival tissues.

Blinking-like mechanical motions were generated by moving a 3D-printed thin shell structure connected to a miniature motor over the scaffold surface (Fig. 3). This system made it possible to actuate the eyelid layer at physiological blinking frequencies and speeds previously reported in the literature [4]. Our ongoing studies focus on leveraging this model system to recapitulate the dynamics of the tear film spreading and ocular surface hydration.

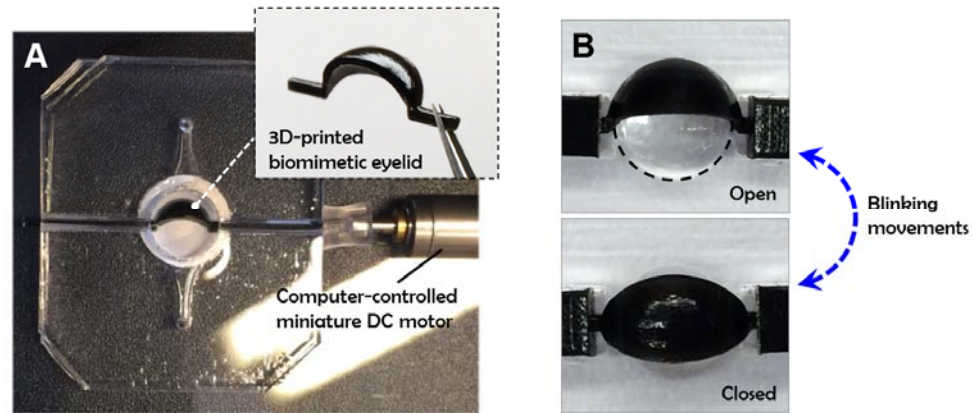


Figure 3. (A) A fully assembled eye-on-a-chip device that integrates PDMS microchannels, 3D cell culture scaffold, 3D-printed biomimetic eyelid, and DC motor-based actuation system. (B) Movements of the 3D-printed biomimetic eyelid over the dome-shaped 3D scaffold surface.

CONCLUSION

Our biomimetic microdevice provides new opportunities to develop novel *in vitro* eye models that allow for replication, visualization, and analysis of key biological processes involved in a wide variety of physiological and pathological situations in the human eye. We believe that the human blinking eye-on-a-chip system offers the promise to address critical technical barriers to progress in ophthalmology and many related areas. Moreover, this approach may enable the development of human disease models that represent cost-effective and more predictable alternatives to conventional animal models for the identification and development of new therapeutic approaches.

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