A Human Blinking 'Eye-on-a-chip'

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Introduction: The cornea is a critical component of the eye that serves as a protective layer and provides a smooth refractive surface for the transmission of incident light. It consists of a stratified squamous epithelium and a non-proliferative endothelium separated by an approximately 500 μm-thick dome-shaped stromal tissue containing keratocytes [1]. The corneal surface is also subjected to dynamic microenvironment created by spontaneous eye-blinking movements and concomitant spreading of the tear film that permits hydration and lubrication of the ocular surface. Despite the critical role of the cornea in health and disease of the human eye, studying its physiology and pathology has been greatly hampered due to the absence of physiologically relevant *in vitro* models. Here we describe a microengineered human blinking eye model that replicates 3D architecture, physiological functionality, and dynamic microenvironment of the cornea.

Materials and Methods: The 'eye-on-a-chip' is generated by forming fully differentiated human corneal epithelium and endothelium separated by a living stromal tissue (Fig. 1A). This is enabled by long-term co-culture of human corneal epithelial cells, keratocytes, and endothelial cells in a microengineered three-dimensional (3D) scaffold that retains the curvature of the native cornea. To recapitulate the dynamic mechanical microenvironment due to eye blinking, this microengineered corneal tissue is integrated with a 3D-printed biomimetic eyelid that can be electromechanically actuated to mimic blinking motions and to spread tear simulants over the corneal surface (Fig. 1B). Physiological patterns and kinematics of eye blinking are simulated by controlling the amplitude, duration, and frequency of movements using computer-controlled miniature motors.

Results and Discussion: We first demonstrated replica molding of hydrogels to produce optically transparent, porous, and thin-walled spherical shell scaffolds for cell culture (Fig. 1C). The functional tissue-tissue interface was also formed by co-culturing primary human corneal keratocytes with epithelial cells in physiological 3D structures (Fig. 1D). Furthermore, integration of the microengineered tissue structure with a 3D-printed biomimetic eyelid enabled recapitulation of cyclic blinking motions over the 3D cell culture scaffolds (Fig. 1E). Our on-going studied focus on the integration of these critical components in single microdevices and the automation of microfluidic cell culture and blinking actuation.

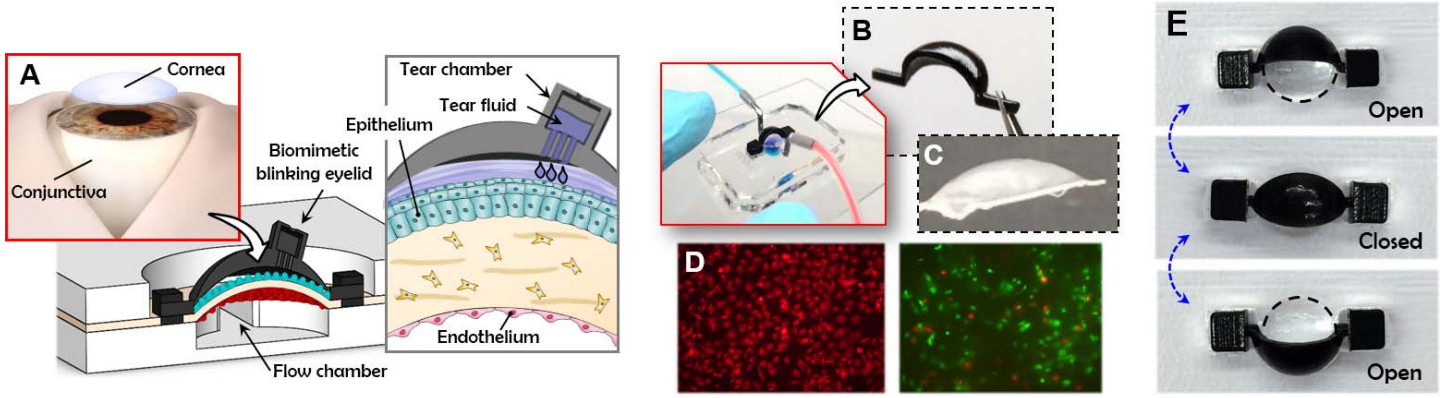

Figure 1 (A) A microengineered blinking 'eye-on-a-chip' that recapitulates the microenvironment and the multi-layered tissue structure of the human cornea. (B) A 3D-printed biomimetic eyelid. (C) Thin-walled spherical shell scaffold for reconstituting multilayered corneal tissue. (D) Co-culture of human primary corneal epithelial cells (left) and corneal keratocytes (right). (E) Blinking motions of the biomimetic eyelid.

Conclusions: Our biomimetic system holds great potential to serve as an innovative platform for replication, visualization, and analysis of physiological and pathological situations in the human eye. We believe that this human blinking eye-on-a-chip may address technical barriers to progress in ophthalmology and many other related areas. Furthermore, this system will provide new opportunities to develop specialized human disease models that represent cost-effective and more predictable alternatives to conventional animal models for the development of new therapeutic approaches.

References

1. "Prelude to corneal tissue engineering – Gaining control of collagen organization," J. W. Ruberti, J. D. Zieske, Prog Retin Eye Res (2008), **27**, 549-577.