Human cornea-on-a-chip – opportunities and challenges

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Purpose

Cornea is a tight barrier restricting topically administered drugs' penetration into the eye. The aim of the study was to create a tight epithelial barrier both with primary and with transformed human corneal cells on a 96-well microfluidic platform.

Methods

The primary human corneal epithelial cells (pHCEC) and transformed cell line (HCE-T) were seeded on AKITA® Plate96-Single 1 µm pore size microfluidic platform (Finnadvance) (Figure 1). After confluent cell layer was formed in three days in vitro, an air-liquid interface in an open-top chamber was established for an additional four days. Permeability experiments were performed for five fluorescent reference compounds, FITC-dextran 70 kDa (FD70), FD 4 kDa (FD4), 6-carboxyfluorescein (6-CF), Rhodamine 123 (Rho 123) and Rhodamine B (Rho B) from apical to basolateral (AB) direction, i.e. corneal surface to anterior chamber. Rho 123, a substrate for active transport processes, was tested from basolateral to apical (BA) direction as well.

Results

HCE-T cells created 7- to 10-fold lower apparent permeability coefficient (P_{app}) values compared to pHCEC cells with low permeability reference compounds. The P_{app} values for high permeability compound, Rho B, was 1.4-fold higher in pHCEC cells. P_{app} values across HCE-T cells are in line with rabbit corneal and *in vitro* values reported in literature previously[1] (Table 1). The active transport of Rho 123 was not detected in efflux ratio, P_{app}(BA)/P_{app}(AB). However, Rho 123 had much lower mass balance (%) in BA direction compared to AB direction (pHCEC 75% vs. 89% and HCE-T 66% vs. 104%, t-test P<0.001) which may indicate accumulation of Rho 123 into cells but lack of active transporter in the apical side.

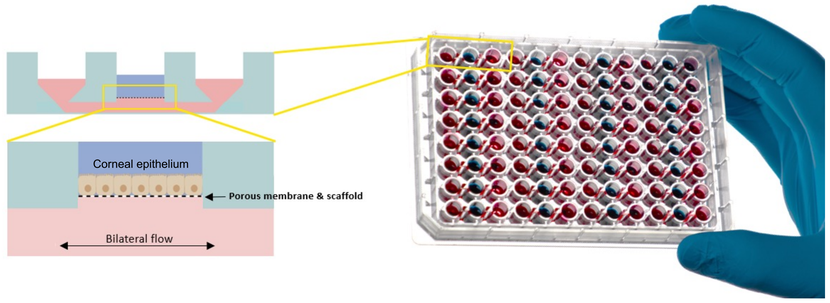

Figure 1. The AKITA® Organ-on-Chip Platform.

Table 1. Comparison of permeability P_{app} values between literature reported values and cornea-on-a-chip model with HCE-T cells.

Test molecule	P_{app} (cm/s) x 10^6		
	Rabbit Cornea	HCE-T cells grown in inserts	Cornea-on-a-chip model
FD70	Impermeable	0.07	Impermeable
FD4	0.06-0.4	1.43	1.02
Rho123 (AB)	0.15-0.42	2.22	2.56
6-CF	0.46-1.4	2.87	2.06
RhoB	9.1-18.1	40.22	29.08

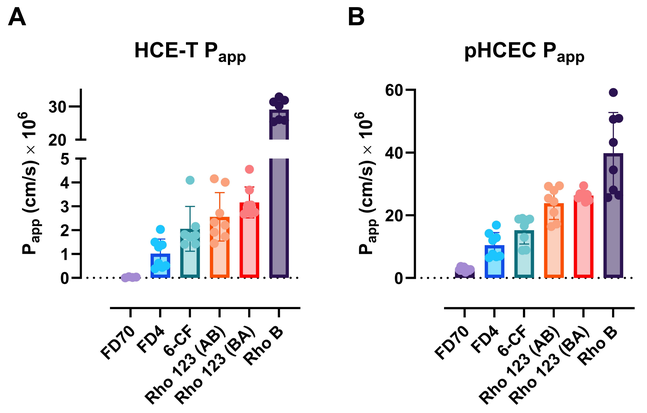

Figure 2. HCE-T (**A**) and pHCEC (**B**) cell barrier permeability P_{app} values for five fluorescent reference compounds on AKITA® Plate96-Single microfluidic platform.

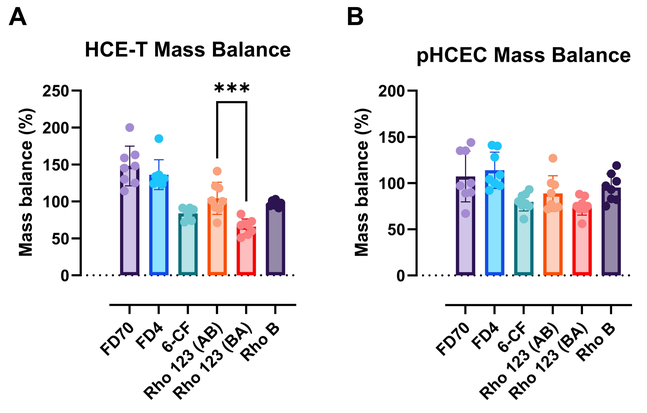

Figure 3. HCE-T (**A**) and pHCEC (**B**) cell barrier mass balance values for five fluorescent reference compounds on AKITA® Plate96-Single microfluidic platform.

Conclusion

HCE-T cells formed 7- to 10-fold tighter epithelial barrier in cornea-on-a-chip microfluidic platform compared to pHCEC cells. Thus, HCE-T cells are more suitable for cornea-on-a-chip model for drug permeability testing. Also, we observed a 4-fold faster maturation in the AKITA® microfluidic platform compared to cells grown in inserts. Further studies are needed to check expression of active transporters and their functionality in cornea-on-a-chip model.

Disclosures
DL, OV: none
THN: Finnadvance Ltd. (I,S,P)
SM: Finnadvance Ltd. (I,S)
JJH: Experimentica Ltd. (I,S)

References
[1]Žiniauskaitė A *et al.* Introducing an Efficient *In Vitro* Cornea Mimetic Model for Testing Drug Permeability. Sci. 2021;3(3),30.

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