Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

A microengineered human corneal epithelium-on-a-chip for eye drops mass transport evaluation

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Section S1.

Chip Validation: To ensure leakage-free operation, the membrane-embedded microchip was tested with two different food coloring dyes. Using a syringe pump (Chemyx Inc), two color dyes, red and blue, were flown in the apical and basal channels and the chip was monitored for any leakage around channels areas.

Particle translocation tests: To ensure corneal epithelial chip functionality in terms of translocation of molecules through the membrane, preliminary tests were carried out on porous PC membrane. Tests were carried out using the set-up shown in **Figure 1**; the Rhodamine B (1 mg mL^{-1}) and Rhodamine B loaded suspension (1 μm size, 1 mg mL^{-1}) were flown into the apical channel while distilled water was flown into the basal channel. The increase in the fluorescence intensity with time at the outlet of the lower channel was correlated to the diffusion into the channel. Diffusive variation in the basal channel at different time points were imaged by fluorescence microscope (Nikon Eclipse Ti, Nikon, Japan) using Nikon NIS-Element software and micrographs were analyzed by the ImageJ (http://rsbweb.nih.gov/ij/) software. Additionally, the samples at the outlet were analyzed by a microplate reader (Genesys, Tecan).

Section S2.

Cell culture: The hTCEpi cells were obtained from the Dr. Jester laboratory at the Gavin Herbert Eye Institute, Irvine, California, and were maintained and cultured in Epilife® medium (Invitrogen, Carlsbad, CA) supplemented with Epilife® defined growth supplement (EDGS, Invitrogen, Carlsbad, CA) and 1 % penicillin/streptomycin (Gibco, Carlsbad, CA) at 37 °C, 5 % CO_2, and were subcultured at least once a week. Experiments were used between passages 10~20. The medium was changed every 2 days and maintained for studies.

Membrane surface treatment for optimization and mimicry of the Bowman's Layer surface topography: Three different surface treatments were analyzed: fibronectin (50 μg mL^{-1} in PBS), gelatin (0.1 % in PBS) and rat tail collagen (0.05 mg mL^{-1} in 0.02 N acetic acid) as well as an untreated control. Prior to the surface treatment, the chip and all the fluidic

connectors were sterilized by 70% ethanol followed by UV germicidal irradiation (8 W Lamp, G30T8, Sankyo Denki) in a biosafety cabinet for 1 hour. The chip was injected with a volume of 10 μL of each protein solution and incubated at 37 °C for 2 - 3 hours. Excess protein was aspirated and the membranes allowed to air dry. Confluent hTCEpi cells in a flask were then washed with 1 × PBS and then were detached by incubation with Trypsin/EDTA for 4 minutes. This was neutralized with Trypsin neutralization solution and cells collected by centrifugation. Cells were then seeded on ECM treated membranes at a concentration of 1×10^5 cells mL^{-1}. Cell viability was evaluated after 5 days by Calcein-AM (Life Technologies) cell viability assay, as well as a well plate assay. Using this method, the fibronectin was selected as the most suitable ECM protein for this study.

Section S3.

Immunofluorescence imaging of corneal epithelial tissue construct: The corneal epithelium was constructed as stated above section. Prior to culture, as a 1st layer, the hTCEpi cells were prelabeled with CellTracker™ Red CMTPX dye (25 μM) (Life Technologies) in the dark for 30 min and seeded into the chip. The 2nd layer of hTCEpi cells was prelabeled with CellTracker™ Green CMFDA dye (25 μM) (Life Technologies) in dark for 30 min and cast on top of the 1st layer of the hTCEpi monolayer. The following layers of cells were alternative prelabeled with CellTracker™ dyes and constructed up to a 5 layer epithelium. In order to evaluate the monolayer and tight junction formation, the cells were washed with 1 × PBS thrice before being fixed with 4 % paraformaldehyde in PBS for 30 min. They were washed with 1 × PBS and immersed in Triton X-100 solution (0.2 % in 1 × PBS) for 15 min to permeabilize the cell membrane. The permeabilized cells were then washed thrice with 1 × PBS and were incubated for 30 min with 1 % bovine serum albumin (BSA) in 1 × PBS to block unspecific bindings. After blocking, double staining was performed to show the tight junctions and the actin cytoskeleton. Briefly, the monolayer cultures were incubated for 2 hours with primary (Zo-1 rabbit polyclonal antibody (Abcam Inc), 2.5 μg mL^{-1}) antibody, washed with PBS, then incubated 1 hour with secondary antibodies (Alexa Flour 488 conjugated Donkey Anti-Rabbit secondary antibody, 1 μg mL^{-1} rhodamine phalloidin (100

nM) (Abcam Inc) at room temperature. Finally, cells were counterstained with Hoechst 33342, trihydrochloride, trihydrate (Nucleus staining) for 10 min, and then washed thrice with 1× PBS and imaged under a fluorescence microscope.

In order to evaluate the multi cell layers constructions, each prelabeled cell construct (1 to 5 layer) were washed and fixed with 4% paraformaldehyde in PBS, that was washed with PBS and then Triton X-100 solution (0.1% in 1× PBS) was added for 15 min to permeabilize the cell membrane and were washed thrice with 1× PBS. Finally, nuclei were counterstained with Hoechst 33342 and washed thrice with 1× PBS. Then, the membrane-cell layers were cut out from the chip and mounted on the cover glass for confocal imaging by using Nikon TE2000 confocal microscope (Nikon, Japan). Image processing and visualization were done using the NIS-Elements Advanced Research software (Nikon, Japan) and ImageJ.

Barrier measurements: The barrier integrity of the corneal epithelial culture on chip was evaluated by measuring the transepithelial electrical resistance (TEER) measurements were also conducted using an EVOM2 system (World Precision Instruments, United States). The TEER was monitored longitudinally in inserts to determine media suitability. TEER values were calculated by subtracting the control resistance measured in the absence of cells and multiplying by the cell culture area.

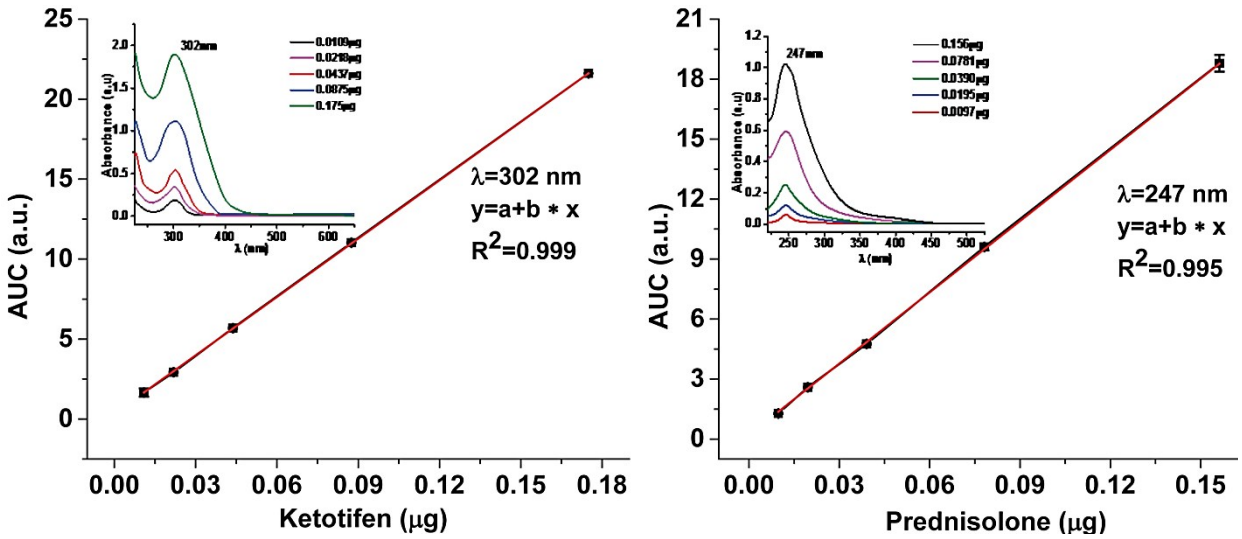

Figure S1. Calibration curve of drugs validated by UV-visible spectroscopic methods. This was found by plotting various concentrations of drugs versus absorbance with linear correlation coefficient. **(A)** The calibration curve was linear over the Pred Forte concentration range tested (0.1562, 0.0781, 0.0390, 0.0195, 0.0097 μg mL^{-1}), **(B)** The calibration curve was linear over the Zaditor concentration range tested (0.175, 0.0875, 0.0437, 0.0218, 0.0109 μg mL^{-1}). Results are explicit as (mean ± SD) of 3 independent experiments.

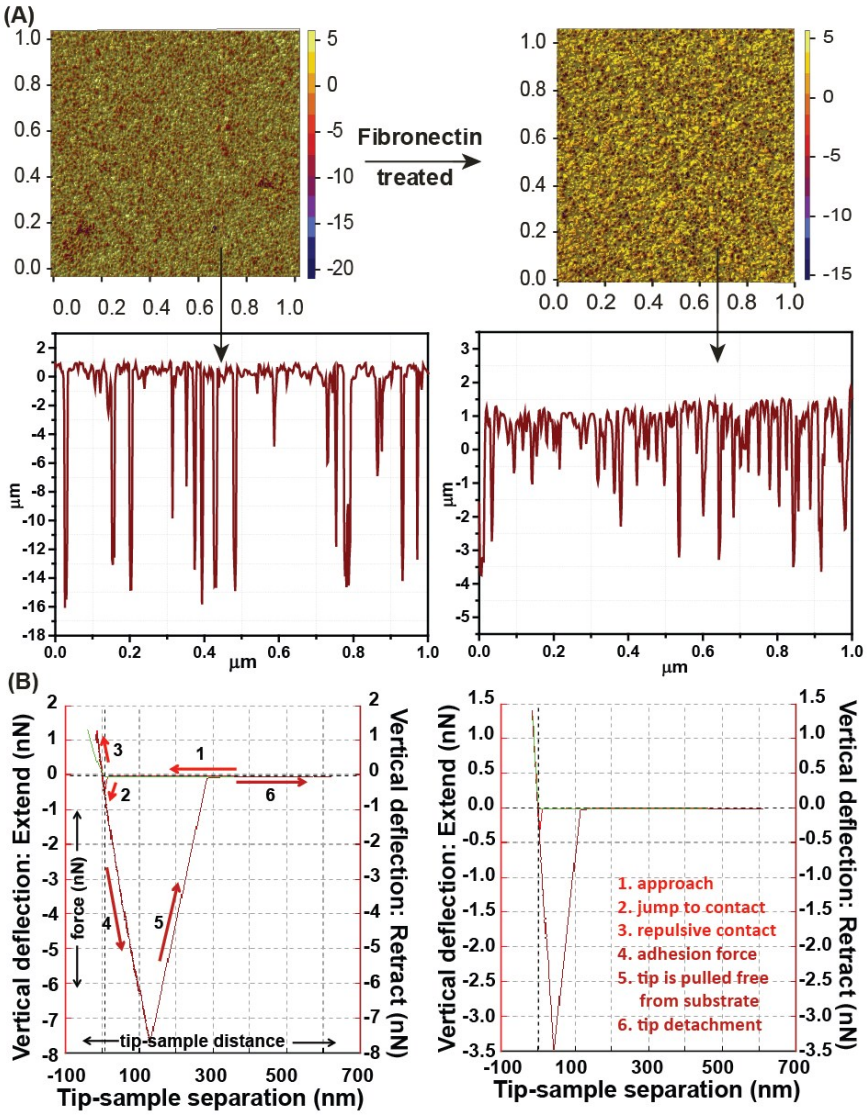

Figure S2. PC membrane functionalization and characterization. **(A)** 2D surface roughness profile of untreated PC (left) and Fibronectin modification/coating (right) obtained by an optical profilometer. **(B)** Force spectra (FS) of the untreated PC membrane **(left side)** and fibronectin treated PC membrane **(right side)**. The obtained Roughness and Young's modulus of untreated and fibronectin modified PC membrane values are given in the main text.

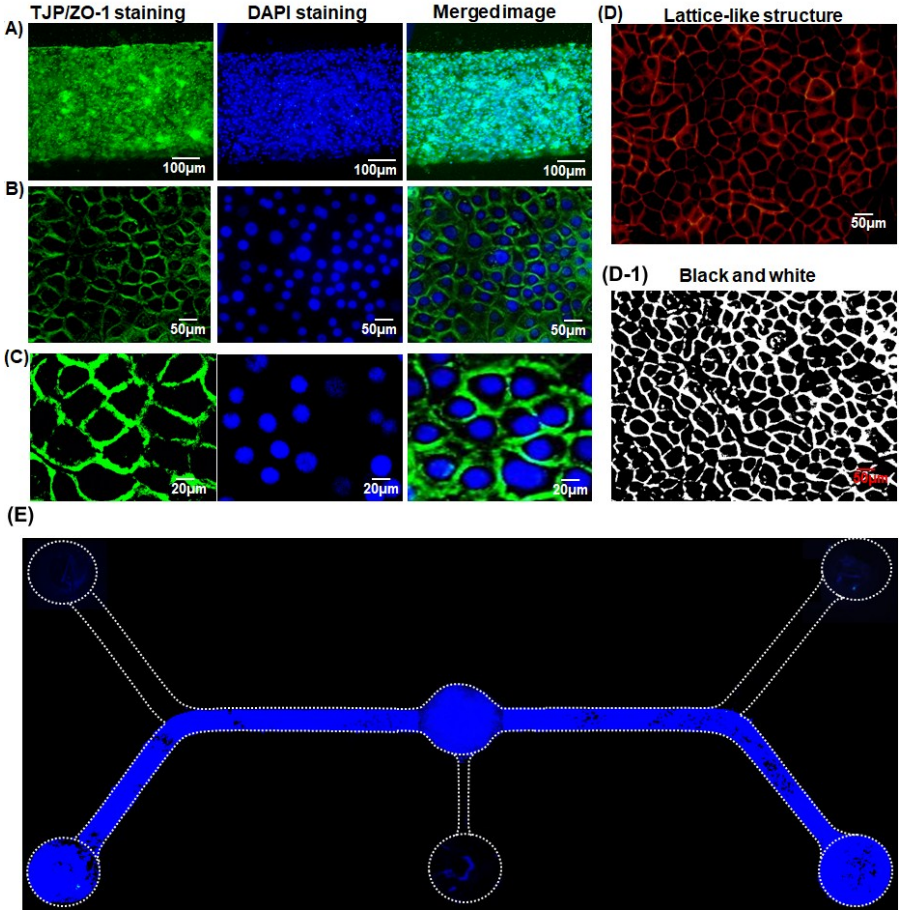

Figure S3. Corneal epithelial cells layer characterization in the corneal epithelial chip. Immunohistochemical analysis of tight-junction protein expression. The cells were cultured in the corneal epithelial chip and allowed to form a monolayer. After incubation for 5 days, cells were fixed and immunofluorescence staining was performed with antibodies against ZO-1 and counterstained with Hoechst. (A) 10 × magnification image. (B) 20× magnification image of a confluent monolayer of cells morphology show the typical tight junction between cells. (C) 40 × magnification image of epithelial culture shows the tight junction between cells. (D) The immunofluorescence staining with antibodies against ZO-1and F-actin show the integrity of epithelium and from the B/W thresholded image (D-1) clearly show the lattice-like structure of the epithelial tight junctions. (E) Confluent living cells on the chip stained with Hoechst dye blue fluorescent staining specific for DNA to show the cell distribution.

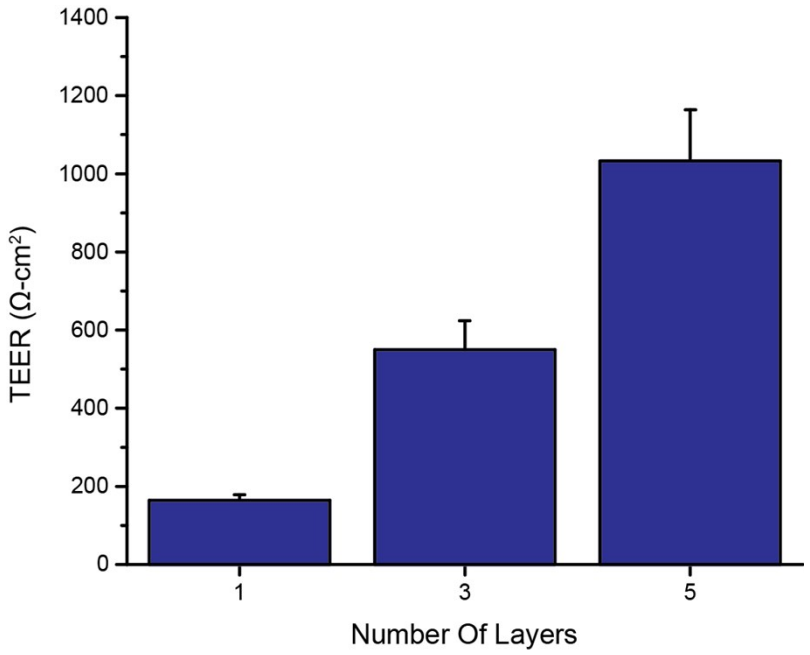

Figure S4. Barrier function was determined by measurement of trans-epithelial electrical resistance in different layer thickness of epithelium (n = 3).

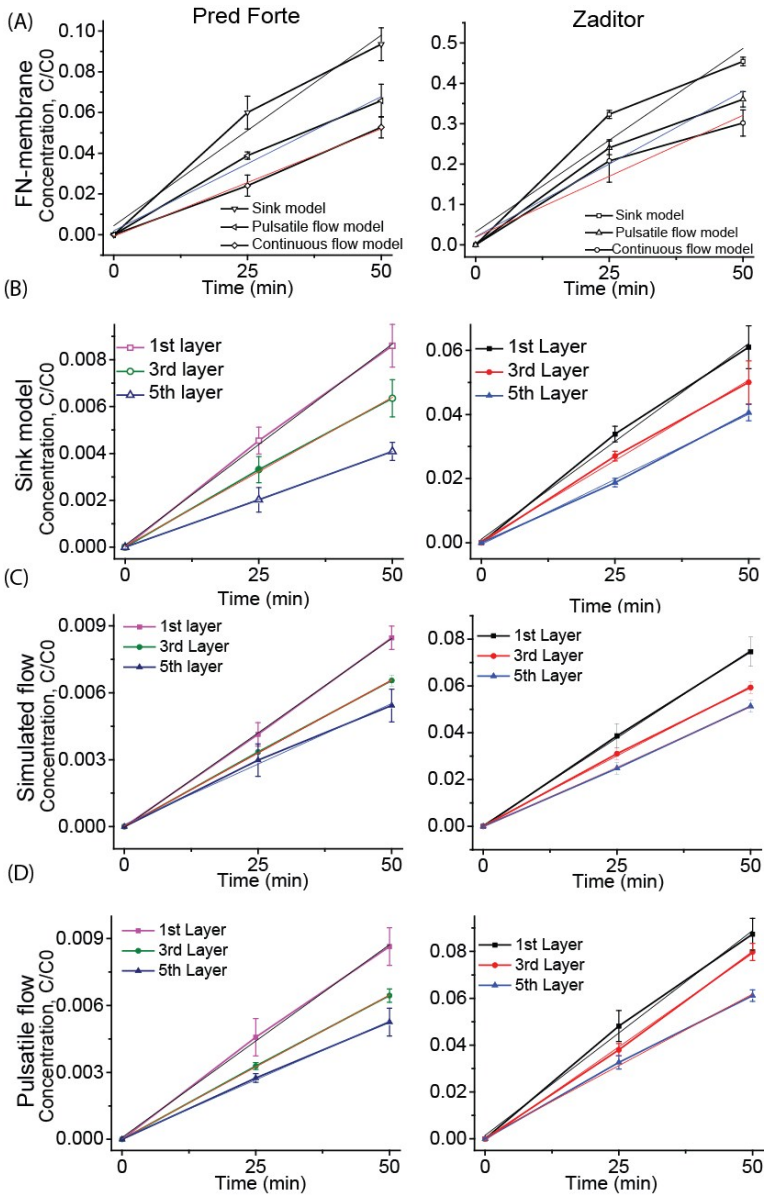

Figure S5. Results of drug mass transport experiments for membrane and cell layers under different condition. Graphs on the left (Pred Forte) report show details of short-term permeation (up to 50 min), graphs on the right (Zaditor) show details of short-term permeation (up to 50 min). This flushing causes the measured concentration to reach a steady state after that time. Each point represents the mean ± SD of three cultures of the corneal epithelial chip in a representative experiment.

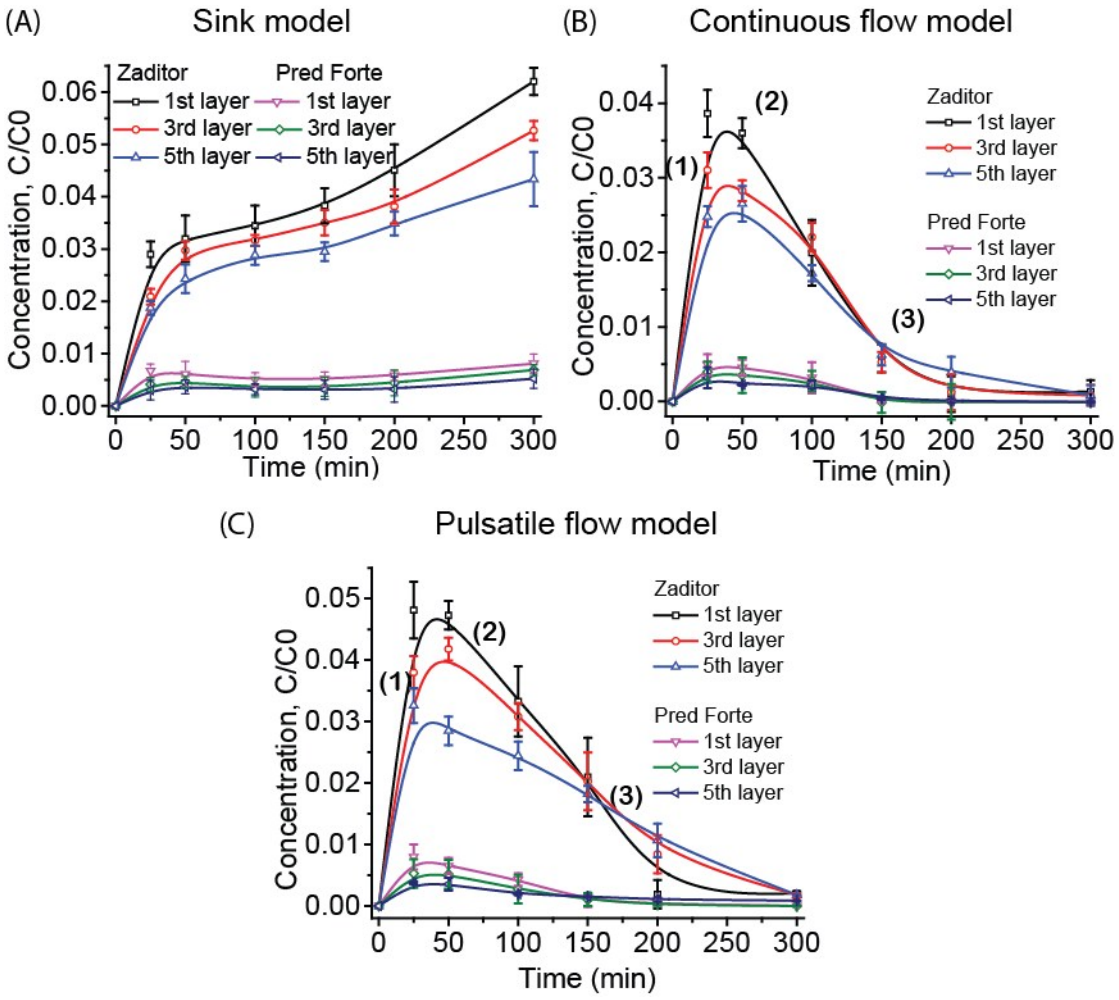

Figure S6. The fraction of drug concentrations in the corneal interstitial compartment with different experimental models. The appearance of Pred Forte and Zaditor in the receiver (corneal interstitial concentration) compartment during the predetermined time of experiment with cultured corneal epithelial cell layers on the corneal epithelial chip under different models. (A) Drug appearance under static condition. (B) Drug appearance under continuous flow condition. (C) Drug appearance under blinking associated pulsatile flow condition. The flow plot shows that the initial tear fluid drug application concentration is almost completely flushed after 100 minutes and the profile depicted the absorption phase (1) post absorption phase (2) and elimination phase (3). This flushing causes the measured concentration to reach

a steady state after that time. Each point represents the mean ± SD of three cultures of the corneal epithelial chip in a representative experiment.

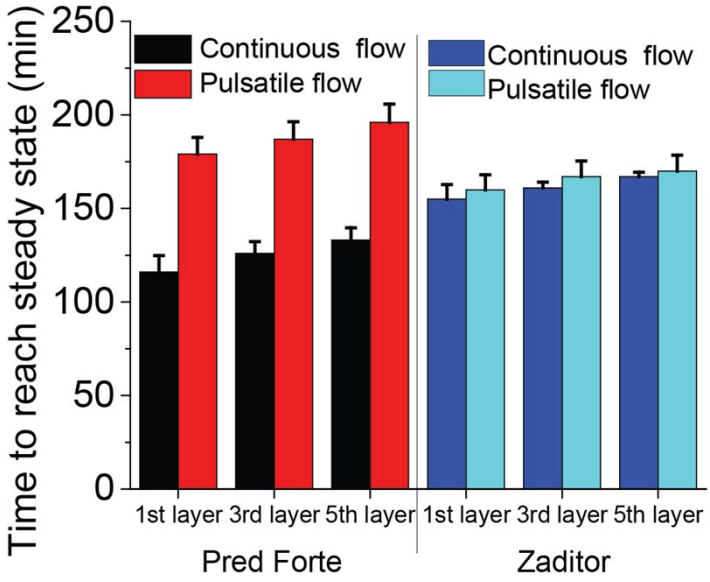

Figure S7. The continuous flow model is significantly faster than pulsatile flow model for reaching the steady state concentration of drugs in the cell layers permeability. Results are explicit as (mean ± SD) of 3 independent experiments.