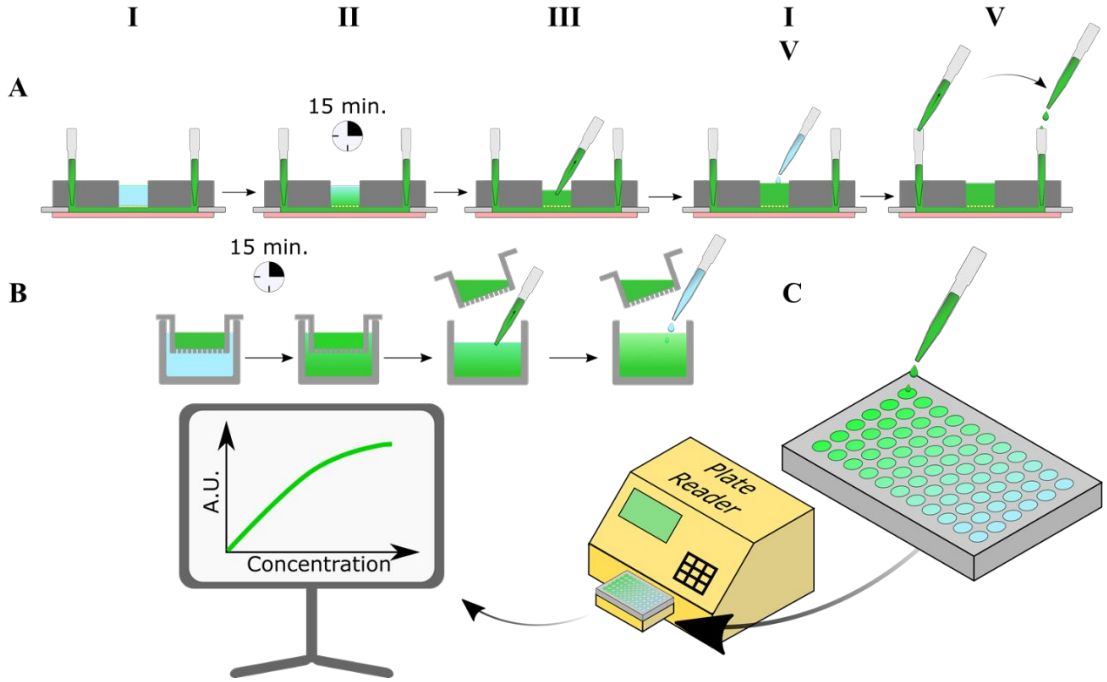
Supplemental Information for

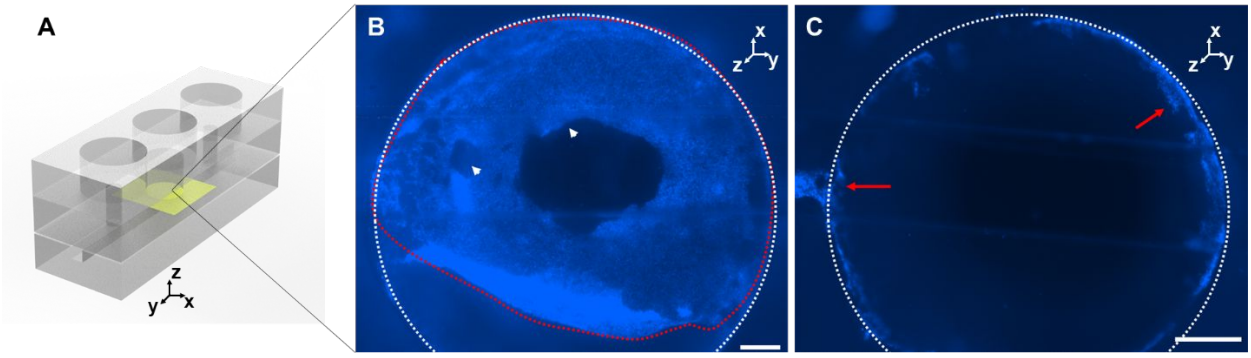
Collagen-I Based Enzymatically Degradable Membranes for Organ-on-a-chip Barrier Models

*Yusuf B. Arık[1,2]‡, Aisen de sa Vivas[1,2]‡, Daphne Laarveld[1], Neri van Laar[1], Jesse Gemser[1], Thomas Visscher[1], Albert van den Berg[2], Robert Passier[1,3], Andries D. van der Meer[1]**

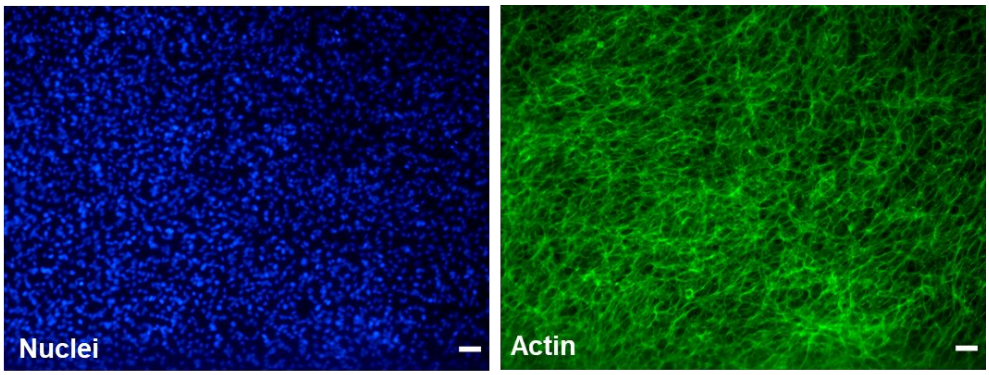
Results:



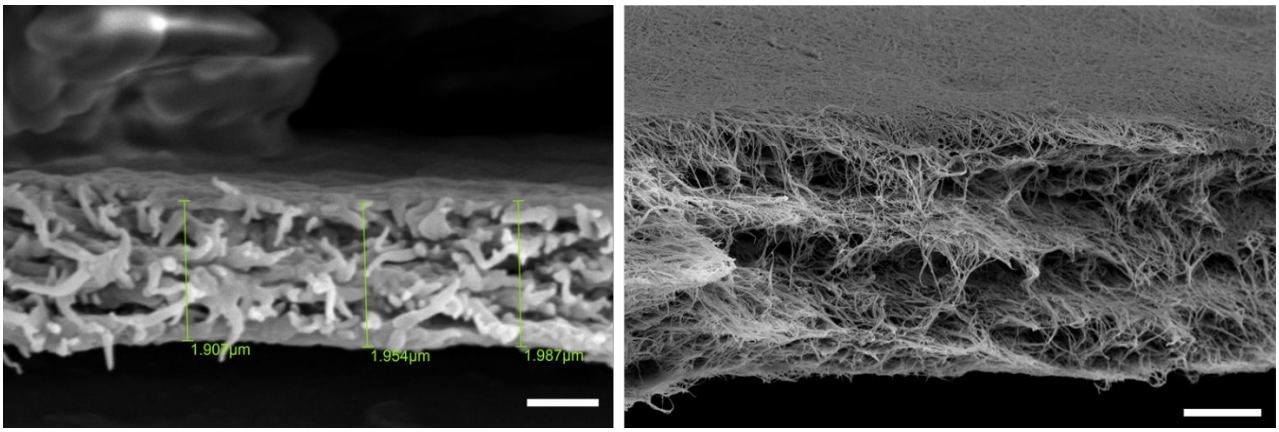
Supplementary Figure 1: Overview of the permeability assay. Permeability of membranes was measured by means of fluorescein diffusion. **(A)** On-chip permeability assay first starts with filling the culture chamber with PBS **(i)**. Second, fluorescein sodium salt diluted in PBS was pipetted into the microchannel **(ii)**, and every 15 min, 5µl of sample was collected from the culture chamber **(iii)**. The levels were normalized by adding PBS afterwards **(iv)**. In order to avoid heterogeneity in dye concentration along the microchannel, dye transferred from one inlet to the other after every sampling. **(B)** Permeability assay in transwells were used for comparison. (From left to right) First, fluid levels were normalized in insert (containing fluorescein) and bottom well (containing PBS). Afterwards, permeability assay was performed by collecting samples every 15min and normalizing the levels subsequently by adding PBS. **(C)** From both systems, the samples were collected and amount of fluorescein were measured using a plate reader. The fluorescent intensity profile was matched to concentration to calculate the permeability of the membranes.



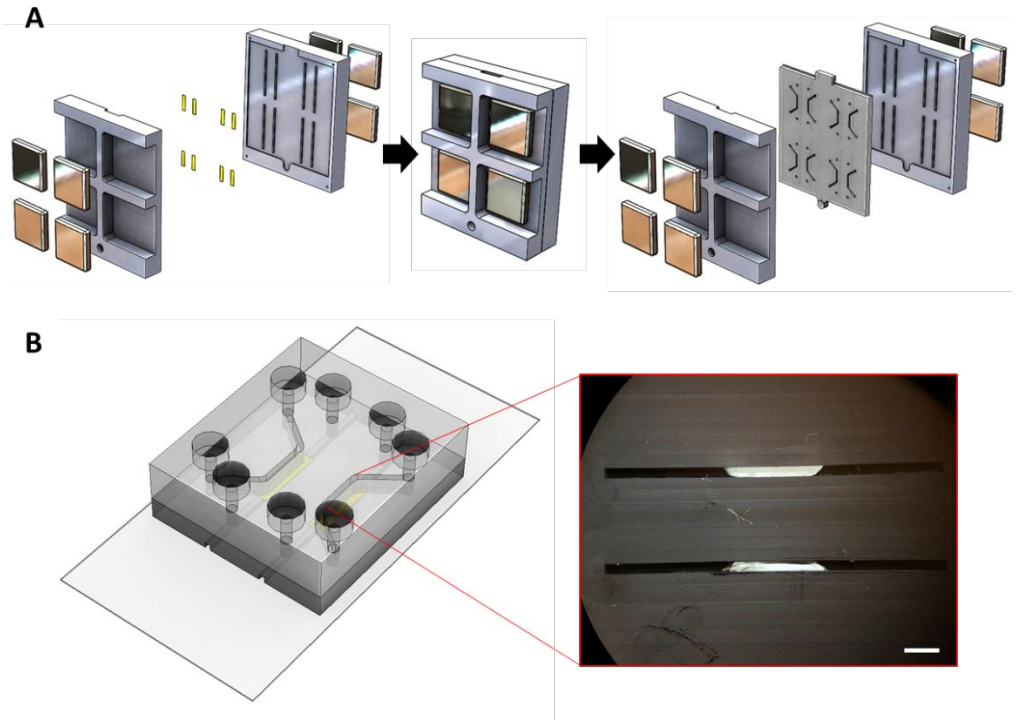
Supplementary Figure 2: High concentrations of collagenase treatment may cause ruptures on collagen membranes. (A) Organ-on-a-chip device schematics where membrane was integrated. **(B-C)** Two separate ARPE-19 seeded membranes stained for nuclei (DAPI, blue) were treated with 120 U/ml collagenase-2 3 days after cell seeding. Upon treatment membranes (red dashed lines) were detached from the culture chamber borders (white dashed lines). In addition, unstable membranes were punctured (white arrow heads) or left fragments (red arrows). Scale bars: 300 µm.



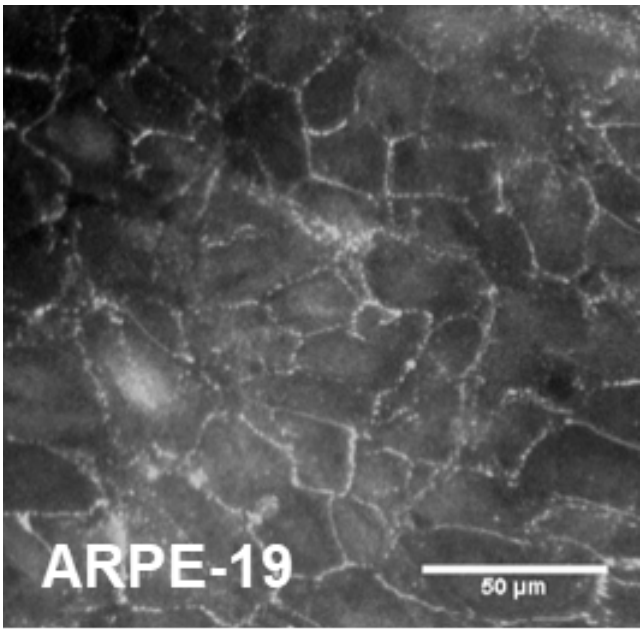
Supplementary Figure 3: Morphology and confluency of ARPE-19 cultured on collagen membranes in the organ-on-a-chip device. ARPE-19 stained for nuclei (DAPI, blue, left) and actin cytoskeleton (phalloidin, green, right) reveals a homogeneous distribution of a healthy population of ARPE-19. Scale bar: 50 µm



Supplementary Figure 4: SEM image of a sing and multi layered collagen-I membrane. (A) A fibrillar, and ~2 µm thick membrane was obtained after the fabrication process. Scale bar: 1 µm. (B) A multi-layered membrane containing 4 and 3 mg/ml collagen-I from top to bottom respectively. Scale bar: 10 µm



Supplementary Figure 5: Devices integrating collagen-I membranes can potentially be fabricated using injection molding design which eliminates the labor intensive assembly of devices. (A) Schematic overview of the fabrication of devices using injection molding. **(A-left)** Empty assembled mold prior to PDMS injection. **(A-middle)** assembled mold after PDMS injection. **(A-right)** Resulting PDMS devices following bake of PDMS. **(B-left)** Schematic overview of the final assembled device. **(B-right)** Resulting devices contain membranes free of PDMS where both channels intersect. Black PDMS was utilized to provide clarity and better illustration of the clean membrane area. Scale bar: 1mm



Supplementary Figure 6: ARPE-19 cell grown on collagen-I coated glass cover slips. These cells express their respective cell-cell adhesion markers (ZO-1) indicating a healthy population. Scale bar: 50µm.