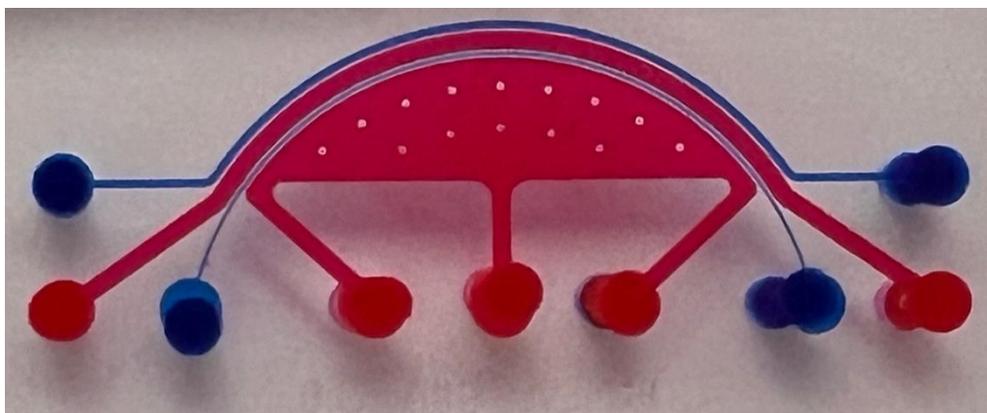




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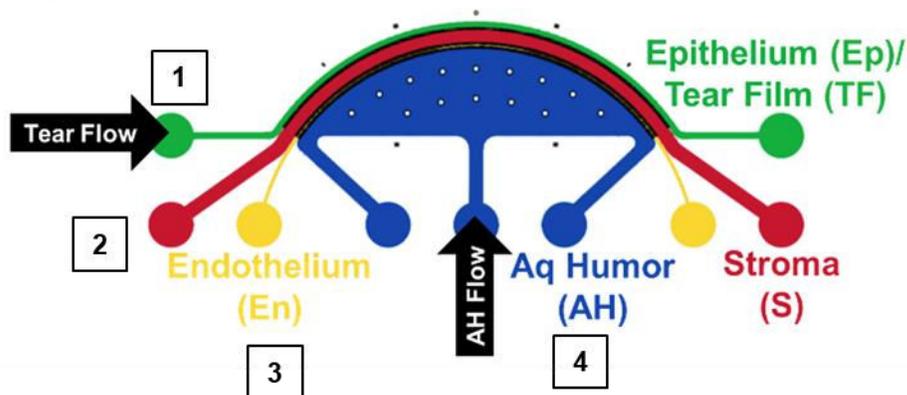


## SynOcu Cornea Model

For Chips and Starter Kits:  
**Catalog #s:** 109001, 501000

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## Introduction



*Schematic of the SynVivo SynOcu device; numbers denote channel identification*

The SynVivo SynOcu chip is a miniaturized, microfluidic device that emulates the structural, functional, and physiological characteristics of the human cornea. Physiologically, the cornea consists of a tear film over an epithelium, the structural Bowman's layer, a stromal layer, and the endothelium. Traditional 2D cell culture assays have significant limitations such as lack of physiological shear stress, real-time visualization capability and large number of consumables. The SynOcu recreates the in vivo microenvironment by mimicking a histological slice of corneal tissue cells. SynOcu is the only in vitro corneal model that allows:

- Accurate in vivo hemodynamic shear stress
- Real-time visualization of cellular and barrier functionality
- Significant reduction in cost and time
- Robust and easy to use protocols

## Workflow

The following workflow charts shows the timing of establishing SynVivo Cornea-on-Chip model:



*SynOcu Daily Workflow*

## SynVivo SynOcu Protocols

### Day 1: Establishing the Extracellular Matrix

#### Principle:

The extracellular matrix (ECM) is a structural framework of proteins that provides a scaffold for cells comprising a tissue. This protocol will use human fibronectin as the ECM, but the protocol can be adapted for other types of ECM, such as collagen or Matrigel.

#### Equipment:

- SynVivo SynOcu Devices (SynVivo #109001)
- Tygon tubing (0.02 inch ID x 0.06 inch OD, or 0.05 cm ID x 0.15 cm OD; SynVivo #201005)
- Blue slide clamps (SynVivo #202001)
- 1 mL syringe with Luer-Lock tip (25 pack; SynVivo #203005)
- 24-gauge blunt-tipped needles (0.5 inches or 1.27 cm long; SynVivo #204003)
- Pneumatic Primer (SynVivo #205001)
- Forceps
- Scissors

#### Reagents:

- 1X PBS without calcium or magnesium
- Human Plasma Fibronectin
- Human Corneal Epithelial Cell (HCEC) media

**Protocol:**

1. Dilute human fibronectin to a final concentration of 200 µg/ml in 1X PBS.
2. Attach a 24 G needle to a 1 mL syringe, and fit a ~6" length of Tygon tubing onto the end of the needle. Fill the Tygon tubing with the diluted fibronectin and insert the end of the Tygon tubing to Channel 1.
3. Perfuse Channel 1 with the fibronectin. Cut the inlet tubing using scissors. Repeat steps 2 and 3 for Channels 2 and 3.
4. Place all devices in the incubator (37°C) for **2 hours**.
5. After the incubation period, perfuse all channels with complete HCEC media. Clamp all the inlet and outlet ports except one.
6. Connect the one unclamped port to the Pneumatic Primer and prime under 7 PSI pressure for 15 minutes. (see **Pneumatic [Primer-Technical Manual](#)** for details).

## Day 1: Establishing the Human Corneal Epithelium

### Equipment:

- 1 mL BD plastic syringes or other 1 mL syringes (SynVivo #203004)
- 24-gauge blunt tip needles (SynVivo #204002)
- Tygon microbore tubing, 0.02" ID X 0.06" OD (SynVivo #201005)
- Clamps (SynVivo #202003)
- Forceps
- Syringe Pump capable of flow rates from 10 nL/min to 10  $\mu$ L/min
- Hemocytometer

### Reagents:

- Primary human corneal epithelial cells (HCECs) in a T75 flask (90% confluent). Suggested source: Cellprogen #36045-16, Millipore Sigma #SCCE016
- Human Corneal Epithelial Cell Media
- 0.05% Trypsin-EDTA
- Trypsin Neutralizing Solution
- 1X PBS without calcium or magnesium

## Establishing HCECs

### Protocol:

#### *Dissociating HCECs from the flask*

1. Rinse the flasks with 3 mL of 1X PBS to remove the FBS.
2. Add 3 mL of pre-warmed 0.05% trypsin-EDTA and incubate for 5-7 minutes at 37°C. Neutralize the trypsin with equal parts Trypsin Neutralizing Solution and pipette the cells into a conical tube for centrifugation.
3. Remove a sample for a cell count. Centrifuge the cells for 5 minutes at 200 g at room temperature.
4. Aspirate the supernatant and resuspend the cells to  $4 \times 10^7$  cells/mL in complete HCEC media.

#### *Seeding HCECs*

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp the outlet port of Channel 1. Leave the other ports clamped.
3. Place a drop of liquid beside the tubing for the Channel 1 inlet port and remove the Tygon tubing.
4. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
  - a. Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
  - b. Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3  $\mu$ L/min.
7. Once Channel 1 is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel. **Make sure that the cells are densely packed to establish a good lumen.**
8. Carefully cut the inlet tubing, keeping the length of the inlet tubing as small as possible.
9. Place the device in a 37 °C incubator for 4 hours before proceeding to **Day 1: Establishing Flow** below.

## Day 1: Establishing Flow

### Protocol:

1. After 4 hours of incubation at 37 °C, check the SynOcu device under the microscope for cell attachment.
2. For each experiment, prepare a 1-mL syringe with complete HCEC media. Attach a length of Tygon tubing to the syringe that is long enough to reach from the syringe pump to the device inside the tissue culture incubator.
3. Mount the syringes onto the syringe pump and perfuse the Tygon tubing completely, ensuring no air bubbles are in the tubing.
4. Place a drop of water at the base of the Channel 1 inlet port tubing to be removed and remove the tubing with forceps.
5. Remove the clamp on the outlet port of Channel 1. Keep all other ports clamped. To capture the effluent during the media change, a small tube can be placed under the outlet tubing to serve as a waste reservoir.
6. While holding the end of the Tygon tubing above the level of the SynOcu device, use the fast perfuse function on the syringe pump to push the media forward until a droplet forms at the end of the tubing.
7. Insert the tubing into the inlet port. The drop of water will prevent air from entering the device as the tubing is inserted.
8. Clean the fluid from the surface of the device.
9. Start the syringe pump program (see **Pump Programming** below)
10. Incubate the device overnight in a 37 °C incubator, 5% CO<sub>2</sub>.

### *Pump Programming*

Step 1: 3 µL/min Infuse for 5 minutes

Step 2: 0.1 µL/min for 24 hours

## Day 2: Establishing Human Corneal Fibroblast Cells

### Equipment:

- 1 mL BD plastic syringes or other 1 mL syringes (SynVivo #203004)
- 24-gauge blunt tip needles (SynVivo #204002)
- Tygon microbore tubing, 0.02" ID X 0.06" OD (SynVivo #201005)
- Clamps (SynVivo #202003)
- Forceps
- Syringe Pump capable of flow rates from 10 nL/min to 10  $\mu$ L/min
- Hemocytometer

### Reagents:

- Human Corneal Fibroblast Cells (HCFs) in a T75 flask (90% confluent).
- HCF Complete Media
- HCF serum-free basal media
- 0.05% Trypsin-EDTA
- Trypsin Neutralizing Solution
- Matrigel
- 1X PBS without calcium or magnesium

## Establishing Human Corneal Fibroblast Cells

### Protocol:

#### *Dissociating HCFs*

1. Rinse the flask with 3 mL of 1X PBS to remove the FBS.
2. Add 3 mL of pre-warmed 0.05% trypsin-EDTA and incubate for 5-7 minutes at 37°C. Neutralize the trypsin with equal parts Trypsin Neutralizing Solution and pipette the cells into a conical tube for centrifugation.
3. Remove a sample for a cell count. Centrifuge the cells for 5 minutes at 200 g at room temperature.
4. Mix 800  $\mu$ L of HCF basal media with 200  $\mu$ L of ice-cold Matrigel. Keep the solution on ice.
5. Aspirate the supernatant and resuspend the HCFs to  $1 \times 10^7$  cells/mL in the diluted Matrigel solution. **Keep the cell suspension on ice until ready to seed.**

#### *Seeding HCFs*

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp the outlet port of Channel 2. Leave the other ports clamped.
3. Place a drop of liquid beside the tubing for the Channel 2 inlet port and remove the Tygon tubing.
4. Quickly load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
  - a. Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
  - b. Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3  $\mu$ L/min.
7. Once the Channel 2 is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel. **Make sure that the cells are densely packed to establish the 3D stroma.**
8. Carefully cut the inlet tubing, keeping the length of the inlet tubing as small as possible.
9. Place the device in a 37 °C incubator. The Matrigel will solidify.
10. After 15 minutes, re-attach the HCECs to the syringe pump and flow 0.1  $\mu$ L/min overnight.

## Day 3: SynOcu Device Maintenance

**Note: Channel 1 will remain flowing at 0.1 $\mu$ L/min with HCEC media. This protocol establishes a continuous media exchange in Channel 3 with HCF media.**

### Protocol:

1. Perfuse Channels 2, 3, and 4 with freshly-prepared HCF complete growth media.
2. For each experiment, prepare a 1-mL syringe with complete HCF media. Attach a length of Tygon tubing to the syringe that is long enough to reach from the syringe pump to the device inside the tissue culture incubator.
3. Mount the syringes onto the syringe pump and perfuse the Tygon tubing completely, ensuring no air bubbles are in the tubing.
4. Place a drop of water at the base of the Channel 3 inlet port tubing to be removed and remove the tubing with forceps.
5. Remove the clamp on the outlet port of Channel 3. Keep Channels 2 and 4 clamped. To capture the effluent during the media change, a small tube can be placed under the outlet tubing to serve as a waste reservoir.
6. While holding the end of the Tygon tubing above the level of the SynOcu device, use the fast perfuse function on the syringe pump to push the media forward until a droplet forms at the end of the tubing.
7. Insert the tubing into the inlet port. The drop of water will prevent air from entering the device as the tubing is inserted.
8. Clean the fluid from the surface of the device.
9. Start the syringe pump program (see **Pump Programming** below)
10. Incubate the device overnight in a 37 °C incubator, 5% CO<sub>2</sub>.

### *Pump Programming*

Step 1: 0.01  $\mu$ L/min Infuse for 24 hours

## Day 4: Establishing Human Corneal Endothelial Cells

### Equipment:

- 1 mL BD plastic syringes or other 1 mL syringes (SynVivo #203004)
- 24-gauge blunt tip needles (SynVivo #204002)
- Tygon microbore tubing, 0.02" ID X 0.06" OD (SynVivo #201005)
- Clamps (SynVivo #202003)
- Forceps
- Syringe Pump capable of flow rates from 10 nL/min to 10  $\mu$ L/min
- Hemocytometer

### Reagents:

- Human Corneal Endothelial Cells (HCENs) in a T75 flask (90% confluent). Suggested source: Cellprogen #36081-13
- HCEN Complete Media
- 0.05% Trypsin-EDTA
- Trypsin Neutralizing Solution
- 1X PBS without calcium or magnesium

## Establishing Human Corneal Endothelial Cells

### Protocol:

#### *Dissociating HCENs*

1. Rinse the HCEN flask with 3 mL of 1X PBS to remove the FBS.
2. Add 3mL of pre-warmed 0.05% trypsin-EDTA and incubate for 5-7 minutes at 37°C. Neutralize the trypsin with equal parts Trypsin Neutralizing Solution and pipette the cells into a conical tube for centrifugation.
3. Remove a sample for a cell count. Centrifuge the cells for 5 minutes at 200 g at room temperature.
4. Aspirate the supernatant and resuspend the HCENs to  $4 \times 10^7$  cells/mL in HCEN media.

#### *Seeding HCENs*

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp the outlet port of Channel 3.
3. Place a drop of liquid beside the tubing for the Channel 3 inlet port and remove the Tygon tubing.
4. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
  - a. Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
  - b. Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3  $\mu$ L/min.
7. Once the Channel 3 is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel. Carefully cut the inlet tubing, keeping the length of the inlet tubing as small as possible.
8. Place the device in a 37 °C incubator for 4 hours before proceeding to **Day 4: Flow Continuity.**

## Day 4: Flow Continuity

### Protocol:

1. After 4 hours of incubation at 37 °C, check the SynOcu device under the microscope for cell attachment.
2. Re-attach Channel 1 to the HCEC media and continue flow at 0.1 µL/min for 24 hours.
3. For each experiment, prepare a 1-mL syringe with complete HCEN media. Attach a length of Tygon tubing to the syringe that is long enough to reach from the syringe pump to the device inside the tissue culture incubator.
4. Mount the syringes onto the syringe pump and perfuse the Tygon tubing completely, ensuring no air bubbles are in the tubing.
5. Place a drop of water at the base of the Channel 4 inlet port tubing to be removed and remove the tubing with forceps. Keep the central Channel 4 port clamped.
6. Remove the clamp on the outlet port of Channel 4. To capture the effluent during the media change, a small tube can be placed under the outlet tubing to serve as a waste reservoir.
7. While holding the end of the Tygon tubing above the level of the SynOcu device, use the fast perfuse function on the syringe pump to push the media forward until a droplet forms at the end of the tubing.
8. Insert the tubing into the inlet port. The drop of water will prevent air from entering the device as the tubing is inserted.
9. Clean the fluid from the surface of the device.
10. Start the syringe pump program (see **Pump Programming** below)
11. Incubate the device overnight in a 37 °C incubator, 5% CO<sub>2</sub>.

### *Pump Programming*

Step 1: 3 µL/min Infuse for 5 minutes

Step 2: 0.1 µL/min for 24 hours

**On Day 5, SynOcu is ready for assay!**

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