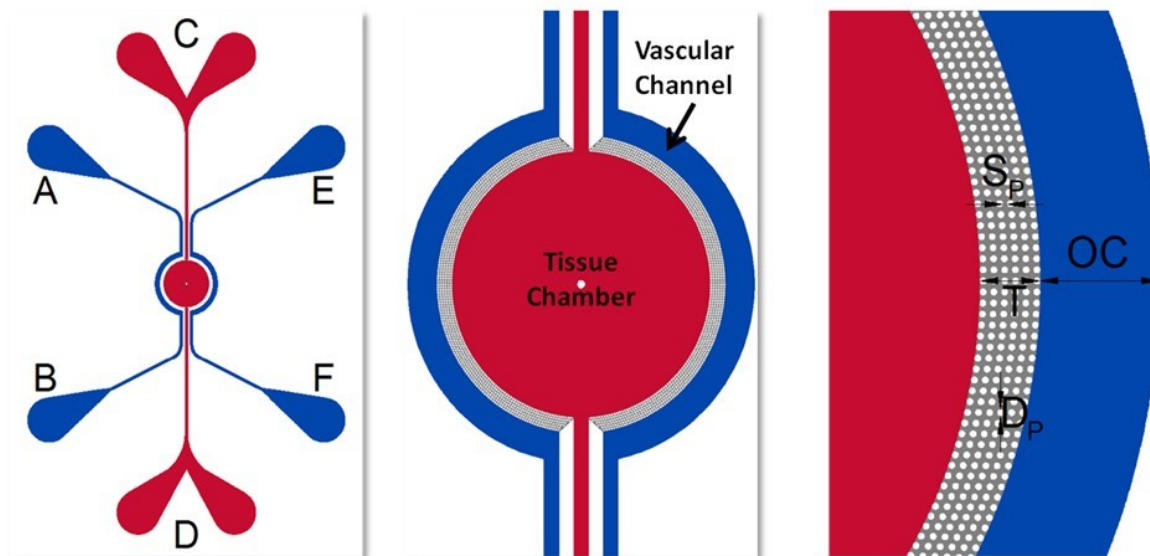


3D Tumor Assays Using SynTumor DESIGN 1 OR 2 Chips – Technical Manual

Catalog #s SY-102004, SY-102012, SY-103002 and SY-103004



Schematic of the Tumor Model Chip: Vascular channels are for culture of endothelial cells) while the central chamber is for culture of tumor cells. Porous architecture enables communication between the vascular and tumor cells and migration.

Overview of Assay

Tumor drug delivery is a complex phenomenon affected by several elements in addition to drug or delivery vehicle's physico-chemical properties. Tumor microvasculature has many unique features including unusual transport characteristics, high interstitial pressure, and enhanced permeability and retention (EPR) effect. Current static *in vitro* models of tumor drug delivery do not account for transport across the vascular endothelium; do not reproduce the complex network structure and fluid shear observed in the *in vivo* tumor microenvironment; rely exclusively on diffusion of the drugs to permeate the tumors, and do not allow real-time visualization to study the delivery of the drug or the drug carrier due to the use of semi-permeable membrane. Therefore, results usually show poor correlation with *in vivo* performance.

The Tumor assay has overcome these limitations to provide an entirely new system for studying the drug/endothelium, drug/tumor interaction in a realistic and dynamic tumor microenvironment. By emulating a histological slice of co-cultured tissue and/or tumor cells with a lumen of endothelial cells, the platform delivers a physiologically realistic model including flow and shear in a platform and enables real-time tracking of drug carrier binding and extravasation processes.

Materials Needed

- Tumor Chips* (Catalog #s 102004, 102012, 103002 or 103004)
- Pneumatic Primer Device (Catalog #205001) - optional
- 1 mL BD plastic syringes or other 1 mL syringes (Catalog #203004)
- 24 gauge blunt tip needles (Catalog #204002)
- Tygon microbore tubing, 0.02" ID X 0.06" OD (Catalog #201005)
- Clamps (Catalog #202003)
- Forceps
- Syringe Pump capable of flow rates from 10nl/min to 10ul/min
- Fibronectin
- Matrigel
- Endothelial Cells
- Tumor Cells

*** Store at Room Temperature. Shelf life is 6 months at room temperature**

Priming the Device Using Pneumatic Primer (Cat# 205001)

1. Place approximately 1 inch long segments of Tygon tubing into the outlet ports of the device.
2. Draw PBS into a 1 mL syringe. Using additional 1inch long segments of tubing, fill the device with liquid by inserting the primed tubing into the inlet and pushing the solution through until the outlet tubing is filled.
3. Do this for all but one inlet port. For this last port, use tubing approximately 2-3 inch long. When the device is filled, unlock the needle from the syringe, leaving the needle attached to the tubing.
4. Clamp all tubing below the liquid line, except for the tubing with the needle attached.
5. Connect the device to the Pneumatic Primer by locking the needle into the LuerLock connector
Note: Multiple devices can be primed simultaneously using the multiple port manifold (cat # 207001)
6. Turn the knob on the controller box and adjust the pressure to ~5-7 psi. Apply the pressure for ~5-20 minutes. Devices will take at least 15 minutes to completely fill.
7. Turn off the pressure and cut the Tygon tubing connected to the Pneumatic Primer.
8. Allow the device to incubate at 37° for a minimum of 1 hour before use.
9. Flush fresh media into device just before seeding endothelial cells.

Matrigel Core Setup

1. Clamp off both vascular channels to prevent the Matrigel from crossing the barrier.
2. Thaw Matrigel on ice followed by dilution to 1:2 in serum free media
3. With the device on a hot plate or in the incubator, quickly inject the Matrigel into the center chamber of the device until 2 drops come out of the outlet tubing.
4. Place the device in the incubator for at least 15 minutes to allow Matrigel to polymerize.

Tumor Cell Seeding and Assay Setup

1. Dissociate tumor cells and resuspend them approximately $5-8 \times 10^6$ cells/ml.
2. Thaw Matrigel on ice and dilute Matrigel 1:5 in serum free media.
3. Resuspend pelleted tumor cells in 50-100ul of the matrigel dilution. Keep suspension on ice.
4. Clamp off all but one vascular channel and seed cells into the open vascular channel. Cells should be dense within the slurry. Clamp the inlet tubing to stop flow
5. Set the device in the incubator for 2 hours to allow the Matrigel to polymerize and the cells to acclimate.
6. Unclamp the center chamber and the empty vascular channel.
7. 20% serum media or VEGF-rich media or any other signaling compound can be used as a chemoattractant for the tumor cells.
 - a. Using a programmable syringe pump, set up the chemoattractant in the empty vascular channel.

- b. Program the pump to flush out the vascular channel without cells every 3 hours at 2 μ l/min for 3 minutes to refresh the chemoattractant.
 - i. Program summary: Media Change
 1. Step 1: Constant Rate
 - a. Mode: Infuse
 - b. Set rate: 2 μ l/min
 - c. Time: 0:03:00 (3 minutes).
 2. Step 2: Pause
 - a. Mode: Pause
 - b. Target time: 3:00:00 (3 hours)
 3. Step 3: Repeat from Step 1
8. Image the cells every few hours or set up in stage-top incubator for regular imaging. Assay could take over 24 hours to see cell movement into center chamber.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses.

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