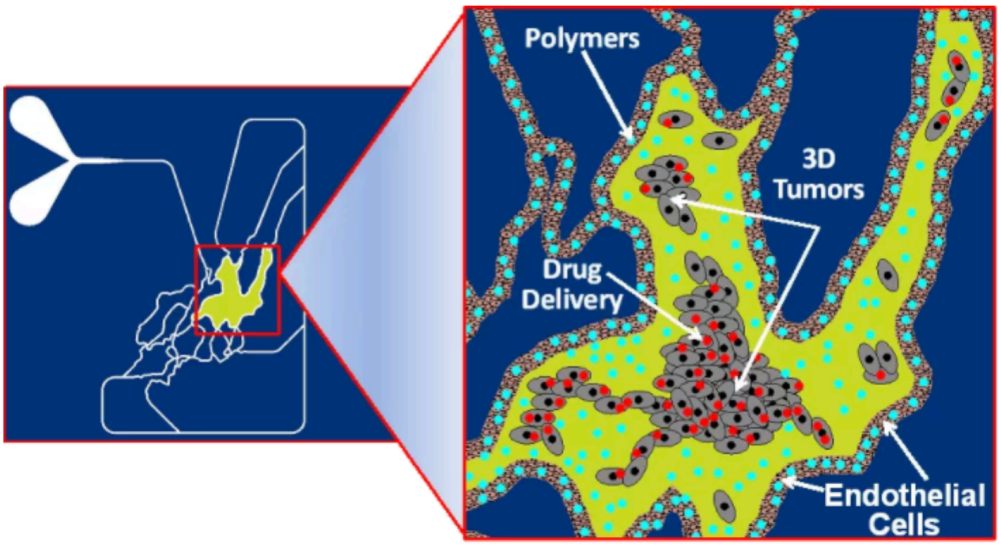
3D Tumor Assays Using SynTumor Microvascular Network Kits and Chips –Technical Manual

Catalog #s 403004, 403008, 403003, 403007, 105007-STu, 105015-STu



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.

SynVivo's SynTumor 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 SynVivo platform delivers a physiologically realistic model including flow and shear in a platform and enables real-time tracking of drug carrier binding and exravasation processes.

Materials Needed

- SynTumor Chips (Catalog # 40304)
- SynVivo Pneumatic Primer Device (Catalog #205001)
- 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
- Endothelial Cells
- Tumor Cells

Coating The Device with Endothelial Cell Culture Matrix

It is very important to remove all air from the device, including within the pores, for the assay to be carried out successfully. Use of Pneumatic Primer (SynVivo Cat# 205001) – is highly recommended for this procedure.

1. Place approximately 1-inch-long segments of Tygon tubing into the outlet ports of the device.
2. Draw 200ug/ml human fibronectin into a 1 mL syringe.
3. 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.
4. Do this for all but one inlet port. For this last port, use a tubing approximately 2-3 inches long. When the device is filled, unlock the needle from the syringe, leaving the needle attached to the tubing.
5. Clamp all tubing below the liquid line, except for the tubing with the needle attached.
6. 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, available from SynVivo (cat # 207001)*
7. Turn the knob on the controller box and adjust the pressure to ~5-7 psi. Apply the pressure for ~5-20 minutes. Tumor devices will take at least 15 minutes to completely fill.
8. Turn off the pressure and cut the Tygon tubing connected to the Pneumatic Primer.
9. Allow the device to incubate at 37°C for a minimum of 1 hour before use. Alternatively, incubate at 4°C overnight.
10. Allow the device to incubate at 37°C for 5 min before use.

Culture of Endothelial Cells in the Device

1. Prepare the endothelial cell suspension for seeding. Endothelial cells should be dissociated, centrifuged and concentrated to approximately 5-8x10^6cells/ml in cell specific media.
2. Place a drop of water at the base of the inlet port tubing to be removed and gently remove the tubing.
3. Remove the clamp on the outlet port. All other ports should be clamped except for one of the outlets.
4. Prepare a syringe and tubing with the previously prepared cell suspension and mount onto the programmable syringe pump.
5. Ensure that the tubing is free of air bubbles and the cell mixture is flush with the end of the tubing.
6. Insert the tubing into the port - the drop of water will prevent air entering the device as the tubing is inserted.
7. Clean the fluid from the surface of the device.

8. Begin the injection at 4-7 ul/min.
9. Watch the device as the cells are flowing. Once the vascular channel is filled with cells, stop the flow and clamp the outlet tubing.
10. Carefully and quickly cut the inlet tubing, keeping the length of all the tubing equal.
11. Allow the cells to attach for at least 2 hours before changing media.
    a. To set up a media change program using a syringe pump, use the following steps:
        i. Program the pump to flush out the vascular channel without cells every 3 hours at 2µl/min for 3 minutes to refresh the media.
            1. Program summary: Media Change
                a. Step 1: Constant Rate
                    i. Mode: Infuse
                    ii. Set rate: 2 µl/min
                    iii. Time: 0:03:00 (3 minutes).
                b. Step 2: Pause
                    i. Mode: Pause
                    ii. Target time: 3:00:00 (3 hours)
                c. Step 3: Repeat from Step 1

12. Culture the cells with appropriate cell culture media using a syringe pump.
    a. Example syringe pump programs are shown (Tables 1 and 2).
        i. The program examples use the "Step" feature of the pump, which infuses media at a user-defined flow rate over a specific period of time.
        ii. Examples will create a step-up in flow rate over 16−17 hours.
            1. Table 1 is an example of a syringe pump program that can be used with endothelial cells immediately after seeding.
                a. This program is best to use when testing primary cells or cell lines for their tolerance to shear stress.

Table 1: Step programming from 0.02 to 0.1 µL/min over 16 hours

SEQ	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.02	4:00:00	INFUSE
2	0.05	4:00:00	INFUSE
3	0.075	4:00:00	INFUSE
4	0.1	4:00:00	INFUSE

Table 2 is an example of a syringe pump program that can be used with endothelial cells immediately after seeding and when the optimized target flow rate for the cell line has already been determined.

a. Place the target flow rate in Step 7.
b. Place the low-volume starting flow rate in Step 1.
c. Plot the slope between the low-volume flow rate and the target flow rate.
d. Add the slope to the low-volume flow rate and all subsequent steps to create the step intervals required to reach the target flow rate.

Table 2: Step programming from 0.02 to 1 µL/min over 17 hours

SEQ	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.02	2:00:00	INFUSE
2	0.05	2:00:00	INFUSE
3	0.075	2:00:00	INFUSE
4	0.1	2:00:00	INFUSE
5	0.3	3:00:00	INFUSE
6	0.8	3:00:00	INFUSE
7	1	3:00:00	INFUSE

Culture of Tumor Cells in the Device

1. Dilute Matrigel 1:5 with chilled serum free media.
   Note: Matrigel dilution can be varied depending on desired conditions.
2. Trypsinize and collect cells to be seeded into the tissue chamber.
3. Concentrate the cells to approximately 2-5x10^6 cells/ml in the 1:5 dilution of Matrigel.
4. Keep suspension on ice throughout seeding process.
5. Place a drop of water around the device tubing in the top punch and gently remove the tubing
6. Using a syringe pump, draw the cell/Matrigel suspension into a small segment of tubing that will just reach the inlet port – it is recommended to only draw up what is necessary for one device (about 20ul). This is to prevent Matrigel from polymerizing in the syringe and excess tubing.
7. Quickly insert tubing into the top port, making sure the tubing is free of air, and begin the injection at 4ul/min
   Note: Flow rate can be changed as the cells flow in to the chamber. As the chamber fills, lower the flow rate to 1-2 ul/min so that the cells begin to form the 3D mass desired.
8. Watch the device as the cells are flowing. Once the tissue chamber is filled with cells, stop the flow and clamp the top inlet tubing.

9. Gently flush the vascular channels with endothelial cell media to flush out any cancer cells that exited the tumor chamber. Once finished, cut the tubing to separate device and syringe.
10. Carefully cut the inlet tubing just above the clamp to separate the device from the syringe.
11. Incubate the device at 37 °C and allow the cells to attach for at least 3 hours before changing media. Media may need to be changed to cell specific media the day of seeding. Many cells may not thrive in serum-free media overnight, though metastatic cell lines such as HeLa and MDA-MB- 231 will continue to grow in serum-free media. When changing media, be sure not to disturb the tumor mass.

Option 1: Quantify Liposome Binding and Extravasation

Prepare liposome sample at a concentration of 50 -250 µM in culture media or buffer solution.
Note: If using buffer solution, make sure it contains Ca^{2+}/Mg^{2+} . $Ca^{2+}/Mg^{2+}$is essential to maintain cell attachment.

1. Draw liposome into a 1 mL syringe.
2. Place a drop of water at the base of the inlet port tubing to be removed and gently remove the tubing.
3. Remove the clamp on the outlet port. All other ports should be clamped except for one of the outlets.
4. Prepare a syringe and tubing with the prepared liposome solution and mount on syringe pump.
5. Ensure that the tubing is free of air bubbles and liposome solution is flush with the end of the tubing.
6. Insert the tubing into the port - the drop of water will prevent air entering the device
7. Clean the fluid from the surface of the device.
8. Begin the injection at 1 µl/min for 30 min.
9. Stop the flow, carefully and quickly cut the inlet tubing.
10. Wash the device with buffer at 1 µl/min for 10 min using steps described previously.
11. Fix cells using 10% natural buffered formalin at 1 µl/min for 10 min using steps described previously.
    Note: Counter staining such as DAPI, Hoechest 33342 can be performed in this step. Just mix appropriate amount of dye with formalin.
12. The device is now ready for imaging and further analysis.

Option 2: Quantify Endothelium Permeability Using Dextran

In this experiment, device has to be imaged every 1 min for 180 hours. Therefore,
1) a microscope stage with compatible software such as Nikon Element is required, and
2) a stage warmer that can maintain the device at 37 ℃ is required

1. Prepare dextran at a concentration of 25 µM in culture media or buffer solution.
   Note: If using buffer solution, make sure it contains Ca^{2+}/Mg^{2+}. Ca^{2+}/$Mg^{2+}$is essential to maintain cell attachment.
2. Draw dextran into a 1 mL syringe.
3. Mount the device into the microscope stage.
4. Make necessary adjustments so that the device is appropriately imaged and ready for recording.
5. Place a drop of water at the base of the inlet port tubing to be removed and gently remove the tubing.
6. Remove the clamp on the outlet port. All other ports should be clamped except for one of the outlets.
7. Prepare a syringe and tubing with the previously prepared dextran solution and mount onto a syringe pump.
8. Ensure that the tubing is free of air bubbles and the liposome solution is flush with the end of the tubing.
9. Insert the tubing into the port - the drop of water will prevent air entering the device as the tubing is inserted.
10. Clean the fluid from the surface of the device.
11. Begin the injection at 1 µl/min for 180 min.
12. Start recording in the imaging software.
13. The video is now ready for analysis.
14. The following equations were used to calculate permeability (P) of dextran across endothelium in the vascular channel:
    P = (1/Iv0)*(V/S)*(dIt/dt) (1)
    where It is the average intensity in the tumor compartment at a given time point, Iv0 is the maximum fluorescence intensity in the vascular channel, V/S is the ratio of vascular channel volume to its surface area and is constant for the each network.

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