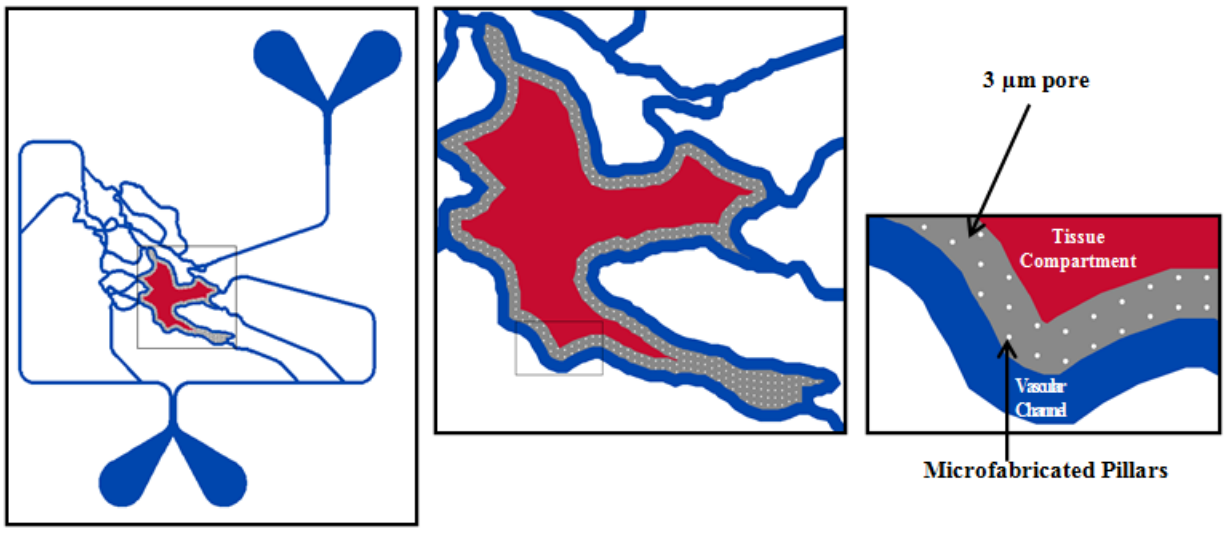
Rolling, Adhesion and Migration Assay Using SynRAM Microvascular Network Kits and Chips – Technical Manual

Catalog #s 401004, 401003, 105001-SR



Overview of Assay

Immune cells play a key role in early response to tissue injury/infection resulting from physical, chemical or biological stimuli. Due to the significance of the immune cells and endothelium interactions, several in vitro models have been developed to study different aspects of the inflammation pathway including rolling, adhesion and migration. Flow chambers are used to study rolling and adhesion phenomena, and Transwell chambers are used for migration studies. However, current flow chamber designs are oversimplified, lack the scale and geometry of the microenvironment and cannot model transmigration. Similarly, Transwell chambers do not account for fluid shear and size/topology observed in vivo, the end point measurements of migration are not reproducible and do not provide real-time visualization.

SynVivo's SynRAM assay has overcome these limitations to provide an entirely new system for studying the entire inflammation pathway in a realistic and dynamic environment. 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 rolling, adhesion and migration processes. This assay has been successfully validated against in vivo studies showing excellent correlation with rolling velocities, adhesion patterns and migratory processes (Lamberti et al 2014, Soroush et al 2016).

Materials Needed

- SynRAM Chips (Catalog # 105001-SR): Use Ports A, C and E as Inlets and B, D and F as Outlets
- 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
- Immune Cells
- TNF-α or IL-1β
- Chemoattractant (e.g. fMLP, MCP-1, etc.)

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. 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° for a minimum of 1 hour before use.
10. Flush fresh media into device just before performing assay.

Culture of Endothelial Cells in the Device

1. Prepare the endothelial cells 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 a 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 4 hours before changing media.
    a. Many endothelial cells can be incubated overnight before a media change is required.
12. Allow the cells to grow approximately 24 hours with at least 1 media change before running migration assay. Cells should be confluent for a successful experiment.
    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

Activation of Endothelial Cells to Initiate the Inflammation Response

1. Dilute inflammatory agent (e.g. TNF-α, IL-1β) in cell-specific media at desired concentration (typical range is from 10ng/ml to 100ng/ml).
2. Endothelial cells are activated with inflammatory agent for 4 hours under static conditions.
3. Selectin molecules, which are responsible for rolling peak between 2-6 hours, hence rolling, adhesion and migration assays should be performed under 6 hours following activation.
4. Integrins responsible for adhesion peak at 24 hours and last until 72 hours, hence adhesion and migration assays can be conducted until 72 hours if needed.

Injection of Chemoattractant (e.g. fMLP, MCP-1)

This step should be done 5-10 minutes before introducing immune cells.

1. Prepare the chemoattractant solution.
    a. Dilute chemoattractant to desired concentration (10ng/ml -1000ng/ml or 10nM to 1000nM) in serum free media.
2. Place a drop of water around the inlet tubing of the tissue compartment and gently remove the tubing with fine-tip forceps. Make sure one inlet and one outlet port is open.

3. Draw up a small amount of the chemoattractant mixture into a ~3” tubing attached to a syringe. Only draw up what is needed (about to the needle).
4. Insert the tubing into the open port, insuring no air is present in the tube, and slowly infuse the chemoattractant mixture until 2 drops come out of inlet/outlet tubings. Do not infuse excessive amounts, as this can disrupt the endothelium integrity in the channel.
   Note: Infusion can be done carefully by hand or using a syringe pump at 1ul/min
5. Cut the inlet tubing to the syringe.

Introduction of cells for Assay

1. Suspend immune cells (e.g. neutrophils, monocytes, etc.) at concentration of ~5 x 10^6/ml.
2. Place a drop of water around the inlet tubing for the vascular channels and gently remove the tubing.
3. Mount a syringe onto a syringe pump and draw up cell suspension into a syringe with tubing long enough to reach the device.
4. While observing on a microscope, attach cell-primed tubing to the inlet port of the device and infuse the cells into the media-only vascular channel.
5. Continue flowing the cells into the vascular channel at 1-10 µl/min and image in real time or at specific interval using the inverted microscope.