BIOPICO SYSTEMS

ADVANCING DRUG DISCOVERY

Application Note: Immunofluorescent Visualization of Vasculature in Biopico's OrganRX™ Plate

Table of Contents

A. Introduction

The formation and function of vascular networks are fundamental to tissue homeostasis, drug delivery, and disease modeling. Traditional 2D culture systems fail to replicate the complexity of in-vivo vascular environments, limiting their utility for translational research. Biopico's OrganRX™ plate enables physiologically relevant 3D co-culture under passive unidirectional flow, offering a robust platform for studying vascular development, angiogenesis, and vascular-endothelial interactions in a controlled microenvironment.

This application note describes the use of the OrganRX™ plate to develop and visualize a vascularized 3D tissue model using human umbilical vein endothelial cells (HUVECs) and lung-derived fibroblasts (MRC-5). The study demonstrates the workflow from seeding to immunofluorescent staining and confocal imaging of vascular networks.

B. Objective

Biopico's OrganRX™ plate offers a flexible platform for replicating in-vivo physiological processes through 3D co-culture systems. In this study, we demonstrate:

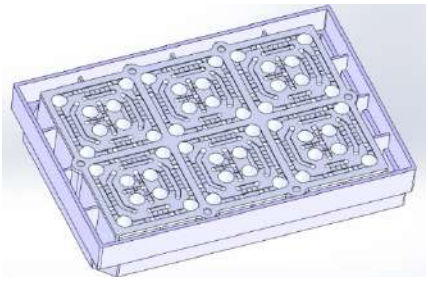
- The generation of a vascularized 3D tissue model by co-culturing endothelial and stromal cells.
- The immunofluorescent visualization of vascular structures within the OrganRX™ plate, enabling assessment of network formation and spatial organization.

C. Materials

OrganRX™ Plate Configuration

- Format: 6 independent units per plate
- Design: Each unit contains dedicated tissue and media reservoirs, enabling gravity-driven unidirectional perfusion
- Compatibility: Transparent bottom for live cell imaging and confocal microscopy

A



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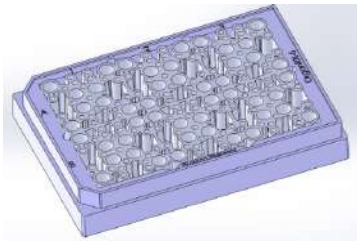

Figure 1. Description of OrganRX™ Plate. **A.)** Bottom view of OrganRX™ wells and channels design, showing all 6 units. **B.)** Top view of the OrganRX™ plate, showing the overall layout visible during cell seeding and medium exchanges.

Multiorgan Recirculation OrganRX™ System

The multi-organ recirculation system (OrganRX™) provides passive gravity-driven unidirectional shear flow to multi-organ culture (Fig. 1B). The OrganRX™ plate can accommodate multiple micro-organs such as liver, gut, kidney and brain. Our OrganRX™ System provides recirculation of media across multiple organs with a specified fluidic shear stress for the recapitulation of organs. The OrganRX™ system is made up of a stainless steel enclosure for easy sterilization and is operated by a smart device control app.

A **B**

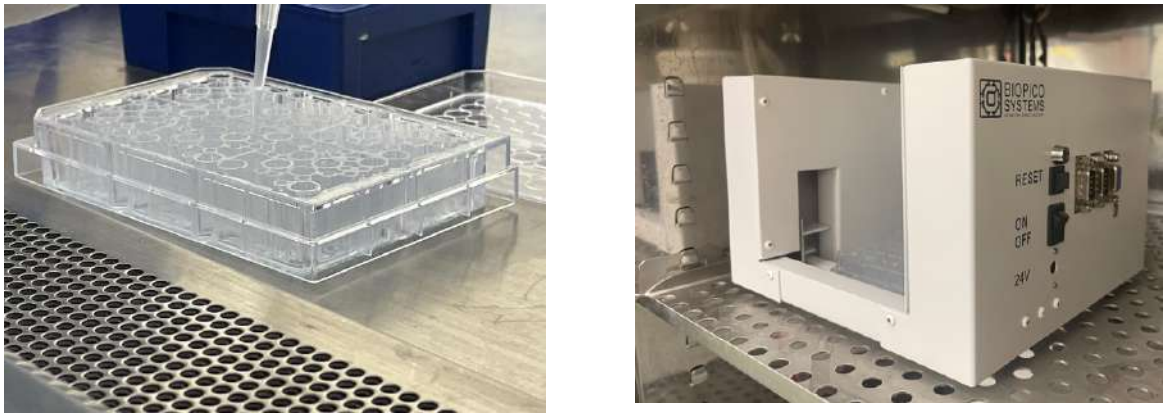

Figure 2. Description of all the OrganRX™ products required for this protocol. **1A.)** OrganRX™ culture plate for performing multiorgan experiments and assays. **1B.)** OrganRX™ system for inducing shear flow in cell cultures.

D. Methods

Model Overview

To mimic aspects of the blood-brain barrier and support vascular formation, Human Umbilical Vein Endothelial Cells (HUVECs) and MRC-5 lung fibroblasts were co-cultured within a fibrin-based 3D matrix in the blood-brain barrier region of the OrganRX plate (6-unit format).

Seeding Procedure

1. Cell Composition: HUVECs and fibroblasts were co-seeded at a 2:1 ratio.
2. Cell Density per Unit:
    a. HUVECs: 1.25 million cells

    b. MRC-5 Fibroblasts: 625,000 cells
3. 3D Matrix Composition:
    a. Fibrinogen: 6 mg/mL
    b. Thrombin: 4 U/mL
4. Temperature Control: During gel polymerization and seeding, the OrganRX plate was placed on a chilled aluminum block over ice to prevent premature gelation.
5. Polymerization Time: ~30 minutes at 37°C post-seeding

Culture Maintenance and Monitoring

1. Media: Endothelial Cell Medium supplemented with:
    a. 25 mL Fetal Bovine Serum (FBS)
    b. 5 mL Endothelial Cell Growth Supplement
    c. 5 mL Penicillin-Streptomycin
2. Media Exchange: Every other day
3. Imaging: Daily brightfield imaging at 4× and 20× magnification using an inverted microscope to monitor morphology and growth.
4. Culture Duration: 7 days

Preparation of Fibrinogen/Thrombin gel

To prepare the thrombin working solution, reconstitute the stock vial by adding 1000 µL of sterile PBS, then aliquot into 7.5 µL portions and store at −80 °C until use. For the fibrinogen working solution, dissolve 11.25 mg of fibrinogen and 1.875 mg of phenol red in 937.5 µL of sterile PBS in a 50 mL tube, incubating the mixture at 37 °C in a water bath for approximately 3 hours until fully dissolved. Once dissolved, filter-sterilize the solution using a 0.2 µm filter and aliquot into 10 tubes containing 93.75 µL each. Store all fibrinogen aliquots at −80 °C and thaw at 4 °C for up to 10 days before use. During device seeding, keep thawed fibrinogen on ice and gently mix before beginning the procedure. The summary of reagents used for preparation of Fibrinogen/thrombin gel is shown in Table 1.

Reagent / Ingredient	**Concentration**	**Volume**	**Purpose / Notes**
Thrombin	4 U/mL	*Calculated*	Main gel matrix; keep on ice
Fibrinogen	6 mg/mL	*Calculated*	Gel matrix; keep on ice
1X PBS with phenol red	1X	*As needed*	Maintain ionic strength; check for homogenous red color
Deionized Water	N/A	*Calculated*	Bring mixture to final volume; keep on ice

Table 1. Summary of reagents used for preparation of Fibrinogen/thrombin gel.

Preparation of Cells from T-flask

To prepare cells from a T-flask, first harvest the cells and count the total number of viable cells obtained. Enter this number into the designated spreadsheet to calculate the required resuspension volume based on experimental needs. Next, centrifuge the cell suspension to pellet the cells and carefully remove the supernatant. Finally, resuspend the cell pellet in the calculated volume of fresh media as indicated by the spreadsheet, ensuring the cells are evenly dispersed for downstream applications.

Seeding cells within Gel in OrganRX™ Plate

To begin seeding, resuspend the cells according to the calculated volume in the worksheet. Add an appropriate amount of the resuspended cell suspension and additional media as calculated in the worksheet to a thawed thrombin aliquot and gently mix. Immediately combine this thrombin-cell mixture with the fibrinogen solution, ensuring the gel mixture is homogeneous. Place the OrganRX™ plate inside a biosafety cabinet, and using regular pipette tips, dispense 25 µL of the gel-cell solution into each of the six BBB ports.

Figure 3. Gel protocol for seeding cells in fibrinogen/thrombin gel

Once seeded, incubate the plate for 30 minutes at 37 °C on a flat surface to allow gel polymerization. After gelation, add 2 mL of media into one of the inner and outer wells of the plate, then place the plate in the recirculating flow system inside of the incubator in order to support vascular network development and dynamic cell interactions. Image the plate as needed to monitor cell growth and gel distribution. Maintain the plate at 37 °C, performing daily medium changes until the experiment concludes.

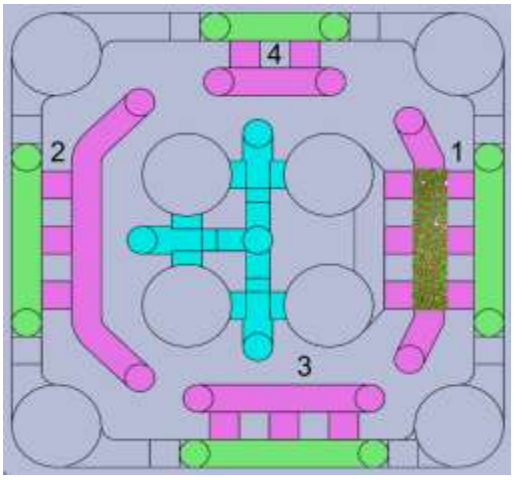

Figure 4. Description of OrganRX™ Plate with cell seeding position for the vascular network.

Organ	Volume
1 (BBB)	25 µL
2 (Liver)	30 µL
3 (Kidney)	25 µL
4 (Gut)	20 µL

Table 2. Volume of 4 different organ channels (pink) from Fig. 5.

Immunohistochemistry

Every day, the OrganRX™ plate was imaged using both 4× and 20× objectives to monitor cell morphology, growth, and vascular network formation. On day 7 of culture, vascularized organoid constructs were fixed and stained for VE-Cadherin, a key endothelial tight junction marker, to assess the integrity and localization of cell-cell junctions within the 3D microenvironment. The

staining was performed using standard immunofluorescence protocols, with primary antibody incubation conducted for 1.5 hours at room temperature. Immediately following staining, the organ chips were imaged using the NyOne imaging station, capturing high-resolution fluorescence images across multiple fields of view.

E. Results

At 4× magnification, wide-field views allowed visualization of overall cell distribution, gel integrity, and structural uniformity across the six BBB ports. At 20× magnification, higher-resolution images captured cell-cell interactions, vascular sprouting, and early signs of lumen formation in the co-culture. Daily imaging enabled qualitative assessment of culture health and temporal progression of tissue maturation. Representative images from selected days were analyzed for confluency, network density, and morphological features consistent with endothelial tubulogenesis and stromal support. Imaging data provided visual confirmation of successful seeding, gel stability, and dynamic cellular remodeling throughout the culture period.

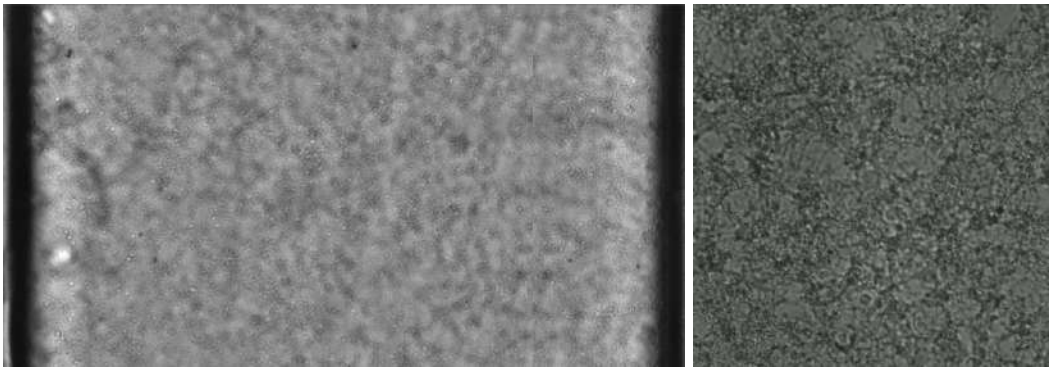

Figure 5. HUVEC monitoring in organ plate. brightfield image on 6th day (a) 4x (b) 20x.

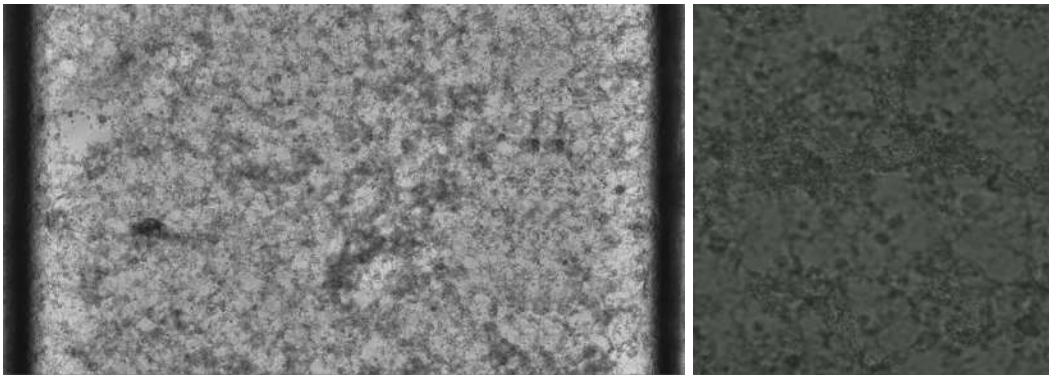

Figure 6. HUVEC/MRC-5 monitoring in organ plate. brightfield image on 6th day (a) 4x (b) 20x

Imaging results revealed distinct VE-Cadherin expression localized at cell-cell borders, indicating successful formation of endothelial junctions and vascular network organization. The spatial pattern of VE-Cadherin staining correlated with previously observed network structures in brightfield imaging, confirming structural and functional maturation of the endothelium. These findings demonstrate that the co-culture system supports endothelial differentiation and junctional complex formation under static and flow conditions in the OrganRX™ platform.

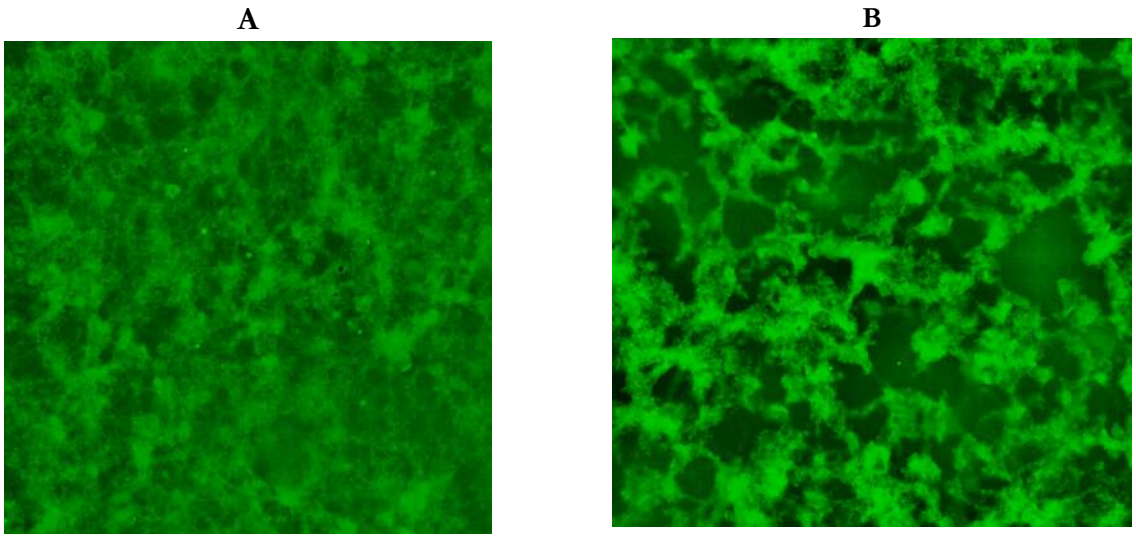

Figure 7. Immunofluorescent staining of (a) HUVEC cells alone (b) HUVEC and MRC-5 co-culture in the organ plate.

F. Other Applications

Multiple cells are seeded for studying vascular interaction with immune cells and other cells as shown in Fig. 7. The following applications show a few examples for studying using the organ system in this configuration.

1. Transplant and Alloreactivity Models

Vascular organ-on-a-chip systems provide a human-relevant platform to model immune rejection in transplantation. By combining HLA-mismatched T cells with vascularized organoids derived from donor tissue, researchers can simulate graft-versus-host–like responses and assess the dynamics of immune infiltration, cytokine release, and tissue damage. This model also enables the testing of immunosuppressive monoclonal antibodies, such as anti-CD3 or CTLA-4-Ig, allowing

preclinical evaluation of therapeutic strategies to mitigate rejection. Additionally, the platform can be adapted to study loss of grafts by incorporating human islets and autologous PBMCs, offering a predictive tool for early-phase islet transplantation studies.

2. Senescence and Radiation Response

These organ-on-a-chip systems are well-suited for modeling radiation-induced vascular injury and senescence, especially in endothelial cells lining perfused microchannels. Following exposure to ionizing radiation, the chip can be used to assess senescence-associated secretory phenotypes (SASP), including pro-inflammatory cytokine release and barrier dysfunction. Co-culture with immune cells such as T cells or macrophages allows the investigation of bystander immune killing and chronic inflammation driven by senescent tissues. The model is also valuable for screening senolytic compounds or radioprotective biologics, helping identify therapies that mitigate radiation damage in sensitive tissues like gut, bone marrow, or vasculature.

3. Infection and Pathogen Response Modeling

Vascularized organ-on-chip systems are ideal for modeling host-pathogen interactions at the endothelial and epithelial interface. These models have been used to study infections like SARS-CoV-2, Zika, and Dengue, where immune infiltration and epithelial barrier function are critical. The inclusion of circulating immune cells such as T cells, monocytes, and macrophages enables tracking of immune recruitment and activation during infection. Moreover, they support evaluation of barrier integrity, tight junction disruption, and the dynamics of infection-induced inflammation. These systems also provide a platform for antiviral drug and antibody testing, helping assess both therapeutic efficacy and inflammatory risk in human tissue contexts.

4. Inflammation and Cytokine Storm Models

The platform's ability to integrate immune cells with perfused tissue barriers makes it ideal for modeling inflammatory cascades such as cytokine storms. Using PBMCs or activated T cells, researchers can simulate IL-6 or TNFα–driven inflammation, as seen in severe infections or autoimmune flares. Vascular endothelial activation can be measured via ICAM-1 and VCAM-1 expression, reflecting immune cell adhesion and transmigration. Multiplex cytokine profiling can be used to monitor immune responses under mAb treatment or infection. These models are valuable for screening anti-inflammatory biologics and understanding tissue-specific effects of systemic immune activation.

5. Vascular Permeability and Drug Delivery

Vascularized organ-on-chip systems provide a dynamic and physiologically relevant environment to study drug permeability and delivery kinetics. Researchers can quantify monoclonal antibody or nanoparticle transport across endothelial layers into organoid tissues, simulating in vivo drug delivery challenges. Vascular leakiness, often driven by VEGF or inflammation, can be induced

and measured in real time. The system is also applicable for blood-brain barrier (BBB) transport studies, assessing the penetration of biologics or immune cells under disease or therapeutic conditions. These applications are critical for evaluating delivery efficiency, especially for CNS or tumor-targeting agents.

6. Autoimmune and Inflammatory Disease Models

Organ-on-chip platforms can be used to model autoimmune diseases like colitis, psoriasis, and systemic fibrosis, especially when combined with activated T cells and inflammatory stimuli. These models are ideal for testing therapeutic monoclonal antibodies such as anti-IL-17 or anti-TNFα under physiologically relevant flow. They also offer predictive tools for checkpoint inhibitors–induced autoimmune toxicity, enabling assessment of off-target effects in healthy vascularized organoid models. By integrating immune cells and tissue-specific organoids, these systems provide a modular and scalable platform to replicate complex inflammatory diseases in vitro.

7. Neuroimmunology Applications

In the central nervous system context, vascular organ-on-chip platforms can recreate a human-relevant BBB using brain microvascular endothelial cells, astrocytes, and microglia. These systems allow researchers to study T-cell transmigration, neuroinflammation, and barrier dysfunction in diseases like multiple sclerosis or Alzheimer's disease. They also support testing of anti-inflammatory monoclonal antibodies or immune checkpoint modulators within a perfused CNS environment. This setup provides a valuable tool for neuroimmunology research, especially where immune surveillance and CNS penetration of therapeutics are critical concerns.

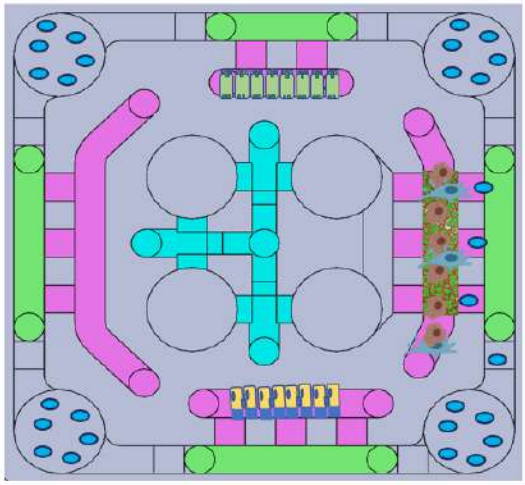

Fig. 7. Multiple cells are seeded for studying vascular interaction with immune cells

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