Fabrication of Microchip Devices for Organ-on-a-Chip and Lab-on-a-Chip Applications

CNF Project Number: 2857-19
Principal Investigator(s): Esak (Isaac) Lee, Ph.D.
User(s): Renhao Lu, Yansong Peng, Shufan Yin

Affiliation(s): Meinig School of Biomedical Engineering, Cornell University
Primary Source(s) of Research Funding: National Institutes of Health (NIH) R01 HL165135 and R01 CA279560
Contact: el767@cornell.edu, rl839@cornell.edu, yp255@cornell.edu, sy766@cornell.edu
Research Group Website: https://leelab.bme.cornell.edu/
Primary CNF Tools Used: Heidelberg Mask Writer DWL2000, ABM Contact Aligner, MVD100

Abstract:

Organ-on-a-chip is a microfluidic cell culture platform, integrated circuit (chip) that simulates the activities, mechanics, and physiological response of an entire organ or an organ system. Our lab aims to create organ-on-a-chip devices to study the mechanism of various diseases. In the past year, we mainly focused on two projects: (1) A 3D biomimetic model of lymphatics reveals cell–cell junction tightening and lymphedema via a cytokine-induced ROCK2/JAM-A complex; (2) Piezo1 regulates meningeal lymphatic vessel drainage and alleviates excessive CSF accumulation.

Summary of Research:

Project 1: A 3D biomimetic model of lymphatics reveals cell–cell junction tightening and lymphedema via a cytokine-induced ROCK2/JAM-A complex [1].

Impaired lymphatic drainage and lymphedema are major morbidities whose mechanisms have remained obscure. To study lymphatic drainage and its impairment, we engineered a microfluidic culture model of lymphatic vessels draining interstitial fluid. This lymphatic drainage-on-chip revealed that inflammatory cytokines that are known to disrupt blood vessel junctions instead tightened lymphatic cell–cell junctions and impeded lymphatic drainage. This opposing response was further demonstrated when inhibition of rho-associated protein kinase (ROCK) was found to normalize fluid drainage under cytokine challenge by simultaneously loosening lymphatic junctions and tightening blood vessel junctions. Studies also revealed a previously undescribed shift in ROCK isoforms in lymphatic endothelial cells, wherein a ROCK2/junctional adhesion molecule-A (JAM-A) complex emerges that is responsible for the cytokine-induced lymphatic junction zippering. To validate these in vitro findings, we further demonstrated in a genetic mouse model that lymphatic-specific knockout of ROCK2 reversed lymphedema in vivo. These studies provide a unique platform to generate interstitial fluid pressure and measure the drainage of interstitial fluid into lymphatics and reveal a previously unappreciated ROCK2-mediated mechanism in regulating lymphatic drainage.

Project 2: Piezo1 regulates meningeal lymphatic vessel drainage and alleviates excessive CSF accumulation [2]

Piezo1 regulates multiple aspects of the vascular system by converting mechanical signals generated by fluid flow into biological processes. In this project, we utilize the lymphatic-on-chip devices to study the role of Piezo1 in lymphatics and its drainage functions. Together with our collaborators, we find that Piezo1 is necessary for the proper development and function of meningeal lymphatic vessels and that activating Piezo1 through transgenic overexpression or treatment with the chemical agonist Yoda1 is sufficient to increase cerebrospinal fluid (CSF) outflow by improving lymphatic absorption and transport. The abnormal accumulation of CSF, which often leads to hydrocephalus and ventriculomegaly, currently lacks effective treatments. We discovered that meningeal lymphatics in mouse models of Down syndrome were incompletely developed and abnormally formed. Selective overexpression of Piezo1 in lymphatics or systemic administration of Yoda1 in mice with hydrocephalus or Down syndrome resulted in a notable decrease in pathological CSF accumulation, ventricular enlargement and other associated disease symptoms. Together, our study highlights the importance of Piezo1-mediated lymphatic mechanotransduction in maintaining brain fluid drainage and identifies Piezo1 as a promising therapeutic target for treating excessive CSF accumulation and ventricular enlargement.

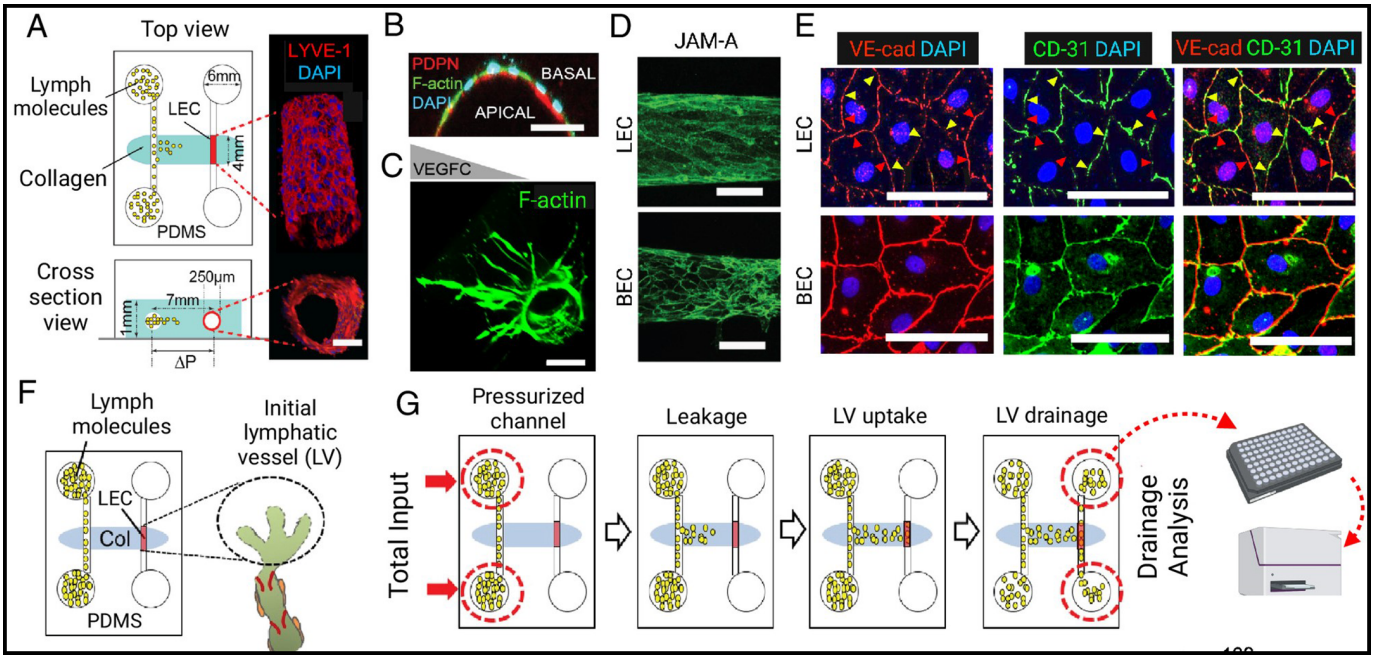

Figure 1, above: Lymphatic drainage-on-chip recapitulates lymphatic structure, drainage, and dysfunction. (A) A schematic of the lymphatic drainage-on-chip platform. (B) Apical podoplanin (PDPN) expression on the luminal side of the vessel. (C) Lymphatic sprouting in response to VEGFC stimulation. (D) Immunostaining of LEC-generated lymphatic vessels and BEC-generated blood vessels with a tight junction marker, JAM-A. (E) Immunostaining of lymphatic vessels and blood vessels with an adherens junction marker, VE-cadherin (VE-cad), and CD31. Red and yellow arrows indicate exclusive expression of VE-cad and CD31, respectively, showing interdigitated, discontinuous expression of VE-cad in LECs. (F) A schematic of a biomimetic lymphatic drainage-on-chip model system. The engineered lymphatic vessel (LV) in the right-side channel functions as an initial LV to drain interstitial lymph fluid that is introduced through the left-side channel. (G) Transport of lymph fluid. The left-side channel pressured with lymph fluid induces fluid transport. The pressure gradient between two channels results in fluid convection from the left channel to the engineered LV. The lymph fluid is drained by the engineered LV and accumulated in two right-side reservoirs. Total drained fluid is analyzed to obtain the number of drained lymph molecules.

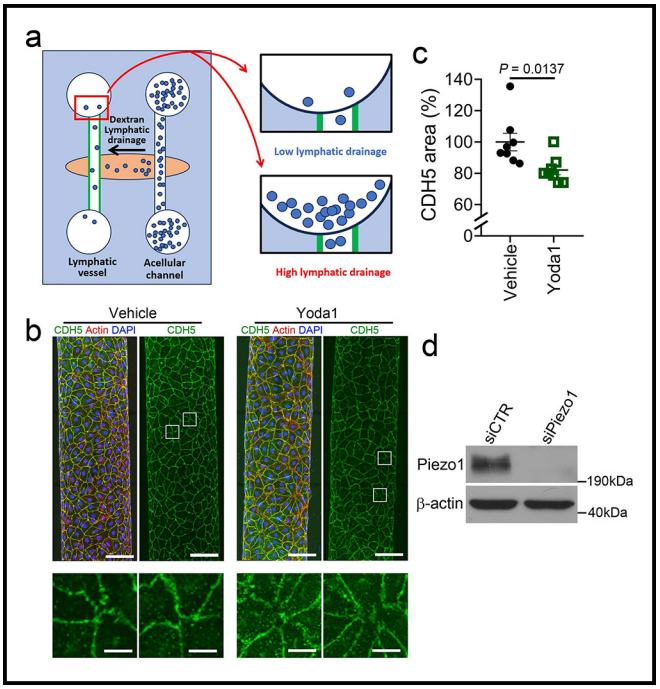

Figure 2, left: (a) Schematic illustration of the 3-D lymphatic vessel model used for this study. The outcome of the Yoda1-induced drainage increase is presented in Fig. 4c. (b) Fluorescence confocal images of the engineered lymphatic vessels stained for F-actin and CDH5. Enlarged CDH5 images (boxed) show more discontinuous junctions in the Yoda1-treated group than in the vehicle group. Scale bars: 100 .m (10 .m, enlarged images). Acellular channels are not shown. (c) The relative area of CDH5-stained cellular junctions (n.=.8 independent experiments). Statistics: two-tailed t-test. (d) Western blot assays verifying the efficient knock-down of Piezo1 in LECs prepared for the drainage measurement shown in Figure 4c (n.=.4 independent samples). Data are presented as mean values +/- SEM.

References:

[1] Lee E, et al. A 3D biomimetic model of lymphatics reveals cell-cell junction tightening and lymphedema via a cytokine-induced ROCK2/JAM-A complex. Proc Natl Acad Sci U S A. 2023 Oct 10;120(41):e2308941120. doi: 10.1073/pnas.2308941120. Epub 2023 Oct 2. PMID: 37782785; PMCID: PMC10576061.

[2] Choi D, et al. Piezo1 regulates meningeal lymphatic vessel drainage and alleviates excessive CSF accumulation. Nat Neurosci. 2024 May;27(5):913-926. doi: 10.1038/s41593-024-01604-8. Epub 2024 Mar 25. PMID: 38528202; PMCID: PMC11088999.