Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Materials

A human initial lymphatic chip reveals distinct mechanisms of primary lymphatic valve dysfunction in acute and chronic inflammation

Samantha E. Kraus[1], Esak Lee[1,*]

[1] Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14853, USA

***Corresponding author:**

Esak Lee, Ph.D.

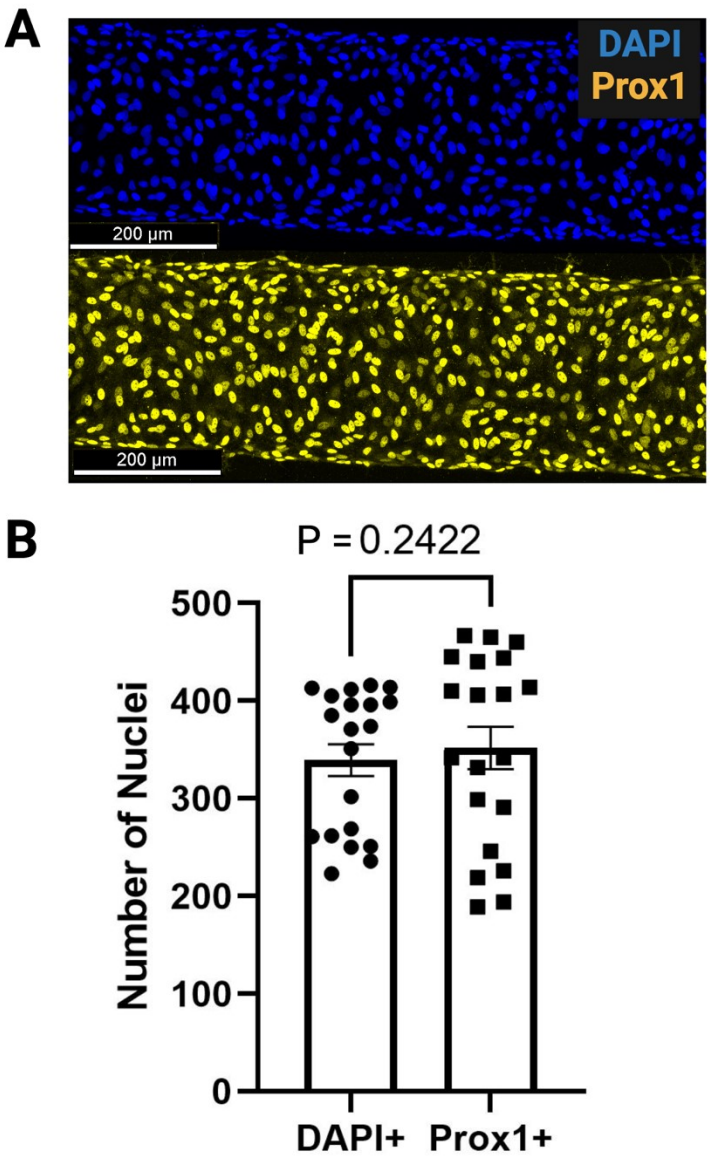
Assistant Professor and Meinig Family Investigator in the Life Sciences

Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University

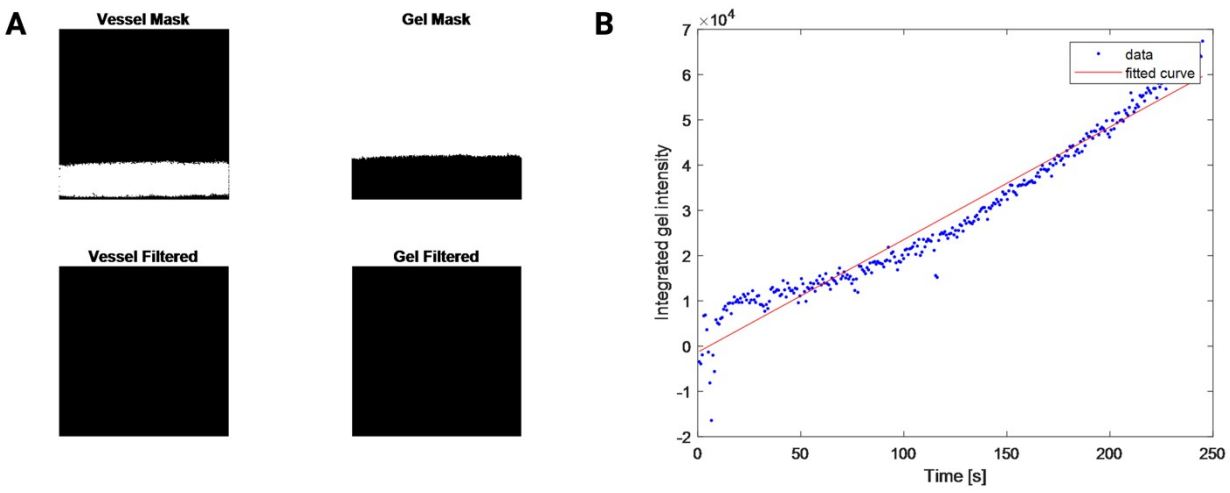
302 Weill Hall, 237 Tower Rd, Ithaca, NY 14853, USA

Phone: 607-255-8491, E-mail: el767@cornell.edu

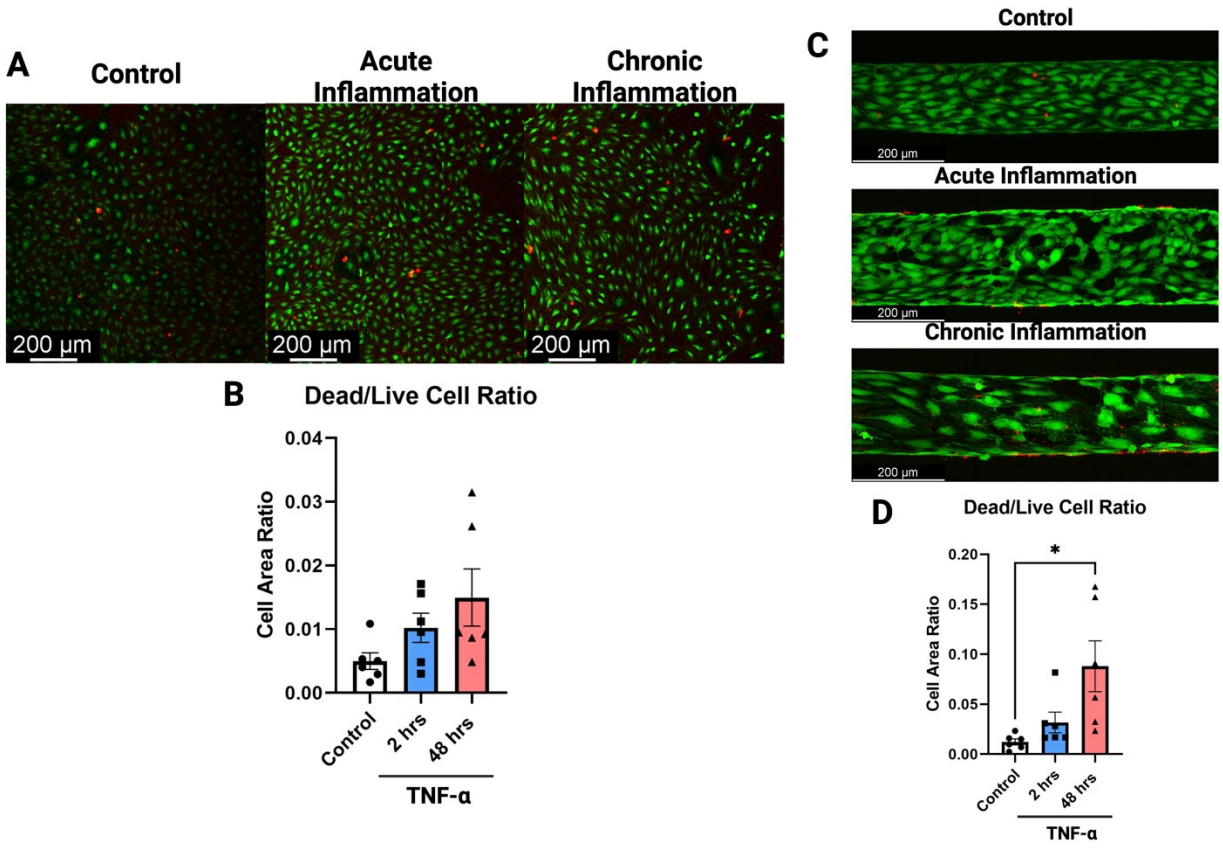
Contents



Supplementary Figure 1. Quantification of DAPI+ and Prox1+ nuclei in engineered lymphatic vessels. (A) Comparison of DAPI and Prox1 channels showing significant overlap between DAPI+ and Prox1+ cells, confirming the LEC identity of the cultured cells. **(B)** Quantification of DAPI+ and Prox1+ nuclei in engineered lymphatic vessels for all control and treatment groups. p = 0.2422; Two-tailed paired Student t- test, n = 20 for each group. Data are expressed as mean ± S.E.M.



Supplementary Figure 2. An example of the MATLAB output generated from the calculation of diffusive permeability for lymphatic vessels. Permeability is calculated by determining a linear fit for the integrated density of fluorescence signal in the interstitium (gel) over time and taking the slope of that fit as the diffusive permeability in cm/s.



Supplementary Figure 3. Live/Dead staining of LECs in inflammation in 2D and 3D cultures. **(A)** Fluorescent images of LECs in 2D 6-well plate culture under acute (2 hr) or chronic (48 hr) inflammation induced by 10-20 ng/mL of TNF-α stained with live green and dead red. **(B)** Quantification of dead red cell area as a ratio over live green cell area for all control and treatment groups in 2D culture. **(C)** Fluorescent images of LECs in 3D vessel culture in microfluidic chips under acute (2 hr) or chronic (48 hr) inflammation induced by 10-20 ng/mL of TNF-α stained with live green and dead red. **(D)** Quantification of dead red cell area as a ratio over live green cell area for all control and treatment groups in 3D culture. * p = 0.0111; One-way ANOVA with Tukey's HSD tests, n = 6 for each group. Data are expressed as mean ± S.E.M.