# Supporting Information

**Interstitial flow promotes the formation of functional microvascular networks *in vitro* through upregulation of matrix metalloproteinase-2**

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Analytical solution of interstitial flow across fibrin gel

Matrix permeability of the fibrin gel was measured by the displacement of media in syringe reservoirs. The Darcy permeability ($K$) of acellular fibrin gel was calculated by Darcy's law:

$$\frac{Q(t)}{A} = \frac{K}{\mu}\frac{\Delta P(t)}{L} \quad (1)$$

where $Q$ is the volumetric flow rate, $A$ is cross-sectional surface area of gel normal to the flow direction, $\mu$ is the viscosity, $\Delta P$ is the pressure difference across the gel, and $L$ is the length of gel in the direction of interstitial flow.

The pressure difference in the syringe reservoirs is determined by the difference in the height of the liquid $\Delta z$, assuming identical syringes were used in inlet and outlet

$$\Delta P(t) = \rho g \Delta z(t) \quad (2)$$

$$\Delta z(t) = \Delta z_0 - \frac{1}{A_r}\int Q(t)\,dt - \frac{1}{A_r}\int Q(t)\,dt \quad (3)$$

where $\Delta z_0$ is the initial difference in liquid levels, $A_r$ is the cross-section area of syringe, $\rho$ is density of media, $g$ is the gravitational constant and the two integrals represent the contributions from the upstream and downstream reserviors.

Equations (1) – (3) give

$$Q(t) = \frac{AK\rho g \Delta z_0}{\mu L} e^{-\frac{2AK\rho g}{\mu L A_r}t} \quad (4)$$

thus $V(t)$, the reduction in liquid volume in the upstream syringe, is calculated as

$$V(t) = \int Q(t)\,dt = \frac{1}{2}\Delta z_0 A_r\left(1 - e^{-\frac{2AK\rho g}{\mu L A_r}t}\right) \quad (5)$$

By transforming equation (5), The Darcy permeability ($K$) is

$$K = -\frac{\mu L A_r}{2\rho g A t}\log\left(1 - \frac{2V(t)}{\Delta z_0 A_r}\right) \quad (6)$$

Based on this calculation and our initial measurements, the Darcy permeability ($K$) of acellular fibrin gel (fibrinogen 3mg/ml) used in this experiment is K = $1.2\times10^{-13}$ $m^2$.

**Single channel device design**

Our aim is to design a single channel microfluidic device that can maintain relatively stable interstitial flow over 24 hours, which is the time interval of replenishing culture media. Based on the analytical solution and measured Darcy permeability, the device is designed with gel region length $L = 15$ mm, height $H = 0.5$ mm, and width $W = 2$ mm. With those parameters, the ratio of pressure difference at $t = 24h$ and $t = 0$ , expressed as a percentage, is

$$\frac{\Delta P(t = 24h)}{\Delta P(t = 0)} \text{x } 100 = 74.55\%$$

which means a relatively stable interstitial flow can be maintained in such devices.

**Primers used for RT-PCR detections:**

| | |
|---|---|
| GAPDH-F | ACCACAGTCCATGCCATCAC |
| GAPDH-R | TCCACCACCCTGTTGCTGTA |
| PLAT-F | TGGTGCTACGTCTTTAAGGCGG |
| PLAT-R | GCTGACCCATTCCCAAAGTAGC |
| PLAU-F | GGCTTAACTCCAACACGCAAGG |
| PLAU-R | CCTCCTTGGAACGGATCTTCAG |
| PLAUR-F | CCACTCAGAGAAGACCAACAGG |
| PLAUR-R | GTAACGGCTTCGGGAATAGGTG |
| SERPINE1-F | CTCATCAGCCACTGGAAAGGCA |
| SERPINE1-R | GACTCGTGAAGTCAGCCTGAAAC |
| ANXA2-F | TCGGACACATCTGGTGACTTCC |
| ANXA2-R | CCTCTTCACTCCAGCGTCATAG |
| S100A10-F | AACAAAGGAGGACCTGAGAGTAC |
| S100A10-R | CTTTGCCATCTCTACACTGGTCC |
| THBD-F | AACGACCTCTGCGAGCACTTCT |
| THBD-R | CCAGTATGCAGTCATCCACGTC |
| MMP1-F | ATGAAGCAGCCCAGATGTGGAG |
| MMP1-R | TGGTCCACATCTGCTCTTGGCA |
| MMP2-F | GCGACAAGAAGTATGGCTTC |
| MMP2-R | TGCCAAGGTCAATGTCAGGA |
| MMP3-F | CACTCACAGACCTGACTCGGTT |
| MMP3-R | AAGCAGGATCACAGTTGGCTGG |
| MMP9-F | GCCACTACTGTGCCTTTGAGTC |
| MMP9-R | CCCTCAGAGAATCGCCAGTACT |
| MMP14-F | CAACACTGCCTACGAGAGGA |
| MMP14-R | GTTCTACCTTCAGCTTCTGG |
| HIF1A-F | TATGAGCCAGAAGAACTTTTAGGC |
| HIF1A-R | CACCTCTTTTGGCAAGCATCCTG |
| COL1A1-F | GATTCCCTGGACCTAAAGGTGC |
| COL1A1-R | AGCCTCTCCATCTTTGCCAGCA |
| CD31-F | AAGTGGAGTCCAGCCGCATATC |
| CD31-R | ATGGAGCAGGACAGGTTCAGTC |
| VE-CAD-F | GAAGCCTCTGATTGGCACAGTG |
| VE-CAD-R | TTTTGTGACTCGGAAGAACTGGC |
| PXN-F | CTGATGGCTTCGCTGTCGGATT |

| | |
|---|---|
| PXN-R | GCTTGTTCAGGTCAGACTGCAG |
| VCL-F | TGAGCAAGCACAGCGGTGGATT |
| VCL-R | TCGGTCACACTTGGCGAGAAGA |
| KDR-F | GGAACCTCACTATCCGCAGAGT |
| KDR-R | CCAAGTTCGTCTTTTCCTGGGC |
| VCAM1-F | GATTCTGTGCCCACAGTAAGGC |
| VCAM1-R | TGGTCACAGAGCCACCTTCTTG |
| VEGFA-F | CGCAGCTACTGCCATCCAAT |
| VEGFA-R | GTGAGGTTTGATCCGCATAATCT |
| IGFBP2-F | CGAGGGCACTTGTGAGAAGCG |
| IGFBP2-R | TGTTCATGGTGCTGTCCACGTG |
| DLL4-F | CTGCGAGAAGAAAGTGGACAGG |
| DLL4-R | ACAGTCGCTGACGTGGAGTTCA |
| HMOX1-F | CCAGGCAGAGAATGCTGAGTTC |
| HMOX1-R | AAGACTGGGCTCTCCTTGTTGC |

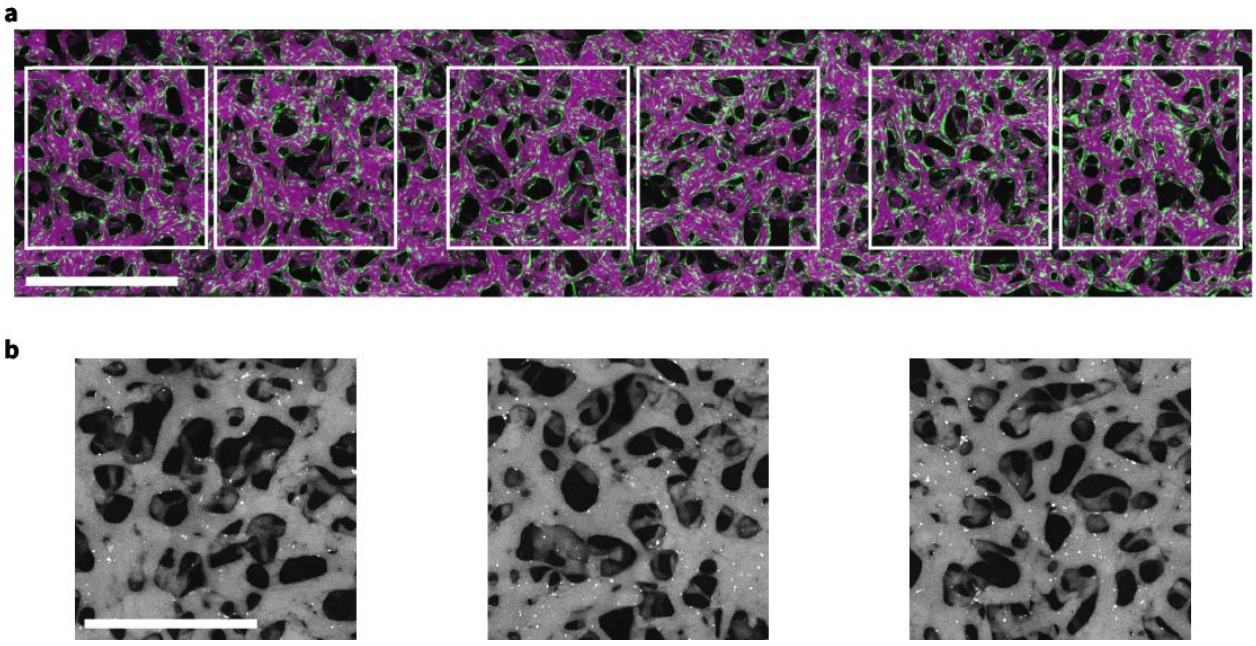


**Figure S1.** (a) Confocal projected z-stack image of perfusable MVN formed after 6 days culture under IF. Green – GFP HUVEC, magenta – 40 kDa Texas Red dextran. White rectangles are the ROIs for statistical analysis of morphological parameters in upstream (close to inlet on the left), middle and downstream (close to outlet on the right) regions. (b) Zoomed-in view of MVNs in the ROIs of different regions along the single channel microfluidic device. Scale bar is 1 mm for both (a) and (b).

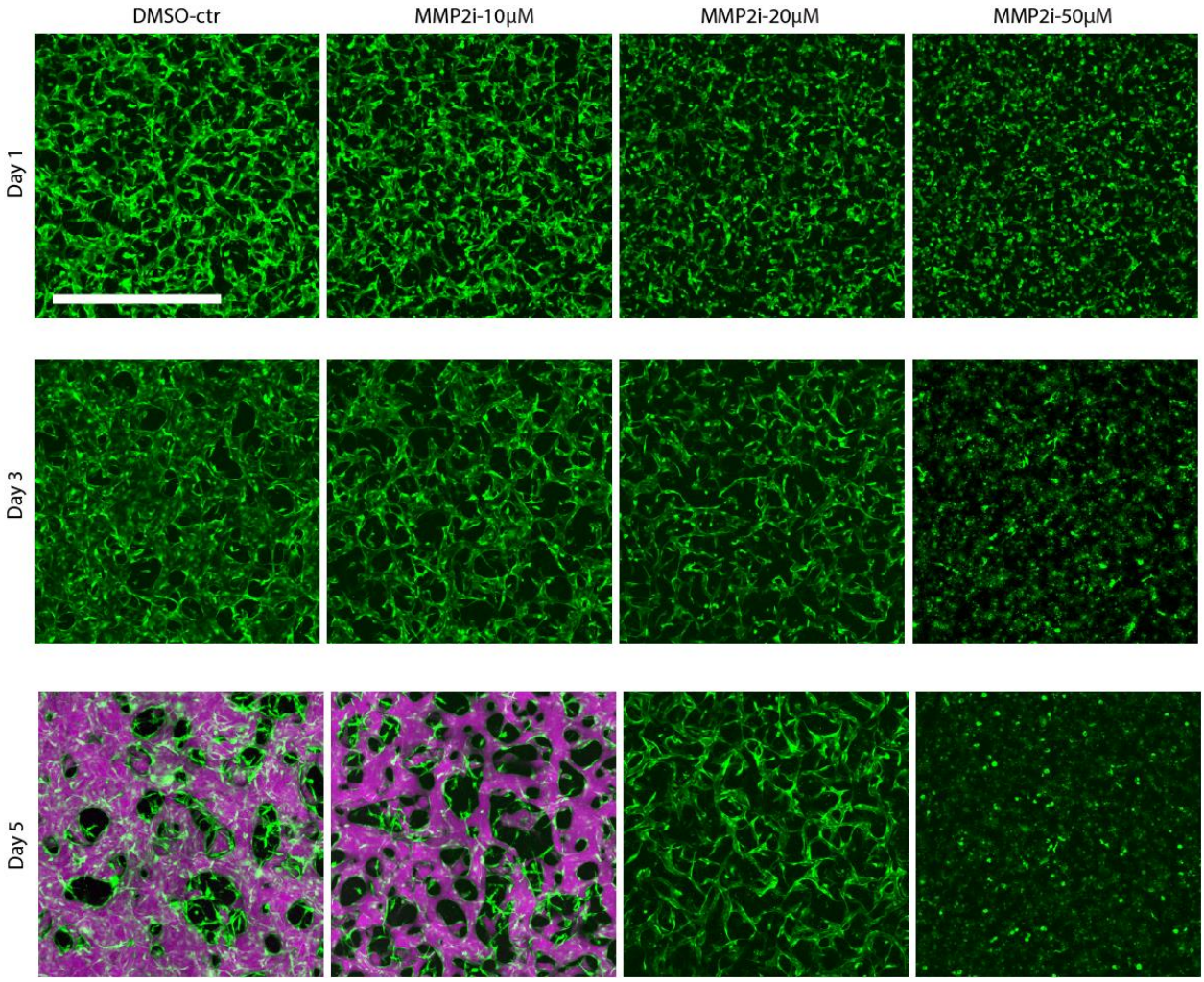


**Figure S2.** Development of MVNs with HUVEC monoculture under IF, supplemented with various concentrations of ARP-100. Images are captured on days 1, 3, and 5 during vessel development. Green – GFP HUVEC, magenta – 40 kDa Texas Red dextran. Scale bar is 1 mm.

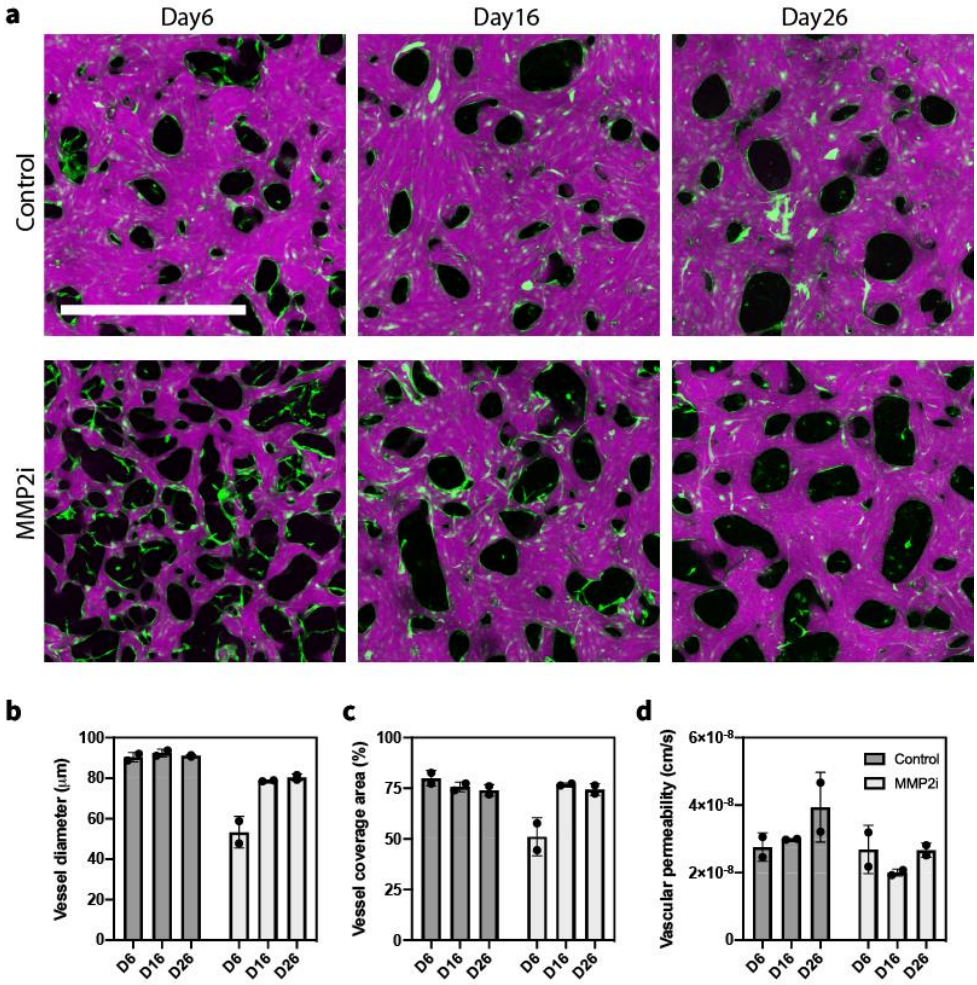


**Figure S3.** (a) Confocal projected z-stack image of MVNs formed with HUVEC monoculture under IF, with or without supplementing 10 μM ARP-100, on days 6, 16 and 26. Inhibitors were only included in the culture media during the first 6 days during vessel development. After the networks become perfusable, ARP-100 was removed from the culture media. Scale bar is 1 mm. (b-d) Time evolution of some morphological parameters and vascular permeability for MVNs formed with HUVEC monoculture under IF, with or without supplementing 10 μM ARP-100.

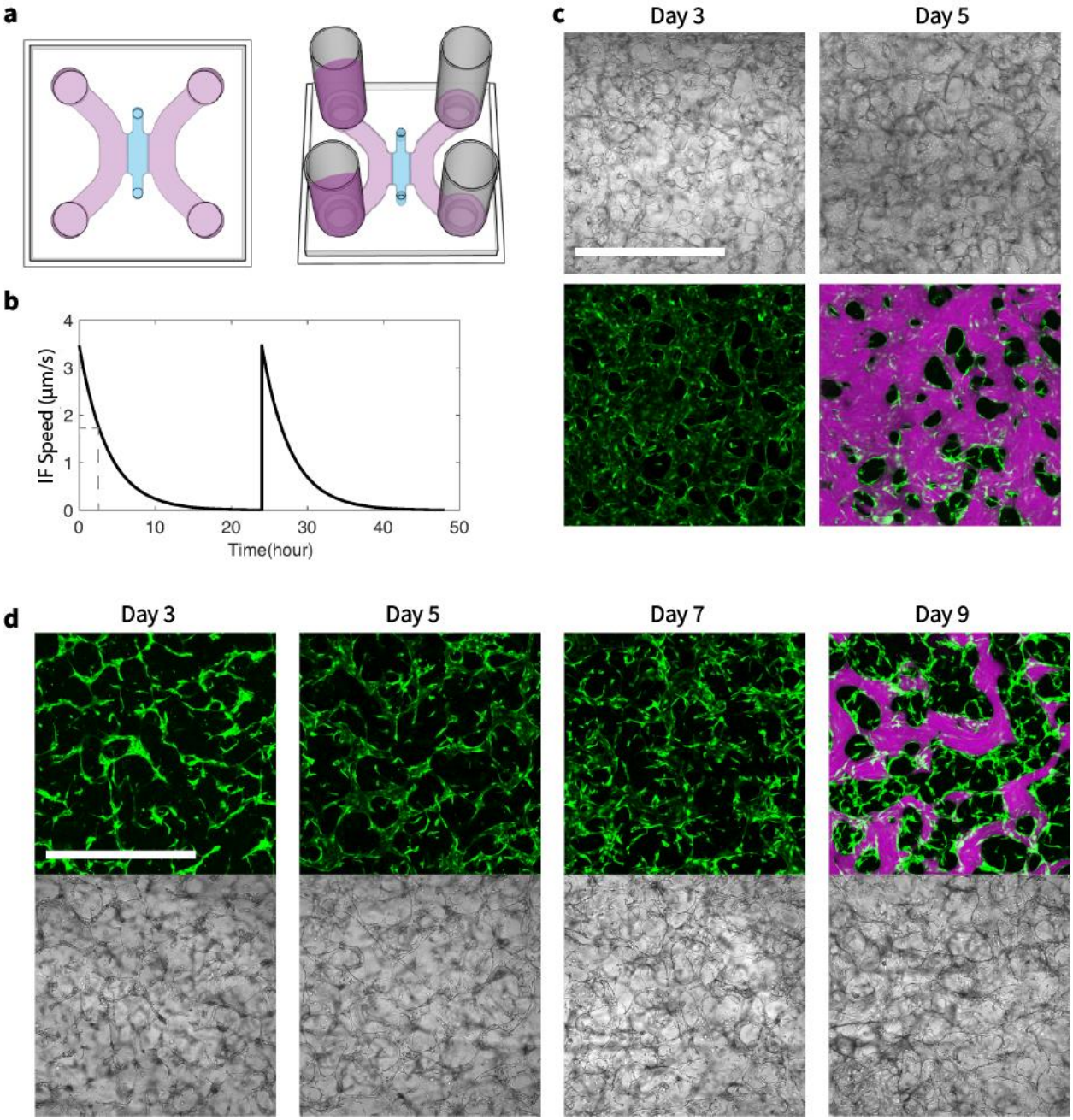


**Figure S4.** Effects of transient IF. (a) Schematics showing the setup of implementing transient IF on the 3-channel device. (b) Time evolution of IF speed across the gel region with this setup, assuming the pressure gradient will be restored at 24 hours with media change. IF was computed with analytical solution and measured matrix permeability of acellular fibrin gel. (c) Development of MVNs formed with HUVEC monoculture, under transient IF. Images were captured on days 3 and 5. Scale bar is 1 mm. (d) Development of MVNs formed with HUVEC monoculture, under transient IF implemented from day 3. Images were captured on days 3, 5, 7 and 9. Scale bar is 1 mm.

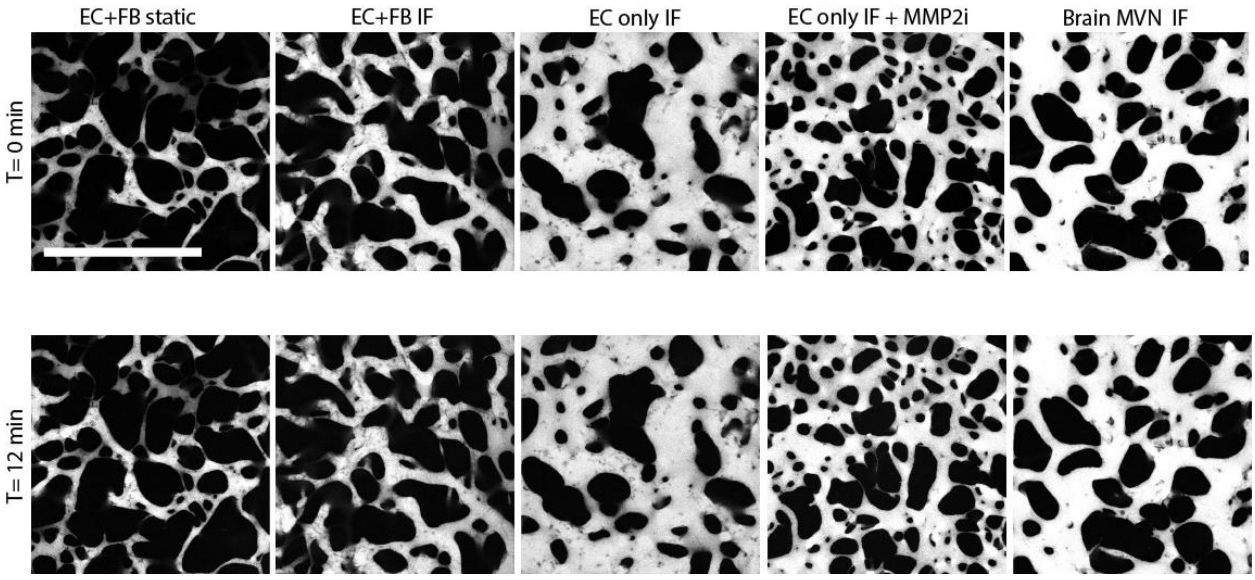


**Figure S5.** Vascular permeability measurements for various perfusable MVNs engineered in this study, including: co-cultured HUVECs and HLFs under static conditions, co-cultured HUVECs and HLFs under IF, HUVEC monoculture under IF, HUVEC monoculture under IF supplemented with MMP2i, and tri-culture of BECs, PCs and ACs under IF. Confocal z-stack images of perfusable MVNs with 40 kDa Texas red dextran were acquired with 12 min time interval. Collapsed z-stack images are shown. Good barrier function can already be seen from the small change in the intensity level in the interstitial space.