A Self-Assembly and Cellular Migration Based Fabrication of High-Density 3D Tubular Constructs of Barrier Forming Membranes

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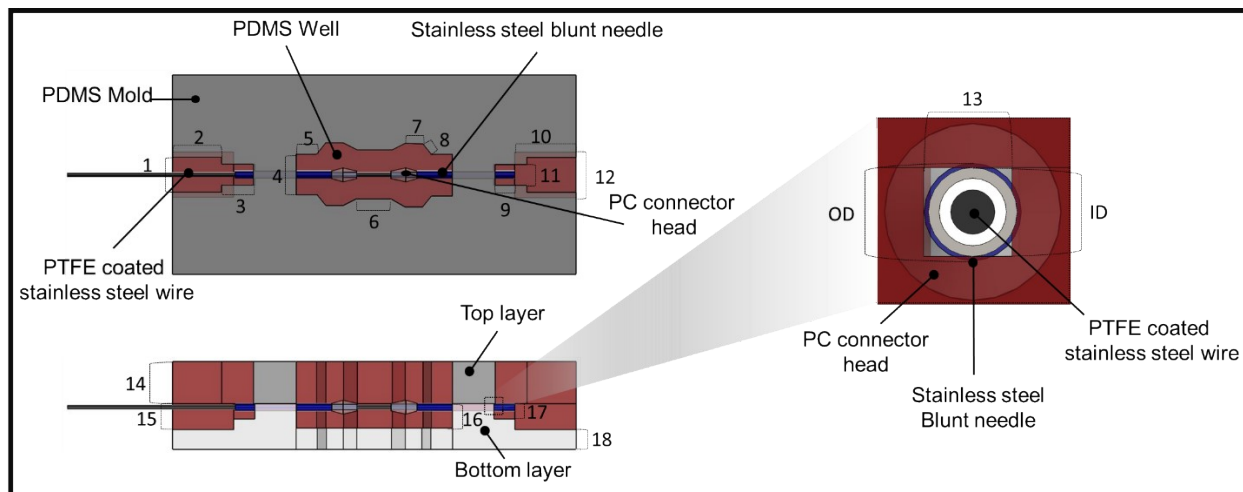
Supplementary Information

Fig. S1. Schematic view of the different components of the final PDMS mold.
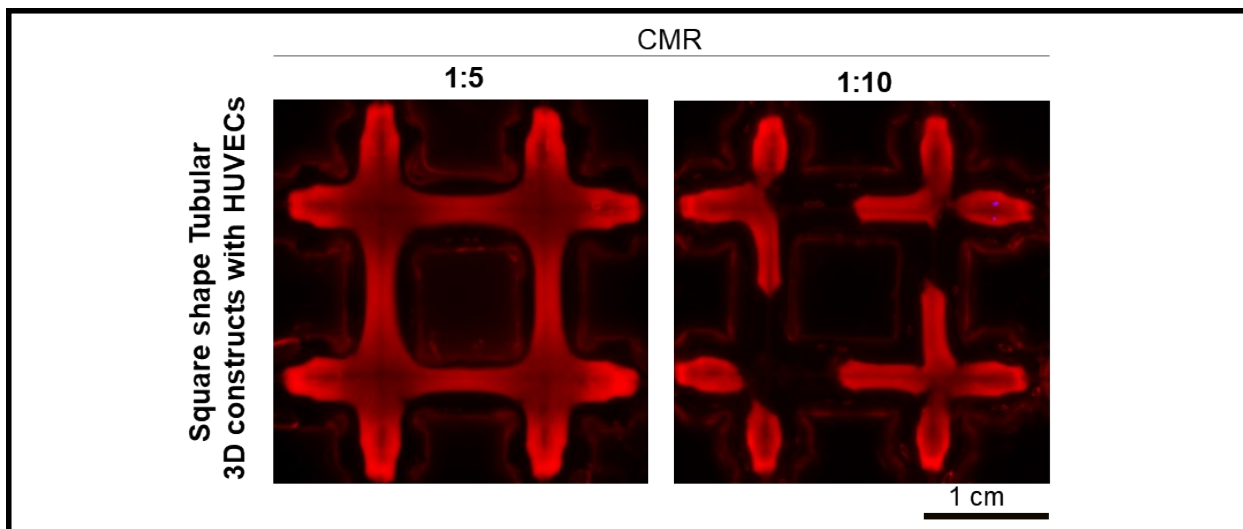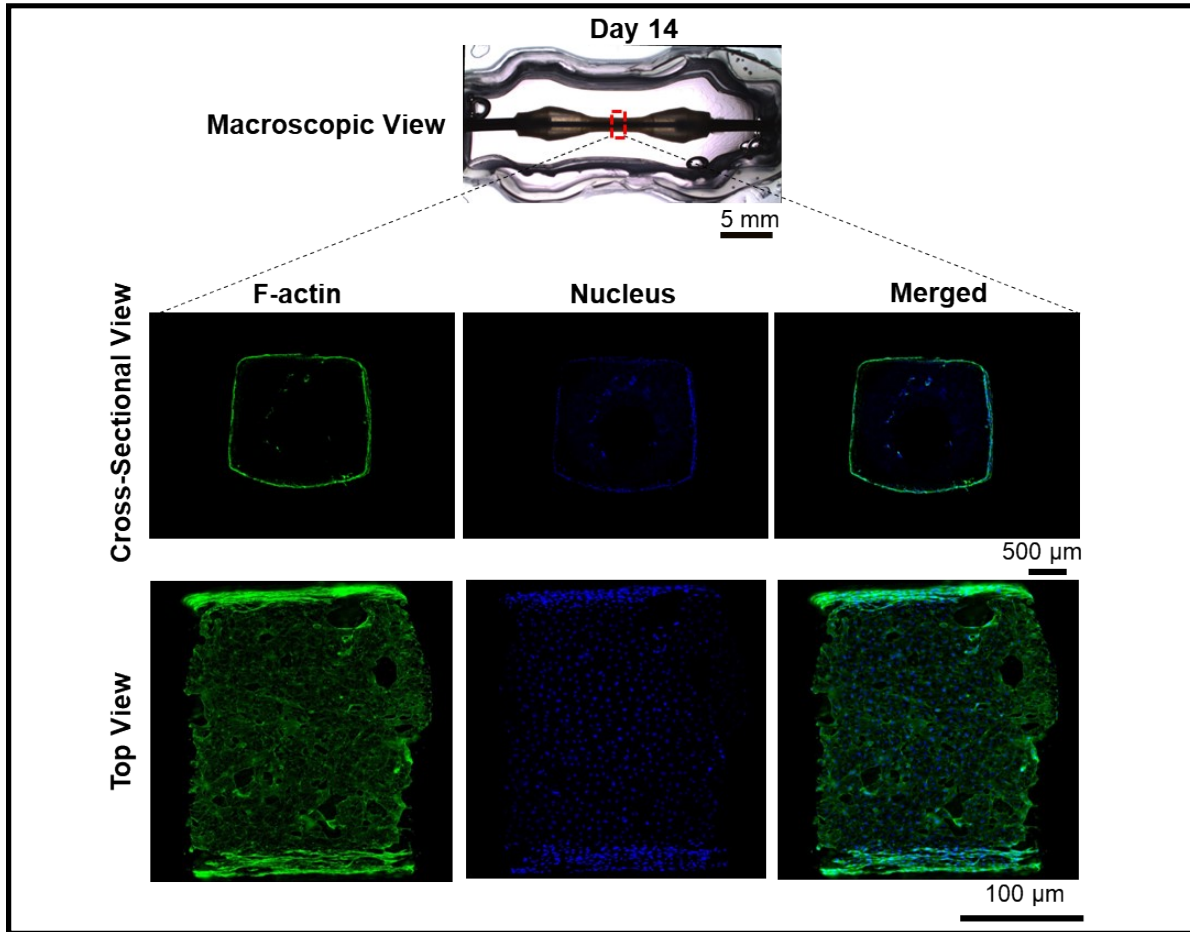

Fig. S2. The effect of CMR on the formation of squared shape tubular constructs. Low collagen amount led to construct rupture, especially at the branching sites, where the collagen structure becomes thinner compared to other sections.

Fig. S3. The HUVECs tubular construct on day 14. The brightfield image does not show a significant change in the overall construct shape after 14 days of culture. The fluorescence image of the cross-section and periphery of the constructs suggest that after 14 days, microvasculatures inside the ECM regress further over time. In addition, the results suggest that the periphery coverage of the endothelial cells also decreases after reaching a peak after 7 days which can be due to the absence of mural cells and fluid flow shear stress.
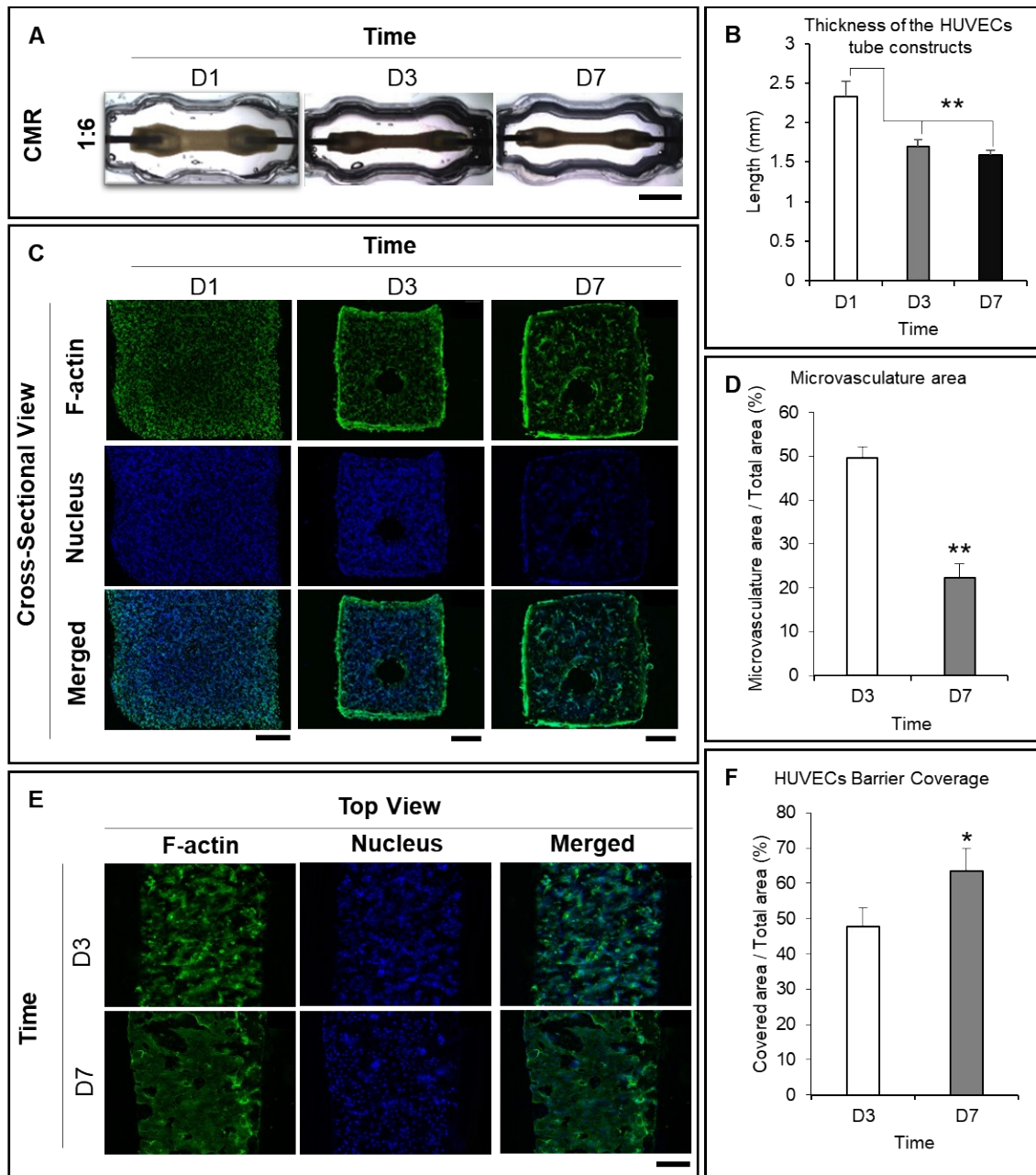
Fig. S4. The effect of 1:6 CMR on the evolution of the HUVECs tubular constructs and the endothelial cells barrier formation. A) Brightfield images of the HUVECs constructs shrinkage over 7 days. Scale bar: 5 mm. B) Significant decrease of constructs thickness after 3 days of culture. C) Fluorescence images of the HUVECs tubular constructs cross-section illustrating HUVECs' actin filaments, nuclei, and overall tissue alterations over 7 days period. Scale bar: 500 μm. D) Significant reduction of microvasculature area within the HUVECs tubular constructs after 7 days. There was no significant difference in relative microvasculature area between the 1:6 and 1:10 groups. E) Fluorescence images of HUVECs tubular constructs periphery illustrating HUVECs' actin filaments, nuclei, and endothelial cells layer formation on the periphery of the tubular construct over 7 days period. Scale bar: 100 μm. F) Significant increase in endothelial cells coverage of the constructs' periphery after 7 days.
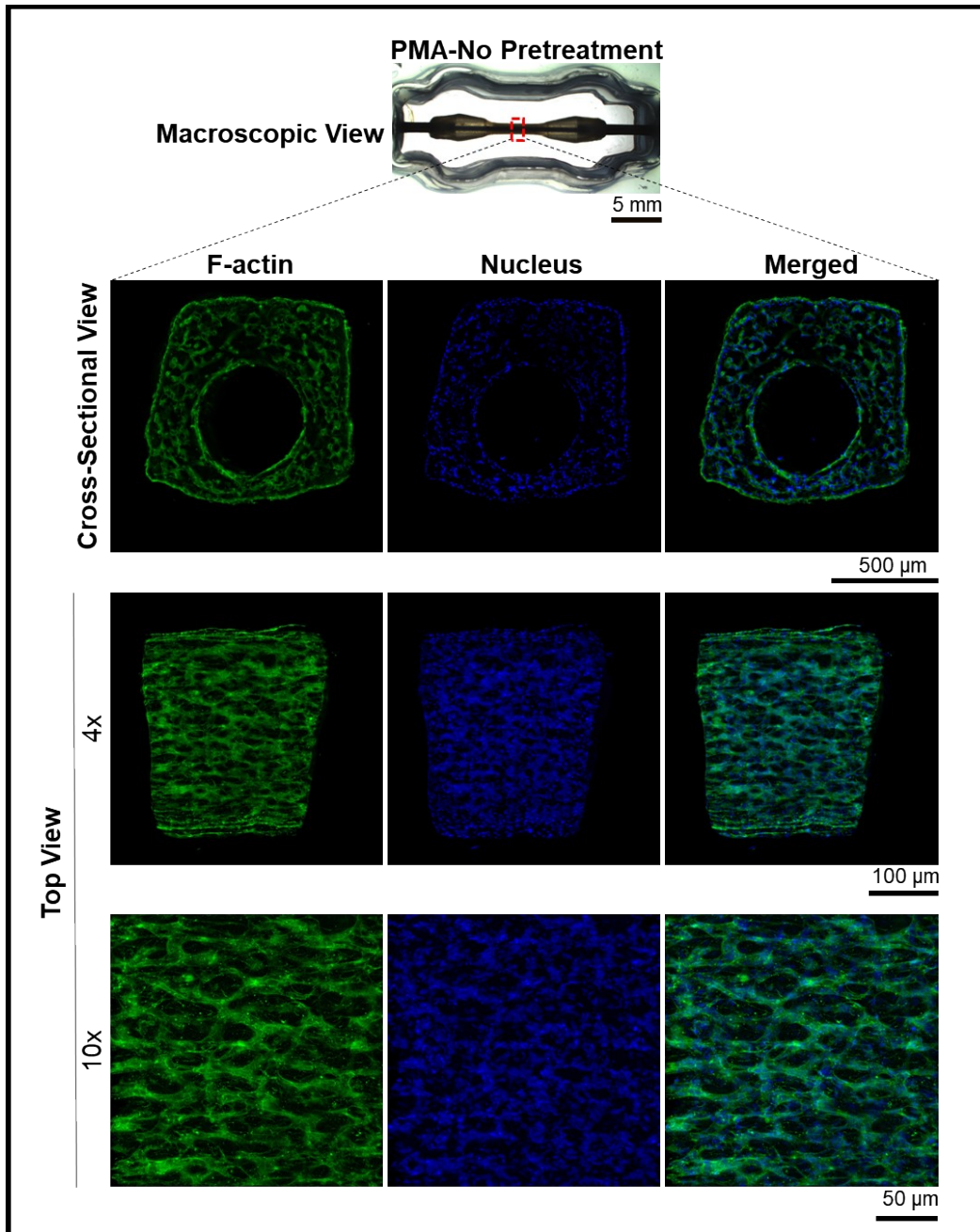
Fig. S5. The effect of PMA treatment without pretreatment on HUVECs tubular constructs after 7 days. The fluorescence images of actin filaments and nuclei suggest that PMA treatment without pretreating HUVECs prior to the biofabrication process will promote the formation of microvasculature-like structures and further stabilize them inside the constructs.

Table S1. Dimensions of the various PDMS devices. All values are presented in millimeters.

Device	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6	Section 13
D1	5	7	4.5	6	3	5	1
D2	5	7	4.5	7.3	3.75	5	1.45
D3	5	7	4.5	8	3.75	5	1.7
D4	5	7	4.5	6	3	10	1
D5	5	7	4.5	6	3	20	1

Device	Section 7	Section 8	Section 9	Section 10	Section 11	Section 12	Section 14
D1	2.5	2	3	8.75	3	6.5	6
D2	2.5	2	3	8.75	3	6.5	6
D3	2.5	2	3	8.75	3	6.5	6
D4	2.5	2	3	8.75	3	6.5	6
D5	2.5	2	3	8.75	3	6.5	6

Device	Section 15	Section 16	Section 17	Section 18	Needles inner diameter	Needles outer diameter	Wire diameter
D1	3.7	3.5	2.2	2.8	0.81	1.07	0.5
D2	3.7	5.5	2.2	2.8	0.96	1.27	0.79
D3	3.7	5.7	2.2	2.8	1.22	1.47	1
D4	3.7	3.5	2.2	2.8	0.81	1.07	0.5
D5	3.7	3.5	2.2	2.8	0.81	1.07	0.5

Table S2. Optimized values of bioink compounds for different tubular constructs designs.

Device	D1	D2	D3	D4	D5	Y shape	Square shape	Square shaped large network
CMR	1:10/1:6	1:6	1:6	1:6	1:6	1:6	1:6	1:6
Total volume (ml)	1.15	2	2	1.5	2	2.5	7	24
Number of HUVECs (×10^6)	2	2	2	2	3	2.2	6	18

Endothelial barrier apparent permeability calculations:

The transport through the microchannel to the medium outside involves transport through the aqueous bound layer to the ECM in the microchannel (P_{aq}), transport through the porous ECM structure (P_{ECM}), and transport through the cellular barrier (P_{CB}). These steps are in series and the apparent permeability (P_{app}) can be calculated using the following formula [1–4]:

$$\frac{1}{P_{app}} = \frac{1}{P_{aq}} + \frac{1}{P_{ECM}} + \frac{1}{P_{CB}} \quad (1)$$

Based on this, by measuring the apparent permeability of a cell-free construct we can calculate the $\frac{1}{P_{aq}} + \frac{1}{P_{ECM}}$ part of the equation and subsequently we can calculate the contribution of the cellular barrier to the barrier properties of the tissue barrier model. Based on this for 4 kDa FITC-dextran, which we measured the cell free apparent permeability as well, we have:

$$\frac{1}{1.13} = \frac{1}{3.11} + \frac{1}{P_{CB}}$$

Thus, P_{CB} would be 1.78 × 10^{-5} cm/s. Based on these calculations, the endothelial barrier is responsible for approximately 64% of the HUVECs tubular constructs and the ECM is forming only 36% of the resistance to the transport of the compounds. This shows that the measured apparent permeability of the HUVECs tubular constructs is majorly dependent on the endothelial barrier, and not the ECM.

References:

1   A. D. Wong, M. Ye, A. F. Levy, J. D. Rothstein, D. E. Bergles and P. C. Searson, *Front. Neuroeng.*, 2013, **6**, 1–22.

2   J. Karlsson and P. Artursson, *Int. J. Pharm.*, 1991, **71**, 55–64.

3   V. Van Duinen, A. Van Den Heuvel, S. J. Trietsch, H. L. Lanz, J. M. Van Gils, A. J. Van Zonneveld, P. Vulto and T. Hankemeier, *Sci. Rep.*, 2017, **7**, 1–11.

4   D. Kim, S. Eom, S. M. Park, H. Hong and D. S. Kim, *Sci. Reports 2019 91*, 2019, **9**, 1–11.