Optimization of a lab-on-a-chip device and method for quantification of extravasation

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Background

Metastasis comprises of intravasation, extravasation and new tumor formation at a secondary site in the body. Understanding the mechanism of metastasis and developing new platforms to study metastasis plays a critical role in both the diagnosis and the treatment of cancer.

Questions

Extravasation on a chip has been modeled before. Yet, the approach requires further optimization.

Methods

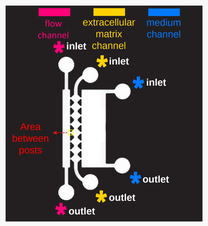


Figure 1: Design of LOC device. Left to the right: flow channel, matrix channel and medium channel.

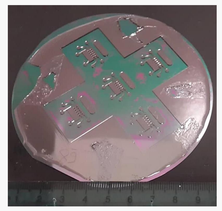


Figure 2: Master mold fabricated by UV litography technique.

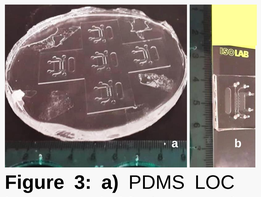


Figure 3: a) PDMS LOC image and **b)** Image of PDMS LOC device after permanent bonding onto glass slide by UV/ozone treatment.

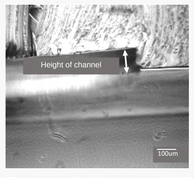


Figure 4: Height of PDMS LOC device.

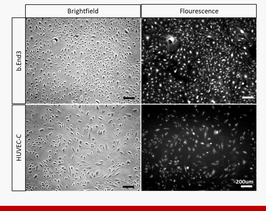
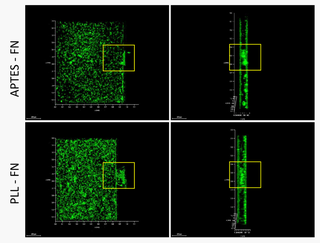


Figure 5: Brightfield and flourescent images of endothelial cells.

Results

APTES coating provides the appropiate surfaces for binding of protein in a short time compare to PLL (Poly-L-lysine).



LAM was better at promoting endothelial monolayer formation compared to FN and COL.

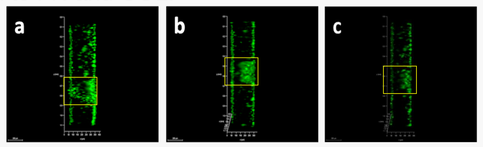


Figure 6: a) APTES-0.005 mg/ml type 1 COL, **b)** APTES-0.0125 mg/ml LAM, **c)** APTES – 0.0125 mg/ml FN.

Number of endothelial cells to be seeded was optimized to be 19736 cells / height of the blood vessel channel (μm) to form a continuous monolayer in APTES-Lam coated LOC device.

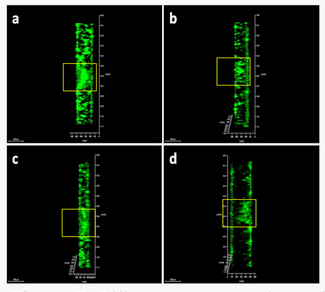


Figure 7: Different concentrations of b.End3 cells depending on different heights.
a)5.8X10^6/ml, **b)** 2.6x10^6/ml,
c) 2.3x10^6/ml, **d)** 7.5x10^6/ml

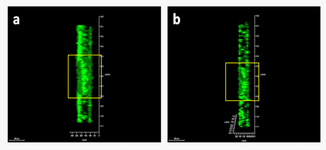
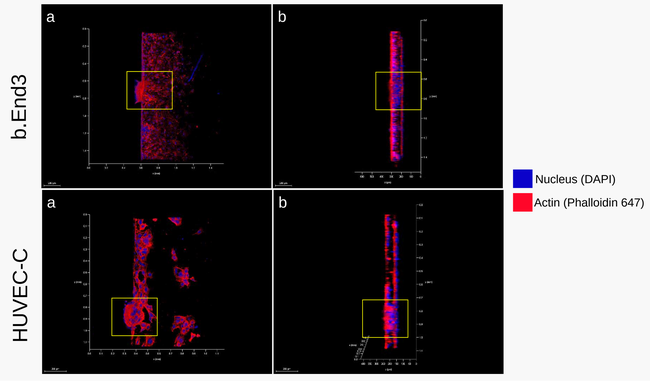


Figure 8: Different endothelial cells after loading
a) HUVEC-C (2.6x10^6/ml),
b) b.End3 (2.3x10^6/ml)



Presence of dextran in the endothelial cell suspension prevented formation of cluster.

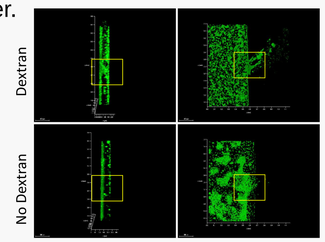


Figure 9: 3D images of endothelial cells after overnight incubation on post side. Then, 3x10^6/ml concentration of cells was loaded into the LOC device.

Real time imaging and analysis showed that 70 kDa fluorescent dextran did not diffuse from the flow channel into the matrix.

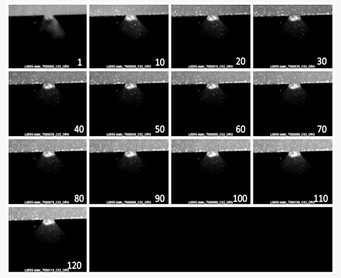


Figure 10: Fluorescent images of b.End3 cells were taken per 10 minutes in 2 hours after dextran 70kDa loading into the flow channel. Shear stress is 0.5 dyne/cm².

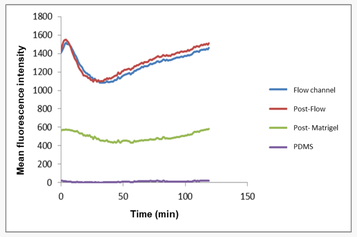
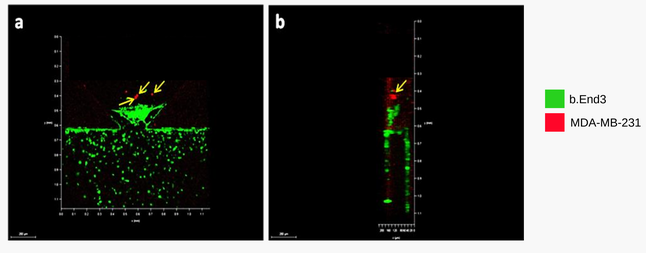


Figure 11: Time-dependent average fluorescence signal plot.

Fluorescently labeled breast cancer cells (MDA-MB-231) interacted with b.End3 cells and they extravasated through the endothelial monolayer into the matrix channel.



Conclusions

We have optimized surface coating, cell density and medium composition for successful mimicking of a blood vessel in a LOC device. The optimized method enables the determination of extravasation of cancer cells.

References and acknowledgements

References: [1] Myers, D.R., Sakurai, Y., Tran, R., Ahn, B., Hardy, E.T., Mannino, R., Kita, A., Tsai, M., Lam, W.A. J. Vis. Exp. (64), e3958, DOI : 10.3791/3958 (2012).
[2] Siddique A, Meckel T, Stark RW, Narayan S. Colloids Surf Biointerfaces 1;150:456-464 (2016).
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