Stroke-on-a-Chip

BMEn 5151

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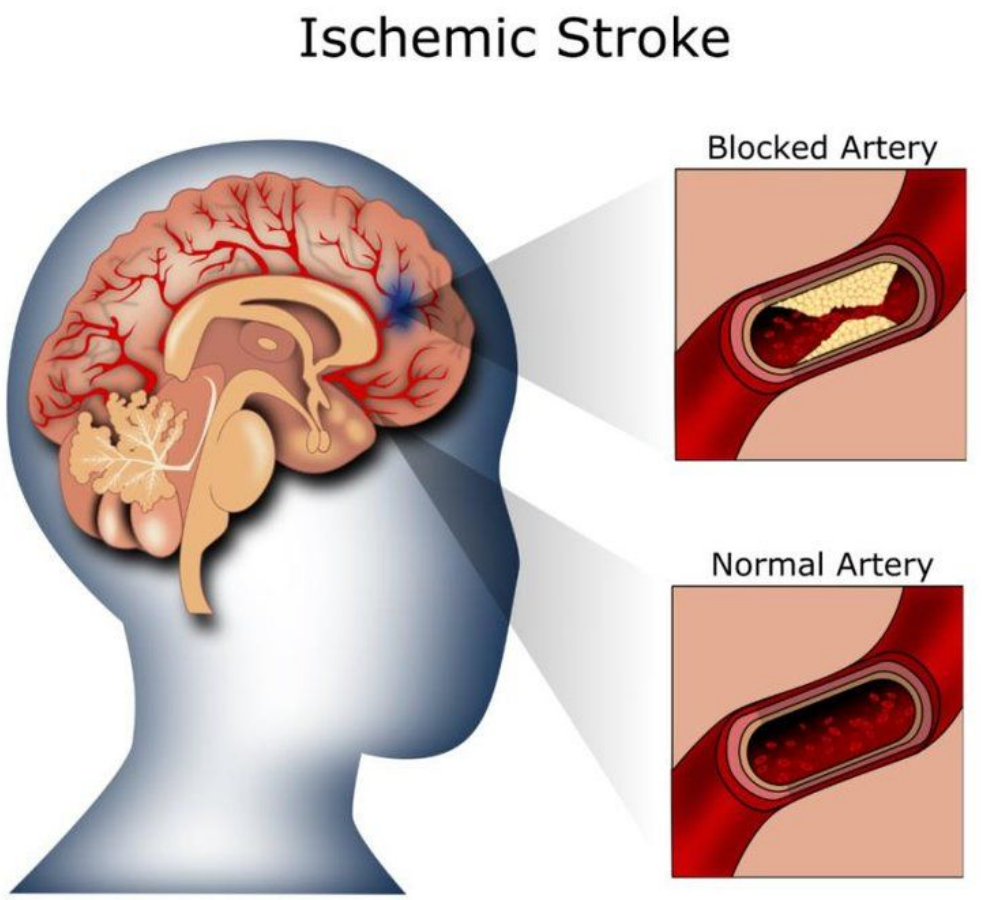
Stroke Background

What is an Ischemic Stroke?

- Blood supply to part of the brain is blocked or reduced preventing the brain from getting oxygen and nutrients causing that part of the brain to die

Causes of strokes

- Blood clots
- Atherosclerosis, a disease that causes the narrowing of arteries over time
- Diabetes
- High Blood Pressure
- High Cholesterol



Impact of Strokes

101 million people are living with the stroke aftermath

1 in 4 people over the age of 25 will experience a stroke in their lifetime.

132 million healthy years of life lost to stroke each year

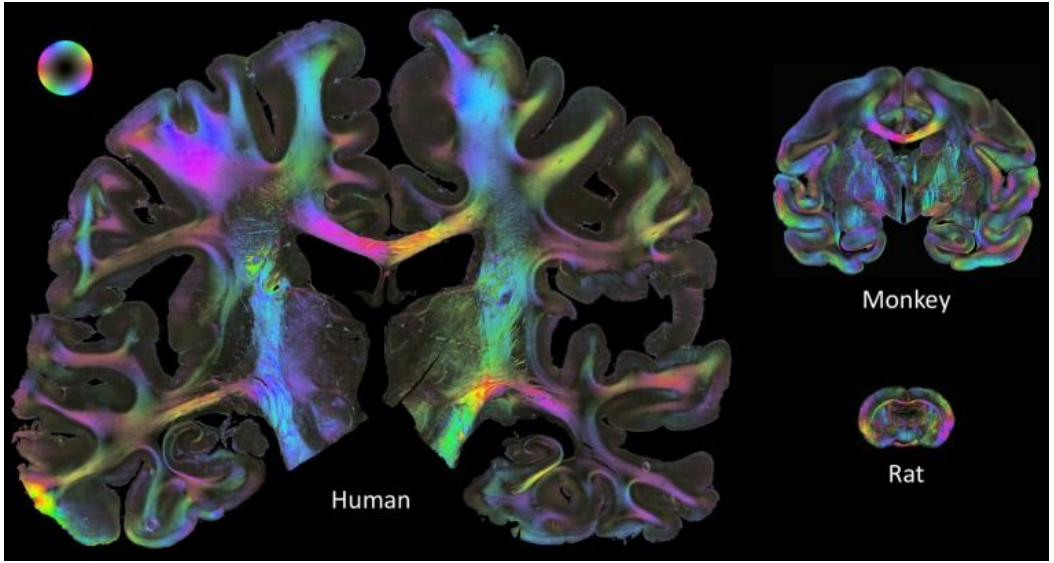
12.2 million new strokes each year

90% of all strokes are linked to 10 modifiable risk factors

Current Experimental Models of Ischemic Strokes

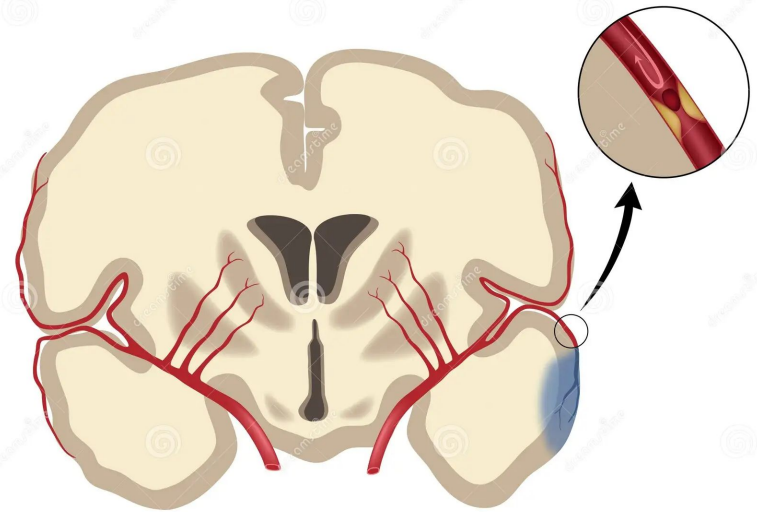
Animal Models

- Absolute volume of brains vary per animal
- Rats lack the “Penumbra” which is the reversibly injured brain tissue around the ischemic core which is the target for the treatment of acute stroke
- Unable to control the location and size of dead tissue resulting from failure of blood supply

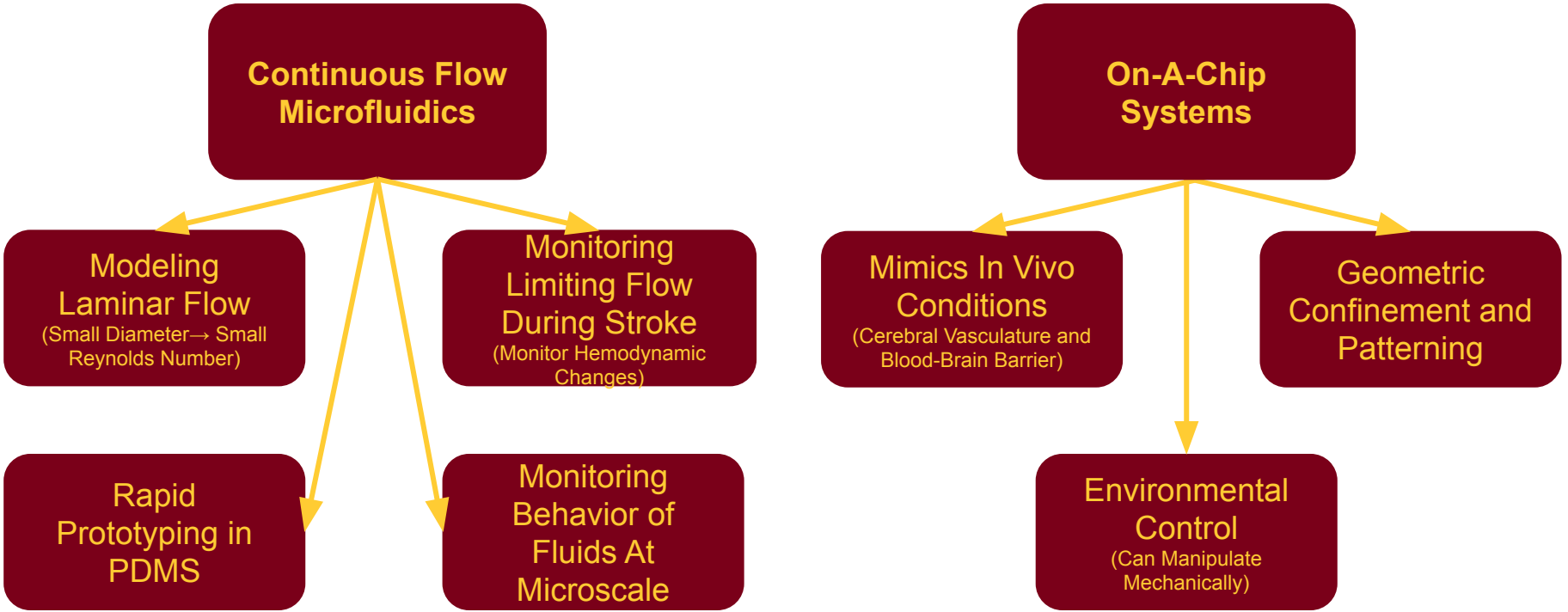


Purpose of Stroke-on-a-Chip

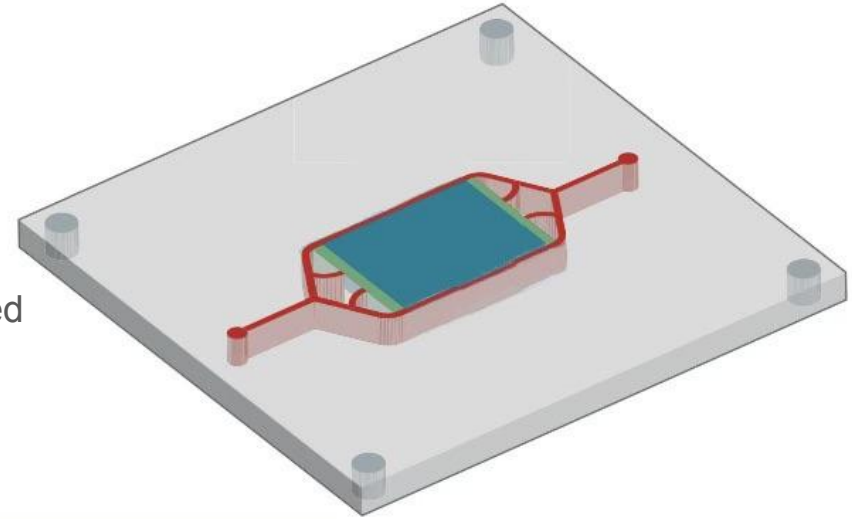
1. Understanding Stroke Mechanisms
   - Interactions between neurons, glial cells, and the vascular system
2. Drug Screening and Development
   - Understanding drug sensitivity in different patient subsets
3. Personalized Medicine
   a. Investigate Effects of Risk Factors/Environmental Effects
      i. High Blood Pressure
      ii. Increased Blood Sugar from Diabetes
      iii. High Red Blood Cell Count
4. Biomarker Discovery for Early Diagnosis, Prognosis, and Monitoring of the Disease
5. Ethical Reduction in Animal Use



Features of Stroke-on-a-Chip



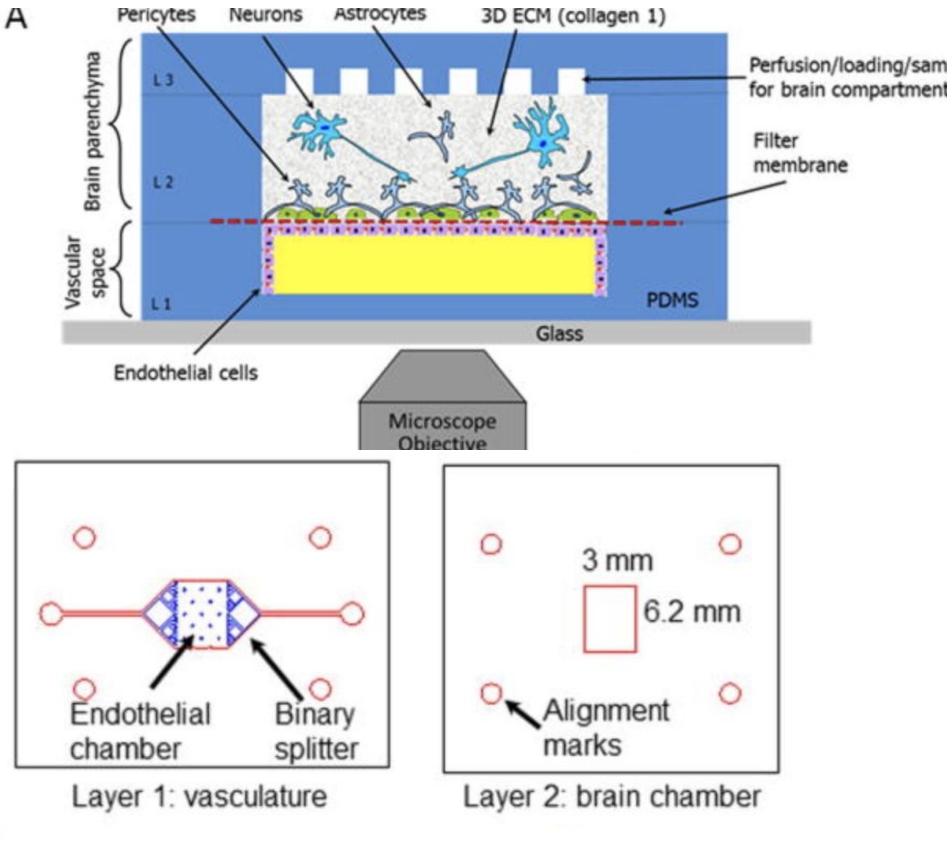
Fabrication - PDMS



- Three PDMS layers using soft-lithographic replica molding
- 1st and 3rd layer produced with PDMS poured into photolithographically created mold
- 2nd layer, the NVU, formed by curing PDMS compressed between polycarbonate plates with block in between
- Removed from molds, trimmed, washed, dried
- Plasma oxidation to bond the three layers
- Red channels support flow of blood-like fluid to the neurovascular unit

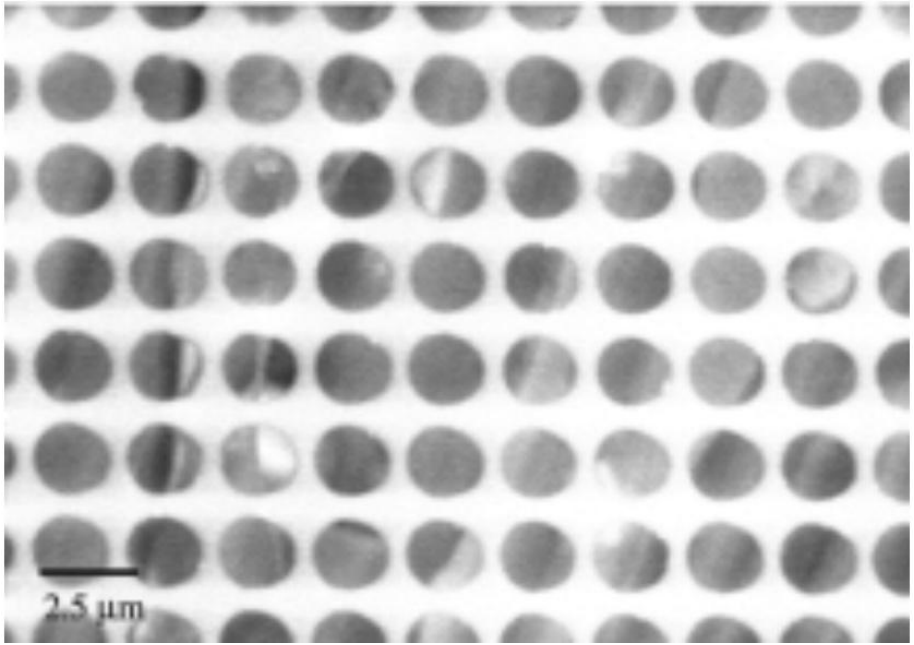
Fabrication - NVU

- Empty NVU coated with laminin to promote cell differentiation and adhesion
- Day 0, human brain-derived microvascular endothelial cells (hBMVEC) loaded into vascular chamber
- Day 1, media perfusion and hBMVEC started 12 day growth (to establish tight junctions)
- Day 12, 2:1 mix of human astrocyte and pericyte loaded and given 1-2 days to grow
- Day 14, collagen containing iPSC-derived neurons & co-differentiating astrocytes loaded atop astro and pericytes



Fabrication - Filter Membrane

- Plastic (SiN) membrane to serve as barrier and interface of brain tissue
- Membrane grown using LPCVD
- Series of membranes and pores formed with lithography
- Serves as membrane between the PDMS microfluidic channels and the NVU

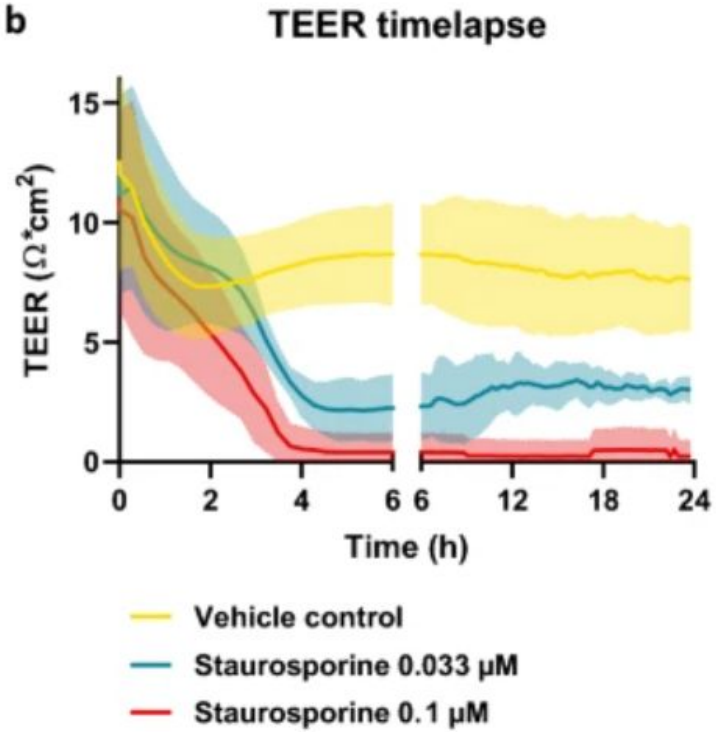


Testing: Overview

- Simulating Stroke Conditions:
  - Limit the flow of nutrients to cells
  - Expose cells to staurosporine which disrupts the blood brain barrier and induces apoptosis
- Testing Techniques:
  - Immunoflorescent Staining
  - Measuring Trans-Epithelial Electrical Resistance (TEER)
- Record data for controls

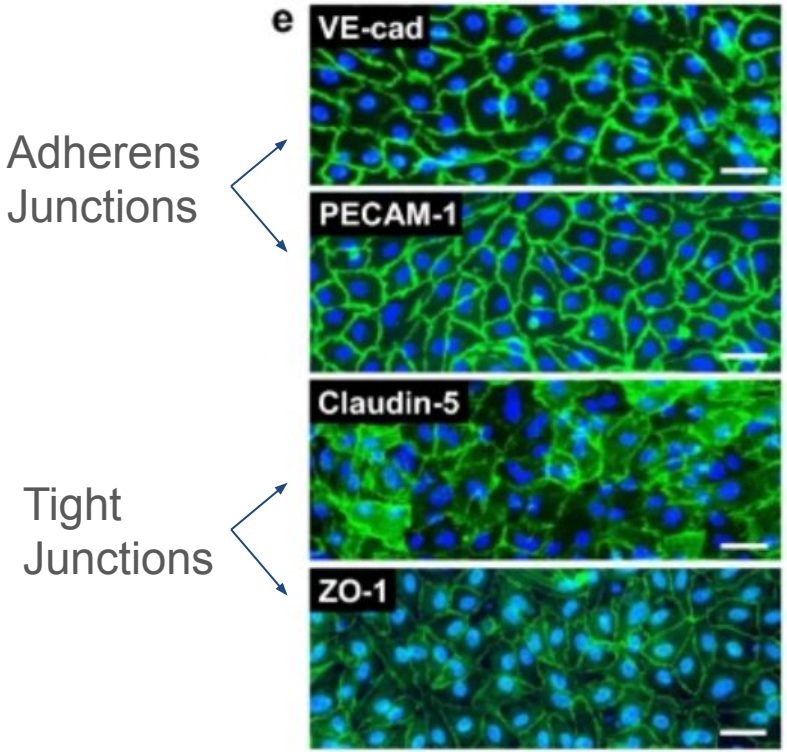
Testing: TEER

- Expose cells to staurosporine to simulate stroke conditions
- Measure Trans-Epithelial Electrical Resistance (TEER) over time at different concentrations



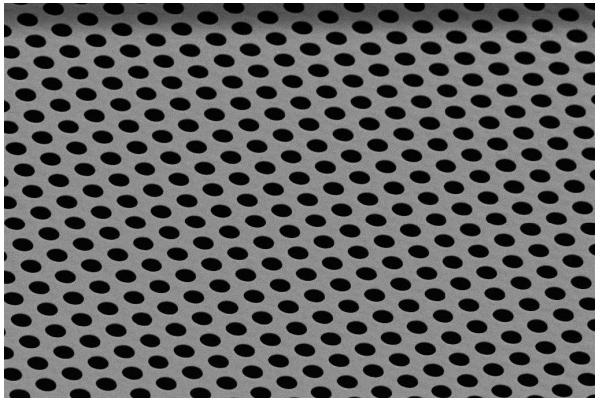
Testing: Immunofluorescent Staining

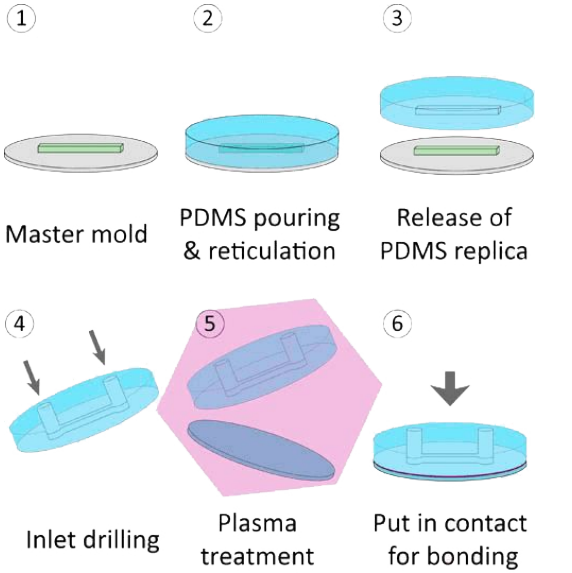
- Staining adherens and tight-junction proteins
  - Presence indicates the formation of barrier structures within the model
- Staining nuclei
  - Changes in morphology could indicate cell damage or apoptosis



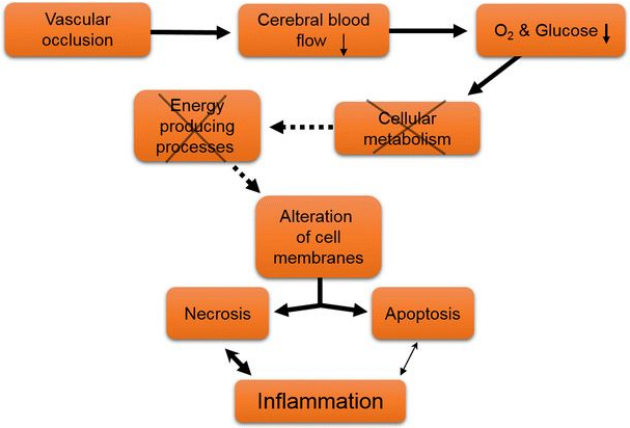
Biocompatibility

- Silicon Nitride Membrane
  - SiN to be used as membrane between vascular area and parenchymal area of the NVU
  - SiN is biocompatible and manufactured to be porous so it can act as a membrane
- PDMS
  - Chemically inert, gas permeable, resistant to protein adsorption through surface activation
  - Widely used in on-a-chip and other microfluidic devices





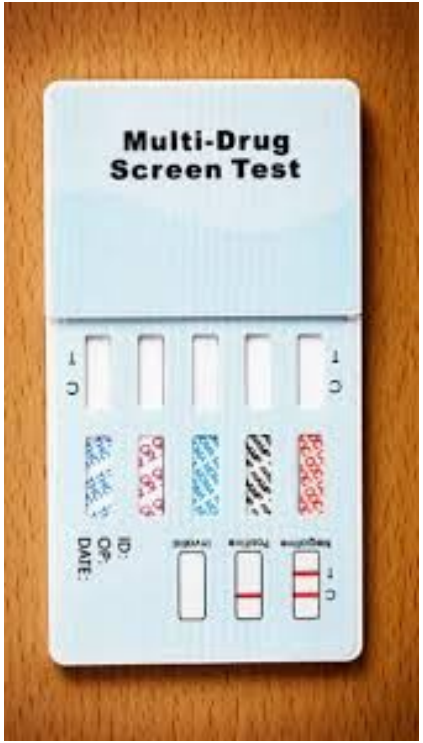
Limitations



- Pathophysiological conditions of ischemic stroke
- Bidirectional flow
  - Cerebral blood flow in vivo is unidirectional
- Lack of adequate shear stress
  - Capillaries typically experience shear stress ≥6 dynes/cm^2 (7)
  - Other models for NVU on-a-chip report a shear stress of ~1.2 dynes/cm^2 (7)
  - Options to increase shear stress include the use of pumps and syringes, however, this decreases ease of use

Future Applications

- Integration with analogous organ-on-a-chip systems to further understand the effects of ischemic stroke
- Development of the current NVU system to better understand other neurological diseases
- Apply the NVU system to investigate potential restorative therapies to fight neurological disorders
- The integration with other “on-a-chip” devices allows for potential drug screening applications



Summary

- Microfluidic OOC device modeling an ischemic stroke
- Fabrication
  - Channels made from PDMS using soft photolithography and replica molding
  - NVU using hBMVECs in vascular chamber, SiN as the filter membrane, and human astrocytes, pericytes, and neurons
- Testing
  - Transepithelial electrical resistance (TEER)
  - Immunofluorescent Staining
- Limitations
  - Shear stress not accurately represented
  - Bidirectional flow is not representative of *in vivo* conditions

References

1. Harris, S.G., Shuler, M.L. Growth of endothelial cells on microfabricated silicon nitride membranes for an*in vitro* model of the blood-brain barrier. *Biotechnol. Bioprocess Eng.* **8**, 246–251 (2003). https://doi.org/10.1007/BF02942273
2. Zhang, B., Korolj, A., Lai, B.F.L. *et al.* Advances in organ-on-a-chip engineering. *Nat Rev Mater* 3, 257–278 (2018). https://doi.org/10.1038/s41578-018-0034-7
3. Vernetti, L., Gough, A., Baetz, N. *et al.* Functional Coupling of Human Microphysiology Systems: Intestine, Liver, Kidney Proximal Tubule, Blood-Brain Barrier and Skeletal Muscle. *Sci Rep* 7, 42296 (2017). https://doi.org/10.1038/srep42296
4. *Impact of Stroke*. (n.d.). World Stroke Organization. https://www.world-stroke.org/world-stroke-day-campaign/about-stroke/impact-of-stroke
5. Sommer, C. J. (2017). Ischemic stroke: experimental models and reality. *Acta Neuropathologica*, *133*(2), 245–261. https://doi.org/10.1007/s00401-017-1667-0
6. Lecture slides
7. Wevers, N. R., Arya Lekshmi Nair, Fowke, T. M., Pontier, M., Kasi, D. G., Spijkers, X. M., Hallard, C., Gwenaëlle Rabussier, Remko van Vught, Vulto, P., Helga, & Lanz, H. L. (2021). Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. *Fluids and Barriers of the CNS*, *18*(1). https://doi.org/10.1186/s12987-021-00294-9
8. Team, E. (2022, December 8). PDMS: A Review. https://www.elveflow.com/microfluidic-reviews/general-microfluidics/the-polydimethylsiloxane-pdms-and-microfluidics/
9. Koh, Seong-Ho & Park, Hyun-Hee. (2017). Neurogenesis in Stroke Recovery. Translational Stroke Research. 8. 10.1007/s12975-016-0460-z.

Any Questions?