Multiple Sclerosis Specific Brain-on-a-Chip

Anthony, Alanna, Carina, Jordan

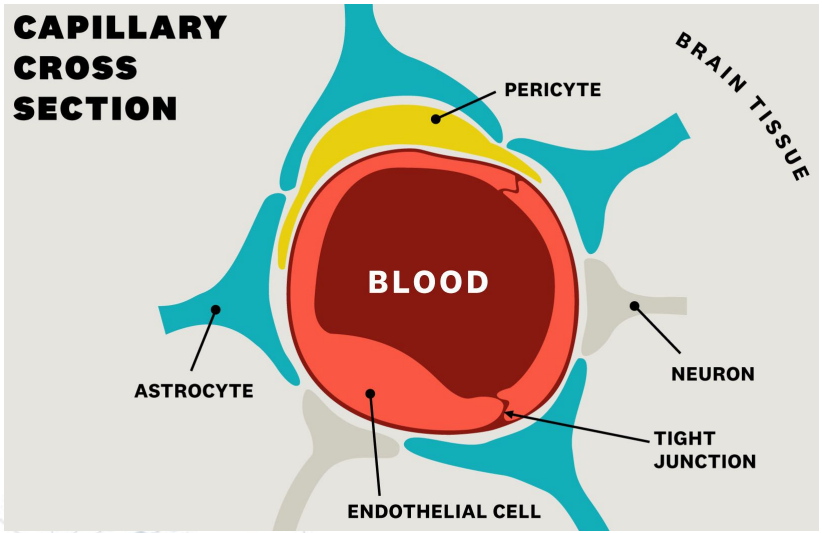
What is Multiple Sclerosis (MS)?

An autoimmune disease that affects the central nervous system.

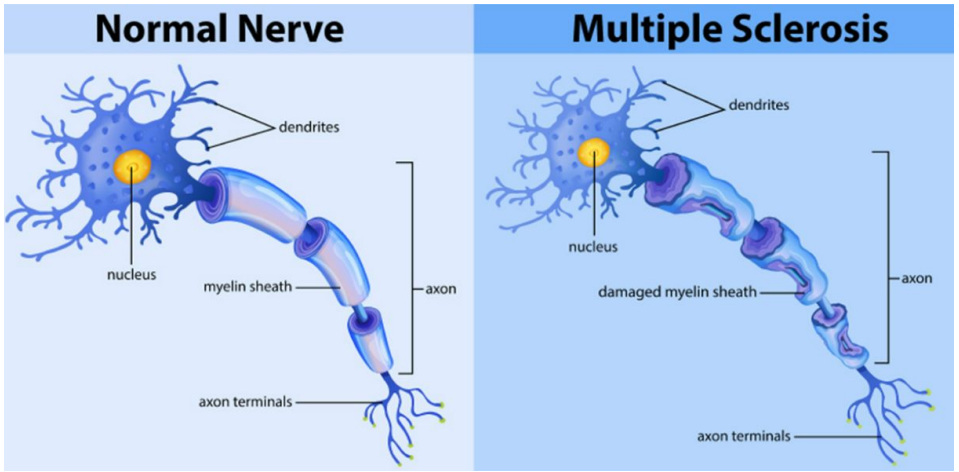
How Does MS Manifest?

1. Blood brain barrier (BBB) broken down (many causes)

2. Immune cells attack myelin



Illustration/Diana Molleda



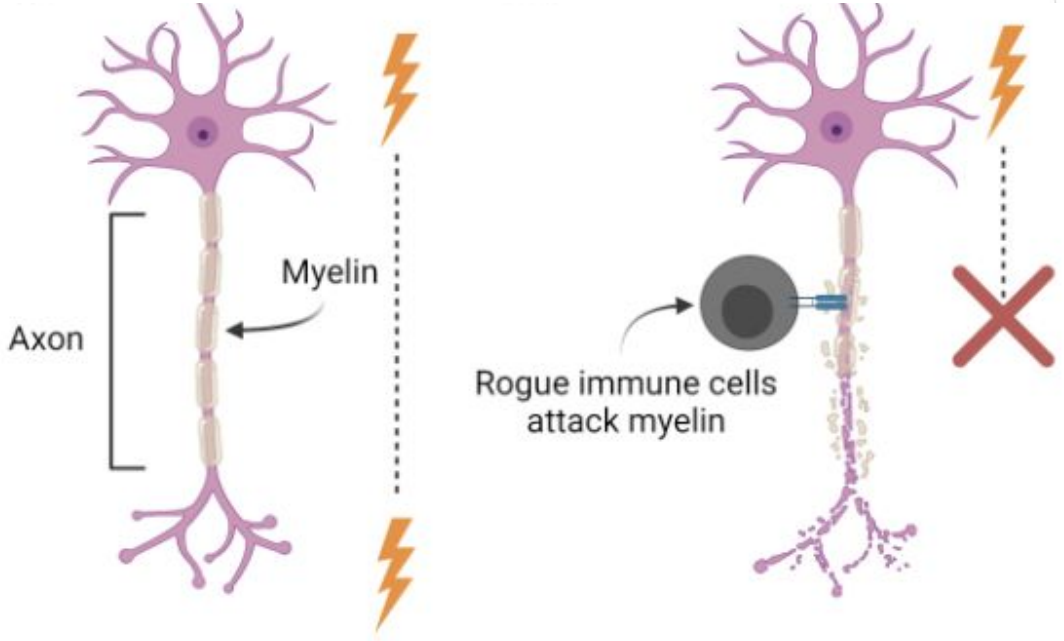
http://www.shieldhealthcare.com/community/incontinence/2019/08/29/the-mysterious-roots-of-multiple-sclerosis/attachment/normal-nerve-and-multiple-sclerosis/

How Does MS Manifest?

3. Electrical signals disrupted as myelin is degraded

Common Symptoms:

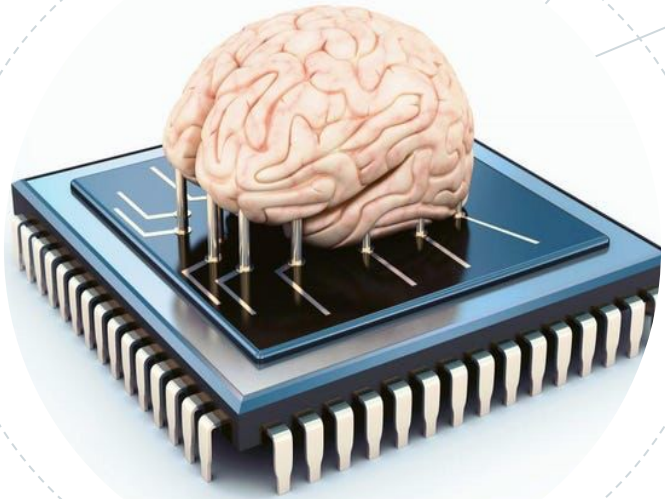
- Walking problems
- Vision problems
- Bladder problems
- Cognitive changes
- Emotional changes



https://immunobites.com/2021/05/10/saving-the-immune-system-from-itself-a-multiple-sclerosis-vaccine/

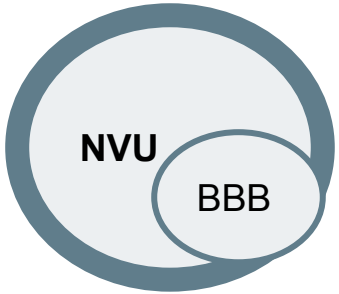
Why Would an MS Brain-on-a-Chip be Useful?

Novel MS treatments can be tested in an environment that truly mimics the disease state.

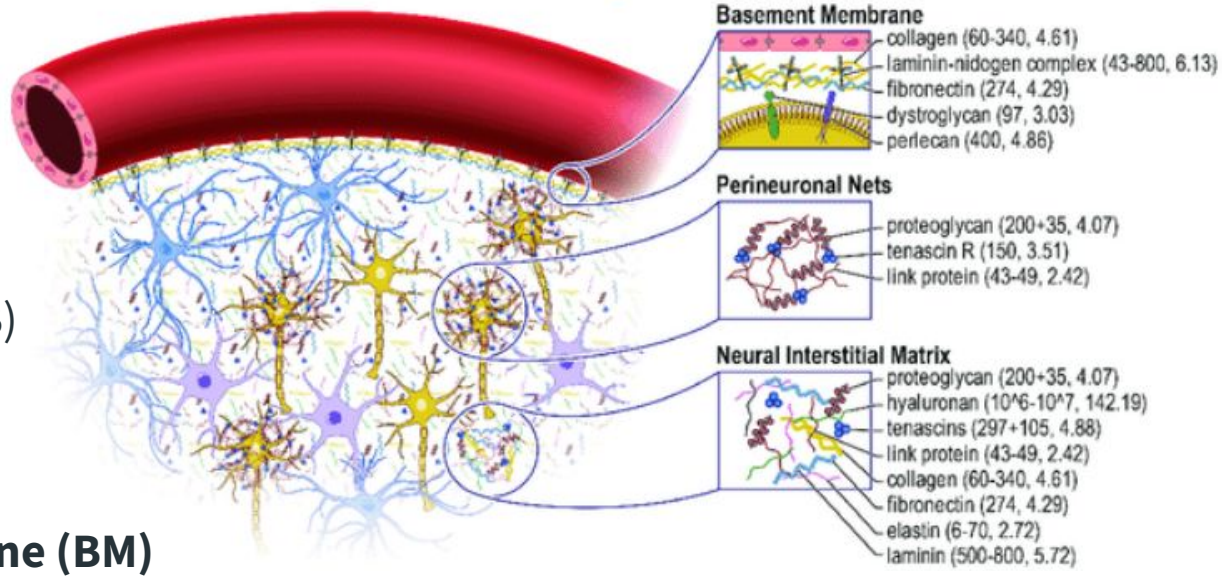


https://www.forbes.com/sites/cognitiveworld/2019/02/21/the-rise-of-the-silicon-brain/

NVU and BBB-on-a-Chip



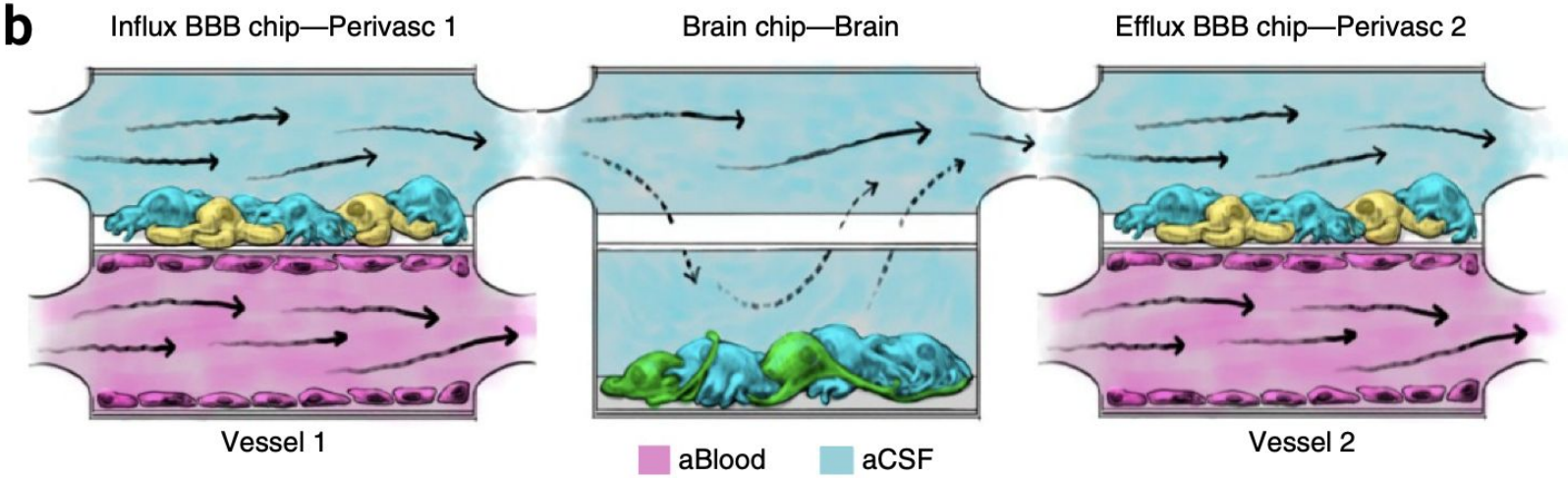
- Neurovascular Unit (NVU)
  - Brain Microvessel Endothelial Cells (BMECs)
  - Astrocytes
  - Pericytes
  - **Neurons**
  - **Microglia**
- Blood Brain Barrier (BBB)
  - BMECs
  - Pericytes
  - Astrocytes
  - **Basement Membrane (BM)**



Salmina, A. B., Kharitonova, E. V., Gorina, Y. V., Teplyashina, E. A., Malinovskaya, N. A., Khilazheva, E. D., Mosyagina, A. I., Morgun, A. V., Shuvaev, A. N., Salmin, V. V., Lopatina, O. L., & Komleva, Y. K. (2021). **Blood-Brain Barrier and Neurovascular Unit In Vitro Models for Studying Mitochondria-Driven Molecular Mechanisms of Neurodegeneration.** *International journal of molecular sciences*, *22*(9), 4661. https://doi.org/10.3390/ijms22094661

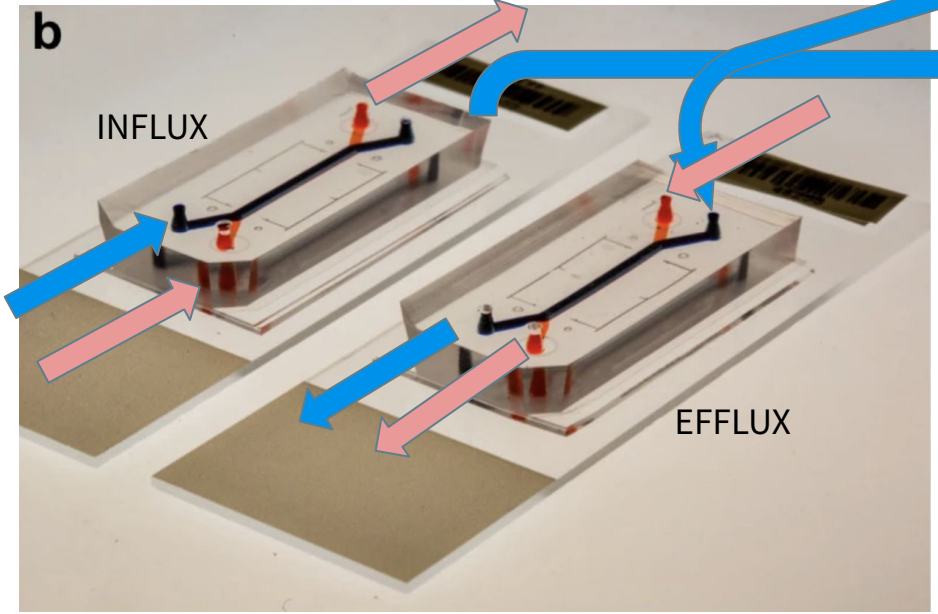
Previous Research

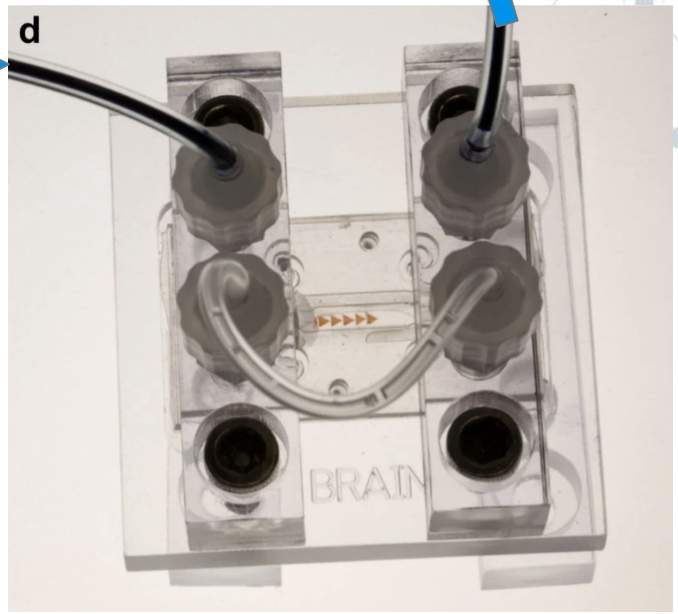
Brain-on-a-Chip Model



- Used for testing the effects of methamphetamine on the NVU
- Combines 3 BBB chips

Maoz, Ben M., et al. "**A Linked Organ-on-Chip Model of the Human Neurovascular Unit Reveals the Metabolic Coupling of Endothelial and Neuronal Cells.**" *Nature Biotechnology*, vol. 36, no. 9, 9, Oct. 2018, pp. 865–74. *www.nature.com*, https://doi.org/10.1038/nbt.4226.



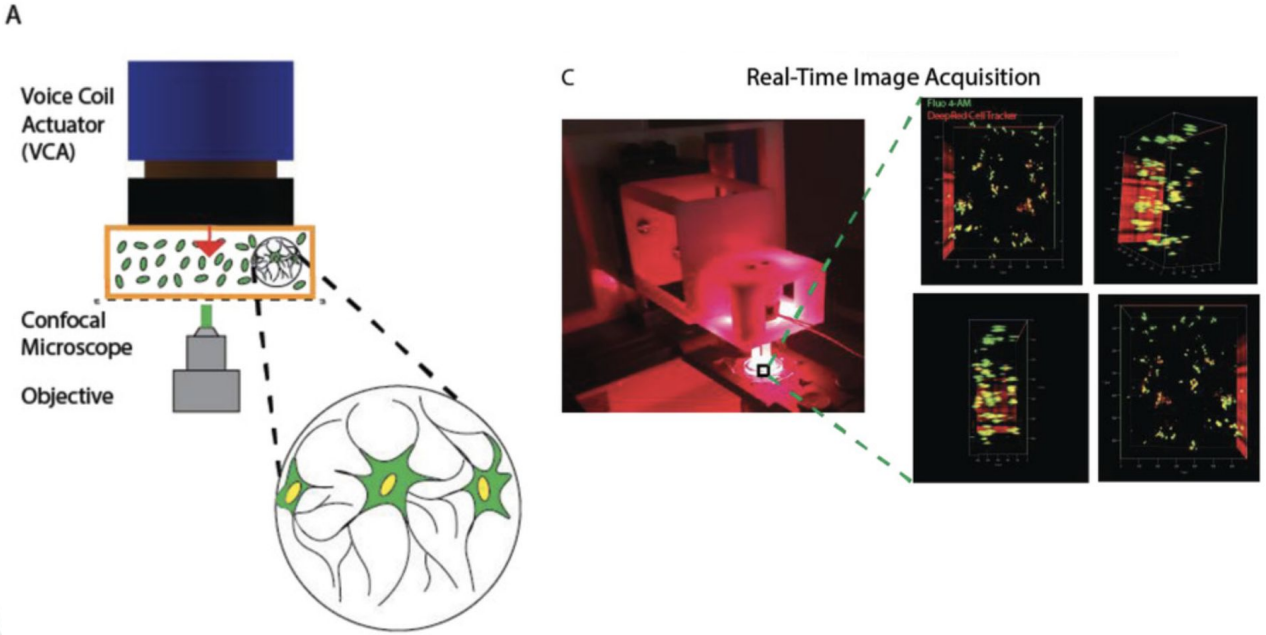


BBB Chips in Parallel

Brain Chip

Maoz, Ben M., et al. "**A Linked Organ-on-Chip Model of the Human Neurovascular Unit Reveals the Metabolic Coupling of Endothelial and Neuronal Cells.**" *Nature Biotechnology*, vol. 36, no. 9, 9, Oct. 2018, pp. 865–74. *www.nature.com*, https://doi.org/10.1038/nbt.4226.

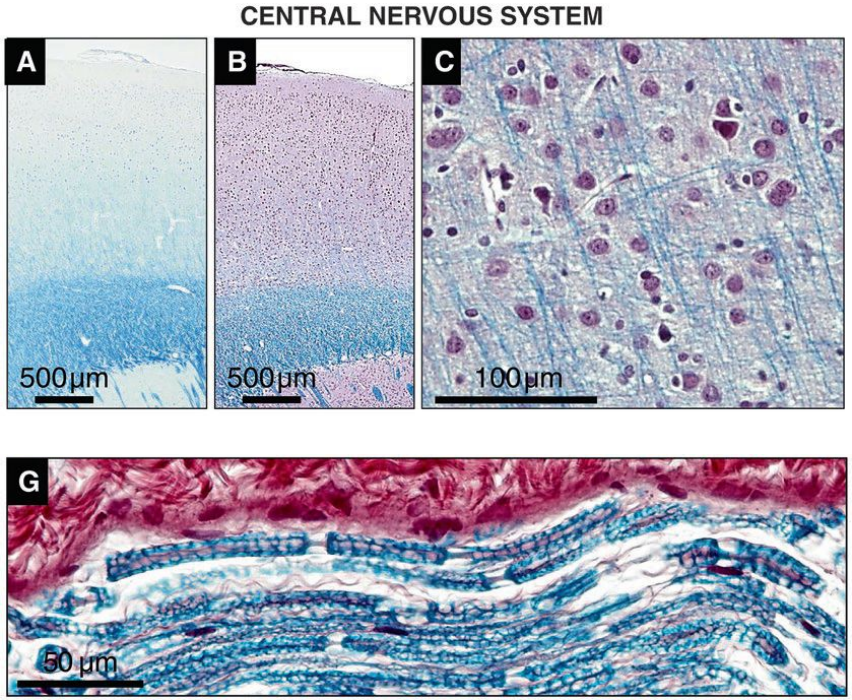
3D Matrix



- 3D extracellular matrix (ECM) with integrated neurons
- Spinning disk confocal microscopy

Bobo, J., Garg, A., Venkatraman, P., Puthenveedu, M., LeDuc, P. R., **3D In Vitro Neuron on a Chip for Probing Calcium Mechanostimulation**. *Adv. Biosys.* 2020, 4, 2000080. https://doi-org.ezp3.lib.umn.edu/10.1002/adbi.202000080

Method of Staining and Imaging Neurons

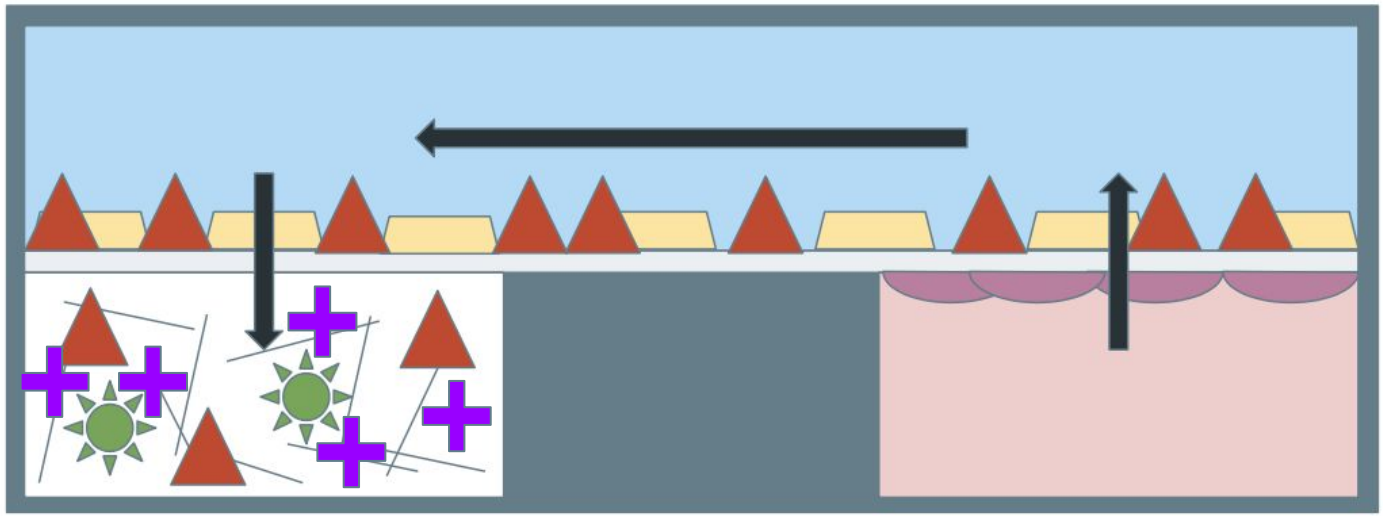


- Used on fixed tissue
- Figure B and C use MCOLL method of staining
- Visible using polarized light microscopy

Carriel V., Campos A., Alaminos M., Raimondo S., Geuna S. (2017) **Staining Methods for Normal and Regenerative Myelin in the Nervous System.** In: Pellicciari C., Biggiogera M. (eds) Histochemistry of Single Molecules. Methods in Molecular Biology, vol 1560. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-6788-9_15

Our Design

2D Model - Side View



LEGEND

PDMS Chip

PET Membrane

Endothelial Cell

Neuron

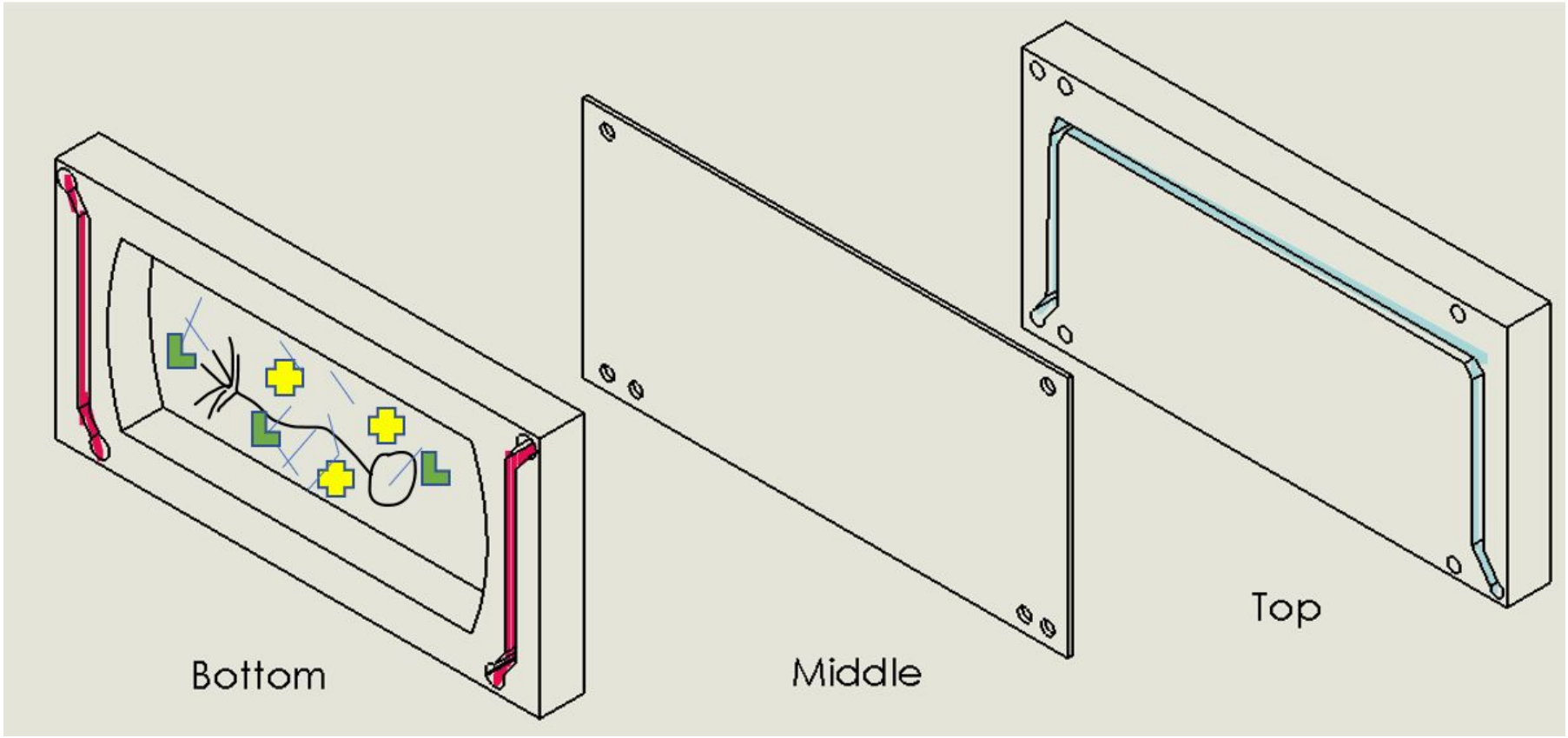
Pericyte

Astrocyte

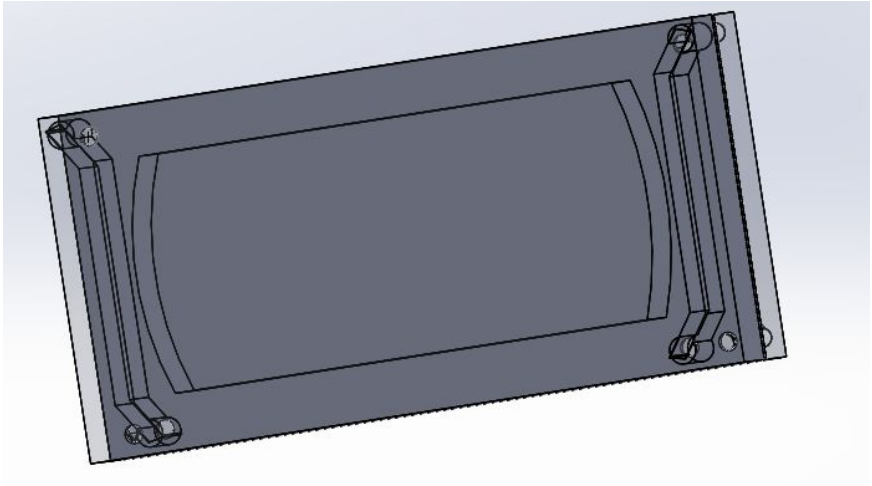
ECM

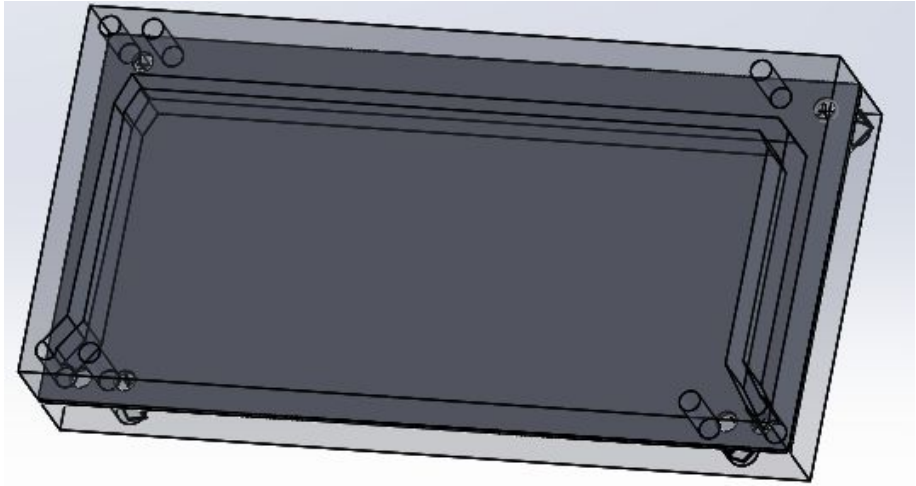
Oligodendrocytes

3D CAD Model - Disassembled



3D CAD Model - Assembled





Microchip Fabrication

Fabrication Materials

Chip Body

- Silicon Wafer
- Polydimethylsiloxane (PDMS)

BBB Membrane

- Polyethylene terephthalate (PET)
- Pores
  - 0.4 um diameter
  - 4×10^6 pores/cm^2

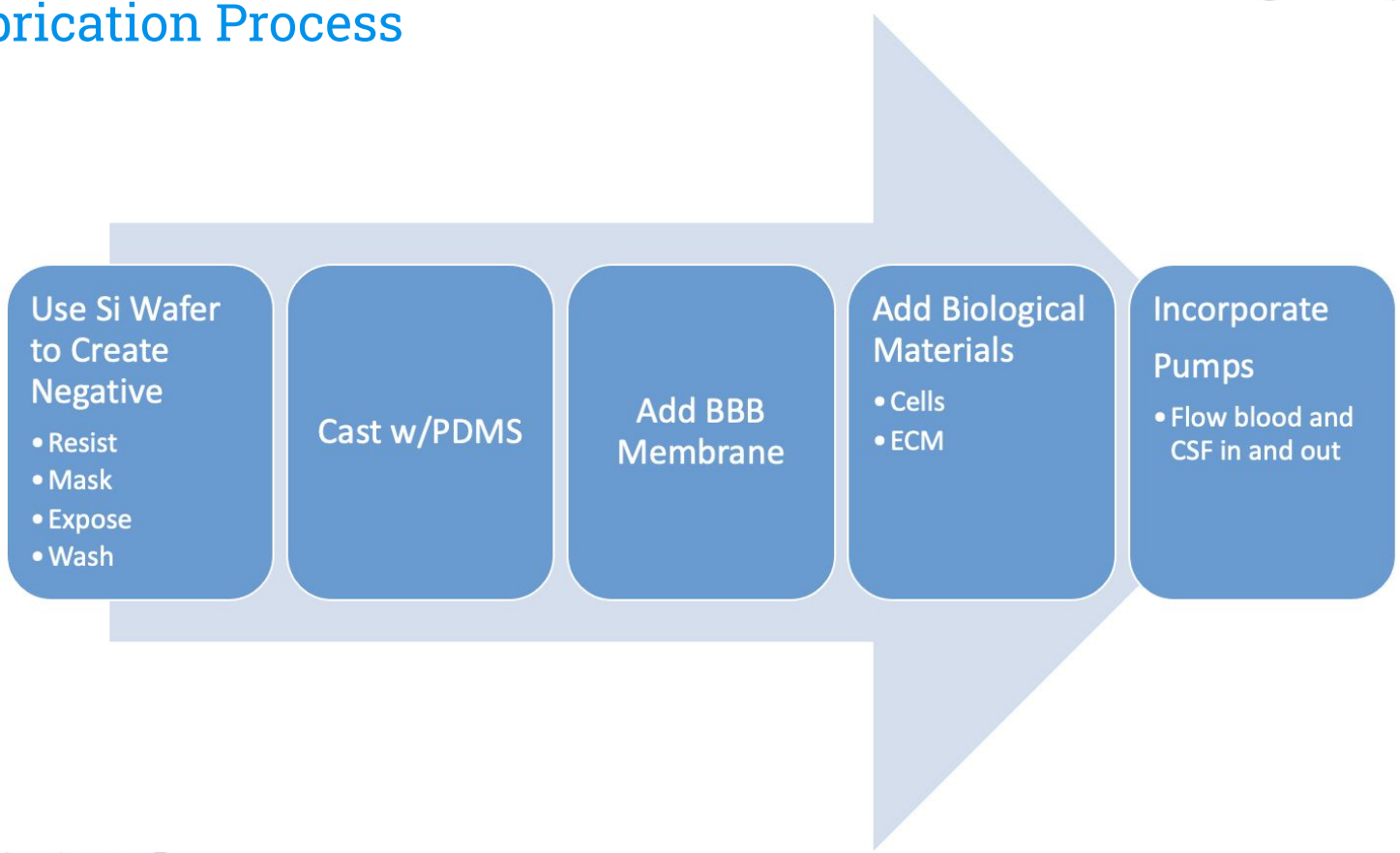
Biological Materials

- Brain decellularized ECM
- Cells
  - Neurons, astrocytes, pericytes

Fluids

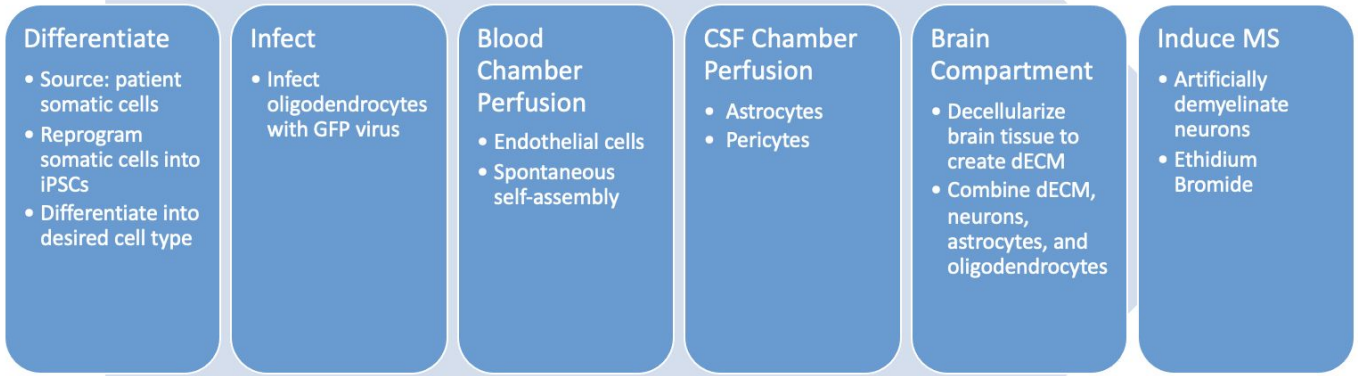
- Artificial Blood
- Artificial cerebral spinal fluid (CSF)
- Peristaltic micropumps

Fabrication Process

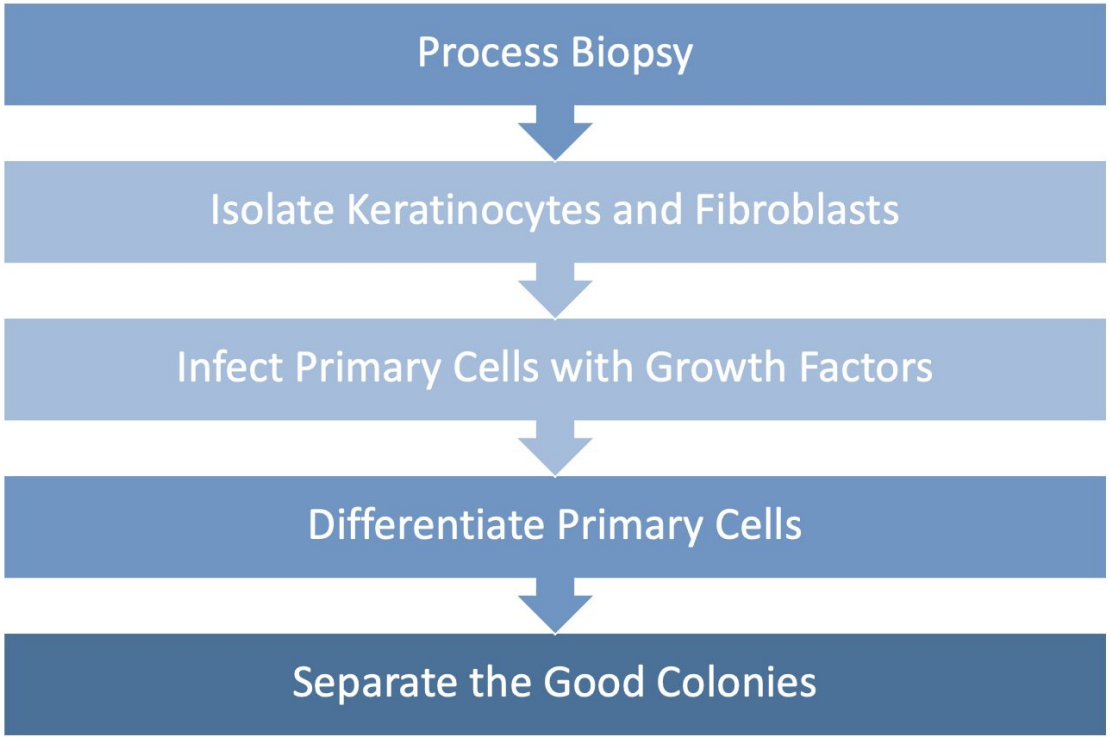


Incorporating Cells

Incorporating Cells into Design



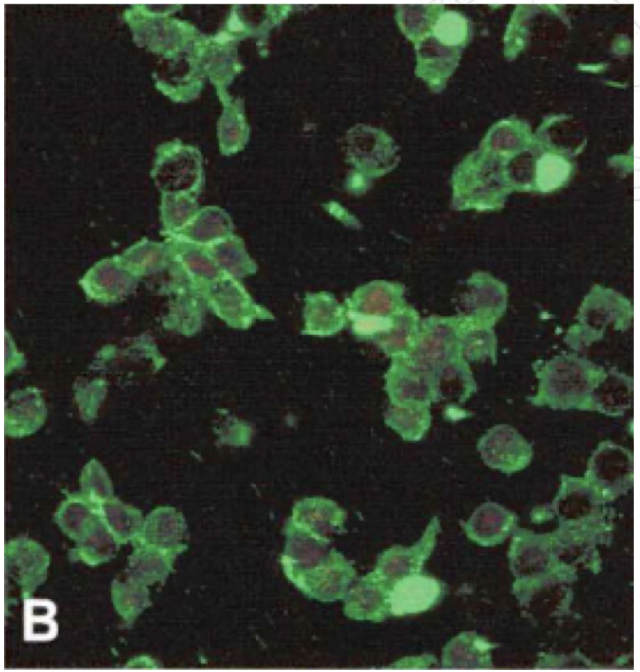
Cell Differentiation Process



Lorenzo, I.M., Fleischer, A. & Bachiller, D. **Generation of Mouse and Human Induced Pluripotent Stem Cells (iPSC) from Primary Somatic Cells**. *Stem Cell Rev and Rep* 9, 435–450 (2013). https://doi.org/10.1007/s12015-012-9412-5

Incorporating GFP into Myelin

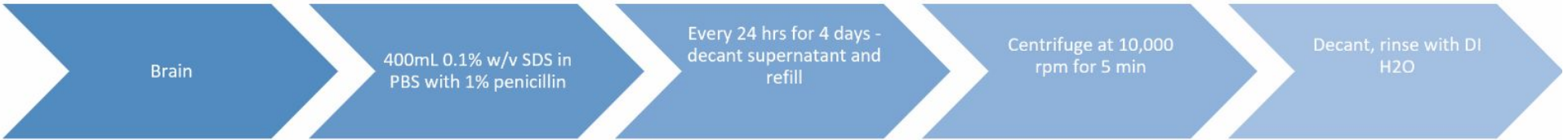
- Virus infection of cells using Measles Virus with Green Fluorescence Protein (GFP) expression
- GFP fluorescence at 488 nm
- Confocal Fluorescence Microscopy
- ↑ Green = ↑ Drug Effectiveness



Plumb, J., Duprex, W. P., Cameron, C. S., Richter-Landsberg, C., Talbot, P., & McQuaid, S. (2002). **Infection of human oligodendroglioma cells by a recombinant measles virus expressing enhanced green fluorescent protein**. *Journal of neurovirology*, *8*(1), 24-34.

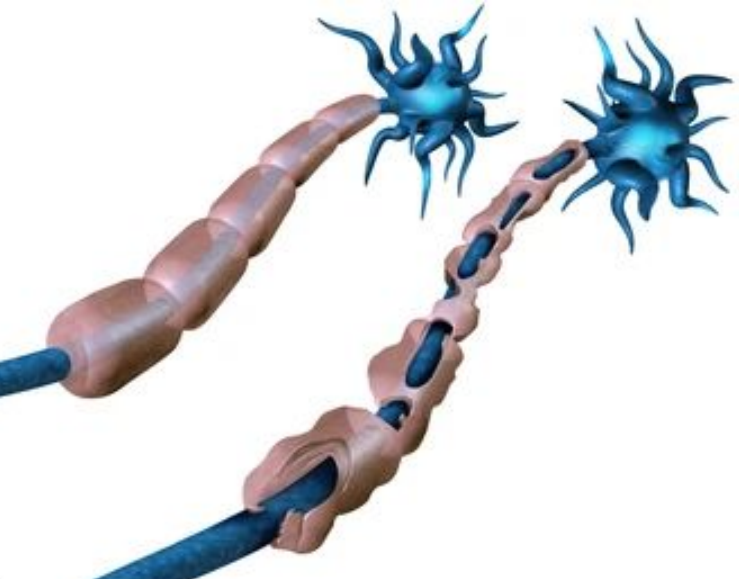
dECM Fabrication Technique

- Human brain dECM - Improved in vivo representation compared to Synthetic alternatives
- dECM preserves ECM proteins and Growth Factors
- Use Methylene Blue to ensure all SDS is removed



DeQuach, J. A., Yuan, S. H., Goldstein, L. S., & Christman, K. L. (2011). **Decellularized porcine brain matrix for cell culture and tissue engineering scaffolds**. *Tissue engineering. Part A*, *17*(21-22), 2583–2592. https://doi.org/10.1089/ten.TEA.2010.0724

Artificially Inducing MS



- Artificially induce demyelination of neurons seen in MS
- Ethidium bromide used to selectively demyelinate neurons
  - Creates a localized lesion

Torre-Fuentes, L., Moreno-Jiménez, L., Pytel, V., Matías-Guiu, J. A., Gómez-Pinedo, U., & Matías-Guiu, J. (2020). **Experimental models of demyelination and remyelination.** *Neurología (english Edition)*, *35*(1), 32-39.

Fluid Flow

- Artificial CSF and Blood will be used to simulate human CSF and Blood
- Purpose for blood flow is to transport oxygen to cells and remove carbon dioxide

Artificial CSF Makeup:

- 8.66g NaCl, 0.224g KCl, 0.206g $CaCl_2$-$2H_2O$, 0.163g $MgCl_2$-$6H_2O$, 0.214g Na_2HPO_4-$7H_2O$, and NaH_2PO_4-H_2O in 1000 mL pyrogen-free sterile water

Artificial Blood Makeup:

1. Perflurocarbons (Carbon and Flouride)
2. Hemoglobin based

https://www.alzet.com/guide-to-use/preparation-of-artificial-csf/

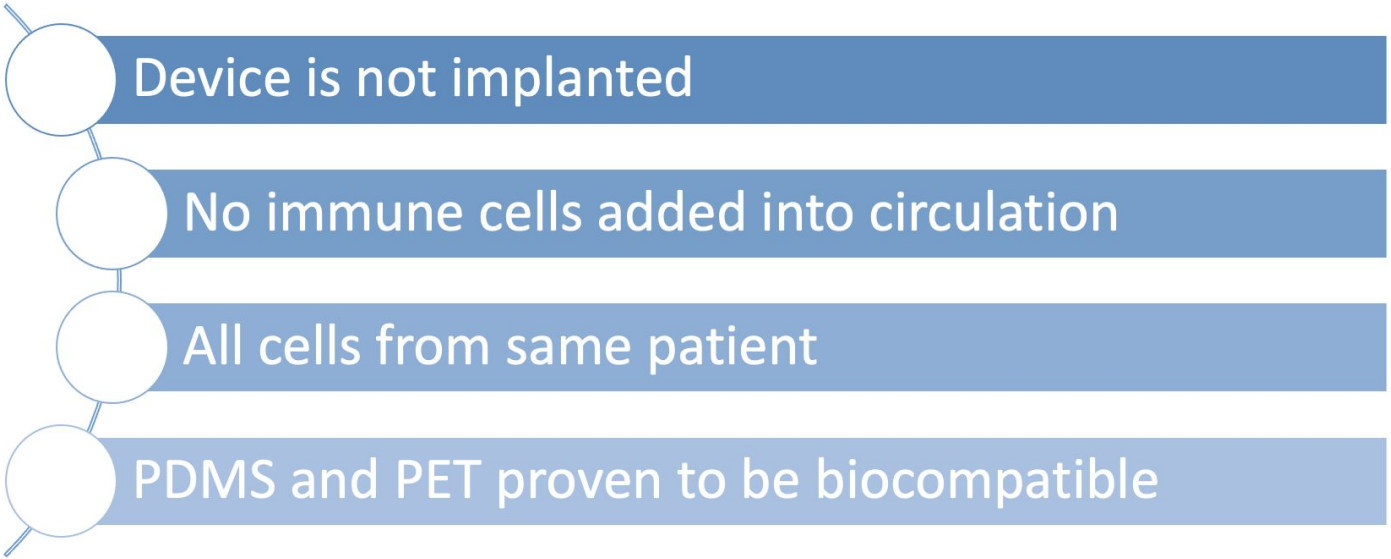
Continuing the Project

Testing

Mechanical	Biological
• Pressure • Flow • Pumps	• Imaging of biological material placement • Cell survival • Does BBB function as intended? • Does demyelination occur?

Biocompatibility & Histocompatibility

No compatibility issues expected because:



Limitations	Future Directions
Does not model diseased BBB	Design chip that models diseased BBB *and* axons
Demyelination was artificially induced	Transplant patients' own diseased neurons or add immune cells to demyelinate
Used artificial blood and CSF	Use patients' own fluids

Thank you!

Questions or Comments?