BIOLOGICAL ENGINEERING

Utah State University

Microfluidic Device for Decellularization of Murine Brain Slices

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I. Introduction

In the body, nearly all cells in tissues reside in an extracellular matrix (ECM) consisting of a complex three-dimensional (3D) microenvironment created through cell-cell and cell-ECM interactions that maintain specificity and homeostasis of the tissue. Accurate representation of microenvironments is pivotal in furthering our understanding of several fields, including disease mechanisms, drug discovery, tissue engineering, and regenerative medicine. Current 3D tissue models are limited in their ability to reproduce tissue-specific biochemical and mechanical cues present *in vivo*[1].

Decellularization is the chemical, physical, or enzymatic means of stripping cells from a tissue, producing a bare 3D ECM that is tissue-specific and representative of native microenvironments. Current decellularzation protocols successfully reduce cellular content at the cost of irreparable damage to the ECM[2]. In a novel approach, decellularization is conducted in a microfluidic environment for the purpose of minimizing ECM damage during DNA removal by the unique properties of microfluidic systems.

II. Objectives and Prototype Criteria

- Allow for decellularization of native neural tissues in a reproducible manner
  - Accommodate complete coronal brain sections
    - ≤ 1mm thickness
  - Decellularized tissue must contain ≤ 50 ng DNA per mg dry tissue
- Produce decellularized brain tissue in a manner and method conducive to cell culture studies
  - Decellularized tissue must retain extracellular component concentrations ≥70% of native tissue
    - Soluble collagen
    - Glycosaminoglycans (GAGs)

1. Corning Matrigel Membrane Matrix:Cell Culture:Antibiotics, Buffers and. (n.d.). Retrieved March 23, 2017, from https://www.fishersci.com/shop/products/corning-matrigel-membrane-matrix-12/p-90960
2. 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. doi:10.1089/ten.tea.2010.0724
3. Crapo, Peter M., Thomas W. Gilbert, and Stephen F. Badylak. "An overview of tissue and whole organ decellularization processes." Biomaterials32.12 (2011): 3233-243. Web.

III. Design Considerations

Table 1. Design decision table for fluid flow direction and device configuration.

Criteria Considered	Tangential Flow 3D Printed Molds	Perpendicular Flow 3D Printed Molds	Tangential Flow Standard Photolithography	Perpendicular Flow Standard Photolithography
Cost (0-7)	5	5	1	1
Fabrication: Technical Difficulty (0-10)	7	8	4	4
Fabrication: Time (0-10)	9	9	3	3
Fabrication Integrity (0-10)	4	4	6	6
Intrinsic Material Limitations (0-7)	6	6	3	3
Tissue Accessibility (0-15)	6	13	6	13
Tissue Visibility (0-5)	3	4	3	4
Shear Stress Generation (0-12)	10	5	10	5
Recellularization Potential (0-15)	8	10	8	10
Design Simplicity (0-5)	4	5	1	2
Total	62	**69**	45	51

(Column group header: Device Design Options)

Table 2. Design decision table for tissue sectioning

Criteria Considered	Vibratome	Microtome	Stamp
Technical dificulty (0-10)	6	4	10
Cost (0-5)	2	2	4
Optimal Thickness (0-15)	15	3	10
Temperature Control (0-3)	2	1	3
Pre-Fixed Tissue (0-5)	4	3	5
Total	29	13	**32**

(Column group header: Tissue Sectioning Modalities)

Table 3. Design decision table for solution surfactant[3]

Criteria Considered	Sodium Dodecyl sulfate	Sodium deoxycholate	Triton X-100
Decellularization (0-7)	7	4	4
ECM denaturation (0-7)	5	4	3
Ultrastructure disruption (0-7)	4	3	5
Precedence (0-10)	8	5	7
Availability (0-5)	5	1	3
Total	**29**	17	22

(Column group header: Surfactants)

IV. Final Design

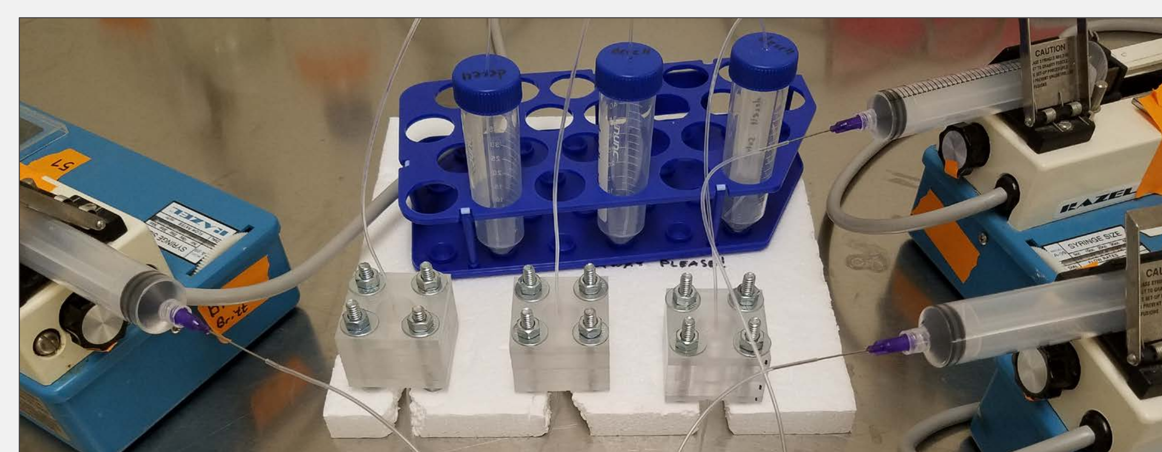

Figure 1. Three microfluidic decellularization cells attached to 30 ml syringe pumps.

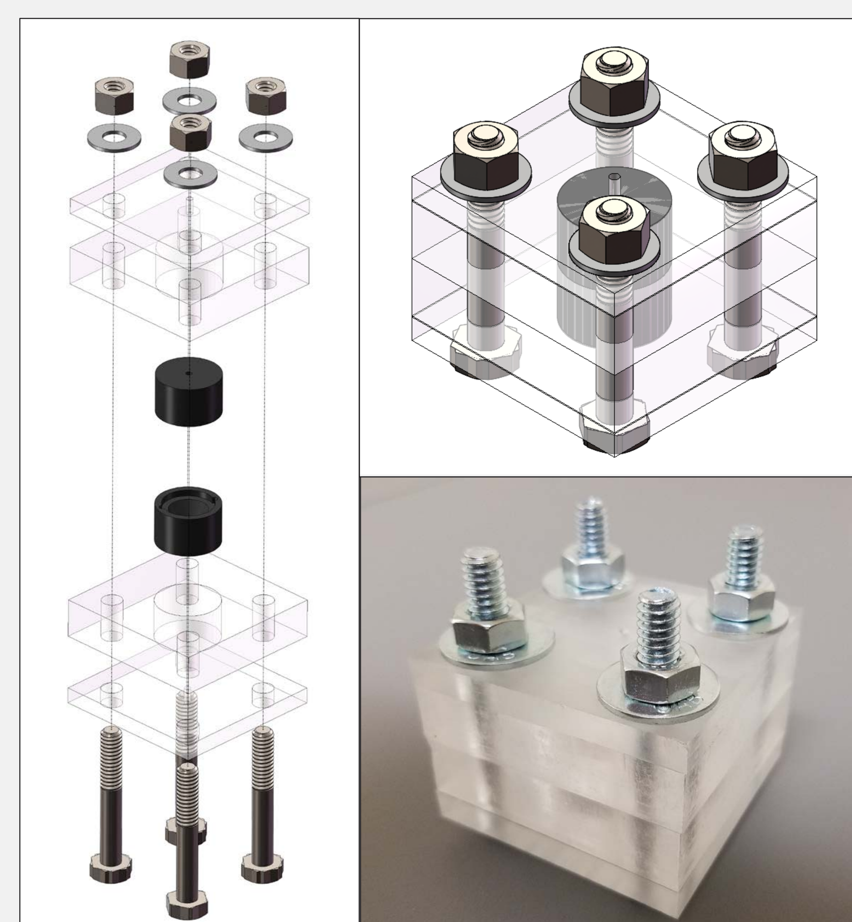

Figure 3. Left: Device and clamping assembly schematic (exploded view). Top Right: Device and clamping assembly schematic (assembled). Bottom Right: Fabricated device and clamping assembly.

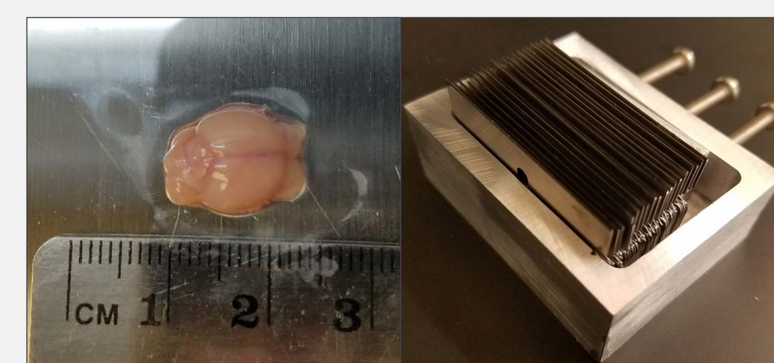

Figure 2. Left: Whole brain pre-slicing. Right: Razor blade cutting block used for slicing (slice thickness 0.95 ± 0.12 mm

Figure 4. Fabricated PDMS microfluidic device.

IV. Evaluation of Criteria

- **Allow for decellularization of native neural tissues in a reproducible manner**

Lightening of tissue was observed upon visual inspection denoting decellularization of murine brain slices in the device for 12, 24, and 48 hour runs.

Figure 5. 60 mL of decellularization fluid over 48 hours. Left: Pre-decellularization. Right: Post-decellularization.

- **Decellularized tissue must contain ≤ 50 ng DNA / mg dry tissue**

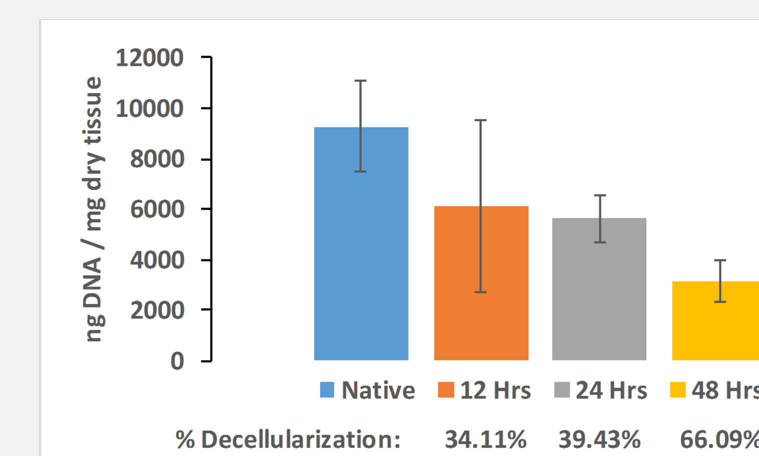

Figure 6. Evaluation of tissue DNA concentrations of native and decellularized brains receiving 60 ml surfactant solution over 12, 24, or 48 hours. Percent reduction of native DNA thresholds are also reported.

- **Decellularized tissue must retain extracellular component concentrations ≥70% of native tissue**

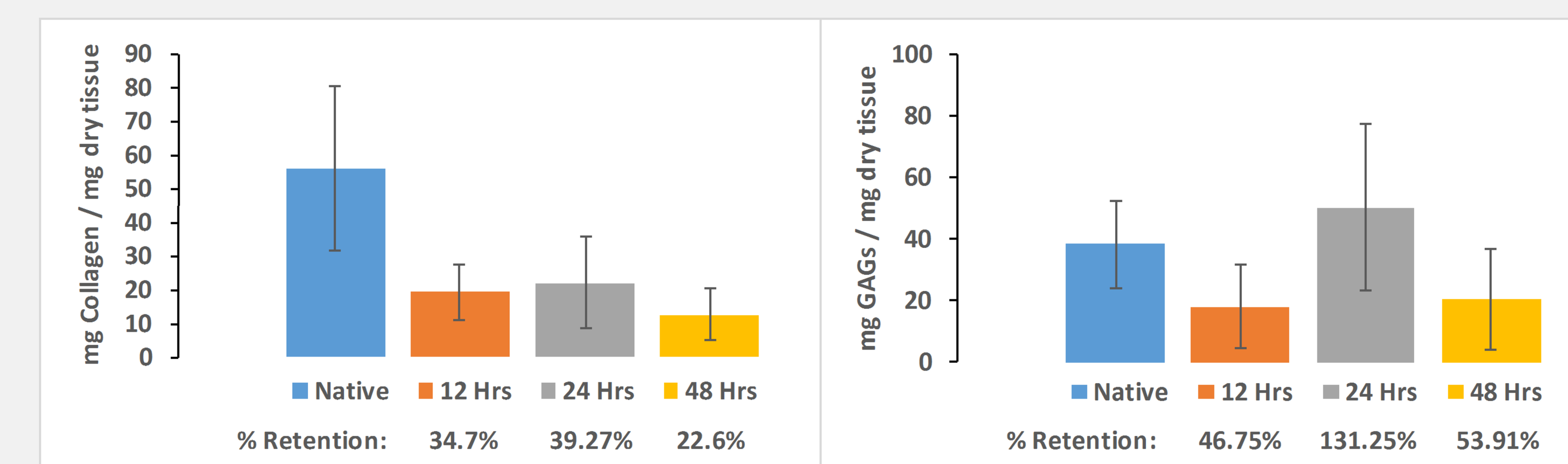

Figure 7. Characterization of extracellular matrix components of native and decellularized tissues. Left: soluble collagen concentration and post-decellularization retention. Right: extracellular glycosaminoglycan concentration and post-decellularization retention.

V. Conclusions

- A microfluidic device for decellularization of murine tissue was successfully designed and fabricated.
- Evaluation criteria specified were not achieved although the results indicated the possibility of complete decellularization in the device with further process optimization.
- ECM characterization data suggests competing factors effecting decellularization. Sheer stress removal of bulk tissue and protein solubility have been identified as potential confounding variables.