Decellularizing Spinach Leaf Scaffolds with Microfluidics to Model Angiogenesis

Colin Webb, Caitlin Veneto, Trey Loving, Sydney Lane, Linqing Li
Department of Chemical and Bioengineering, University of New Hampshire

University of New Hampshire
College of Engineering and Physical Sciences

CIBBR Center of Integrated Biomedical and Bioengineering Research

INTRODUCTION

- Current organ-on-a-chip devices for various vascular testing **lack hierarchical structures** that are found in human vessel systems
  - There is no differentiation between artery, vein, and capillary sizes
- Spinach leaves contain a wide range of vessel diameters, that could be utilized as a more **physiologically relevant *in vivo* model**
- Plant cells must be removed by decellularization before mammalian cell culture
  - This will allow room for the cells to adapt to the lumen structures of the leaf scaffold
- The cultured leaves can be integrated into a PDMS microchip for a functioning **vein-on-a-chip device**

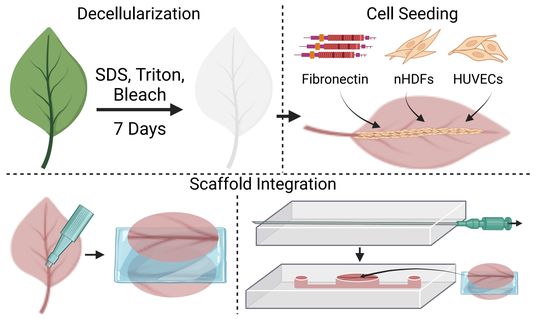

Figure 1: Schematic of general project outline.

METHODS

1. Decellularization
   - Leaves submerged in 10% sodium dodecyl sulfate (SDS) for 6 days
   - Leaves transferred to 5% Bleach and 0.1% Triton X-100 solution for 24 hrs
   - Decellularized leaves kept in PBS w 1% P/S in 4°C
2. Leaf Drying
   - Place samples in 30% ethanol for one hour, at each hour increase concentration by 10% until at 100%
   - Critical point drying executed 24 hours post-ethanol shock
3. Cell Seeding
   1. Leaves were coated with 100ug/ml of fibronectin
   2. Neonatal human dermal fibroblasts (nHDF) were seeded at 1e6 cells/ml on leaf surfaces
   3. Scaffold was cultured in DMEM containing 10% FBS and 1% P/S
4. Scaffold Integration
   - Leaf scaffolds inserted into the PDMS chip
   - Arranged seeded scaffolds between fibrin gel
   - Soaked leaves in VEGF to promote angiogenesis in scaffold

DECELLULARIZATION

- Cleaned the organic spinach leaves in PBS with 1% Penicillin-Streptomycin
- 10% SDS was initially injected into the stem of the leaves, and subsequently submerged in the SDS solution
  - Leaves were washed in PBS every 24 hours, and the SDS solution was replaced after each cleanse
- On the 6th day, the leaves were placed in 5% bleach and 0.1% Triton for 24 hours
- Finally, the leaves were thoroughly washed in PBS to remove excess bleach

Figure 2: Day 0 - 7 Decellularization of spinach leaves. For days 0-6 the leaves were placed into a 10% SDS solution. After 6 days, the leaves were placed into 5% bleach, and 0.1% Triton solution.

SCANNING ELECTRON MICROSCOPY

- Vascular structures observed by each method of drying-out samples: Oven baking and Critical Point Drying (CPD)
- Oven baking is not a sufficient drying process
  - Induced lots of wrinkles and imperfections on the leaf
- CPD of samples removes the apparent visual of the leaves vascular system
  - The leaf appears flat instead of a dynamic landscape

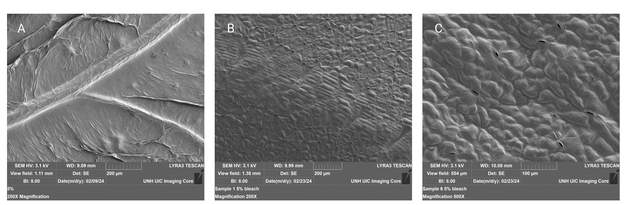

Figure 3: Scanning Electron Microscopy images taken of spinach leaf structure post-decellularization. (**A**) Decellularized spinach leaf "dried" via oven baking at 37°C for 24 hours. (**B**) and (**C**) Decellularized spinach leaf undergone Critical Point Drying

PDMS MICROCHIP

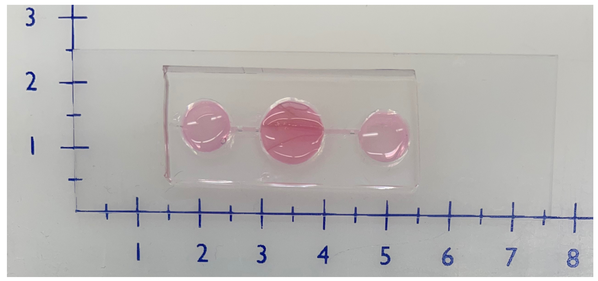

Figure 4: PDMS microchip prototype design.

1. Chemical hardener (Silicone Elastomer Curing Agent) and chemical base (Silicone Elastomer Base) mixed in 1:10 ratio
   a. Mixed thoroughly, degassed in vacuum chamber, and cured on 60°C hot plate
2. Acupuncture needles coated in gelatin were used to create channels within the chip
3. Biopsy punches were used to curate wells in the chip to house leaf system and entrance and exit sources
4. Fibrin gels - created with fibrinogen and thrombin - were used to bind the leaf samples to the bottom of the well
5. The seeded scaffolds were transferred into the center well of the microchip

CONFOCAL MICROSCOPY

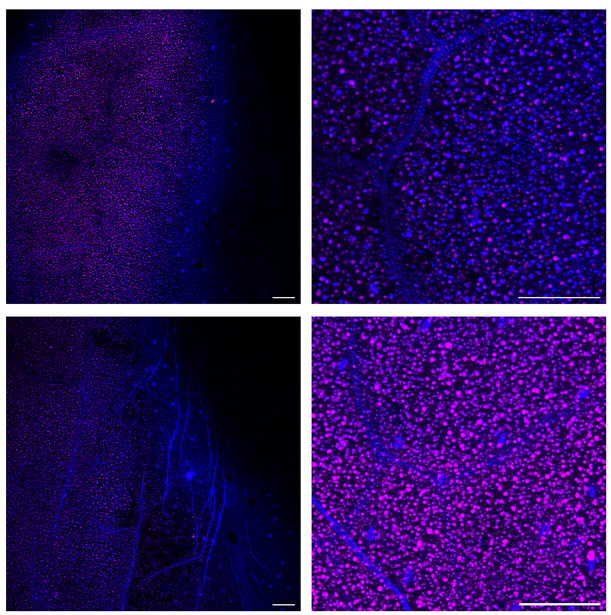

Figure 5: Confocal microscopy of decellularized spinach leaves displaying fluorescence. Scaffolds were stained with DAPI (blue) for nuclei and Phalloidin-647 (pink) for cytoskeleton (f-actin). Scale bars represent 100um.

CONCLUSION

- **Decellularized** scaffolds of organic spinach leaves were successfully achieved
- SEM Imaging verified the presence of in-tact **vascular structures** post-decellularization
- Testing in PDMS microchip presented **successful flow** of trypan blue dye from one side of the chip to the other

FUTURE DIRECTIONS

- Introduce HUVECs with fibroblasts for a **co-culture** on the leaf scaffold
  - Confocal imaging to verify presence of cells on leaves
- **Complete integration** of chip, leaves, and cells in one device for cell culture
- **Angiogenesis assays** to evaluate potential growth and sprouting effects of HUVECs
  - Test proliferation, migration, & growth factor detection and regulation
- **Thrombosis flow modeling** to analyze the flow of blood clots
- Study **diabetic conditions**
  - Culture cells in media with high sugar content
- **Tumor modeling** utilizing various cancer cell lines to observe spheroid morphologies

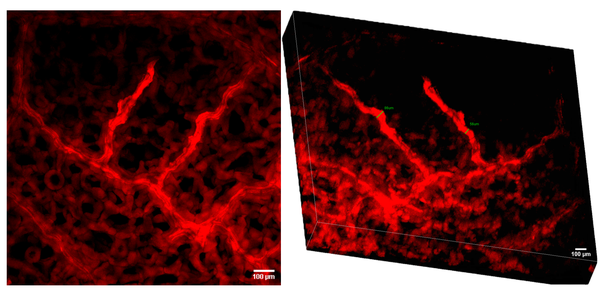

Figure 6: TRITC Autofluorescence Z-stack (left) and 3D Volume render (right) of decellularized spinach.

ACKNOWLEDGEMENTS

We acknowledge the funding support from the National Institute of Health COBRE Center of Integrated Biomedical and Bioengineering Research CIBBR (2P20GM113131) from NIGMS.

REFERENCES

1. J. D. Jones, A. S. Rebello, and G. R. Gaudette, “Decellularized spinach: An edible scaffold for laboratory-grown meat,” Food Bioscience, vol. 41, p. 100986, Jun. 2021, doi: 10.1016/j.fbio.2021.100986.
2. J. R. Gershlak et al., “Crossing kingdoms: Using decellularized plants as perfusable tissue engineering scaffolds,” Biomaterials, vol. 125, pp. 13–22, May 2017, doi: 10.1016/j.biomaterials.2017.02.011.p