Plant Tissue Decellularization for Complex Vascular Scaffold Applications

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I. Background

Decellularized cellulose scaffolds are used for supporting tissue growth, from vascular structures to cartilage, with a capacity to provide increasingly complex, yet natural, structures. *Spinacia oleracea* (spinach) has been the plant of choice for producing these scaffolds thus far due to its vascularized structure.

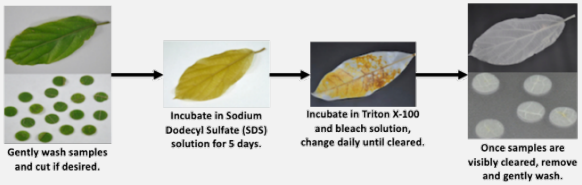

Figure 1. Previously defined decellularization process adapted from [1].

Although vasculature makes spinach a good choice, previous research is limited in identifying alternatives. Other vascular plants such as *Brassica oleracea var. sabellica* (kale) or *Lactuca sativa* (romaine lettuce) may yet provide scaffolds with superior biocompatibility or vascular structure.

II. Approach

AIM: Determine the superior cellulose scaffold of three different vascular plants– spinach, romaine lettuce and kale –through decellularization and biocompatibility assessment, using a cell culture assay.

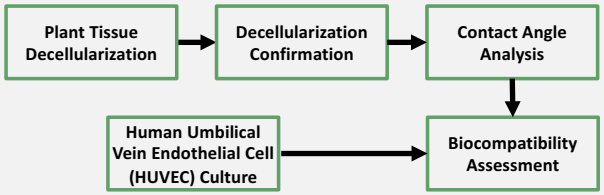

Figure 2. Experimental plan flow chart addressing project AIM.

III. Plant Tissue Decellularization

Decellularization procedure adapted from [1] using detergent-based approach. The process is identical to Figure 1.

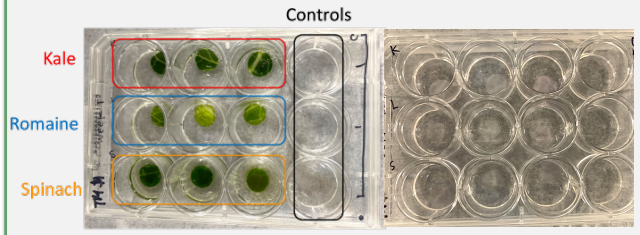

Figure 3. Decellularization progress with well plate configuration with washed 12mm sample disks (left) and completely decellularized samples (right).

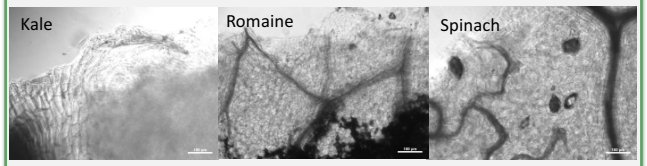

Figure 4. Sample edges after 5-day SDS treatment. Scale bars represent 100 µm.

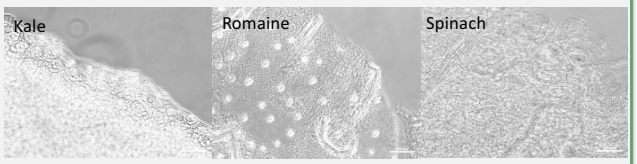

Figure 5. Sample edges after 4-day Triton X-100 and bleach solution treatment. Scale bars represent 100 µm.

References

1. Adamski M, Fontana G, Gershlak JR, Gaudette GR, Le HD, Murphy WL. Two Methods for Decellularization of Plant Tissues for Tissue Engineering Applications. JoVE [Internet]. 2018 [cited 2021 Mar 14];57586.

IV. Confirmation & Contact Angles

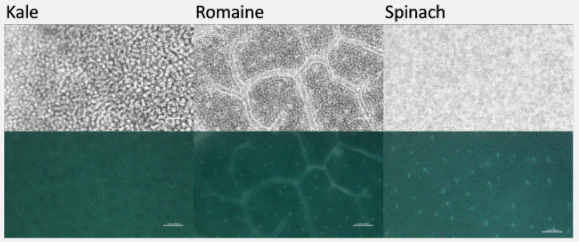

Figure 6. NucBlue-stained bright field images (top) and fluorescence images (bottom) showed no nuclei present, confirming decellularization.

Table 1. Contact Angle Analysis

	Kale	Romaine	Spinach
Mean Contact Angles	6.05°	41.4°	5.80°

Contact angle measurements revealed that the absence of cells left a highly porous surface resulting in water being adsorbed nearly completely at the surface. Both the NucBlue staining and contact angle results confirm decellularization.

V. Biocompatibility & Future Work

The HUVECs were resistant to attaching to both the cellulose scaffolds and the control well plates.

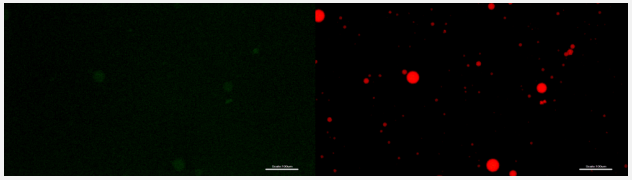

Figure 7. Control well live-dead viability assay florescence images live (left) and dead (right). Live cells are green and dead cells are red.

We expect that the decellularization process affected the well plate itself and plan to transfer scaffolds to new plates and attempt further cell culture.

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