Onion Epithelial Membranes as a Scaffold for Generating Connective Tissue

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Background

Plant-derived scaffolds have proven useful in the field of tissue engineering. Decellularized green onion scaffolds have been successfully used as a scaffold for C2C12 and human skeletal muscle cells [1] and rabbit corneal epithelial cells were successfully cultured on onion epithelial membranes (OEM) [2]. The effect of decellularization of the OEM on cell growth has not been tested. It is possible that decellularized OEM may function as a more effective scaffold for connective tissue. We aim to assess the growth of connective tissue, specifically NIH 3T3 fibroblasts, on decellularized OEM as compared to non-decellularized OEM and tissue culture flasks.

Research Goals

The purpose of this research is to evaluate the efficacy of decellularized OEM as a scaffold for connective tissue.

- Successfully decellularize OEM
- Characterize decellularized and non-decellularized OEM
- Seed decellularized and non-decellularized OEM and tissue culture flasks with NIH 3T3 fibroblasts
- Assess growth, viability, and attachment of cells grown on the evaluated surfaces

Methods

1. Decellularization

OEM were decellularized by a series of detergent washes, followed by soaking in sterilized double distilled water.

2. Sterilization

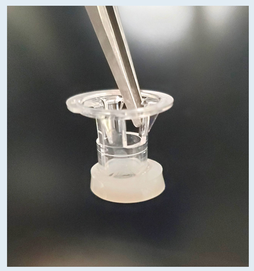
Prior to seeding the membranes and flask, all membranes were sterilized by soaking in ethanol and bleach.

3. Membrane Characterization

Membranes were characterized via NucBlue Live Cell staining and contact angle measurements. This characterization was done to determine cellular imaging and hydrophilicity differences between decellularized and non-decellularized membranes.

Methods (Continued)

4. Membrane Mounting and Cell Seeding



Cells were seeded at a cell density of 6.3 x 10^5 cells/ml.

Cells were cultured for one week in DMEM F-12 10% FBS and kept in an incubator at 37ºC and 5% CO_2. Media was changed twice a week.

Figure 1. OEM mounted on a Transwell insert.

Results

Table 1. Contact angles of decellularized and non-decellularized OEM.

Membrane Treatment	Advancing Angle (°)	Receding Angle (°)
Decellularized, Sterile	27.0 ± 2.7	32.3 ± 10.4
Non-Decellularized, Sterile	53.6 ± 2.5	53.8 ± 5.0
Non-Decellularized, Non-Sterile	38.9 ± 8.9	39.7 ± 9.0

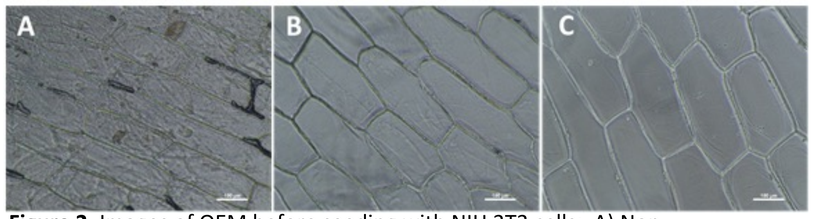


Figure 2. Images of OEM before seeding with NIH 3T3 cells: A) Non-Decellularized, Non-Sterile B) Non-Decellularized, Sterile C) Decellularized, Sterile

Figure 3. Images of unseeded OEM stained with NucBlue Live Cell Stain, which stains DNA: A) Non-Sterilized B) Non-Decellularized C) Decellularized

References
[1] Y.-W. Cheng, D. J. Shiwarski, R. L. Ball, K. A. Whitehead, and A. W. Feinberg, "Engineering Aligned Skeletal Muscle Tissue Using Decellularized Plant-Derived Scaffolds," *ACS Biomater. Sci. Eng.*, vol. 6, no. 5, pp. 3046–3054, May 2020, doi: 10.1021/acsbiomaterials.0c00058.
[2] G. Wang *et al.*, "Onion Epithelial Membrane Scaffolds Transfer Corneal Epithelial Layers in Reconstruction Surgery," *Advanced Healthcare Materials*, vol. 9, no. 14, p. 2000469, 2020, doi: https://doi.org/10.1002/adhm.202000469.

Results (Continued)

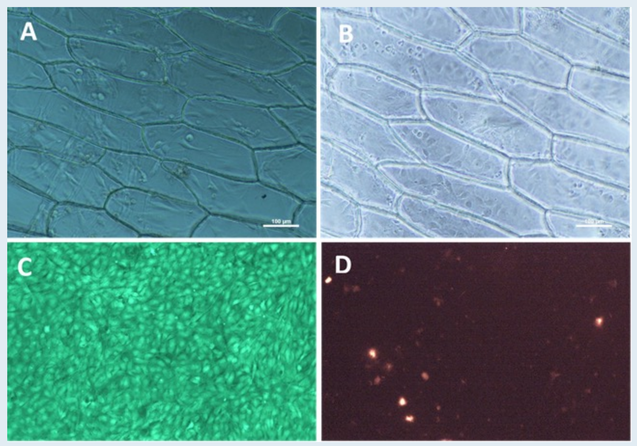


Figure 4. Images of NIH 3T3 growth: A) non-decellularized membrane, 2 days after seeding B) non-decellularized membrane, 4 days after seeding
C) Live cell stain performed on control well, 10 days after seeding
D) Dead cell stain performed on control well, 10 days after seeding

Conclusions

Decellularization was successful, but decellularization and sterilization procedures caused significant weakening of the membranes.

Decellularized membranes were found to be the most hydrophilic (Table 1).

NIH 3T3 fibroblasts grew on non-decellularized and decellularized OEM, but growth was significantly less than on tissue culture well plate controls (Figure 4).

Contamination, likely introduced during media changing procedure, was a significant issue.

Future Work

Refine decellularization and sterilization processes to preserve structural integrity of OEM.

Introduce antibiotics and antimycotics to prevent contamination.

Seed OEM at a higher cell density to shorten growing time and decrease contamination risk.