Supporting Information

# Fast Automated Approach for the Derivation of Acellular Extracellular Matrix Scaffolds from Porcine Soft Tissues

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**List of abbreviations S1**

| Abbreviation | Explanation |
|---|---|
| ECM | Extracellular Matrix |
| Col | Type I Collagen hydrogel |
| TCP | Tissue Culture Plastic |
| sH-ECM | sheet Heart-Extracellular Matrix |
| sH-ECMh | sheet Heart-Extracellular Matrix hydrogel |
| mH-ECM | micronized Heart-Extracellular Matrix |
| mH-ECM-O | micronized Heart-Extracellular Matrix Optimized |
| mH-ECMh-O | micronized Heart-Extracellular Matrix hydrogel Optimized |
| sVFLP-ECM | sheet Vocal Fold Lamina Propria-Extracellular Matrix |
| sVFLP-ECMh | sheet Vocal Fold Lamina Propria-Extracellular Matrix hydrogel |
| mVFLP-ECM | micronized Vocal Fold Lamina Propria-Extracellular Matrix |
| mVFLP-ECMh | micronized Vocal Fold Lamina Propria-Extracellular Matrix hydrogel |

**Method S1 Gelation kinetics**

After hydrogel preparation (Section 2.5) 100 µL of hydrogel/well at least in triplicate were aliquoted in a 96-well plate and kept on ice until measurement. The samples were measured using a BioTek SYNERGYneo2 multimode reader pre-heated to 37 °C. Absorbance measurements were taken at 405 nm every 1 minute for 90 minutes. The data was normalized using the following equation:

$$Normalized\ absorbance_{405\ nm} = \frac{(A_x - A_{min})}{(A_{max} - A_{min})}$$

Where $A_x$ = experimental measurement, $A_{min}$ = minimum absorbance, $A_{max}$ = maximum absorbance. The data was graphed using Prism 8.0.

**Figure S1 Final decellularized product placed inside the inline filtration bioreactor.**

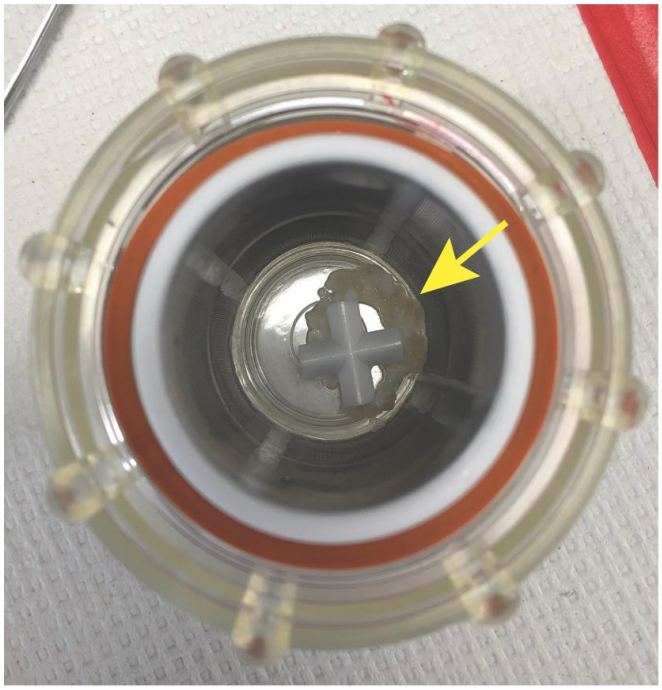
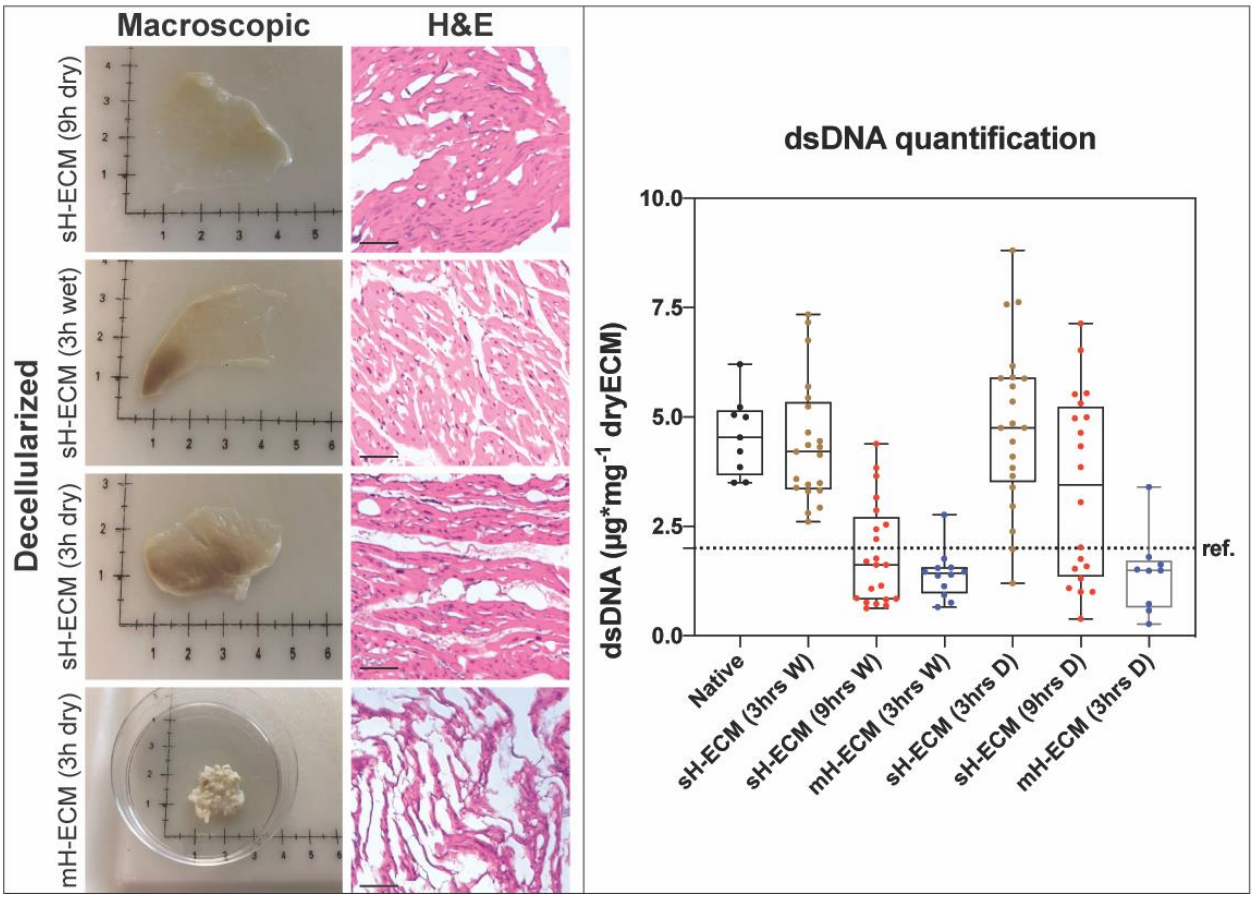
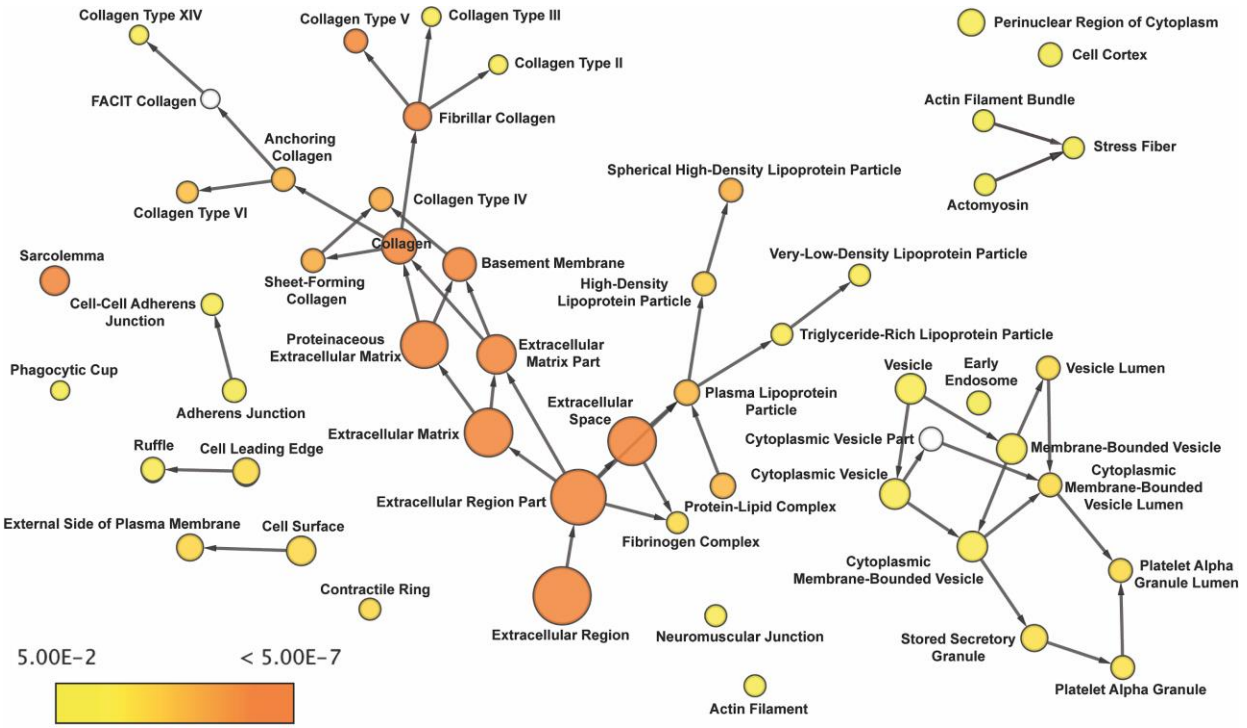


**Figure S2 Histological analysis showing Hematoxylin and Eosin (H&E) stain. Box and Whisker plot showing double stranded DNA (dsDNA) quantification per mg of dry tissue for native, wet micronized (W), and freeze-dried (D) decellularized sH-ECM and mH-ECM after 3 and/or 9 hours.**



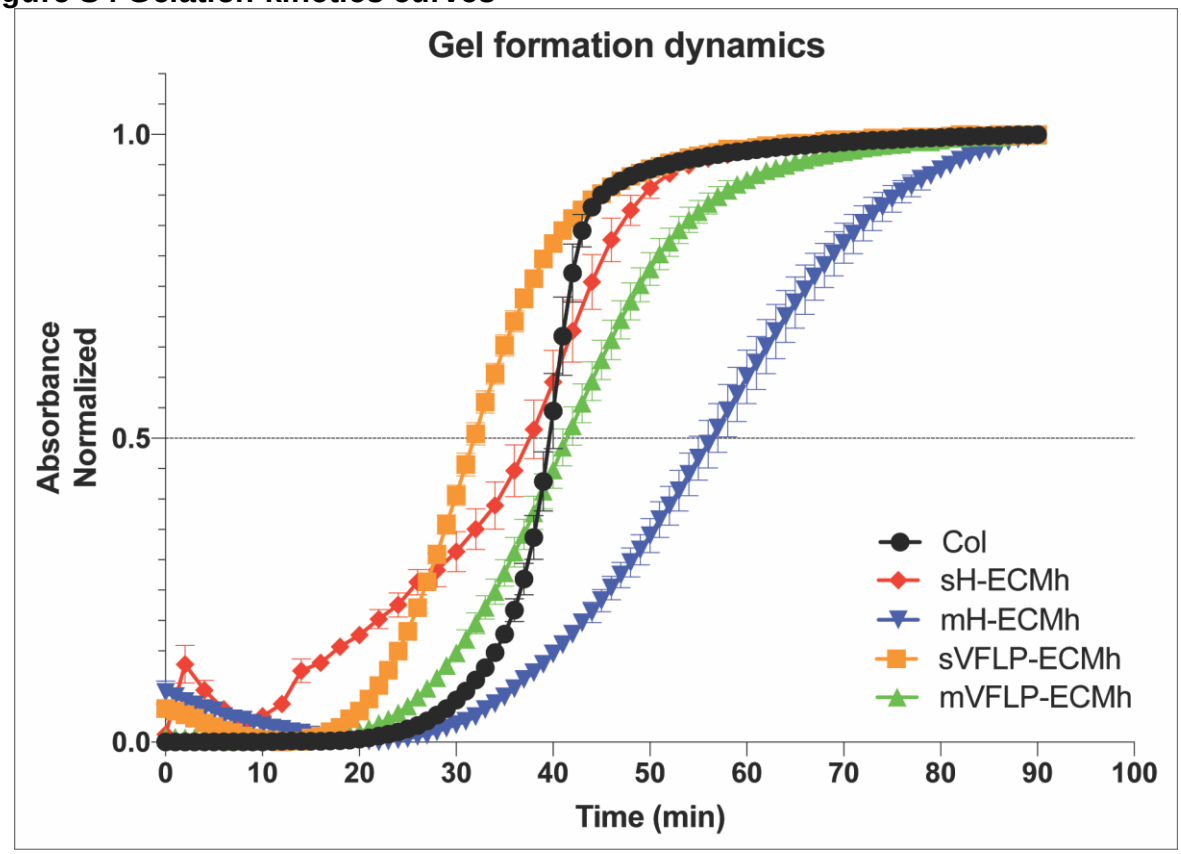

Three independent organs were decellularized at least three independent times (n=9). The error bars represent the standard error of the mean (SEM). ref. ~2 µg/mg (for Urinary Bladder Matrix–UBM). W & D were the pre-treatment conditions for the tissue to be decellularized W = wet micronized, D = freeze-dried. Scale bar = 360 µm.

**Figure S3 Gene Ontology (GO)**



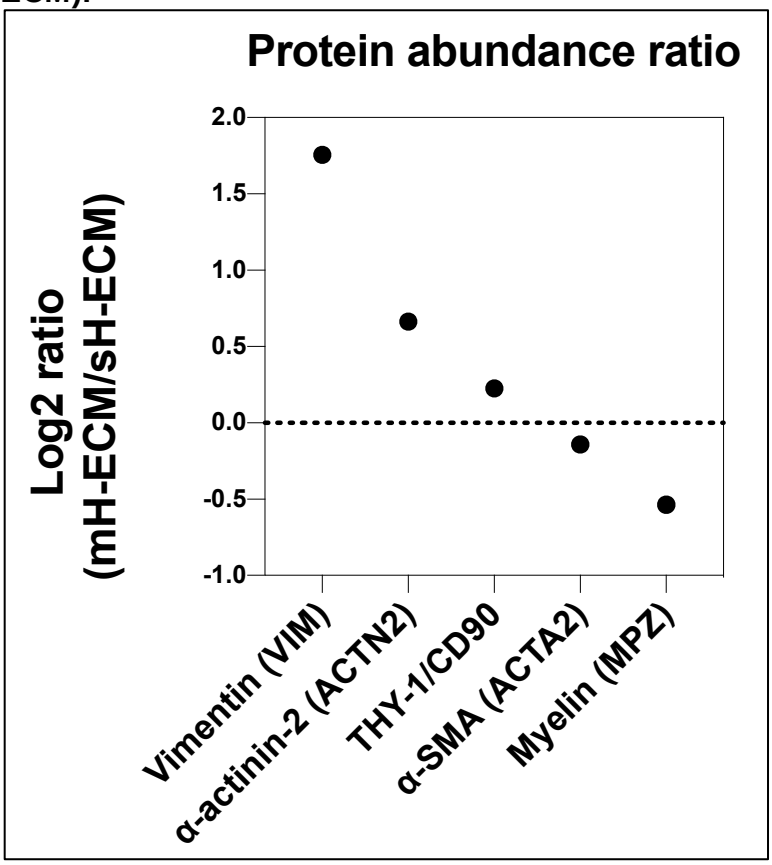

Gene ontology (GO) based on cellular components using the proteins in Figure 5C and extracted from Proteome Discoverer 2.4.0.305 as input in Cystoscope V3.0 and the application Bingo to plot overrepresenting analysis. The scale bar represents the P-value for the nodes.

**Figure S4 Gelation kinetics curves**




The curves were plotted based on the normalized absorbance at 405 nm for sH-ECMh, mH-ECMh, sVFLP-ECMh, mVFLP-ECMh, and collagen type I hydrogel (Col) control. The error bars represent the SEM of at least three replicates.

**Figure S5 Differences in selected cellular proteins between automated (mH-ECM) vs. manual (sH-ECM).**




A Log2 ratio equal to 0 (·····) indicates the 3-hour method (mH-ECM) yielded the same abundance for the protein than the 9-hour method (sH-ECM).

**Table S1** Approximate total number of manual steps calculated based on steps involving tissue preparation and manual reagent changes.

| Decellularization Protocol | Approx. Number of Manual Steps | | Total Number of Manual Steps |
|---|---|---|---|
| | Tissue Preparation | Manual Reagent Changes | |
| mH-ECM* | 4 | 1 | **5** |
| mVFLP-ECM* | 6 | 1 | **7** |
| Sheet-Heart (Freytes et al.) | 3 | 15 | **18** |
| Sheet-Heart (Singelyn et al.) | 2 | 4 | **6** |
| Sheet-Heart (Shah et al.) | 2 | 19 | **21** |
| Sheet-VFLP (Wrona et al.) | 3 | 9 | **12** |
| Sheet-VFLP (Tse et al.) | 3 | 5 | **8** |
| Sheet-VFLP (Xu et al.) | 2 | 5 | **7** |
| Sheet-UBM (Freytes et al.) | 3 | 5 | **8** |
| Sheet-Aorta (Pellegata et al.) | 6 | 1 | **7** |
| Whole-Lungs (Price et al.) | 4 | 1 | **5** |
| Whole-Kidneys (Poornejad et al.) | 4 | 1 | **5** |
| Whole-Heart (Momtahan et al.) | 4 | 1 | **5** |

* = Present Study