

Supplementary Information

Towards a Standardized Multi-Tissue Decellularization Protocol for the Derivation of Extracellular Matrix Materials

*Andreea Biehl^{a,b}, Ana M. Gracioso Martins^{a,b}, Zachary G. Davis^{a,b}, Daphne Sze^{a,b},
Leonard Collins^c, Camilo Mora-Navarro^d, Matthew B. Fisher^{a,b,e}, Donald O. Freytes^{a,b*}*

^aJoint Department of Biomedical Engineering, North Carolina State University & University of North Carolina-Chapel Hill, 4130 Engineering Building III, Campus Box 7115, Raleigh, NC 27695, USA

^bComparative Medicine Institute, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27606, USA

^cMolecular Education, Technology and Research Innovation Center (METRIC), North Carolina State University, Dabney Hall, 2620 Yarbrough Drive, Raleigh, NC 27607, USA

^dDepartment of Chemical Engineering, University of Puerto Rico-Mayaguez, Route 108, Mayaguez, Puerto Rico, USA

^eDepartment of Orthopaedics, University of North Carolina School of Medicine, 102 Mason Farm Road Second Floor, Chapel Hill, NC 27514, USA

Corresponding Author:

*Donald O. Freytes, Ph.D.

Associate Professor

Joint Department of Biomedical Engineering

NC State/ UNC-Chapel Hill

Biomedical Partnership Center

1001 William Moore Drive

Room 28

Raleigh, NC 27607

dofreyte@ncsu.edu

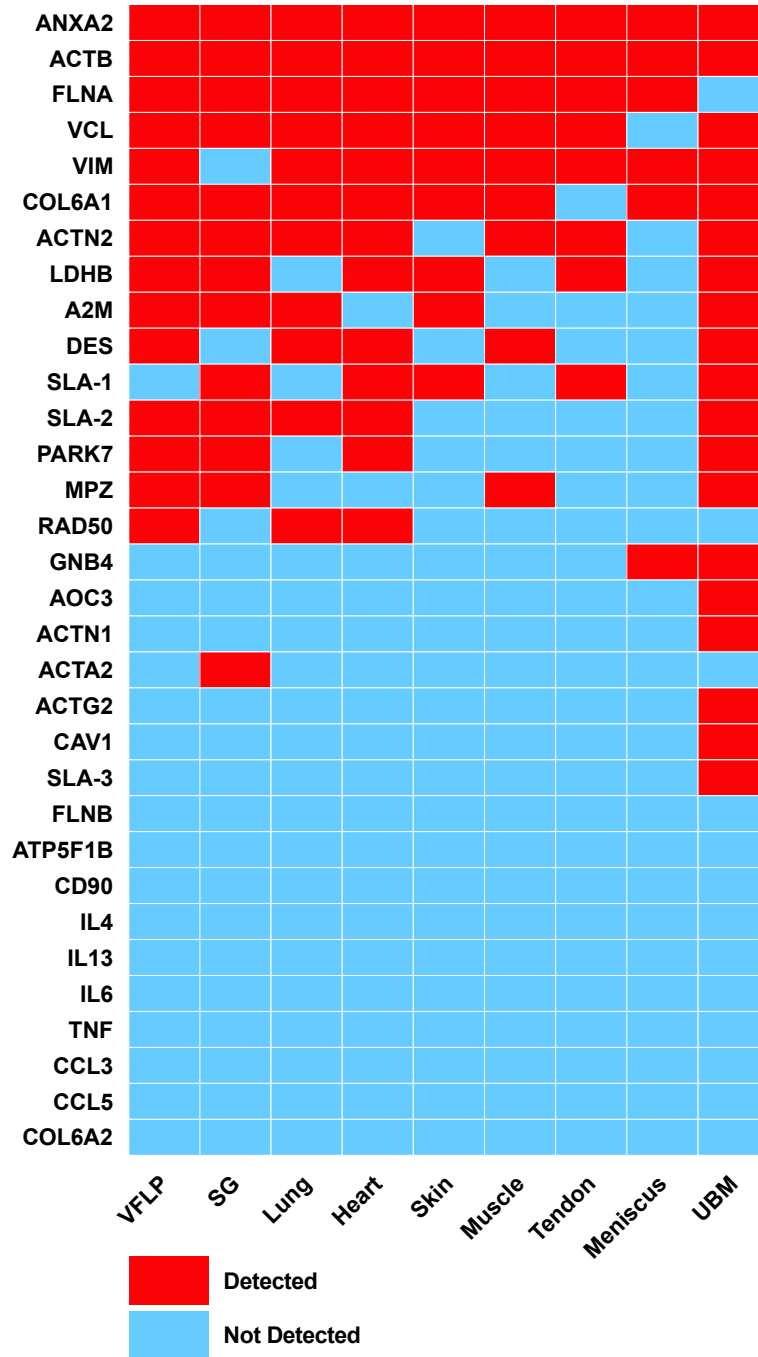
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Supplementary Figures 1-5

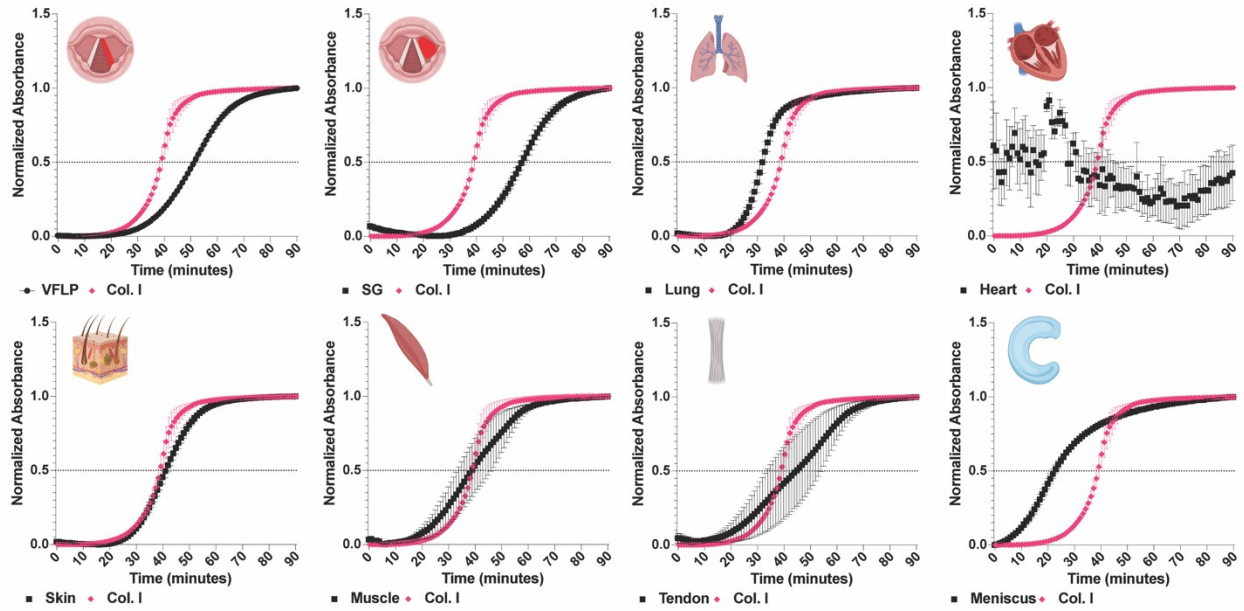
Supplementary Tables 1-2

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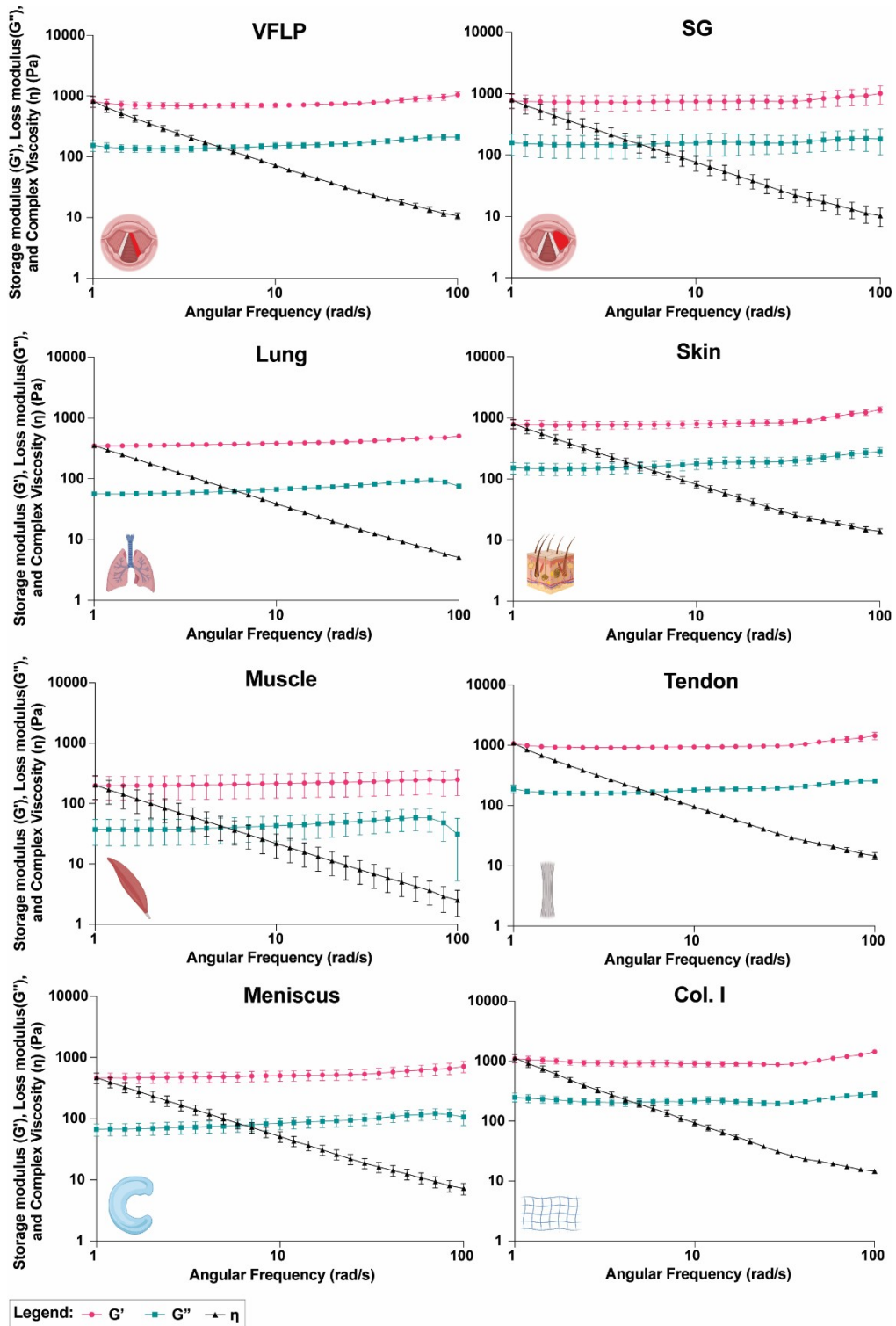
Immunogenic proteins and sub-units



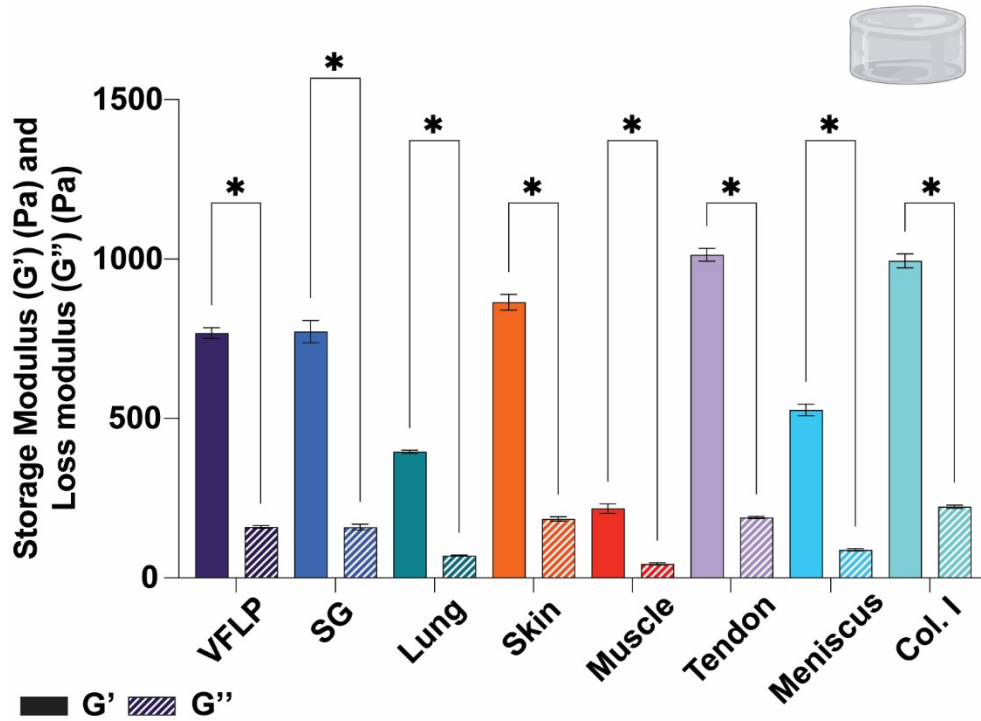
Supplementary Figure 1. Heatmap showing the detection or absence of immunogenic proteins and sub-units per each ECM type.¹⁻³ The database for UBM was retrieved from the study by Mora-Navarro et al. 2020.⁴



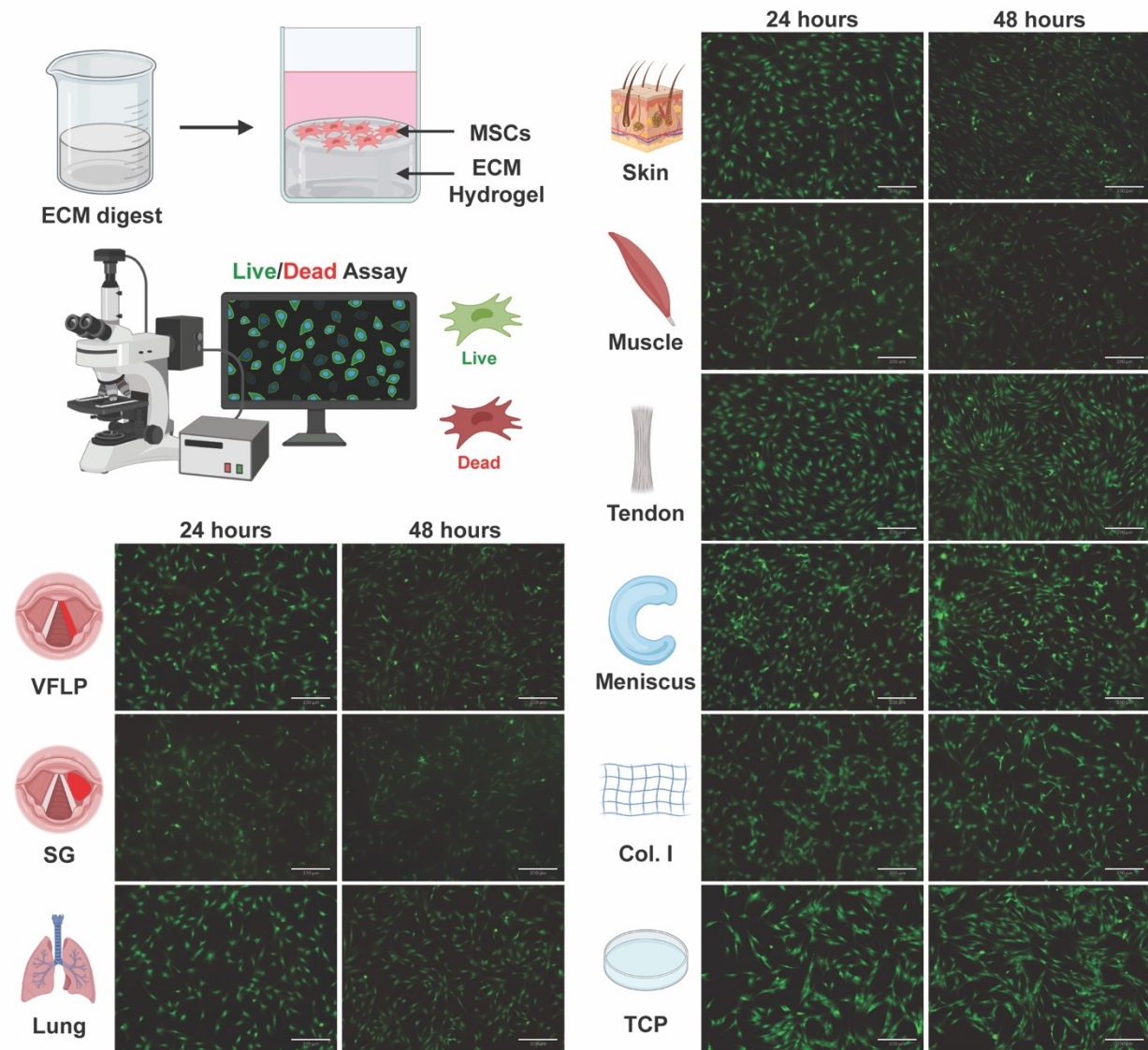
Supplementary Figure 2. Gelation kinetics curves based on the normalized absorbance at 405 nm for VFLP-ECM, SG-ECM, Lung-ECM, Heart-ECM, Skin-ECM, Muscle-ECM, Tendon-ECM, and Meniscus-ECM hydrogels in reference to collagen type I hydrogel (Col. I) control. The errors bars represent the SEM of three replicates.



Supplementary Figure 3. Graphs of storage modulus (G'), loss modulus (G''), and complex viscosity (η) for VFLP-ECM, SG-ECM, Lung-ECM, Heart-ECM, Skin-ECM, Muscle-ECM, Tendon-ECM, Meniscus-ECM hydrogels, and collagen type I hydrogel (Col. I) control plotted over an angular frequency from 1 to 100 rad/s. The errors bars represent the SEM of three replicates.



Supplementary Figure 4. Average storage modulus (G') and average loss modulus (G'') for VFLP-, SG-, lung-, skin-, muscle-, tendon-, and meniscus-ECM, and Col. I control hydrogels prepared at 6 mg/mL. The error bars represent the standard error of the mean (SEM). $n=3$ each. * = statistically significant ($P < 0.05$).



Supplementary Figure 5. Live/Dead viability assay for human mesenchymal stem cells (hMSCs) grown on VFLP-ECM, SG-ECM, Lung-ECM, Heart-ECM, Skin-ECM, Muscle-ECM, Tendon-ECM, Meniscus-ECM hydrogels, collagen type I hydrogel (Col. I) control, and tissue culture plastic (TCP) control. Representative images are shown for 24 and 48 hours in culture. Scale bar = 320 μm .

Supplementary Table 1. Double stranded DNA quantification of native and decellularized ECM tissues (Mean \pm SEM of at least three independent experiments).

Tissue Category	Tissue Type	dsDNA in Native ($\mu\text{g}\cdot\text{mg}^{-1}$ dry material)	dsDNA in Decellularized ($\mu\text{g}\cdot\text{mg}^{-1}$ dry material)
Respiratory	VFLP	10.255 \pm 0.208	1.512 \pm 0.172
	SG	7.580 \pm 0.977	0.286 \pm 0.042
	Lung	18.422 \pm 1.473	0.474 \pm 0.035
Cardiac	Heart	5.160 \pm 0.200	1.264 \pm 0.052
Dermal	Skin	2.367 \pm 0.069	0.467 \pm 0.052
Musculoskeletal	Muscle	2.169 \pm 0.180	0.285 \pm 0.026
	Tendon	2.557 \pm 0.439	0.203 \pm 0.077
	Meniscus	1.607 \pm 0.177	0.350 \pm 0.055

Supplementary Table 2. Mean and standard error of the mean (SEM) of the complex viscosity at 100, 10, and 1 Pa*s and the storage and loss moduli averaged across the frequency sweep from 1-100 Pa*s for each ECM type and 6mg/mL Col. I control hydrogels. * = statistically significantly different from 6mg/mL Col. I control.

			Angular Frequency (rad/s)			Storage Modulus (Pa)	Loss Modulus (Pa)
			100	10	1		
Respiratory	VFLP	Mean	10.7	72.7	826.8	767.5*	159.9*
		SEM	1.2	6.7	166.5	16.7	4.0
	SG	Mean	10.3	76.0	786.4	772.2*	158.8*
		SEM	3.4	21.1	213.1	35.0	3.6
	Lung	Mean	5.1*	38.8*	353.5*	395.9*	69.9*
		SEM	0.2	0.8	7.1	5.031	1.4
Dermal	Skin	Mean	13.9	81.7	801.8	864.7*	184.8*
		SEM	1.5	10.9	135.6	24.9	7.1
Musculo- skeletal	Muscle	Mean	2.5*	21.9*	202.2*	217.5*	44.0*
		SEM	1.2	9.4	85.2	15.0	3.2
	Tendon	Mean	14.6	95.8	1088.4	1014.0	189.2*
		SEM	2.0	6.4	60.4	19.7	3.7
	Meniscus	Mean	7.2*	51.2	467.1*	526.9*	87.6*
		SEM	1.5	9.9	89.2	18.2	3.6
Control	Col. I	Mean	14.7	93.9	1138.7	994.1	223.6
		SEM	1.3	10.7	174.6	21.9	5.1

References

- 1 J. Muhamed, T. Anilkumar, A. Rajan, A. Surendran and A. Jaleel, *Biomed Phys Eng Express*, 2019, 5, 025003.
- 2 J. M. Aamodt and D. W. Grainger, *Biomaterials*, 2016, 86, 68–82.
- 3 U. Boer, F. F. R. Buettner, M. Klingenberg, G. C. Antonopoulos, H. Meyer, A. Haverich and M. Wilhelmi, *Plos One*, 2014, 9, e105964.
- 4 C. Mora-Navarro, A. Badileanu, A. M. G. Martins, E. W. Ozpinar, L. Gaffney, I. Huntress, E. Harrell, J. R. Enders, X. Peng, R. C. Branski and D. O. Freytes, *Acs Biomater Sci Eng*, 2020, 6, 1690–1703.