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One-step decellularization of porcine uterine tissue for developing alginate–decellularized uterine ECM hydrogel for uterine tissue engineering

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One-step decellularization of porcine uterine tissue for developing alginate–decellularized uterine ECM hydrogel for uterine tissue engineering

Running title: Bioactive hydrogel for uterine bioprinting

Supplementary File 1

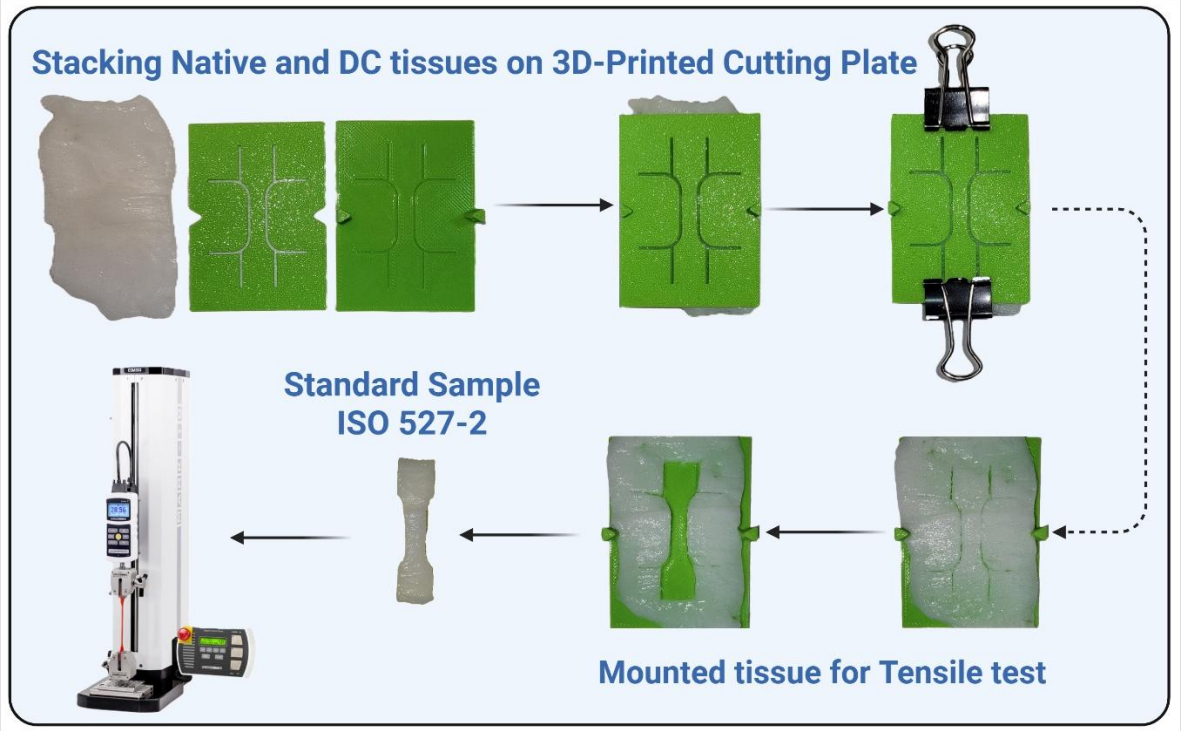

Figure S1: Procedure of specimen cutting and mounting for tensile experiments.

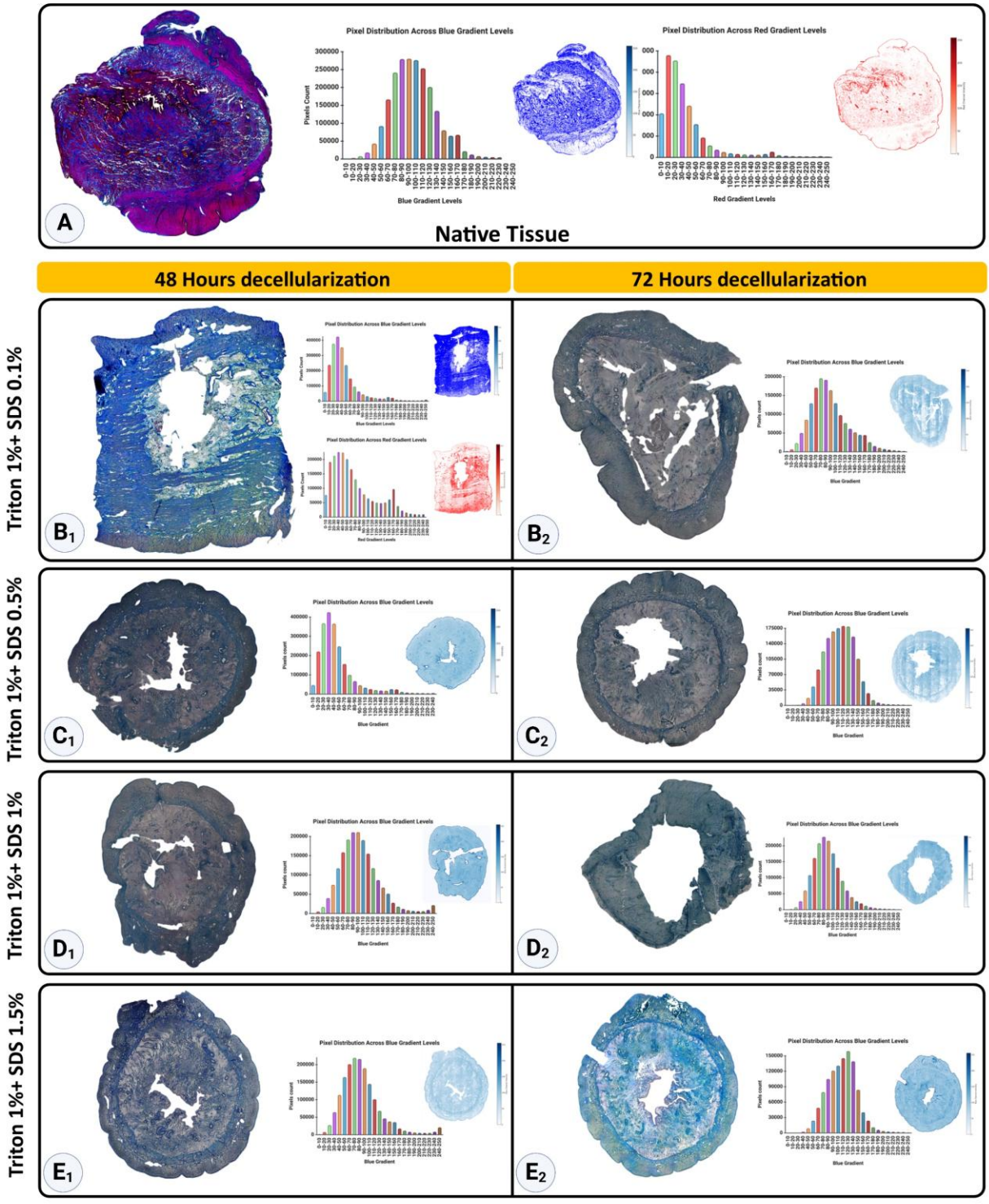

Figure S2: Mason Trichrome staining of native and decellularized uterine tissues, illustrating collagen distribution (blue) and non-collagen components (red). Panel **A** shows the native tissue with the highest collagen content and non-collagen signals, reflected in the dense blue and

red intensities. Panels **B_1–E_1** represent tissues treated with various decellularization protocols for 48 hours, where T1% + S0.1% - 48h (**B_1**) retains a high level of collagen. Collagen loss becomes progressively evident with increased SDS concentration in **C_1** (T1% + S0.5% - 48h), **D_1** (T1% + S1% - 48h), and **E_1** (T1% + S1.5% - 48h). Panels **B_2-E_2** show tissues treated for 72 hours, with the most pronounced collagen degradation observed in **E_2** (T1% + S1.5% - 72h). Non-collagen components (red signals) are primarily seen in **A** (native tissue) and **B_1** (T1% + S0.1% - 48h), reflecting effective decellularization in other protocols.

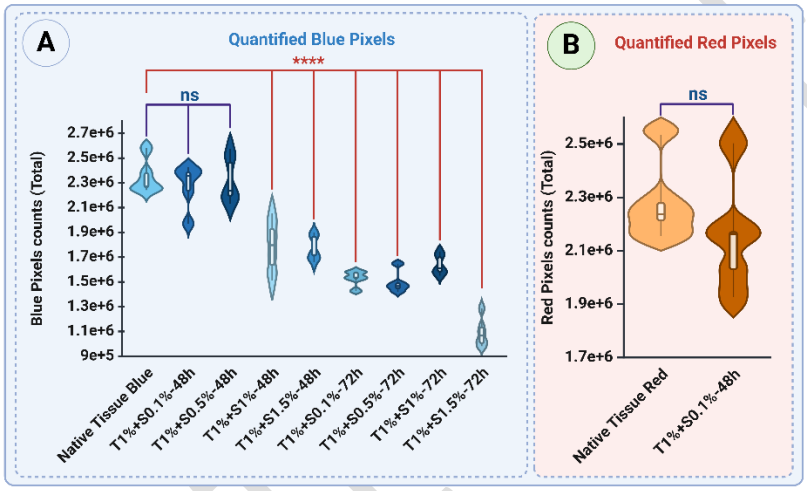

Figure S3: Violin plots quantifying blue (A) and red (B) pixel intensities from Mason Trichrome-stained samples. Panel **A** illustrates collagen content across native and decellularized tissues, with the native tissue showing the highest blue intensity. Among decellularized tissues, T1% + S0.1% - 48h retains the most collagen, while T1% + S1.5% - 72h) demonstrates the lowest. Panel **B** quantifies non-collagen components, with measurable red signals only in native tissue and T1%+S0.1%-48h). Statistical significance is indicated as follows: ns = not significant and *p<0.0001 (****). Data are presented as mean ± SD.

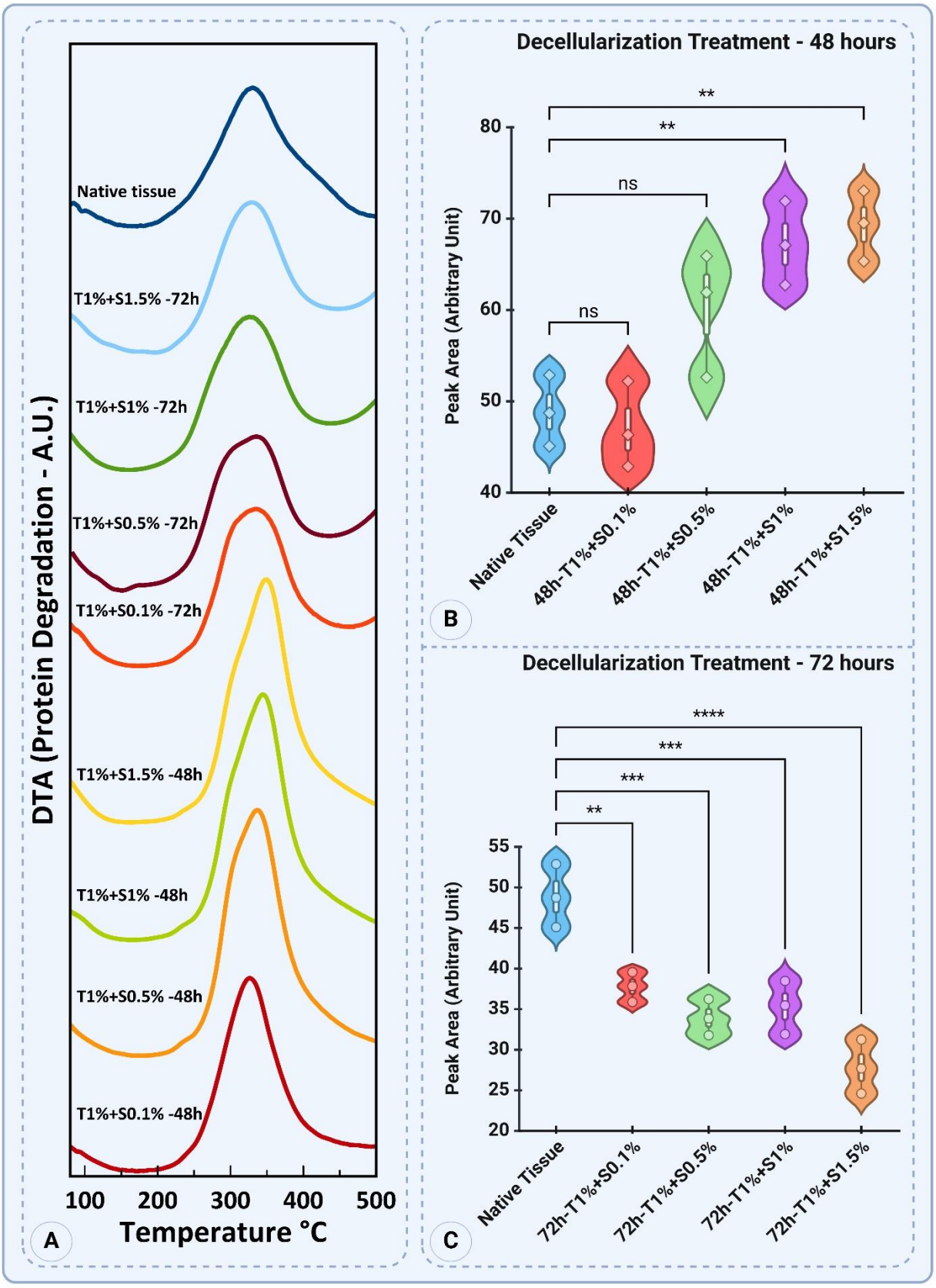
DTA (Protein Degradation - A.U.)
Native tissue
T1%+S1.5% -72h
T1%+S1% -72h
T1%+S0.5% -72h
T1%+S0.1% -72h
T1%+S1.5% -48h
T1%+S1% -48h
T1%+S0.5% -48h
T1%+S0.1% -48h
100
200
300
400
500
Temperature °C
A
Decellularization Treatment - 48 hours
Peak Area (Arbitrary Unit)
80
70
60
50
40
**
**
ns
ns
Native Tissue
48h-T1%+S0.1%
48h-T1%+S0.5%
48h-T1%+S1%
48h-T1%+S1.5%
B
Decellularization Treatment - 72 hours
Peak Area (Arbitrary Unit)
55
50
45
40
35
30
25
20
****
***
***
**
Native Tissue
72h-T1%+S0.1%
72h-T1%+S0.5%
72h-T1%+S1%
72h-T1%+S1.5%
C

Figure S4*:* Thermogravimetric analysis (TGA) and peak area comparison of native and decellularized porcine uterine tissues. (A) TGA profiles showing the thermal degradation of native tissue and samples treated with 1% Triton™ X-100 and varying SDS concentrations (0.1%, 0.5%, 1%, and 1.5%) for 48 and 72 hours. The major degradation phase between 200°C and 500°C indicates the breakdown of proteins, including collagen. **(B)** Peak area comparison of TGA data for native tissue and decellularized samples treated for 48 hours. Lower SDS concentrations (0.1% and 0.5%) exhibit minimal changes compared to native tissue, while higher concentrations (1% and 1.5%) show significant increases in peak areas, reflecting enhanced decellularization. **(C)** Peak area comparison for 72-hour treatments. Extended exposure to SDS leads to significant reductions in peak areas, with the greatest effect observed for SDS 1.5%, indicating substantial protein removal and ECM disruption. Statistical significance is indicated as follows: ns = not significant, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and *$p<0.0001$ (****). Data are presented as mean ± SD.

Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA) results provide insights into the thermal decomposition behavior of porcine uterine tissue and its decellularized counterparts. The profiles reflect the effects of different SDS and Triton™ X-100 concentrations, treatment durations, and their impact on the structural and thermal properties of the extracellular matrix (ECM).

The TGA curves in Supplementary Figure 4A illustrate the thermal degradation profiles of native and decellularized uterine tissues subjected to varying concentrations of SDS and Triton for 48 and 72 hours. The native tissue exhibits a characteristic degradation peak around 330°C, indicating stable protein content and structural integrity. In contrast, decellularized samples demonstrate varied peak temperatures and degradation behaviors depending on the SDS concentration and treatment duration. Notably, the 48-hour treatments at higher SDS concentrations (e.g., 1% and 1.5%) show slightly shifted degradation peaks compared to the native tissue, suggesting alterations in protein composition and thermal stability. Similarly, for the 72-hour treatments, degradation peaks are more distinct and shifted toward lower temperatures, especially at 1.5% SDS, highlighting substantial protein degradation.

Quantitative analysis of peak areas reveals significant differences in thermal degradation behavior (Supplementary Fig. 4B). The native tissue (48.84 ± 3.89) and the T1% + S0.1% - 48 group (47.07 ± 4.72) show comparable peak areas with no significant difference ($p>0.05$). However, as the SDS concentration increases to 0.5%, 1%, and 1.5%, the peak areas significantly rise to 60.09 ± 6.81, 67.18 ± 4.59, and 69.24 ± 3.86, respectively. Statistical analysis indicates that the differences between native tissue and T1% + S1% - 48h and T1% + S1.5% - 48h ($p<0.001$) are significant, suggesting enhanced detergent penetration and increased decellularization efficiency at these concentrations.

The 72-hour treatments exhibit a different trend, with all decellularized groups displaying lower peak areas than the native tissue (Supplementary Fig. 4C). The native tissue maintains a peak area of 48.84 ± 3.89. In contrast, the T1% + S0.1% - 72h group shows a reduced value of 37.69 ± 1.84. The T1% + S0.5% - 72h, T1% + S1% - 72h, and T1% + S1.5% - 72h groups exhibit further reductions in peak areas, with means of 33.89 ± 2.23, 35.22 ± 3.28, and 27.78 ± 3.33, respectively. Statistical analysis highlights significant differences between native tissue and all decellularized groups, with the most substantial reduction observed for T1% + S1.5% - 72h ($p<0.0001$). This trend suggests that prolonged exposure to higher SDS concentrations leads to more extensive protein degradation and structural disintegration.

The observed trends align with previous studies on decellularization protocols, which report that higher detergent concentrations and extended treatment durations enhance the removal of cellular components but also compromise ECM integrity. For instance, it has been reported on SDS-based protocols for soft tissues that protein denaturation and collagen loss are dose-dependent [1]. Furthermore, the thermal stability reductions observed in the 72-hour treatments correlate with the depletion of thermally stable proteins such as collagen and elastin.

Table S1: Semi-quantitative SEM analysis of collagen fiber diameter, orientation, and alignment index in native and decellularized uterine tissues.

Group	Mean ± SD fiber diameter (nm)	Alignment index *
Triton 1% + SDS 0.1%-48 Hours	**56 ± 21**	**~ 0.4**
Triton 1% + SDS 0.5%-48 Hours	**54 ± 23**	**~ 0.5**
Triton 1% + SDS 1%-48 Hours	**57 ± 24**	**~ 0.1**
Triton 1% + SDS 1.5%-48 Hours	**56 ± 17**	**~ 0.2**
Triton 1% + SDS 0.1%-72 Hours	**60 ± 23**	**~ 0.4**
Triton 1% + SDS 0.5%-72 Hours	**49 ± 22**	**~ 0.2**
Triton 1% + SDS 1%-72 Hours	**49 ± 17**	**~ 0.1**
Triton 1% + SDS 1.5%-72 Hours	**48 ± 19**	**~ 0.2**

*Alignment index ranges from 0 to 1, where values closer to 1 indicate stronger preferential fiber alignment and values closer to 0 indicate random orientation.

Table S2: Peak wavenumbers, chemical assignments, vibration types, and structural significance observed in FTIR analysis of decellularized uterine tissue

Peak Wavenumber (cm^{-1})	Chemical Assignment	Vibration type	Structural significance
3500–3200	Hydroxyl (-OH)	Stretching	Represents hydrogen-bonded hydroxyl groups; strong in native tissue, reduced in decellularized samples.
3072	Amide B (-NH)	Stretching	Indicates N-H stretching; minor reduction observed in SDS-treated samples.
2938	Aliphatic (-CH)	Asymmetric Stretching	Reflects lipid components; relatively unchanged across all protocols.
2875	Aliphatic (-CH)	Symmetric Stretching	Represents lipid components; minimal disruption in decellularized samples.
1655	Amide I (C=O stretching)	Stretching	High in native tissue; reflects collagen integrity and secondary structure.

1630	Amide I (C=O stretching)	Stretching	Decreased intensity indicates collagen denaturation or degradation.
1538	Amide II (N-H bending, C-N)	Bending and stretching	Reduced intensity in decellularized samples, suggesting disruption of peptide bonds.
1450	Carboxylate (COO^-)	Symmetric Stretching	Reflects alterations in carboxylic acid groups; varies with protocol severity.
1234	Amide III (N-H bending, C-N)	Bending and stretching	Significantly reduced with increasing SDS concentration, reflecting collagen damage.
1080	C-O-C in polysaccharides	Stretching	Indicates glycosaminoglycan (GAG) presence; loss indicates ECM component degradation.

Reference

1. Fazel Anvari Yazdi, A., et al., *Comparative analysis of porcine-uterine decellularization for bioactive-molecule preservation and DNA removal.* Frontiers in Bioengineering and Biotechnology, 2024. **12**: p. 1418034.