Supplemental data

Residual Detergent Detection Method for Non-Destructive Cytocompatibility Evaluation of Decellularized Whole Lung Scaffolds

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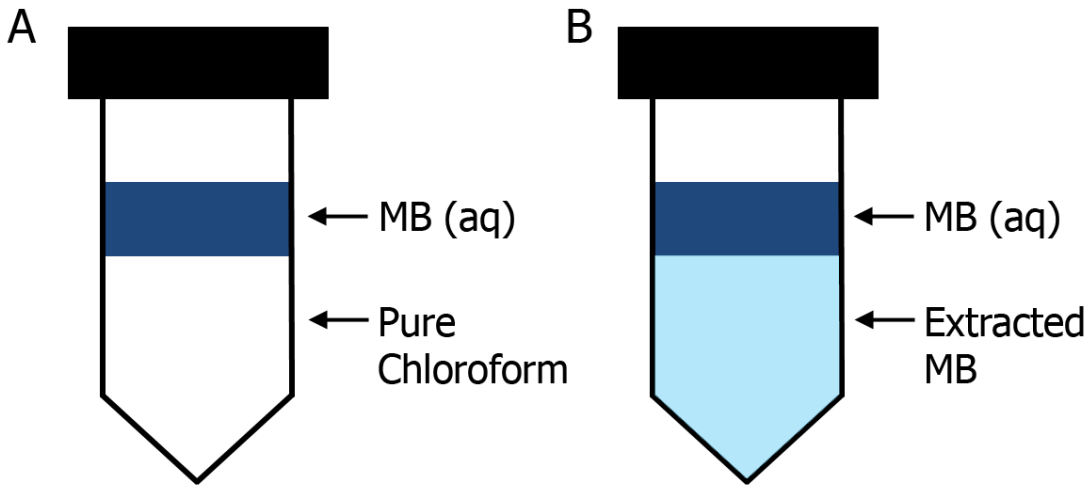
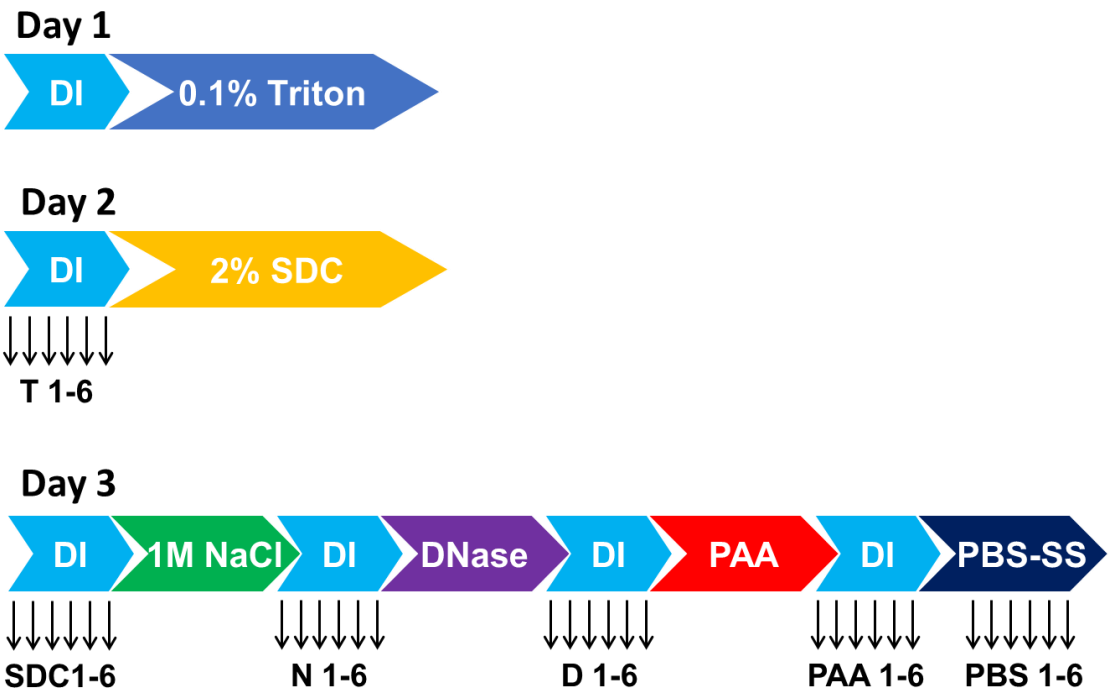
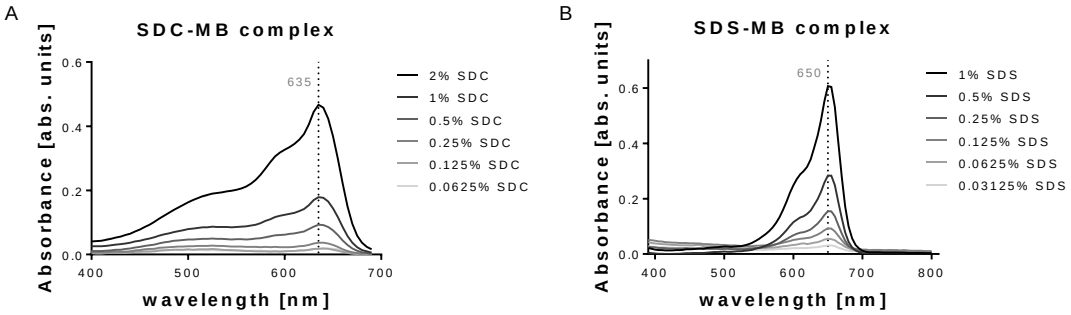


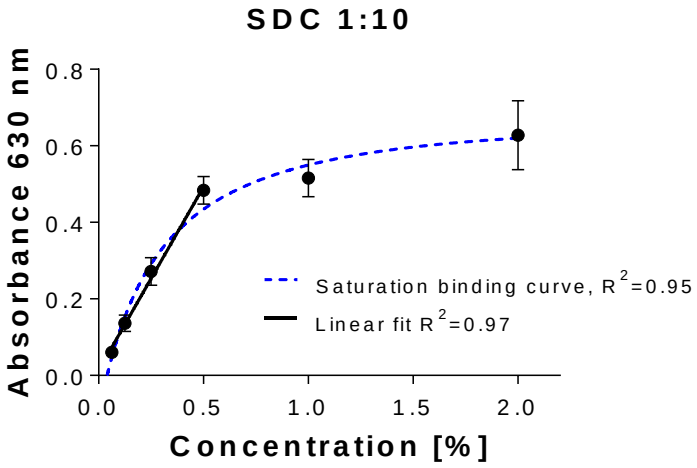
Supplemental Figure 1. Schematic showing detection of sodium deoxycholate (SDC) or sodium dodecyl sulfate (SDS) in a methylene blue (MB) detergent assay optimized for whole organ perfusion decellularization. **(A)** Maintenance of phase separation of methylene blue from chloroform layer following vortexing with pure water (aq=aqueous) due to the absence of an anionic detergent. **(B)** In the presence of an anionic detergent methylene blue complexes with the detergent and is extracted into the chloroform layer for spectrophotometric detection at 630 nm for SDC and 650 nm for SDS.



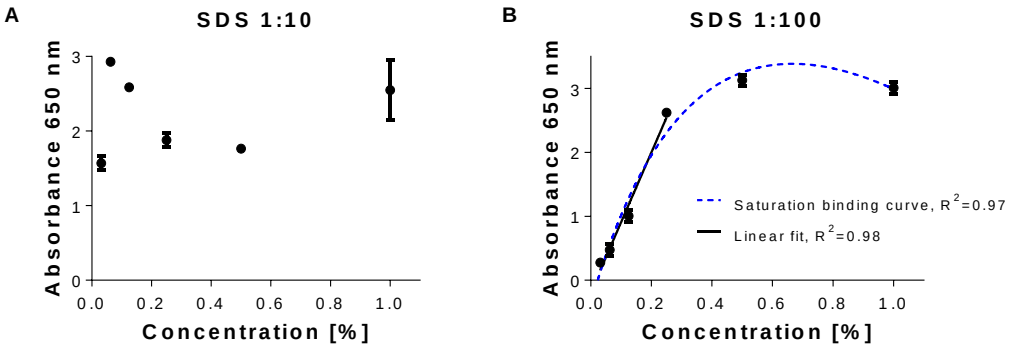
Supplemental Figure 2. Scheme of the effluent sampling during lung decellularization. Effluents were collected during de-ionized water (DI) rinses for detergent assessment. Collection washes were named after the solution that was used for decellularization and numbered. T=Triton, N=NaCl, D=DNAse, PAA=peracetic acid, PBS-SS=PBS storage solution. See methods for more details.



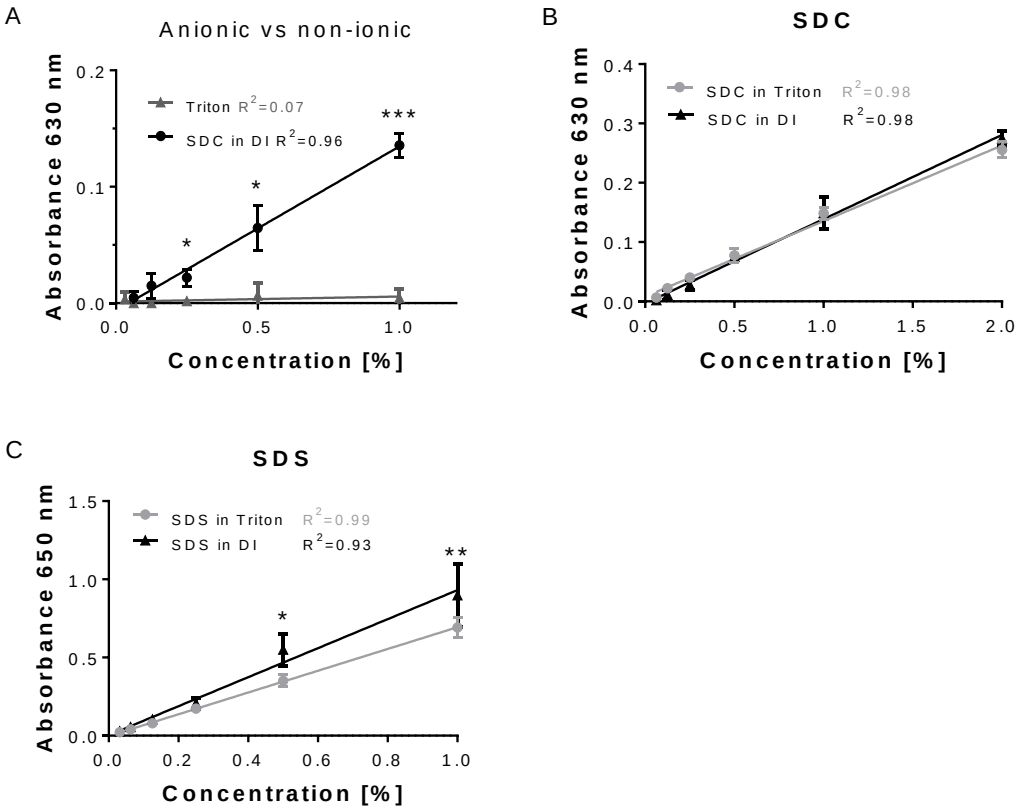
Supplemental Figure 3. Absorption spectra of detergent-MB complex to identify absorption maxima. A) Absorption spectrum of SDC in DI water at various concentrations using the optimized protocol (SDC:MB 1:100). Values are shown as absolute absorbance minus the absorbance of the blank (DI-water). B) Absorption spectrum of SDS in DI water at various concentrations using the optimized protocol (SDS:MB 1:1000), values are shown as absolute absorbance minus the absorbance of the blank (DI-water). 630 nm was chosen for SDC and 650 nm for SDS detection according to configurations with common spectrophotometers.



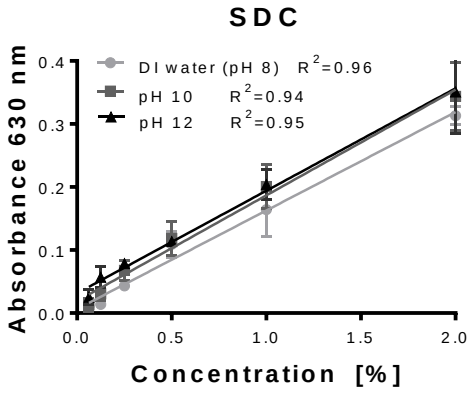
Supplemental Figure 4. SDC in DI water detection using a 1:10 (SDC solution:MB solution) in the MB detergent assay. A saturation binding curve, one-site total non-linear regression, was used to fit all data points while a linear regression curve was used to test values from 0 to 0.5%. All data points represent the mean ± SD, samples measured in triplicates at 630 nm.



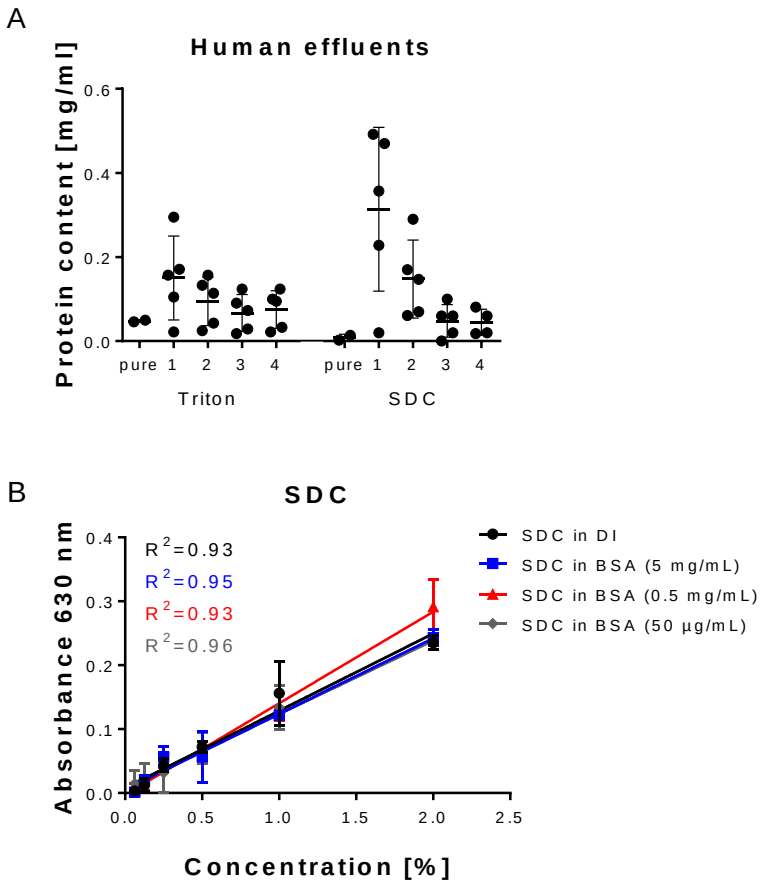
Supplemental Figure 5. Optimization of the detergent detection for SDS in DI water. A) SDS standard using a 1:10 (detergent:MB solution) dilution. B) SDS standard using a 1:100 (detergent:MB solution) dilution. A saturation binding curve, one-site total non-linear regression, was used to fit all data points while a linear regression curve was used to test values from 0 to 0.25%. All data points represent the mean ± SD, samples measured in triplicates at 650 nm.



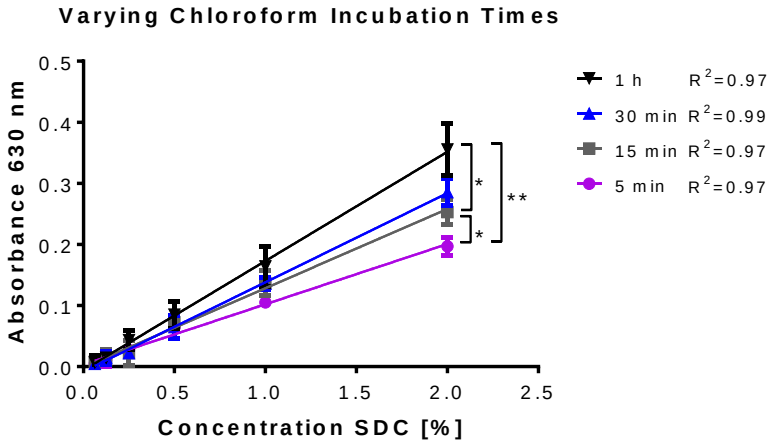
Supplemental Figure 6. Effect of a non-ionic detergent, Triton X-100, on SDC or SDS detection at low concentrations and its ability to be detected by the MB detergent assay. A) Absorbance measurement of different SDC and Triton X-100 concentrations using a 1:100 (detergent:MB solution) dilution in DI water. B) SDC standard using a 1:100 (detergent:MB solution) dilution in DI water or 0.1% Triton X-100 solution. C) SDS standard using a 1:1000 (detergent:MB solution) dilution in DI water or 0.1% Triton X-100 solution. All data points represent the mean ± SD, samples measured in triplicates at 630 and 650 nm, respectively. *: significant compared to Triton at the same concentration; *: $p<0.05$, *** $p<0.001$, unpaired t-test.



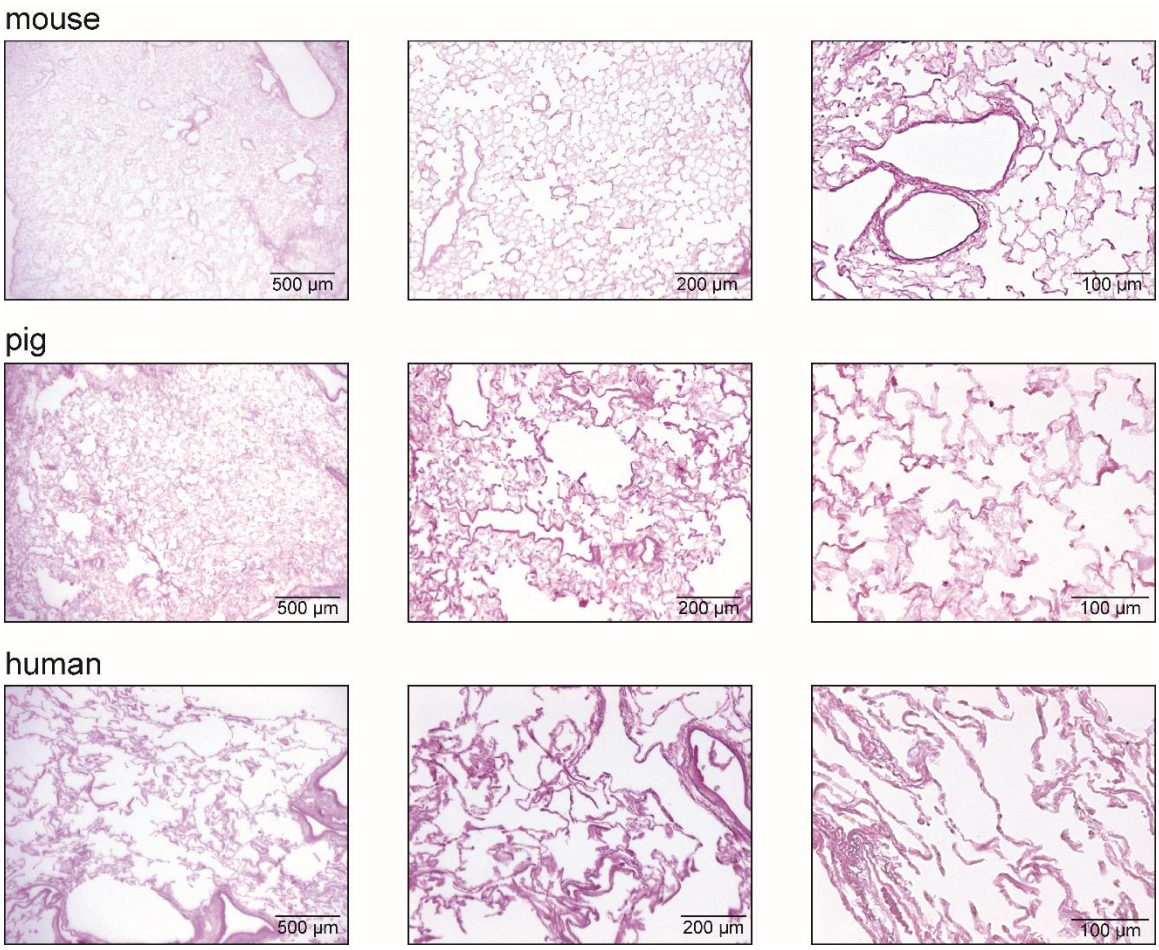
Supplemental Figure 7. Effect of basic pH on the standard curve linearity or absorbance values from those done in DI water. Absorbance measurement of SDC standard curves at pH of 8 (baseline SDC solution in DI water), 10, and 12 using a 1:100 (detergent:MB solution) dilution in DI water. All samples were adjusted for specific basic pH with 1M NaOH. All data points represent the mean ± SD, samples measured in triplicates at 630 nm.



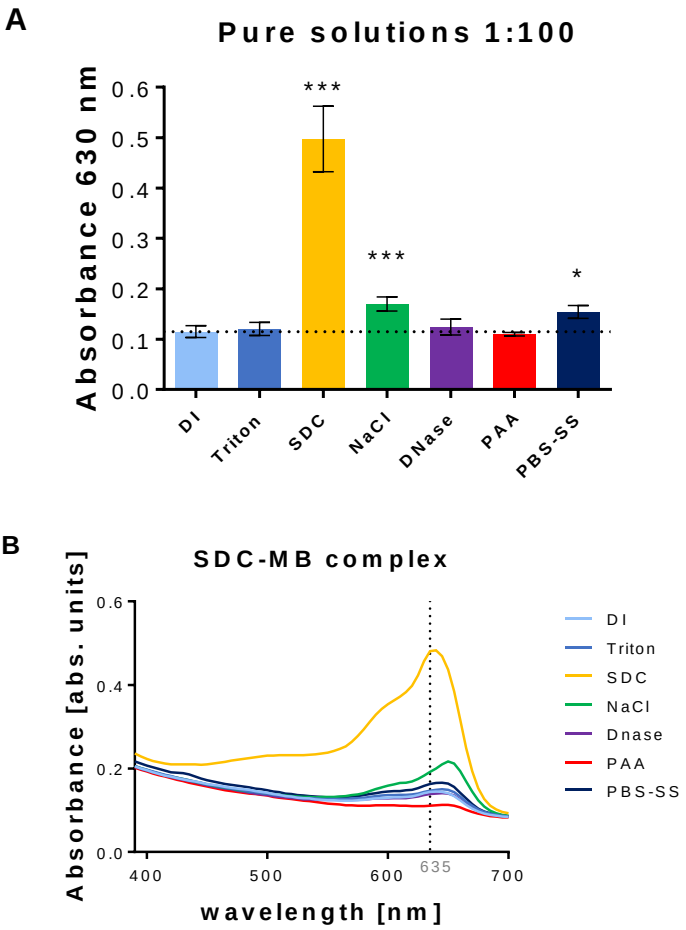
Supplemental Figure 8. Effect of bovine serum albumin (BSA) on absorbance values of SDC solutions. A) Determination of protein concentrations in pure detergent and consecutive DI washes of human lung lobe decellularizations after incubation with 0.1% Triton and 2% SDC. B) Absorbance of SDC solutions were determined in combination with either 5 mg/ml, 0.5 mg/ml, or 50 µg/ml BSA. All data points represent the mean ± SD, samples measured in triplicates at 630 nm.



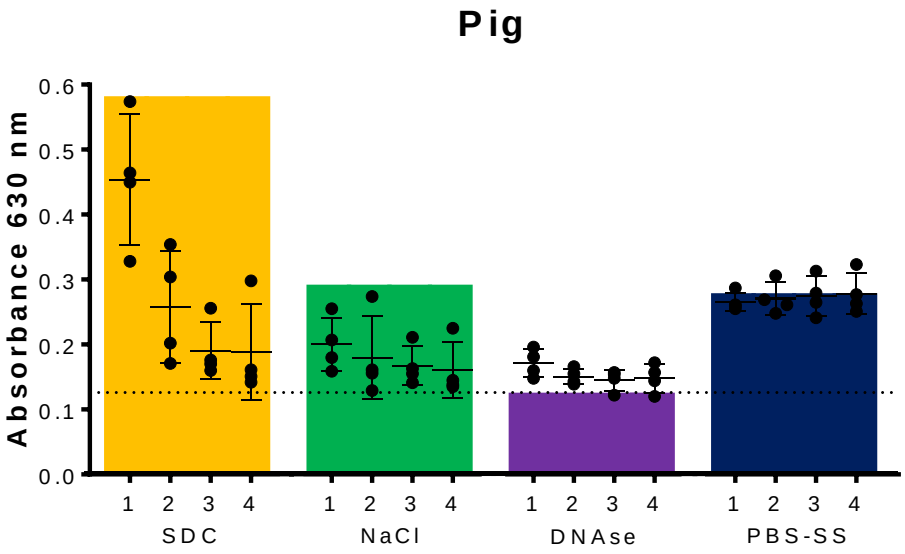
Supplemental Figure 9. Effect of incubation times on absorbance values of methylene blue in a range of SDC samples. All data points represent the mean ± SD, samples measured in triplicates at 630 nm. *: $p<0.05$, **: $p<0.01$.



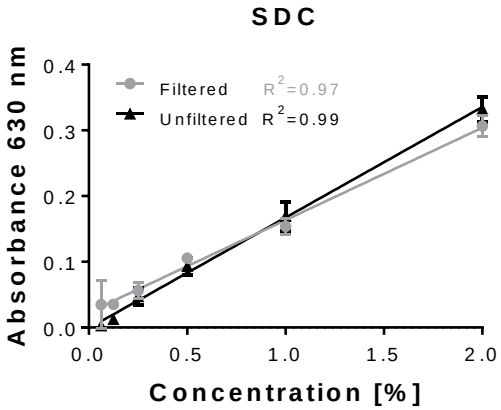
Supplemental Figure 10. Histologic characterization following decellularization in mouse, pig, and human lungs. Decellularization with 0.1% Triton, 2% SDC, 1M PBS and DNase leads to removal of cellular material in mouse, pig, and human lungs. Hematoxylin and Eosin staining confirms loss of cellular bodies and nuclear material with general maintenance of ECM architecture.



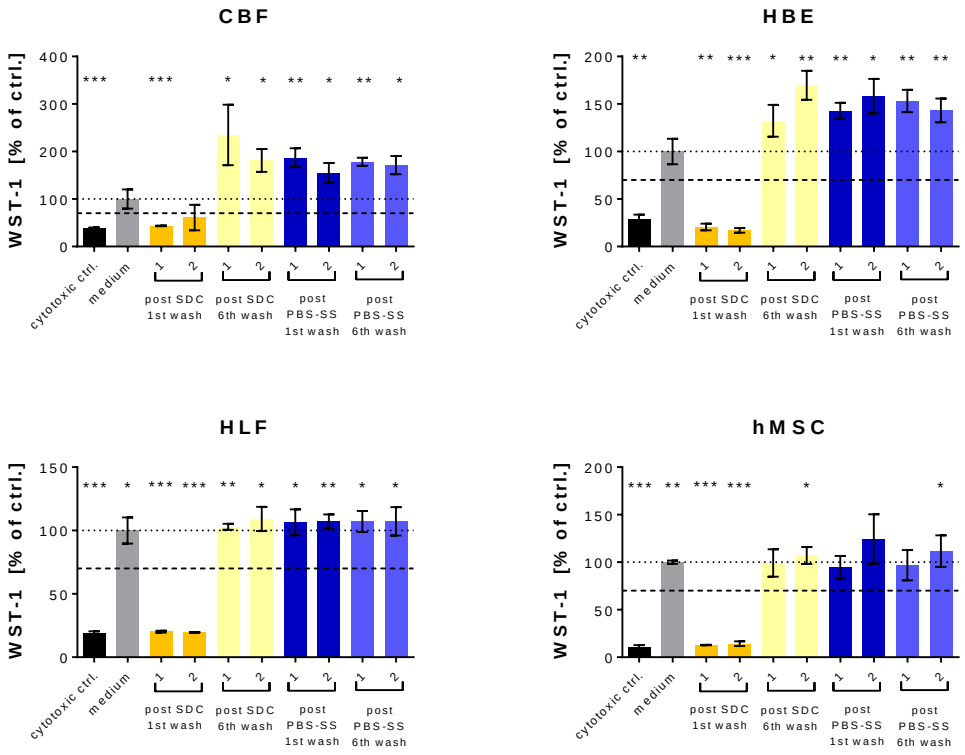
Supplemental Figure 11. A) Raw absorbance measurements of each pure solution used throughout the decellularization process in the optimized assay. Dotted line representing background absorbance of pure chloroform. B) Absorbance spectra of the solutions used during decellularization. n=3, all data points represent the mean ± SD, samples measured in triplicates at 630 nm, *: significant compared to background absorbance, *: $p<0.05$, ***: $p<0.001$, ANOVA with Bonferroni multiple comparison.



Supplemental Figure 12. Effluent collection and detergent detection in porcine lungs decellularized individually, using an SDC and Triton X-100 based detergent decellularization protocol. Effluents of four individual pig lung decellularizations are depicted measured with the 1:10 dilution protocol. The bars represent the average absorbance determined in the pure solutions used during decellularization. The dotted line indicates the value of the DI water.



Supplemental Figure 13. Effect of filtration with a 0.22 µm PES membrane on SDC detection. A standard curve using a 1:100 (detergent:MB solution) dilution was used to assess this potential influence before (unfiltered) and after filtration (filtered). All data points represent the mean ± SD, samples measured in triplicates at 630 nm.



Supplemental Figure 14. Toxicity of different effluents from porcine lung decellularization (n=2) in different cell lines (HBE, CBF, HLF, and hMSC). Viability of different cell lines (HBE, CBF, HLF, and hMSC) was determined by WST-1 conversion after cultivation for 24 h with effluents collected after different DI water wash steps of pig lung lobe decellularizations (effluents mixed 1:4 with medium). 2.5% Triton X-100 served as cytotoxic (positive) control while cultivation medium served as negative control (set to 100%). The threshold for cytotoxicity was set at 70%. Mean ± SD, samples measured in triplicates. One sample t-test *: sign. to 70% viability, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Supplemental Table 1. Measured SDC concentration (%w/v) in the final PBS-SS washes and human decellularized lung lobe homogenates.

sample	Last PBS- Wash	Homogenates
human 1	0.009	none detectable
human 2	none detectable	none detectable
human 3	0.025	none detectable
human 4	none detectable	none detectable
human 5	none detectable	none detectable