ABSTRACTS

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Assessing the Effects of Bladder Decellularization Protocols on Protein Content

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Introduction: The Core Matrisome (CM) encompasses the fundamental structural proteins of the extracellular matrix (ECM); Matrisome Associated (MA) proteins interact with and regulate the ECM. Prior work from our group has shown effectiveness of two diffusion-based decellularization protocols for removal of DNA and cellular content from tissue, and indicated discrepancy in overall protein preservation between protocols (Eggleston et al, EUS 2024). The aim of this subsequent study was to comparatively evaluate specific CM and MA protein preservation in bladders decellularized with these two perfusion-based decellularization protocols, compared to native tissue.

Methods: As previously described (REF), Sodium Dodecyl Sulfate (SDS) and Detergent Enzymatic Treatment (DET) perfusion-based protocols were used for decellularization. 50-100 mg bladder tissue samples were obtained from adult New Zealand White male rabbits (n=3/cohort, pre- and post-decellularization pairs) Protein was extracted (Precellys kit, urea buffer followed by homogenization and centrifugation cycles) as described (Yiu et al, JPU 2024). Mass spectrometry data was processed using MaxQuant; proteins names and symbols were found by inputting protein codes into UNIPROT. Student t-tests were performed in base R to assess protein intensity change between paired native and decellularized samples, operating with a p-value < 0.05.

Results: Of the 81 CM and MA proteins, 21 CM and 5 MA proteins were found to have a significant mean intensity change from baseline, as compared to native bladders (Figure 1). Mean intensity significantly decreased for 50% of CM and 13.3% of MA proteins from samples decellularized with SDS, while 47.72% CM and 6.67% MA proteins decreased significantly for samples decellularized with DET (Figure 1). Figure 2 further expands on intensity change by examining trends of change between CM and MA protein sub-groups categorized by functionality.

Conclusion: SDS and DET decellularization both significantly reduced the mean intensity measurements of a similar proportion of CM and MA proteins, respectively. However, effects on specific proteins differed between the treatment modalities. Future studies that apply bladder acellular matrices should report the details of each decellularization protocol, and users are advised to consider differences in protein content and distribution that may alter observed translational outcomes.

Figure 1: All CM and MA Protein Content Native to Decellularized Volcano Plot

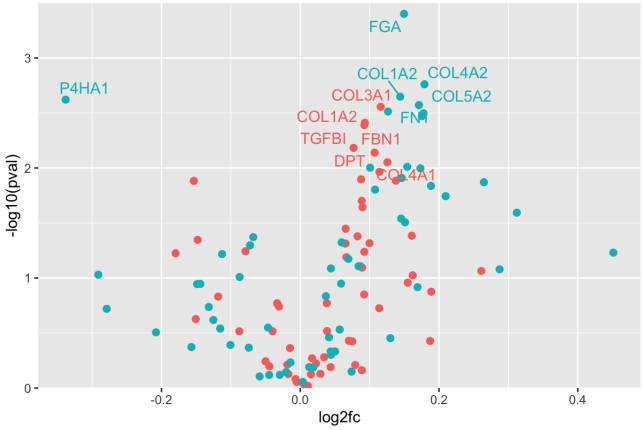

Figure 2: All CM and MA Proteins Native to Decellularized Mean Intensity Change