MALDI Mass Spectrometry Imaging of Human Penile Tissue Scaffolds following Organ Decellularization to Evaluate Extracellular Matrix Preservation

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Abstract

Human penile reconstruction and transplantation is exceedingly complex due to the structure and function of the organ. Furthermore, transplantation for gender confirmation surgery and trauma leads to potential problems including life-long immunosuppression and tissue rejection. We have established a method to remove donor cells and preserve the extracellular matrix (ECM) which can then be seeded with recipient cells for transplantation. However, very little is known about the composition of the ECM of the human penis. In this study, we are using MALDI mass spectrometry imaging (MSI) to map both the fresh-frozen human penis and the decellularized specimen to better understand the ECM using a combination of unsupervised analysis and proteomics. We will use this data to develop tailored approaches to reseed the penile scaffold to improve clinical outcomes.

Introduction

Recently, we have reported the first decellularization of whole-organ human penile specimens for total penile tissue engineering, which are being developed for gender confirming surgery or trauma patients requiring penile reconstruction. Our long-term goal is to utilize whole organ penile extracellular matrix (ECM) scaffolds repopulated by the recipient's own cells to minimize tissue rejection. Standard approaches to evaluating decellularization protocols assess only limited protein subsets to evaluate the relative success of removal of antigenic cellular material versus preservation of key ECM proteins. In this study, we sought to use matrix-assisted laser desorption ionization (MALDI) imaging to evaluate the preservation of the ECM in penile tissue following decellularization as compared to unaltered fresh-frozen penile tissue.

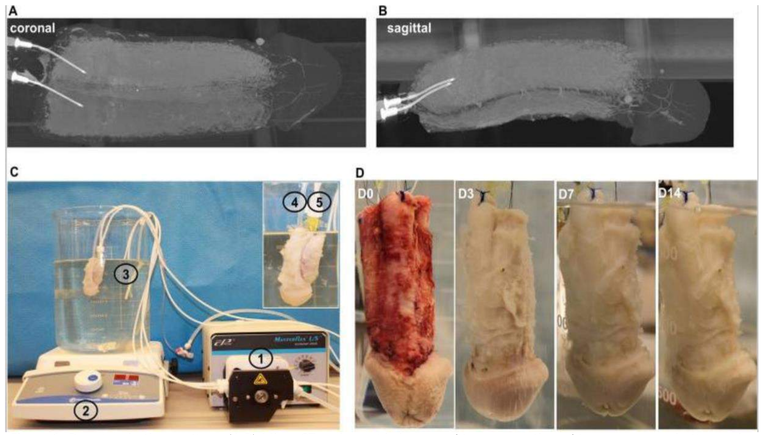
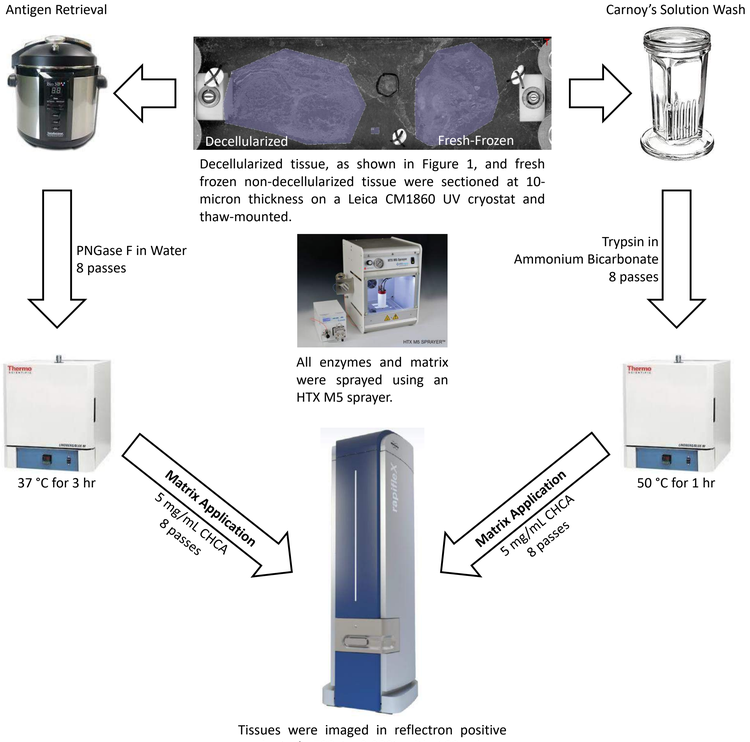


Figure 1: Decellularization method. (A,B) Micro-CT radiographic images of the cannulation of the canvernosa in the penile tissue. (C) Perfusion system consisting of a (1) peristaltic pump, (2) magnetic stirrer plate, and a (3) 4 L glass container. The scaffold has been cannulated with a (4) Foley catheter in the urethra and (5) two angiocatheters in the cavernosal arteries. (D) Photographic representation of the decellularization process at 0, 3, 7, and 14 days, respectively.[1]

Methods



Tissues were imaged in reflectron positive mode (m/z 500 to 3,200) with 100 micron pixel size (200 laser shots) on a Bruker RapifleX MALDI TOF/TOF instrument.

Results: Tryptic Digestion

Figure 2: Full average skyline spectra of the tryptic digestion of both decellularized and fresh-frozen penile tissue (inset).

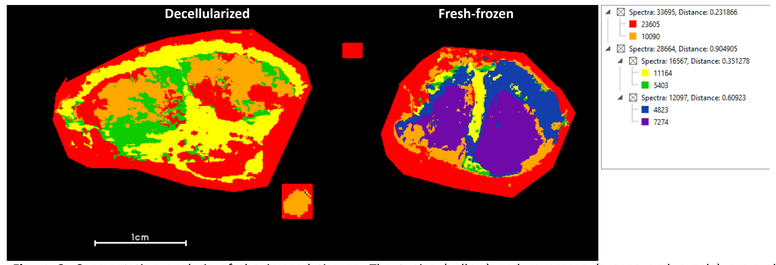


Figure 3: Segmentation analysis of the imaged tissues. The tunica (yellow) and cavernosa (orange and purple) are well differentiated in the tissue. Furthermore, there is a significant difference between decellularized (left) and fresh-frozen (right) tissues due to the removal of cells within the tissue. The decellularized tissue is composed entirely of extracellular matrix (ECM).

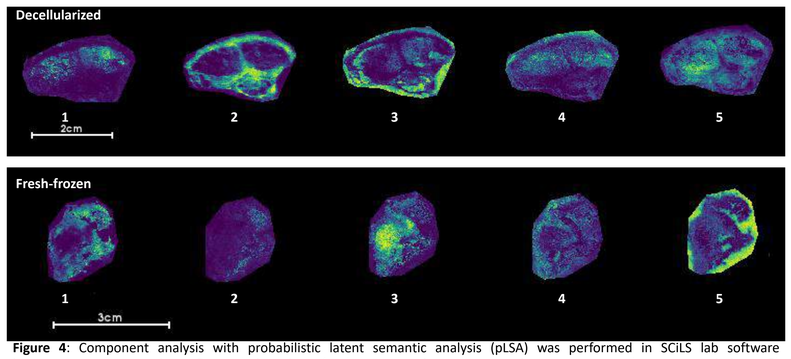


Figure 4: Component analysis with probabilistic latent semantic analysis (pLSA) was performed in SCiLS lab software independently for each tissue. Decellularized tissue (top) showed major components include cavernosa (components 1, 4, and 5) and tunica (components 2 and 3). Fresh-frozen components revealed primarily cavernosa (components 1-4), with one component showing tunica (component 5). Analysis was performed on a random subset of individual spectra with no denoising.

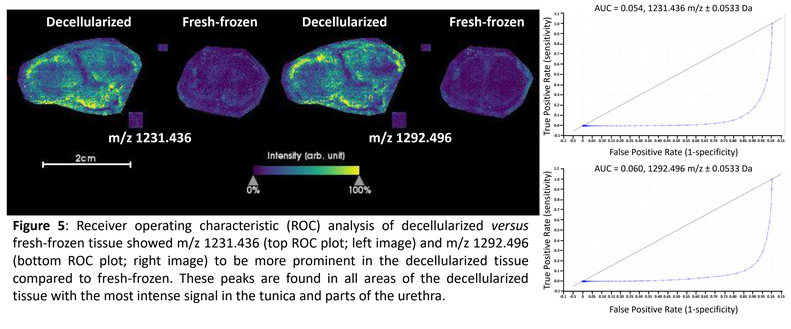


Figure 5: Receiver operating characteristic (ROC) analysis of decellularized *versus* fresh-frozen tissue showed m/z 1231.436 (top ROC plot; left image) and m/z 1292.496 (bottom ROC plot; right image) to be more prominent in the decellularized tissue compared to fresh-frozen. These peaks are found in all areas of the decellularized tissue with the most intense signal in the tunica and parts of the urethra.

Results: PNGase F Digestion

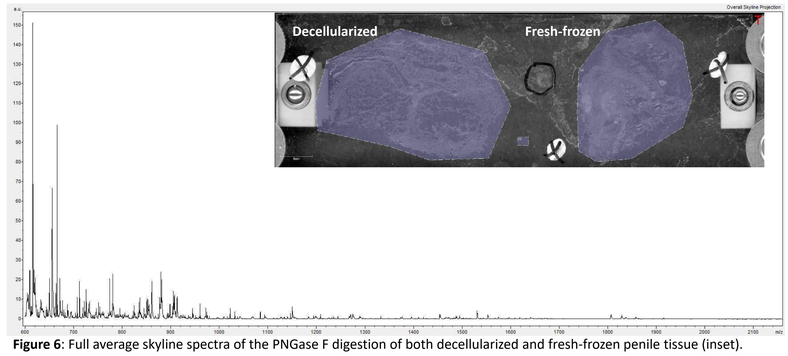


Figure 6: Full average skyline spectra of the PNGase F digestion of both decellularized and fresh-frozen penile tissue (inset).

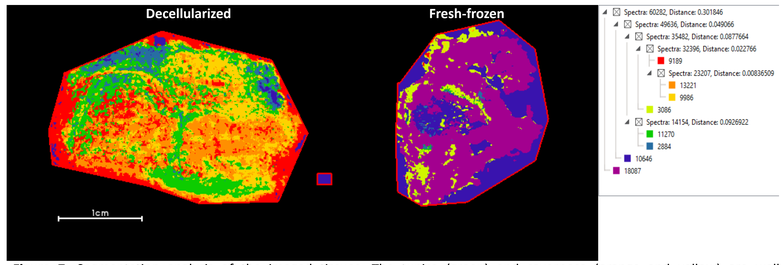


Figure 7: Segmentation analysis of the imaged tissues. The tunica (green) and cavernosa (orange and yellow) are well differentiated in the decellularized tissue. The Fresh-frozen tissue is less well differentiated. Furthermore, there is a significant difference between the decellularized (left) and fresh-frozen tissue (right) due to the removal of cells within the tissue. The decellularized tissue is composed entirely of extracellular matrix (ECM).

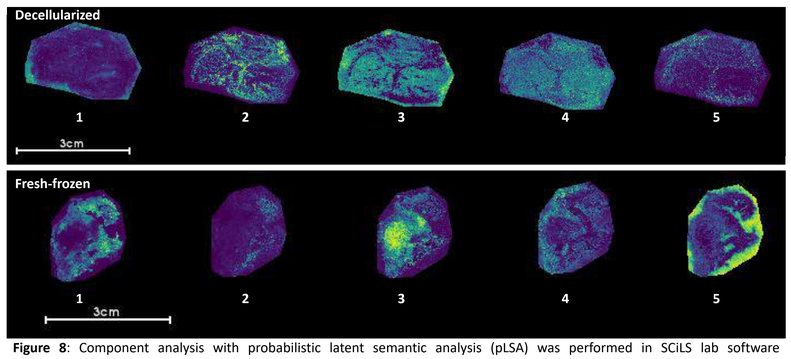


Figure 8: Component analysis with probabilistic latent semantic analysis (pLSA) was performed in SCiLS lab software independently for each tissue. Decellularized tissue's (top) major components included primarily cavernosa (components 2, 3, and 4) and tunica (components 5). Fresh-frozen components revealed primarily cavernosa (components 1-3 and 5), with one component showing tunica (component 4). Analysis was performed on a random subset of individual spectra with no denoising.

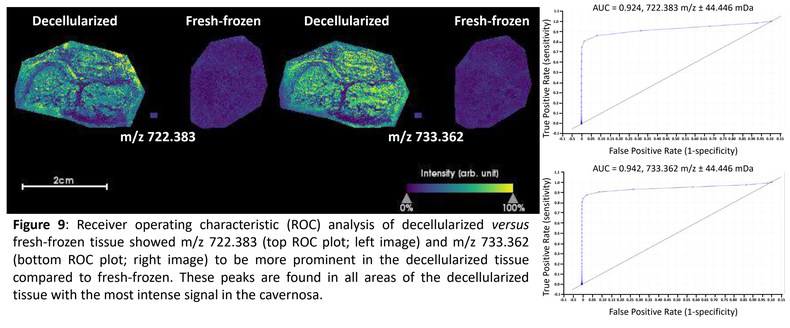


Figure 9: Receiver operating characteristic (ROC) analysis of decellularized *versus* fresh-frozen tissue showed m/z 722.383 (top ROC plot; left image) and m/z 733.362 (bottom ROC plot; right image) to be more prominent in the decellularized tissue compared to fresh-frozen. These peaks are found in all areas of the decellularized tissue with the most intense signal in the cavernosa.

References

1. Tan Y, Landford WN, Garza M, Suarez A, Zhou Z, Coon D. Complete Human Penile Scaffold for Composite Tissue Engineering: Organ Decellularization and Characterization. *Sci Rep*. 2019;9(1):16368. Published 2019 Nov 8. doi:10.1038/s41598-019-51794-6

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Conclusions and Future Directions

- Methods were successfully developed to image tryptic peptides and glycans from both fresh-frozen and decellularized human penile tissue.
- Segmentation analysis results demonstrate that some ion suppression may be present in the fresh-frozen specimen due to cellular components not observed in the decellularized specimen.
- Unsupervised analysis, including segmentation and component analysis, demonstrated that the ECM of the human penis is unique to each structure within the penis (i.e., cavernosa and tunica). This indicates that individualized reseeding techniques may be necessary for tissue engineering, which are specific to the ECM of each specialized penile structure.
- We are currently working on establishing a pipeline for peak identification using MS/MS and LC-MS/MS to determine the identities of proteins and glycans in this tissue.
- We intend to employ hematoxylin-eosin (H&E) and immunohistochemistry (IHC) stains to confirm our MALDI MSI findings.