Optimization and Investigation of Protein Molecular Signatures in Endometriosis Tissues by Mass Spectrometry Imaging

Monica Lin[1], Erin H. Seeley[1], Suzanne Ledet[3], Christina Salazar[3], Michael T. Breen[3], and Livia S. Eberlin[1,2]

(1) University of Texas at Austin, Austin, TX; (2) Baylor College of Medicine, Houston, TX; (3) Ascension Seton Medical Center, Austin, TX

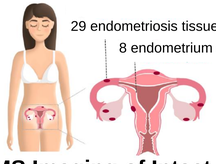
OVERVIEW

- Here, we describe the use of a red blood cell lysis buffer and MALDI MS imaging for the optimization and investigation of protein molecular signatures in ectopic and eutopic endometrial tissues.
- 29 endometriosis and 8 endometrium tissue samples were analyzed by MALDI MS for intact protein (20 endometriosis, 4 endometrium) and tryptic peptide imaging (9 endometriosis and 4 endometrium).

INTRODUCTION

- Endometriosis is a prevalent gynecological condition affecting approximately 10% of women in reproductive age and is characterized by uncontrolled growth of endometrial-like tissue outside the uterine cavity.[1]
- Although highly prevalent, the biological mechanisms of endometriosis are poorly understood, and the disease often misdiagnosed due to current unavailability of pre-operative diagnostic methods.[2]
- Thus, better characterization of molecular markers of endometriosis are critical to improve our current understanding of the disease and management in patients.[3]
- Here, we investigate protein and peptide molecular signatures in eutopic and ectopic endometrial tissues using matrix-assisted laser desorption ionization (MALDI) imaging.
- Signal optimization was performed through application of a red blood cell lysis buffer to reduce ion suppression from hemoglobin proteins and improve molecular coverage for intact protein imaging of blood rich tissues.[4]

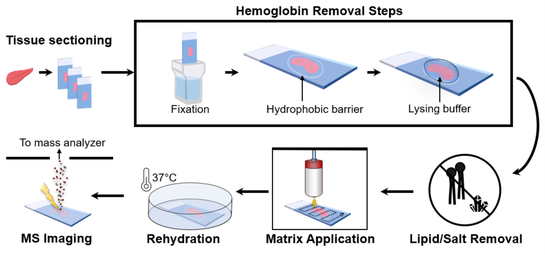
METHODS



- Endometriosis and endometrium tissues were collected from the Seton Medical Center from patients undergoing endometriosis surgeries, flash frozen, and stored at -80°C.
- Tissues were sectioned (12 µm) prior to imaging of intact proteins or tryptic peptides.

MS Imaging of Intact Proteins

- Tissues were fixed in ethanol (70, 90, 95%, 30 s each) prior to application of red cell lysis buffer (10 min). Tissue sections were washed in water (2×30 seconds), ethanol (70%, 100%, 30 s), Carnoy's fluid (6/3/1 ethanol/chloroform/acetic acid), ethanol, water, and ethanol.



- 2-hydroxy-5-methoxybenzoic acid/5-methoxysalicylic acid was used as a matrix.
- MALDI imaging was performed on a Bruker rapifleX MALDI TOF/TOF MS operated in linear positive ion mode from *m/z* 2000-24000 using a spatial resolution of 50 µm.

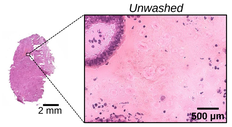
MS Imaging of Tryptic Peptides

- Tissue sections were washed in ethanol (70%, 100%, 30 s), Carnoy's fluid (2 min), ethanol (30 s), water (30 s), and ethanol (30 s).
- Trypsin was applied onto the tissue surface using an HTX M5 Sprayer, then incubated for 4 hours at 37°C prior to matrix application.
- α-cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix.
- MALDI imaging data was acquired on a Bruker timsTOF Flex MS operated in positive ion mode from *m/z* 600-4000 using a spatial resolution of 50 µm.

RESULTS

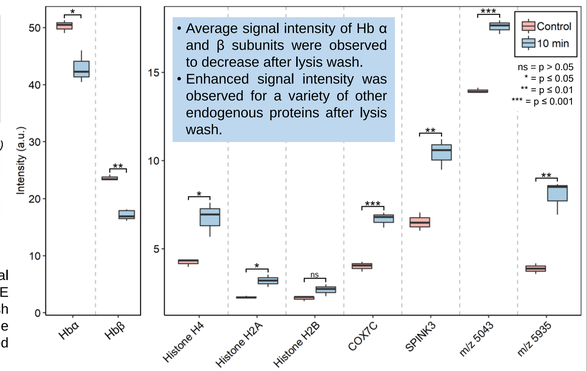
Optimization of Lysis Buffer Wash for Intact Protein Imaging

H&E Stained Images of Endometrium Tissue Before and After Lysis Buffer Wash



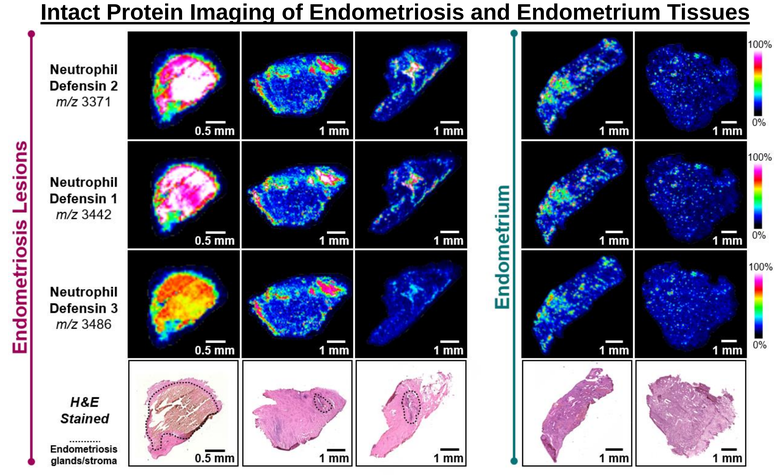
- Minimal disruption to endometrial glands were observed in H&E stained images after lysis wash suggesting high specificity of the lysis buffer for targeting red blood cells in tissue sections.

Signal Intensity of Selected Ions Before and After Lysis Wash of Endometrium Tissue

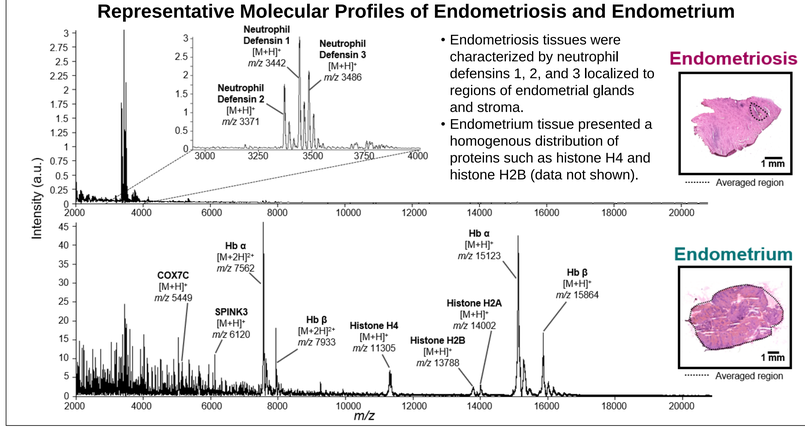


- Average signal intensity of Hb α and β subunits were observed to decrease after lysis wash.
- Enhanced signal intensity was observed for a variety of other endogenous proteins after lysis wash.

Intact Protein Imaging of Endometriosis and Endometrium Tissues



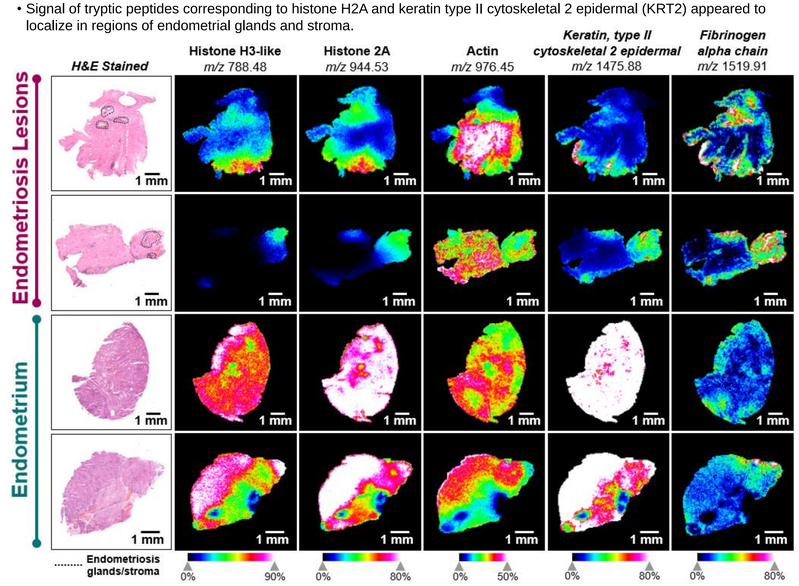
Representative Molecular Profiles of Endometriosis and Endometrium



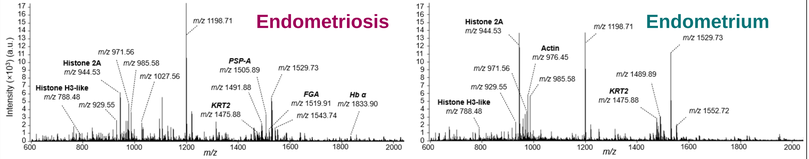
- Endometriosis tissues were characterized by neutrophil defensins 1, 2, and 3 localized to regions of endometrial glands and stroma.
- Endometrium tissue presented a homogenous distribution of proteins such as histone H4 and histone H2B (data not shown).

Imaging of Tryptic Peptides in Endometriosis and Endometrium Tissues

- Improved molecular coverage was observed using a tryptic digest to analyze endogenous peptides.
- Signal of tryptic peptides corresponding to histone H2A and keratin type II cytoskeletal 2 epidermal (KRT2) appeared to localize in regions of endometrial glands and stroma.



Average Molecular Profiles of Endometriosis and Endometrium



- 750 and 16450 pixels corresponding to areas of endometriosis glands/stroma and endometrium were extracted from 9 and 4 tissue samples, respectively. All mass spectra were averaged to obtain molecular profiles representing each tissue type.

CONCLUSIONS

- Treatment with lysis buffer for 10 minutes was observed to improve protein detection by MS imaging in endometrium tissues containing high blood content by reducing the susceptibility of other endogenous proteins to ion suppression from hemoglobin.
- Molecular imaging revealed that endometriosis tissues were characterized by detection of neutrophil defensins 1, 2, and 3 localized to regions of endometrial glands and stroma.
- Improved depth of molecular data obtained was observed by performing a tryptic digest, revealing localization of histone H2A and KRT2 to regions of endometrial glands and stroma.

REFERENCES AND ACKNOWLEDGMENTS

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This work was supported by The Welch Foundation (F-1895-20190330), Cancer Prevention and Research Institute of Texas (CPRIT, Grant RP190617), and NIH (5R01HD101560-03).