Multimodal MALDI Imaging Mass Spectrometry Reveals Spatially Correlated Lipid and Protein Changes in Mouse Heart with Acute Myocardial Infarction

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ABSTRACT: Acute myocardial infarction (MI) is a cardiovascular disease that remains a major cause of morbidity and mortality worldwide despite advances in its prevention and treatment. During acute myocardial ischemia, the lack of oxygen switches the cell metabolism to anaerobic respiration, with lactate accumulation, ATP depletion, Na⁺ and Ca²⁺ overload, and inhibition of myocardial contractile function, which drastically modifies the lipid, protein, and small metabolite profile in the myocardium. Imaging mass spectrometry (IMS) is a powerful technique to comprehensively elucidate the spatial distribution patterns of lipids, peptides, and proteins in biological tissue sections. In this work, we demonstrate an application of multimodal chemical imaging using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-IMS), which provided comprehensive molecular information in situ within the same mouse heart tissue sections with myocardial infarction. MALDI-IMS (at 30 μm per pixel) revealed infarct-associated spatial alterations of several lipid species of sphingolipids, glycerophospholipids, lysosphospholipids, and cardiolipins along with the acyl carnitines. Further, we performed multimodal MALDI-IMS (IMS3) where dual polarity lipid imaging was combined with subsequent protein MALDI-IMS analysis (at 30 μm per pixel) within the same tissue sections, which revealed accumulations of core histone proteins H4, H2A, and H2B along with post-translational modification products, acetylated H4 and H2A, on the borders of the infarcted region. This methodology allowed us to interpret the lipid and protein molecular pathology of the very same infarcted region in a mouse model of myocardial infarction. Therefore, the presented data highlight the potential of multimodal MALDI imaging mass spectrometry of the same tissue sections as a powerful approach for simultaneous investigation of spatial infarct-associated lipid and protein changes of myocardial infarction.

KEYWORDS: imaging mass spectrometry, MALDI, infarcted, heart, lipids, proteins

INTRODUCTION

Acute myocardial infarction (MI) is a cardiovascular disease (CVD) that remains a major cause of morbidity and mortality worldwide despite advances in its prevention and treatment. Coronary heart disease is the most common among the CVDs, which is characterized by buildup of plaques on the inside of the coronary arteries, leading to reduction of blood flow to the heart tissue. The buildup of plaques over time could ultimately lead to a complete blockage of one of the coronary arteries, causing a MI, which prevents oxygen and nutrients reaching part of the heart tissue.¹ Being the most energy-requiring organ of the body, the heart heavily relies on fatty acid oxidation for energy metabolism.² During acute myocardial ischemia, the lack of oxygen switches the cell metabolism to anaerobic respiration, with lactate accumulation, ATP depletion, Na⁺ and Ca²⁺ overload, and inhibition of myocardial contractile function, which drastically modifies the lipid, protein, and small metabolite profile in the myocardium.³ Accumulation of specific lipid species causes impairment of heart function and could ultimately cause heart failure.⁴⁻⁶ On the contrary, dysregulation of epigenetic post-transcriptional modifications of histones in chromatin is thought to be associated with the pathology of CVDs and acetylation of core histones has been found to be associated with myocardial infarction.⁶⁻⁸ Therefore, investigation of lipid and protein changes following myocardial infarction is important for the understanding the life-threatening consequences of a myocardial infarction.

Lipidomics studies using whole heart tissue extracts have been used to investigate lipid profile changes after an infarction.⁹ However, this approach does not provide any spatial information on the sample, which makes it difficult to connect changes of specific species with the infarcted area as well as detecting small, highly localized changes in lipid composition. While immunohistochemistry can provide qualitative informa-
tion and Western blot analysis can determine the relative quantification of proteins, simultaneous probing of multiple proteins can be restricted due to the interference of the chromogens and fluorochromes, as well as different subcellular localization of proteins. Unlike conventional lipidomics and proteomics approaches, mass spectrometry imaging (MSI) provides an ideal approach for the analysis of lipids, peptide, protein, and metabolite compositions by simultaneous investigation of the spatial relative abundances of many ionized species within the tissue sections. Matrix-assisted laser desorption (MALDI), secondary ion mass spectrometry (SIMS), and desorption electrospray ionization (DESI) based MSI have been demonstrated on myocardium to investigate the spatial distributions of lipids, metabolites, and peptides/proteins for probing lipid biochemistry in heart function and dysfunction. Among these methods, MALDI-MSI is a multifaceted tool for probing spatial localizations of molecules including lipids, metabolites, and peptides/proteins in a wide spectral range. While analysis of different mass ranges and ionization polarity of molecules often requires different matrix molecules and multiple analyses on consecutive tissue sections, recent developments in MALDI-IMS methodologies allow for subsequent imaging of several molecules within the same tissue sections and it is possible to perform multimodal IMS analysis on single tissue sections. Dual polarity lipid imaging with MALDI-IMS has been demonstrated using a few tens of micrometers offset in between the two modalities. Nevertheless, recent developments in multimodal MALDI-IMS methodologies allow for subsequent imaging of lipids (on the same pixel points) and proteins within the same tissue sections at high-spatial resolutions (down to 10 μm per pixel). Multimodal MALDI-IMS of a single brain tissue section has been utilized to shed light on the focal lipid and peptide molecular pathology of amyloid plaques in the brain tissue sections of a transgenic Alzheimer’s disease mouse model. Hence, this methodology can be a powerful tool for probing lipid and protein profiles of myocardial infarction where spatial molecular alterations are evident in/around the infarcted tissue.

In this study, dual polarity MALDI-IMS on the same pixel points was performed to dissect the infarct-associated alterations of lipids on mouse heart tissue where an infarction had been surgically induced by permanent ligation of the left coronary artery. MALDI-IMS (at 30 μm per pixel) revealed infarct-associated spatial alterations of several lipid species of sphingolipids, glycerophospholipids, lysophospholipids, and cardiolipins along with the acylcarnitines. Further, we performed multimodal MALDI-IMS (IMIS3), where dual polarity lipid imaging was combined with subsequent protein MALDI-IMS analysis (at 30 μm per pixel) within the same tissue sections, which revealed accumulations of core histone proteins H4, H2A, and H2B along with post-translational modification products, acetylated H4 and H2A, on the borders of the infarcted region. This methodology allowed us to interpret the lipid and protein molecular pathology of the very same infarcted region in a mouse model of myocardial infarction. Therefore, the presented data highlight the potential of multimodal MALDI imaging mass spectrometry of the same tissue sections as a powerful approach for simultaneous investigation of spatial infarct-associated lipid and protein changes of myocardial infarction.

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**METHODS**

**Surgical Preparation of Mouse Heart Tissue.** The mice were anesthetized with isoflurane, orally intubated, and connected to a ventilator (SAR-830, Geneq, Montreal, Canada) providing oxygen, air, and isoflurane (2–3%). An incision was made between the fourth and fifth ribs, revealing the anterior left ventricle (LV) wall and a lower region of the left atrium. MI was induced by ligating the left anterior descending coronary artery right after the branching of the left coronary artery. The success of the procedure was verified immediately using ECG changes, along with akinesis of the LV anterior wall. Twenty-four hours after MI, the mice were sacrificed by an overdose of isoflurane and cervical dislocation. The hearts were snap-frozen in liquid isopentane cooled by liquid nitrogen. All animal studies were approved by the local animal ethics committee and conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

**Section Preparation for Mass Spectrometry Imaging Analysis.** Extracted hearts were sectioned in an argon-purged cryo-microtome (Leica CM1520) at ~20°C. For MALDI-IMS analysis, 10 μm thick tissue sections from both infarcted and noninfarcted mice heart were cut and thaw mounted on ITO-glass slides. Matrix deposition for lipid imaging analysis was carried out using a vacuum sublimation apparatus (Sigma-Aldrich, Stockholm, Sweden) design as previously described in detail elsewhere. Briefly, ~300 mg of 1,5-diaminonaphthalene (1,5-DAN) matrix powder was spread evenly on the outer bottom of the sublimation chamber, which was then attached to the top using an O-ring seal. A vacuum of ~0.8 mbar was provided by a membrane pump, and the cooler was filled with ice slush (≥0°C). We used previously optimized sublimation conditions: 20 min at a temperature of 130°C under a stable vacuum of ~0.8 mbar.

Protein MALDI-IMS experiments were performed as previously described in detail elsewhere. Briefly, prior to analysis after lipid MALDI-IMS, tissue sections were washed two times with 100% ethanol (EtOH) (30 s) to remove the remaining 1,5-DAN matrix molecules on the tissue surface. Lipids and salts on the tissue surface were washed away in sequential washes of 70% EtOH (15 s), Carnoy’s fluid (6:3:1 EtOH/chloroform/acetone acid) (30s), 100% EtOH (15 s), H2O with 0.2% trifluoroacetic acid (TFA) (15 s), and 100% EtOH (15 s). MALDI matrix compound in 60% ACN/2% TFA solution was applied using a spraying system consisting of a TM Sprayer (HTX Technologies, Carrboro, NC) combined with a HPLC pump (Shimadzu LC-10ADVP, Kyoto, Japan) with optimized spraying parameters as previously described.

**MALDI Imaging Mass Spectrometry.** Imaging MS analysis of tissue sections was performed on a MALDI-TOF/TOF UltraflexTreme mass spectrometer equipped with a SmartBeam II Nd:YAG/355 nm laser operating at 1 kHz in TOF/TOF mode and at 2 kHz in TOF mode (Bruker Daltonics, Bremen, Germany). MALDI-IMS analysis of lipids in dual polarity (at 30 μm per pixel spatial resolution) on the pixel points were performed using a few number of laser shots (first 10 for negative and then 30 for positive ion modes) using optimum laser power for better ion yields and minimum matrix cluster detection as previously described in detail elsewhere. Data acquisitions for lipids were performed in reflective ion mode over a mass range 300–2000 Da with a source accelerating...
voltage of +25 kV in positive and −20 kV in negative polarities. External calibration was carried out using peptide calibration standard I (Bruker Daltonics). Image data were reconstructed and visualized (without normalization) using Flex Imaging v3.0 (Bruker Daltonics). For peptide/protein analysis on the same imaging region of the same tissue section, the multimodal MALDI-IMS MALDI-IMS (IMS3) approach was used as previously described.\textsuperscript{30} Protein analysis (at 30 μm per pixel spatial resolution) was performed in linear positive ion mode in a mass range 2000–20000 Da. Approximately 30 laser shots/

Figure 1. Infarct-associated alterations of phospholipids revealed by dual polarity (red and green for negative and positive polarities, respectively) MALDI mass spectrometry imaging (at 30 μm per pixel spatial resolution) of coronal tissue section of mouse heart with myocardial infarction. (A) Brightfield optical image of the analyzed tissue section to reveal the tissue morphology and infarcted region in yellow dashed line area. Ion images of phosphatidylcholines (PC) in positive ion mode (green) reveal accumulations of (B) PC(32:0) (m/z 734.6), (C) PC(34:2) (m/z 758.5), (F) PC(32:0)+Na (m/z 756.6), and (G) PC(34:2)+Na (m/z 780.5) and depletions of (H) PC(38:6)+K (m/z 844.6), (I) PC(38:4)+K (m/z 848.5), and (J) PC(40:6)+K (m/z 872.5) in the infarcted region, while ion images of (D) PC(34:1) (m/z 760.6) and (E) PC(36:4) (m/z 782.5) do not reflect any infarct-associated changes. Ion images of phosphatidylinositol (PI), phosphatidylethanolamines (PE), phosphatidylserines (PS), and phosphatidic acid (PA) in negative ion mode (red) reveal depletions of (K) PI(36:2) (m/z 861.5), (L) PI(38:4) (m/z 885.5), (M) PI(40:6) (m/z 909.5), (N) PS(36:1) (m/z 788.5), and (O) PS(40:6) (m/z 834.5) species and accumulations of (P) PA (34:2) (m/z 671.4), (Q) PA(34:1) (m/z 673.4), (R) PA(36:4) (m/z 695.4), (S) PA(36:3) (m/z 697.4), (T) PA(36:2) (m/z 699.4), (U) PE(36:2p) (m/z 726.5), (V) PE(36:1p) (m/z 728.5), and (W) PE(36:2) (m/z 742.5), while ion images of (X) PE(38:5) (m/z 764.5) and (Y) PE(38:4) (m/z 766.5) do not reveal any infarct-associated changes. Scale bar in panel A is 1000 μm.
raster spot were fired with a 1 kHz repetition rate. Identification of lipids on their spatial locations was performed by examining MS/MS spectra obtained in LID-LIFT-TOF/TOF mode by comparing the diagnostic fragment ions from the LIPID MAPS database (Nature Lipidomics Gateway, www.lipidmaps.org). Identification of histone proteins was previously performed on the border regions of the infarct region of mouse heart with myocardial infarction by Lefcoski et al. Therefore, we annotated core molecular ions of histone proteins H3, H2B, and H2A and molecular ions of acetylated H3 and H2B proteins on the basis of this previously published report.

RESULTS AND DISCUSSION

Accumulated lipids in the infarcted region on mouse heart tissue, where an infarction had been surgically induced by permanent ligation of the left coronary artery, can be determined with classical staining techniques such as oil red O and triphenyltetrazolium chloride (TTC) for staining accumulated neutral lipids or tissue necrosis, respectively. However, these techniques do not provide specific molecular information on an infarcted region, which is required for the investigation of molecular pathology of myocardial infarction.

Therefore, the semiquantitative feature of MALDI mass spectrometry imaging of lipid species can provide infarct-associated alterations of molecular species across the myocardial tissue, which can provide both identification of the infarct region and specific molecular information.

For the inspection of the spatial alterations of lipid species in the infarcted region of the myocardial infarction induced mouse heart tissue section, MALDI mass spectrometry imaging has been previously applied. For example, Menger et al. performed MALDI-IMS in positive polarity and reported depletion of phosphatidylcholines and elevation of lysophosphatidycholines in the infarction region of the tissue sections obtained from mouse heart with acute myocardial infarction. Nevertheless, while the lipid species are abundant components of tissue sections and several of them efficiently ionized with MALDI, the structural variance of lipid molecules limits the number of molecules detected and imaged within a single IMS analysis. Therefore, a repeat MALDI-IMS of lipids in dual polarity of the same tissue sections can provide enhanced molecular coverage of the same tissue section with deeper insights into the lipid biochemistry of histopathological features of the disease. Hence, matrix molecules assisting laser desorption ionization of lipids in dual polarity such as 2,5-DHB, 2,6-DHA, norhamane, and 1,5-DAN are recommended for MALDI-IMS.

It was previously demonstrated that 1,5-DAN is a particularly efficient MALDI matrix for the ionization of lipids in dual polarity compared to several other commercially available matrix molecules. Further, 1,5-DAN requires relatively lower laser power with few laser shots for optimum ionization of lipids.
which facilitates dual polarity MALDI-IMS of lipids on the same pixel points. Therefore, we performed dual polarity MALDI-IMS of lipids for probing infarct-associated lipid changes in mouse myocardial infarction (see the dual polarity lipid spectra in Figure S1). This revealed alterations of acylcarnitines and several species of phospholipids, lysophospholipids, sphingolipids, and cardiolipins in the infarction region of the mouse heart with acute myocardial infarction. The advantage of this methodology is that alterations of the lipid species detected in negative polarity such as phosphatidylethanolamines (PEs), phosphatidic acids (PAs), phosphatidylinositol (PIs), phosphatidylserines (PSs), and cardiolipins (CLs) and the lipid species detected in positive polarity such as phosphatidylcholines (PCs) and sphingomyelins (SMs) can be correlated with the very same infarct region in a single mouse heart tissue section, which allows for associative interpretation of lipid species detected in dual polarity.

Inspection of ion images revealed alterations of several phospholipids in the infarcted region (Figure 1). Protonated and sodium adduct ion images of phosphatidylethanolamines such as PC(32:0) and PC(34:2) reveal their accumulations in the infarct region, whereas \([M+H]^+\) ion images of PC (34:1) and PC (36:4) do not reveal any clear infarct-associated alteration. On the contrary, potassium adduct ion images of phosphatidylethanolamines such as PC(38:6), PC(38:4), and PC(40:6) reveal their clear depletions in the infarct region. Infarct-associated accumulation of sodium adduct ion species and depletion of potassium adduct ion species of the same PCs was also observed (Figure S2). Phosphatidylinositol and phosphatidylinositol deplete in the infarct region as revealed by the ion images of PI(36:2), PI(38:4), PI(40:6), PS(36:1), and PS(40:6) species. Further, ion images of phosphatidic acids including PA(34:2), PA(36:4), PA(36:3), and PA(36:2) reveal their clear accumulation in the infarct region. Ion images of plasmalogen phosphatidylethanolamines PEP(36:2) and PEP(36:1) reveal their accumulations on the border of the infarct region, while ion images of PE(36:2) reveal their clear accumulation in the infarct region. Ion images of PEs such as PE(38:5) and PE(38:4) reveal no clear infarct-associated alterations of these species.

Along with the alterations of phospholipids, we observed accentuated accumulations of several lysophospholipids including lysophosphatidic acid (LPA), lysophosphatidylserine (LPS), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), and lysophosphatidylcholines (LPC) along with the acylcarnitines (CAR) in the infarcted region (Figure 2).

Ion images of cardiolipins (CL) reveal similar alterations to infarct-associated alterations of the PC species. Pseudomolecular, \([M−H]−\), and potassium adduct ion \([M+K−2H]−\) images of CL(72:8) reveal their clear depletions in the infarct region, whereas sodium adduct ion signal of CL(72:8−18:2) is elevated in the infarct region. However, the sodium adduct ion of CL(72:8) appeared relatively evenly distributed across the tissue (Figure 3).

Myocardial damage in MI is mainly due to ischemic necrosis, oxidative stress, and inflammatory mechanisms. During acute myocardial ischemia, the lack of oxygen switches the cell metabolism to anaerobic respiration, with lactate accumulation, ATP depletion, and Na+ and Ca2+ overload. Excess intracellular Ca2+ can trigger the activation of several phospholipases (PL) including phospholipase A2 (PLA2), phospholipase C (PLC), and phospholipase D (PLD), which can result in accentuated alterations of phospholipids within the infarcted heart region and stimulate various cascade toxic reactions within the cells. Additionally, due to the toxicity of the intracellular Ca2+ overload, the cell tries to activate the sodium calcium exchange (NCX) in cardiac muscle to stimulate Ca2+ efflux to sarcoplasmic reticulum, which results in depolarization of membrane potential and hinders Ca2+ efflux. The NCX efflux is also observed during coronary occlusion during acute ischemia, which was suggested to be a result of three mechanisms, namely, inhibition of the Na+/K+ pump (INaK), activation of the IK(ATP), and activation of an inward Na+ current.
this, we observed accumulations of Na adduct ions (Figures 1F,G and 3B) and depletions of K adduct ions (Figures 1H–J and 3E) of phosphatidylecholines and cardiolipins in the infarct region of mouse heart with myocardial infarction. Accentuated accumulations of lysophospholipids (Figure 3) in the infarct region can be explained by PLA2 activity, which hydrolyzes phospholipids to yield fatty acids and lysophospholipids, and this can further trigger inflammatory responses. This is in line with the general depletion of phospholipids (Figure 1). Further, depletions of PIs can be also a result of PLC activity, which can suggest perturbed phosphoinositide metabolism and second messenger dependent cellular mechanisms. Additionally, accumulation of PAs in the infarct region can be a result of PLD activity, which was found to be stimulated during MI.

Along with the alterations of phospholipids, accumulations of sphingolipids including ceramides (Cers), ceramide phosphates (CerPs), and sphingomyelins (SMs) were observed in the infarct region (Figure 4). Accumulation of lipids in heart tissue results in a cellular dysfunction called lipotoxicity. Lipotoxicity is believed to be a contributing cause for impairment of heart function by instigating cardiac cell death. Toxic effects of ceramides have also been proven by using a mouse model of cardiomyocyte death where a reduction of ceramide levels, by inducing ceramidase, was shown to have beneficial effects.

Ceramides are produced by either de novo synthesis or hydrolysis of SM catalyzed by acid and/or neutral sphingomyelin synthase (SMS). Accumulation of cardiac ceramides in the post-ischemic heart is mediated mainly by acid SMS and not by de novo sphingolipid synthesis, and targeting acid SMS has been shown to reduce ceramide accumulation. On the contrary, CerP, the phosphorylation product of ceramides, has opposite effects to ceramide by acting as an intracellular second messenger to promote cell survival. Therefore, accumulations of Cers (Figure 4E) in the infarct region might be associated with cardiac cell death, while the accumulations of CerPs (Figure 4B,C) on the periphery of the infarct regions can suggest a resistance mechanism against pathways leading to cell death. Further, accumulation of SMs on the periphery of the infarct region can be correlated with inflammatory cytokines and indeed can induce inflammatory responses in the ischemic region in MI.

During the ischemia, the oxidative metabolism of fatty acids is impaired. Acylcarnitines are the intermediate esters involved in the transport of fatty acids into mitochondria for β-oxidation mediated production of energy. Hence, accumulations of acylcarnitines on the borders of the infarct region (Figure 2N,O) are likely a result of impaired oxidative metabolism of fatty acids during the ischemia in myocardial infarction.

Using the multimodal MALDI-IMS approach, protein imaging on the same tissue section, following dual polarity lipid analysis, could be performed and was used to image histones that show changes in signal between the infarcted, border, and noninfarcted tissue regions. While the positive and negative ion data can be directly correlated with each other as the data were acquired in the same image sequence in on the same pixel points, precise protein correlation is complicated as the tissue has to be removed from the instrument, washed, and subjected to fresh matrix application. As a result, there are small changes in tissue morphology as can be observed in Figure 5, but these changes did not affect the ability to identify the infarcted, noninfarcted, and border regions in the tissue.

Histones are part of the chromatin and function by packing the DNA into structural units. MALDI imaging has successfully been used for identifying modifications of histones in situ in mouse brain tissue, as well as studying acetylation of histones in cancer tumor tissue. Modification of the histone proteins, e.g., acetylation, deacetylation, and methylation, determines how strong the histones interact with DNA and determines which genes are on/off in a specific cell. Enzymes inhibiting the deacetylation have been shown to influence the progression of myocardial infarction; hence, imaging of the histones and their modification could be an important tool for investigating progression of the disease process as well as evaluating different treatment methods. It has been demonstrated that ischemia induces histone deacetylase (HDAC) activity in the heart with...
deacetylation of histones in vitro and in vivo, and this promotes infarct area expansion. Figure 5 shows the accumulation of histones on the border of the infarct region compared with previously discussed lipid classes.

Interestingly, when taking a closer look at peaks for the unmodified histones and the acetylated forms in various part of the tissue, the ratio between these peaks changes depending on closeness to hypoxic tissue the spectrum is taken from (see Figure 5. Multimodal MALDI mass spectrometry imaging (at 30 μm per pixel spatial resolution) of the same coronal tissue section of mouse heart with myocardial infarction reveals infarct-associated alterations of lipid ions in negative polarity (red) and positive polarity (green) along with the core histone proteins (blue). (A) Brightfield optical image of the analyzed tissue section reveals the tissue morphology and infarcted region in yellow dashed line area. Dual polarity MALDI-IMS revealed infarct-associated alterations of (B) LPA (16:0) (m/z 409.3), (C) SM (34:1-NH₂) (m/z 687.6), (D) PA (34:2) (m/z 671.4), (E) PI (38:4) (m/z 885.5), (F) CL (72:8−18:2+Na) (m/z 1207.8), (G) LPC (16:0) (m/z 496.3), (H) LPC(18:0) (m/z 524.3), (I) PC (34:2)+Na (m/z 780.5), and (J) PC (38:6)+K (m/z 844.6). MALDI-IMS of proteins revealed infarct-associated accumulations of histone proteins (K) histone H4 (m/z 11307), (L) H4 Ac (m/z 11349), (M) H2B (m/z 13775), (N) H2B (13806), (O) H2A (m/z 14007), and (P) H2A Ac (m/z 14047). Scale bar in panel A is 1000 μm.
spectral overlay in the Supporting Information Figure S3). In the spectra from the infarct and border region, the acetylated form is higher, while in the spectrum from the noninfarcted region the unmodified histone peaks are slightly higher. This could indicate that chromatin remodelling is occurring during the progression of the infarction in the tissue, hence, changing gene expression in affected tissue.

**CONCLUSION**

The presented data highlight the potential of multimodal MALDI imaging mass spectrometry of the same tissue sections as a powerful approach for simultaneous investigation of spatial infarct-associated lipid and protein changes of myocardial infarction. The data reveals spatial alterations of various metabolites, lipids, and proteins within the same infarct region, which thus allows for interrelated interpretation of a comprehensive molecular pathology of myocardial infarction. Of particular note is the ability to colocalize positive and negative lipid species to the border of the infarcted tissue area as well as changes in the acylation of histones to the same region. The combination of all of these chemical profiles that represent effects of hypoxia and provide signs of lipotoxicity and of gene activation can be used to provide a better understanding of how the heart is responding to the MI and, in future, how potential treatments or metabolic conditions affect the molecular pathophysiology of the tissue.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.0c00245.

Figures of representative multimodal MALDI-MS spectra, brightfield optical and ion images, and protein spectra of MALDI analysis (PDF)

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**Author Contributions**

The manuscript was written through contributions of all authors. I.K., S.S., J.S.F., and J.B. conceived and designed the study. I.K. conceived the application of IMS3, designed and performed the experiments, analyzed and interpreted the data, and wrote the paper. S.S. analyzed the data and interpreted it and wrote part of the manuscript. J.S.F. and J.B. provided the samples and resources and interpreted the final data. J.S.F., S.S., and J.B. critically read the paper. All the authors approved the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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