Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Trends in mass spectrometry imaging for cardiovascular diseases

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Table S1 Overview of MSI research on cardiovascular

tissue		Species	Tissue	Sample preparation	Instrument and settings	Main results	Histology	Ref*
Lipid	MALDI	Rat	Healthy heart	Frozen Washes DHB, sublimation	Autoflex III MALDI-TOF/TOF, Bruker Daltonics Lateral resolution: 100µm Negative ionization mode	Ventricular myocardium: CL (*m/z* 1447.97)	H&E	[1]
		Rat	Healthy heart	Frozen AgNPs^{-} implantation	MALDI LTQ-XL Orbitrap, Thermo Fisher Lateral resolution: 50µm Positive ionization mode: 600-1100 Da, Negative ionization mode: 600-1700 Da	Vessels region: TAG (+) and PI (-) species Myocardium: PC (+), PE (+&-) and CL (-) species and fragments	n/a	[2]
		Mouse	Healthy heart	Frozen DHB, sublimation	MALDI TOF (homebuilt) Lateral resolution: 9.6µm Positive ionization mode	Hinge: *m/z* 600 Hinge and valve cups: *m/z* 741, 846	H&E	[3]
		Rat	LAD ligation model; heart	Frozen DHB with(out) NaOAc, nebulizer	MALDI LTQ-XL Linear ion trap, Thermo Scientific Lateral resolution: 100µm Positive ionization mode, *m/z* 100-250 and *m/z* 200-2000	Infarcted region: lysoPL Perfused region: Intact PL, creatine (*m/z* 132)	TTC	[4]
		Ovine	Healthy adult aortic valves	Frozen Washes DHB, sublimation	UltrafleXtreme MALDI TOF/TOF, Bruker Daltonics Lateral resolution:25µm Positive ionization mode, *m/z* 450-1800	Differentiate fibrosa and spongiosa: PC(12:0/16:0) *m/z* 678.5, SM(d18:1/18:0) *m/z* 731.7, PC(16:0/16:0) *m/z* 732.6, PE(18:1/20:1) *m/z* 774.7, PC(18:0/22:5) *m/z* 836.7	VVG	[5]
			Healthy pre-natal aortic valves			Amorphous valve structure		
		Human	Aortic valve – pediatric donor tissue 0 to 14 years of age – healthy or diseased	OCT – frozen Washes DHB, sublimation	Apex-9.4-Qe FTICR, Bruker Daltonics Spatial resolution: 20µm Positive ionization mode, *m/z* 450-1600	Fibrosa: SM(16:0) *m/z* 703.6 Spongiosa: SM(18:0) m/z 731.6	H&E	
		Mouse	ApoE$^{-/-}$ - high cholesterol diet – orally dosed with stable isotope labelled cholesterol Atherosclerotic aortic tissue	OCT – frozen DHB, sublimation or CHCA, acoustic droplet ejection	QTOF MALDI Synapt G2 HDMS, Waters Corporation Spatial resolution: 50µm Positive ionization mode, *m/z* 50-950	Plaque and necrotic core: Free cholesterol (*m/z* 369.3514) Heart tissue: CE (*m/z* 649.5944) Lesion arterial wall and atheroma: LPC species (*m/z* 496.3394, 524.3699, 522.3538) Homogeneous: PC (*m/z* 758.5677) → Lp-LPA2 action	H&E	[6]
		Rabbit	Atherosclerosis model - aortic section	Frozen DHB, sprayed	UltrafleXtreme MALDI-TOF, Bruker Daltonics Spatial resolution: 30µm Dual polarity, 500-1200 Da	Calcified region: PA, SM, and PE-Cer species Increased in intima: SFA, SM, PI, PF, and lysolipid species	H&E, AR ORO IHC	[7] [8]
		Mouse	ApoE$^{-/-}$, aortic sinuses	OCT DAN, sublimation or Silver sputtering	UltrafleXtreme MALDI-TOF/TOF, Bruker Daltonics Spatial resolution: DAN dual polarity, 40µm - Silver-assisted LDI, 30µm	Bulk of plaque: LPC (example: *m/z* 496.3) Plaque: Interior wall - PC species (example: *m/z* 804.5, 832.5), Cholesterol rich regions Aortic valve cusps: TAG species	ORO	[9]
		Human	Atherosclerotic carotid artery	Frozen - OCT DAN, sublimation or Silver sputtering	UltrafleXtreme MALDI-TOF/TOF, Bruker Daltonics Spatial resolution: DAN dual polarity, 100µm - Silver-assisted LDI, 100µm	Plaque: LPC species and ceramide species (*m/z* 616.4, 687.6, and 685.6)	H&E	
		Mouse	ApoE$^{-/-}$, atherosclerotic aortic roots	Paraformaldehyde, CMC DHB, airbrush	Ultraflex II MALDI TOF/TOF, Bruker Daltonics Lateral resolution: 25µm Positive ionization mode, *m/z* 400-1000	Lipid rich: CE species (*m/z* 671.6, 673.6) Smooth muscle cells: PC species (m/z 804.5, 832.5) Calcified region: *m/z* 566.9	H&E, ORO IHC	[10]
		Human	Atherosclerotic femoral artery	Frozen DHB, airbrush	Ultraflex II MALDI TOF/TOF, Bruker Daltonics Lateral resolution: 50µm Positive ionization mode, *m/z* 400-1000	Lipid rich: CE (*m/z* 671.6, 673.6) and TAG species (*m/z* 907.7) Smooth muscle cells: PC species (*m/z* 804.5, 832.5) Calcified region: *m/z* 539.0		
		Mouse	ApoE$^{-/-}$, aortic sample	Frozen	Ultraflex II MALDI TOF/TOF, Bruker Daltonics	Atherosclerotic tissue: increase LPC and decrease	H&E,	[11]

		Human	Atherosclerotic femoral artery	DHB, airbrush	Lateral resolution: 50µm Positive ionization mode, *m/z* 400-1000	arachidonyl-PC Arachidonyl-PC region: increase LPCAT3 expression	EVG IHC	
LIPID	SIIMS	Mouse	Healthy heart	OCT - frozen	LA (New wave, Fremont, US) coupled to ICPMS (quadrupole, XSeries2), Thermo Fischer Scientific Spatial resolution: 160µm TOF SIMS IV, IONTOF GmbH - Bismuth liquid metal ion gun Lateral resolution: 33µm, *m/z* up to 800	Right ventricle: higher Zn, Mn, Cu, Mg, and Ca Aorta: higher Fe Endocardium: choline and its fragment (*m/z* 104.21, 84.12), cholesterol fragment (*m/z* 146.97)	H&E	[12]
		Rat	Healthy aorta	Frozen, freeze-fracture, free-dried	TOF SIMS IV & V, IONTOF GmbH Bismuth liquid metal ion gun Ion beam focus: 300nm Dual polarity	Intima and media (between lamellae): choline headgroup (*m/z* 184); Media (lamellae region): potassium, cholesterol (*m/z* 369, 385)	Toluidine blue/azan	[13]
		Human	Atherosclerotic plaque	Frozen		Intima: cholesterol (spots), PC and SM species Media: PC and DAG species	n/a	
		Rat	Healthy heart	Frozen Metallization: gold sputtered	TOF SIMS TRIFT II, Physical Electronics, Gold liquid metal ion gun Positive ionization mode, *m/z* up to 1500	Aorta wall (*m/z* 667 and 840), Pericardium (*m/z* 334), Ventricles (*m/z* 175 and 213) Aorta wall, semilunar valve, endocardium: *m/z* 83 Pulmonary artery, right atrium, atrioventricular valve: *m/z* 145; Atria, aorta wall, atrioventricular valves, coronary artery: cholesterol (*m/z* 369, 385); Atria, aortic wall, aorta valve: *m/z* 86; Atria, aorta, pulmonary artery, atrioventricular and semilunar valves: choline (*m/z* 104)	H&E	[14]
		Mouse	Healthy heart			Mouse similar patterns as rat		
		Human	Ventricle from failing left-ventricular free-wall heart explants			Myocardium: cholesterol choline (*m/z* 369), choline (*m/z* 104); Endocardium: choline (*m/z* 104); Pericardium: DAG species (*m/z* 549, 557) and ceramide (*m/z* 604)	H&E Sirius Red Trichrome	
		Mouse	LDLr$^{-/-}$ model; Atherosclerotic carotid artery	Gelatin – Frozen	TOF-SIMS TRIFT II, Physical Electronics Gold liquid metal ion gun Dual polarity	Necrotic core: LPA species, cholesterol, phosphatidic acids, triglycerides	H&E IHC	[15]
		Rat	Healthy heart	Frozen Silicon substrate	TOF SIMS IV, IONTOF, Gold liquid metal ion gun Lateral resolution: ~3.9µm Dual polarity	Complementary distribution of PL and CL species, 16 and 18-C FA species	n/a	[16]
			Healthy left ventricular cells	Isolated cells 25µl on silicon substrate	TOF SIMS IV, IONTOF, Gold liquid metal ion gun Lateral resolution: ~430nm Positive ionization mode	Cell membrane: phospholipids and cholesterol species		
		Human	Atherosclerotic carotid artery	Frozen	TOF-SIMS IV, IONTOF, Bismuth cluster ion source Spatial resolution: 1-2µm Dual polarity, *m/z* 1-1000	Inner border: cholesterol (*m/z* 385), phosphatidic acids (*m/z* 650-750) and SM fragments (*m/z* 616, 642) Surrounding and thrombus: vitamin E (*m/z* 430) Intima: PI fragments	Masson Trichrome	[17]
LIPID	DESI	Human	Atherosclerotic carotid artery	Frozen CMC for sectioning	DESI stage (lab built) coupled to LTQ (Thermo Scientific) - Normal & Reactive DESI Spatial resolution: 200µm Positive ionization mode, *m/z* 150-1200	Plaque: SM and PC species, and cholesterol Lipid core: CE species surrounded by cholesterol, PC, and SM	H&E	[18]
		Mouse	LAD ligation model; heart	Frozen	DESI stage (custom built) LTQ-Orbitrap XL (Thermo Fisher Scientific) Lateral resolution: 0,2mm Negative ionization mode, *m/z* 50-1200	Infarcted tissue: FA(18:1) *m/z* 281.248, FA(18:0) *m/z* 283.264, FA(16:1) *m/z* 253.217, and FA(16:0) *m/z* 255.232 Perfused tissue: glycerophospholipid species, polyunsaturated FAs, and taurine (*m/z* 124.007) GBDT algorithm: 62 peaks for classification	H&E	[19]

		Human	Vascular graft	Frozen	DESI source (OMNIspray) AmaZon ETD (Bruker Daltonics) - Normal & Reactive DESI Dual polarity, *m/z* 300-1200	Graft: SM, PC species and cholesterol. Graft Wall: SM (24:1), Biofilm on graft wall: PS (38:4), Plaque: cholesterol	n/a	[20]
Peptide/protein	MALDI	Chicken	Healthy heart	Fixation - Embedding Dewaxing Washes DHB, CHCA or SA, nebulizer	Autoflex III MALDI TOF/TOF, Bruker Daltonics, Linear mode Lateral resolution: 75-100µm Positive ionization mode, *m/z* 3000-30000	Myocardium (*m/z* 9492, 11862), Myocardium right ventricle (*m/z* 6614), Atrial myocardium (*m/z* 6643) Outer ventricular wall (*m/z* 6643), Walls major heart vessels layers (*m/z* 7475, 6143, and 11862) Aortic valvular tissue *(m/z* 5308,) Aortic intimal and adventitial tissue (*m/z* 5935) Apex interventricular septum (*m/z* 6669), Bundle of His (*m/z* 9329), Valve structures (*m/z* 12209), Leaflets left AV valve (*m/z* 3915)	n/a	[21]
		Mouse	LAD ligation model; heart	Frozen ANG II	Autoflex III MALDI TOF/TOF, Bruker Daltonics	Infarct area: ANG III (*m/z* 931) and ANG-(2-7) (*m/z* 784) Increase of APA in MI tissue	TTC	[22]
		Mouse	LAD ligation model; heart	Frozen Washes Intact: SA, TM sprayer Peptide: trypsin, SA, TM sprayer	Autoflex Speed MALDI-TOF/TOF, Bruker Daltonics Spatial distribution: 100µm Positive ionization mode Intact: linear mode Peptides: reflectron mode	Healthy cardiac tissue: creatine (*m/z* 132) Remote: altered enzymatic activity Border: high transcription and translation Infarcted: mitochondrial and metabolic enzymes	H&E	[23]
			Healthy heart			Healthy cardiac tissue: *m/z* 908, 1632.96, 2816.25, and 4497.11 (Ephrin A1 peptides)		
		Mouse Human	MI heart samples	FFPE Pre-treatment buffer Trypsin, DHB, chemical inkjet printer	MALDI TOF/TOF (AXIMA Performance and 7090 series; Shimadzu) Positive ionization mode, *m/z* 700-3000	Endocardium: Haemoglobin subunit α (*m/z* 1529.997) Infarcted region: MYH6, MYL3, ATP5A, MYH7, and ACTA2 (*m/z* 1084.64, 1396.96, 1625.09, 1741.12, and 1956.24, respectively)	H&E PTAH IHC	[24]
		Rabbit	Atherosclerosis model - aortic section	Frozen Washes SA, sprayed	UltrafleXtreme MALDI-TOF, Bruker Daltonics Spatial resolution: 30µm Linear positive, 2000-20000 kDa	TMSB4X (*m/z* 4762) upregulated	IHC	[7] [8]
		Human	Heart valve with moderate CAS	Frozen Carnoy procedure SA, sprayed	Autoflex III smartbeam MALDI-TOF/TOF, Bruker Daltonics Spatial resolution: 75µm Positive ionization mode, 1000-30000Da	Calcified area (5059 Da), Collagen-rich (4300 Da), Elastic fibers-rich (13984 Da), Surrounding tissue (14659 Da), Margins calcified area: NDRG-2 (peptide, 3398 Da), Around calcified lesion: CO6A3 (peptide, 4321 Da)	VVG ORO IHC	[25]
		Human	Left atrial appendage specimens Atrial fibrillation (PX, PE, and LSP)	FFPE Dewaxing, Washes, Antigen retrieval Trypsin, CHCA, Image Prep	Autoflex III MALDI TOF/TOF, Bruker Daltonics Lateral resolution: 80µm Positive ionization mode, *m/z* 800-3500	Decreased in PX: ATPA (*m/z* 1000.489), MYL4 (*m/z* 1262.601), H13 (*m/z* 1260.618), AHNK (*m/z* 901.472), CDH13 (*m/z* 1564.840), VIM (*m/z* 1093.555) Increased in LSP and PE: CO1A1 (*m/z* 837.393)	IHC	[26]
Metabolites	MALDI	Mouse	LAD ligation model; heart 13C labelled glucose & lactate injection	FMW fixation Super cryo embedding medium 9AA, manual spray coated	UltrafleXtreme MALDI TOF, Bruker Daltonics Lateral resolution: 100µm Negative ionization mode, *m/z* 50-1000	Ischemic core: increase NADH, lactate, succinate, $^{13}C_3$-glutamate; Non-ischemic region adjacent to core: ATP, ADP; More distant region: higher lactate/pyruvate ratio	n/a	[27]
		Mouse	C3H mice - injected with CVB3 virus (VM hearts) - control	Frozen 9AA, Suncollect	QTOF MALDI SYNAPT HDMS G2Si, Waters Lateral resolution: 100µm Negative ionization mode	VM hearts vs control: decrease ATP, ADP, AMP, total adenine nucleotide, NAD Increase UDP-GlcNAc, PI, AA, cardiolipin	H&E	[28]

* Reference as listed below. Not equal to the main manuscript

Reflectron mode unless stated otherwise, +: positive ionization mode, -: negative ionization mode

AR: Alizarin Red, EVG: Elastica van Gieson, H&E: Haematoxylin and eosin, IHC: Immunohistochemistry, ORO: Oil Red O, PTAH: Phosphotungstic acid-haematoxylin, TTC: 2,3,5-triphenultetrazolium chloride, VVG: Verhoeff-Van Gieson

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