SUPPLEMENTARY MATERIAL

Extracellular vesicles released in the response to respiratory exposures - implications for chronic disease

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Table S1 – Overview of all identified articles in which the effects of CS and related exposures on EV were investigated. AMD, age-related macular degenration; B(a)P, Benzo(a)Pyrene; BALF, bronchoalveolar lavage fluid; CS, cigarette smoke; CSE, cigarette smoke extract; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; EM, electron microscopy; EV, extracellular vesicle; FC, flow cytometry; FCS, fetal calf serum; MS, mass spectrometry; NNK, Nitrosamine 4-(Methylnitrosamino)-1-(3-pyridyl)-1- butanone

Reference	type of study	Exposure	study population (in vivo)/ cell type (in vitro)	EV source	EV isolation	EV charac-terization	EV termi-nology	Inflamma-tion	Hyper-coagulability	Endothe-lial dys-function	tissue re-modelling	Investi-gated pathology
Casey et al. (2004)	human	CS	healthy male non-smokers (n=9) healthy male smokers (n=10)	platelet supernatants	no isolation	direct FC	micro-particles	n.d.	→	→	n.d.	CVD
Xu and Deng (2004)	in vitro	NNK	human lung carcinoma cells	media, 0.1% FCS	100,000 x g	functional assays	(media) vesicles	n.d.	n.d.	n.d.	↑	lung cancer
Heiss et al. (2008)	human	second hand CS	healthy non-smokers (n=10) randomized crossover	platelet-depleted plasma, citrate	no isolation	direct FC	micro-particles	n.d.	n.d.	↑	n.d.	CVD
Wang et al. (2009)	in vitro animal	B(a)P	human retinal pigment epithelial cells murine eyes (n=5)	not applicable	no isolation	exosome markers	exosomes	↑	n.d.	n.d.	n.d.	AMD
Alcazar et al. (2009)	in vitro	Hydro-quinone	human retinal pigment epithelial cells	media, 0.1% FCS	others (100 x g)	fluorescene microscopy exosome markers MS functional assays	membrane blebs	↑	n.d.	n.d.	↑	AMD
Li et al. (2010)	in vitro	CSE	human monocytes and monocyte-derived macrophages	media, FCS-free	no isolation	direct FC	micro-vesicles	n.d.	↑	n.d.	n.d.	CVD
Gordon et al. (2011)	human	CS	non-smokers (n=32/10; *discovery/validation*) smokers (n=41/20; *discovery/validation*) smokers with low DLCO (n=19/15; *discovery/validation*)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-particles	n.d.	n.d.	↑	↑	COPD
Grant et al. (2011)	human in vitro	CS nicotine	healthy volunteers (n=57) including smokers (n=?) monocytes	platelet-depleted plasma, citrate media, FCS-free	10,000 x g	direct FC EM	micro-vesicles	↓	↓	n.d.	n.d.	none
Li et al. (2013)	in vitro	CSE	human monocyte-derived macrophages	media, FCS-free	100,000 x g	direct FC functional analysis	micro-vesicles	n.d.	n.d.	n.d.	↑	CVD COPD
Takahashi et al. (2013)	in vitro	CSE	human lung microvascular endothelial cells human aortic endothelial cells	media, 5% FCS	10,000 x g	direct FC	micro-particles	n.d.	n.d.	↑	n.d.	COPD

Reference	type of study	Exposure	study population (in vivo)/ cell type (in vitro)	EV source	EV isolation	EV charac-terization	EV termi-nology	Inflamma-tion	Hyper-coagulability	Endothe-lial dys-function	tissue re-modelling	Investi-gated pathology
Cordazzo et al. (2014)	in vitro	CSE	human peripheral blood mononuclear cells	media, FCS-free	100,000 x g	direct FC functional assays	micro-particles	↑	↑	n.d.	n.d.	COPD CVD
Badrnya et al. (2014)	human	CS	healthy non-smokers (n=20) healthy smokers (n=20)	platelet-depleted plasma, citrate	10,000 x g	direct FC	micro-vesicles	↓	↓ →	→	n.d.	CVD
Mobarrez et al. (2014)	human	CS	intermittent smokers (n=12)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-particles	→	n.d.	↑	n.d.	CVD
Liu et al. (2014)	animal	CS	Wistar rats; 6 groups, n=10 per group CS-exposed vs. air-exposed for 2, 4 and 6 months	platelet-depleted plasma, citrate	no isolation	direct FC	micro-particles	n.d.	n.d.	↑	n.d.	COPD
Moon et al. (2014)	in vitro	CSE	human bronchial epithelial cells	media, 10% FCS	100,000 x g	EM exosome markers direct FC functional assays	exosomes	↑	n.d.	n.d.	↑	COPD
Bourdonnay et al. (2015)	in vitro	CS	rat alveolar macrophages	media, FCS-free (?)	10,000 x g - microparticles 100,000 x g - exosomes	direct FC fluorescence microscopy functional assays	micro-particles exosomes	↑	n.d.	n.d.	n.d.	no specific disease
Scruggs et al. (2015)	in vitro	CSE	human pulmonary microvascular endothelial cells, pulmonary artery endothelial cells, aortic endothelial cells, pulmonary artery smooth muscle cells	media, FCS-free	100,000 x g	EM direct FC	micro-particles	n.d.	n.d.	n.d.	n.d.	no specific disease
Liu et al. (2016)	in vitro	CSE	human bronchial epithelial cells	media, 10% FCS	precipitation	EM exosome markers functional assays	exosomes	n.d.	n.d.	n.d.	↑	lung cancer
Fujita et al. (2015)	in vitro	CSE	human bronchial epithelial cells	media, FCS-free	100,000 x g	EM exosome markers RNA profiling size distribution functional assays	EV/exo-somes	n.d.	n.d.	n.d.	↑	COPD
Folkesson et al. (2015)	human in vitro	CSE	intraluminal thrombi from patients with abdominal aortic aneurysm (n=21) HL60 neutrophils	media, FCS-free intraluminal thrombi	100,000 x g	direct FC	micro-vesicles	n.d.	n.d.	n.d.	↑	CVD

Reference	type of study	Exposure	study population (in vivo)/ cell type (in vitro)	EV source	EV isolation	EV charac-terization	EV termi-nology	Inflamma-tion	Hyper-coagulability	Endothe-lial dys-function	tissue re-modelling	Investi-gated pathology
Chen et al. (2016)	in vitro	CSE	monocyte-derived macrophages	media, FCS-free	100,000 x g	fluorescence microscopy direct FC	micro-vesicles	↑	n.d.	n.d.	n.d.	no specific disease
Sheller et al. (2016)	in vitro	CSE	human amnion epithelial cells	media, 10% EV-depleted FCS	100,000 x g	EM size distribution exosome markers	exosomes	↑	n.d.	n.d.	n.d.	preterm parturition
Baek et al. (2016)	human	CS	healthy volunteers (n=161) including smokers (n=51)	platelet-depleted plasma, EDTA	no isolation	exosome markers	EV/exo-somes	n.d.	n.d.	n.d.	n.d.	no specific disease
Strulovici-Barel et al. (2016)	human	CS	healthy non-smokers (n=28), healthy smokers (n=61) and smoking COPD patients (n=49) smoking cessation after baseline visit (≥12 months): healthy ex-smokers (n=17), COPD ex-smokers (n=18)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-particles	n.d.	n.d.	↑	↑	COPD
Serban et al. (2016)	human animal in vitro	CS, CSE	human pulmonary miscrovascular and artery endothelial cells non-smokers without COPD (n=8) non-smoking COPD patients (n=9) smoking COPD patients (n=8)	platelet-depleted plasma, citrate media, 2% FCS	100,000 x g	direct FC EM fluorescence microscopy size distribution functional assays	micro-particles	↑	↑	n.d.	n.d.	COPD
Antoniewicz et al. (2016)	human	E-cigarette vapor	healthy intermittent smokers (n=16) randomized crossover (E cigarette vapor/room air)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-vesicles	→	n.d.	↑	n.d.	CVD
Heliot et al. (2017)	human in vitro	CS	healthy non-smokers (n=10) healthy smokers (n=10)	BALF	100,000 x g	size distribution EM exosome markers RNA profiling	EV	↑	n.d.	n.d.	n.d.	lung cancer COPD
Benedikter et al. (2017b)	in vitro	CSE, acrolein, H_2O_2	bronchial epithelial cells	media, 0.1% EV-depleted FCS	100,000 x g	exosome markers EM size distribution	EV/exo-somes	n.d.	n.d.	n.d.	n.d.	no specific disease
Enjeti et al. (2017)	human	CS	healthy volunteers (n=143) including smokers (n=21)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-vesicles	n.d.	↑ ↓	n.d.	n.d.	CVD
Benedikter et al. (2017a)	in vitro	CSE	bronchial epithelial cells	media, 0.1% EV-depleted FCS	100,000 x g / others (SEC)	exosome markers EM MS size distribution functional assays	EV	→	n.d.	n.d.	n.d.	no specific disease

Table S2 - Overview of all identified articles in which the effects of PM and related exposures on EV were investigated. BALF, bronchoalveolar lavage fluid; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; EDTA, ethylenediaminetetraacetic acid; EM, electron microscopy; EV, extracellular vesicles; FC, flow cytometry; FCS, fetal calf serum; MION, magnetic iron oxide nanoparticles; OVA, ovalbumin; PBS, phosphate buffered saline; PM, particulate matter

Reference	type of study	Exposure	study population (in vivo)/ cell type (in vitro)	EV source	EV isolation	EV characterization	EV terminology	inflam-mation	hypercoa-gulability	endothelial dysfunction	tissue remodelling	Investi–gated pathology
Stewart et al. (2010)	human	PM	Type 2 diabetics (n=19)	whole blood, citrate	no isolation	direct FC	micro-particles	n.d.	→	→	n.d.	CVD
Emmerechts et al. (2012b)	human	PM	type 1 or 2 diabetics (n=233)	platelet-depleted plasma, citrate	no isolation	direct FC	micro-vesicles	→	↓↑	n.d.	n.d.	CVD
Zhu et al. (2012)	animal in vitro	MION	BALB/c mice; 6 groups; n=8 per group OVA sensitized vs. Unsensitized intratracheal challenge: OVA vs. Low MION vs. High MION	BALF	100,000 x g	EM exosome markers functional assays	exosomes	↑	n.d.	n.d.	n.d.	occupa–tional asthma
Emmerechts et al. (2012a)	animal	traffic emissions	C57Bl6/j mice; 6 groups; n=7-8 per group young (10 weeks) vs. old (20 months) clean air vs. 0.4 µm-filtered tunnel air vs. unfiltered tunnel air	platelet-depleted plasma, citrate	no isolation	direct FC	micro-vesicles	→	↑	n.d.	n.d.	CVD
Frampton et al. (2012)	human	PM, ozone, carbon monoxide	Type 2 diabetics (n=19)	whole blood, citrate	no isolation	direct FC	micro-particles	n.d.	↑	n.d.	n.d.	CVD
Levanen et al. (2013)	human	PM	healthy controls (n=10) indivduals with mild asthma (n=10)	BALF	100,000 x g	RNA profiling exosome markers	exosomes	→	n.d.	n.d.	n.d.	asthma
Xu et al. (2013)	animal	carbon monoxide	C57BL/6J mice; breathed different CO concentrations for 60 min	whole blood, heparin	100,000 x g	direct FC functional assays	micro-particles	↑	↑	↑	n.d.	Neurologi–cal damage
Bollati et al. (2015)	human	PM	healthy male steel production workers (n=55)	media, 10% FCS platelet-depleted plasma, EDTA	100,000 x g	RNA profiling	micro-vesicles	n.d.	n.d.	n.d.	n.d.	CVD
Frampton et al. (2015)	human	ozone	GSTM1-WT never-smokers (n=12) GSTM1-null never-smokers (n=12) 3x3 crossover trial: filtered air vs. 100 ppb vs. 200 ppb ozone for 3 h	whole blood, citrate	no isolation	direct FC	micro-particles	n.d.	→	n.d.	n.d.	CVD lung function
Rodosthenous et al. (2016)	human	PM	elderly men (n=22)	platelet-depleted plasma, EDTA	100,000 x g	EM exosome markers RNA profiling	EV	↑	n.d.	n.d.	n.d.	CVD
Brostrom et al. (2015)	in vitro	toluene diiso-cyanate	alveolar epithelial cells bronchial epithelial cells employees from 9 isocyanate companies (n=118)	not applicable	no isolation	fluorescence microscopy	micro-vesicles	↑	n.d.	n.d.	n.d.	occupa–tional asthma

Reference	type of study	Exposure	study population (in vivo)/ cell type (in vitro)	EV source	EV isolation	EV characterization	EV terminology	inflam-mation	hypercoa-gulability	endothelia l dysfunctio n	tissue remodellin g	Investi–gated pathology
Liu et al. (2015)	in vitro	PM	human monocyte-derived macrophages	media, FCS-free	precipitation	functional assays	EV	n.d.	n.d.	n.d.	n.d.	Neurologi-cal damage
Neri et al. (2016)	in vitro	PM	peripheral blood mononuclear cells human umbilical vein endothelial cells	media, 5-10% FCS	100,000 x g	size distribution functional assays	micro-particles	↑	↑	n.d.	n.d.	CVD
Pavanello et al. (2016)	human	PM	healthy male steel production workers (n=55)	platelet-depleted plasma, EDTA	100,000 x g	RNA profiling	EV	↑	↑	n.d.	n.d.	CVD neurologi–cal disease
Pope et al. (2016)	human	PM	healthy young non-smokers (n=72)	platelet-depleted plasma, citrate	10,000 x g	direct FC	micro-particles	↑	n.d.	↑	n.d.	CVD
Lim et al. (2016)	in vitro	toluene, xylene, ethyl-benzene	HL-60 promyeloblast cells	media, 10% EV-depleted FCS	precipitation	EM exosome markers RNA profiling	exosomes	↑	n.d.	n.d.	n.d.	sick building sydrome cancer
Lim et al. (2017)	in vitro	toluene	HL60 promyeloblast cells	media, 10 % EV-depleted FCS	precipitation	EM exosome markers RNA profiling	exosomes	n.d.	n.d.	n.d.	n.d.	no specific disease
Bonzini et al. (2017)	human	PM	healhty volunteers, normal weight (n=26) healthy volunteers, overweight (n=24)	platelet-depleted plasma, EDTA	100,000 x g	size distribution direct FC	EV	n.d.	n.d.	↑	n.d.	CVD
Pergoli et al. (2017)	human	PM	overweight and obese subjects (n=1630) discovery cohort n=883/validation cohort n=747	platelet-depleted plasma, EDTA	100,000 x g	direct FC size distribution RNA profiling	EV	↑	↑	↑	n.d.	CVD
Thom et al. (2017)	in vitro	carbon dioxide	primary human and murine neutrophils and monocytes	platelet-depleted plasma, heparin PBS or media, FCS-free	100,000 x g	direct FC functional assays	micro-particles	↑	n.d.	n.d.	n.d.	no specific disease

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