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Comparison of the effect of chemically and biologically induced inflammation on the volatile metabolite production of lung epithelial cells by GC×GC-TOFMS

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MATERIALS AND METHODS

Quality control procedure

Prior to the analysis of volatile organic compounds (VOCs) released by the cells *in-vitro*, the repeatability and the stability of the analytical method were evaluated. This quality control procedure consisted in the injection of the Grob mixture every batch of 20 samples in order to monitor analytical variations and correct bias if required. The Grob mixture (containing 2,3-Butanediol (CAS#: 6982-25-8), Decane (CAS#: 124-18-5), 1-Octanol (CAS#: 111-87-5), Undecane (CAS#: 1120-21-4), Nonanal (CAS#: 124-19-6), 2,6-Dimethylphenol (CAS#: 576-26-1), 2,6-Dimethylaniline (CAS#: 87-62-7), Methyl decanoate (CAS#: 110-42-9), Methyl undecanoate (CAS#: 1731-86-8), Methyl dodecanoate (CAS#: 111-82-0), Dicyclohexylamine (CAS#: 103-83-7)) (Supelco, Bellefonte, PA, USA) injections covered the entire period of samples analysis. Raw peak areas were monitored and relative standard deviations (RSD) below 10 % were obtained for all the standards in the Grob Mix, except for dicyclohexylamine (12 %RSD) and methyl laureate (11.1 %RSD) (Table E1).

This procedure reveals the high stability of the GC×GC system used. This latter insures that the observed *in-vitro* metabolic changes are not due to analytical variations, increasing the confidence in the obtained results. In addition, the stability of the instrumentation over the time of the experiments enables to perform minimal data pre-processing (*i.e.*, auto-scale) prior to further statistical analysis, preserving the integrity of the data.

Table E1. Grob mixture compounds used for the evaluation of the stability and repeatability of the SPME-GC×GC-TOFMS system used in this study.

ID#	Compounds	Chemical formula	CAS#	^1t_R [min]	^2t_R [s]	m/z	RSD [%]
1	2,3-Butanediol	$C_4H_{10}O_2$	6982-25-8	4.83	1.949	45	5.6
2	Decane	$C_{10}H_{22}$	124-18-5	9.832	1.289	57	6.0
3	1-Octanol	$C_8H_{18}O$	111-87-5	11.299	1.671	56	6.6
4	Undecane	$C_{11}H_{24}$	1120-21-4	11.899	1.304	57	6.4
5	Nonanal	$C_9H_{18}O$	124-19-6	11.999	1.728	41	6.1
6	2,6-Dimethylphenol	$C_8H_{10}O$	576-26-1	12.099	0.253	107	6.6
7	2,6-Dimethylaniline	$C_8H_{11}N$	87-62-7	13.299	0.52	106	6.3
8	Methyl caprate	$C_{11}H_{22}O_2$	110-42-9	16.032	1.663	74	7.2
9	Methyl undecanoate	$C_{12}H_{24}O_2$	1731-86-8	17.698	1.663	74	9.6
10	Dicyclohexylamine	$C_{12}H_{23}N$	101-83-7	17.732	1.863	138	12.3
11	Methyl laurate	$C_{13}H_{26}O_2$	111-82-0	19.232	1.677	74	11.1

Sample analysis

Volatile metabolites were extracted using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber from Supelco (Bellefonte, PA, USA). The samples were incubated 10 min at 40 °C prior to the fiber exposure and analytes extraction for 30 min at 40 °C. The fiber was desorbed into the GC injector for 3 min at 250 °C in split mode (1:10). Regarding the analysis, the column set used consisted in a non-polar Rxi-5SilMS (30 m × 0.25 mm id × 0.25 µum d_f) connected to a mid-polar Rxi-17SilMS (2 m × 0.25 mm id × 0.25 µm d_f) (Restek Corporation, Bellefonte, PA, USA). Helium was used as carrier gas, at a flow rate of 1.4 mL/min. The primary oven temperature program was 40 °C (hold 1 min) ramped to 200 °C at a rate of 5 °C/min. A fast temperature ramp of 15 °C /min to 270 °C assured the system cleaning for the successive analysis. The secondary oven offset was +5 °C and the modulator offset was

+20 °C from the primary oven. A modulation period of 2.5 s was used. A mass range of 40 to 500 *m/z* was collected at a rate of 200 spectra/sec. The ion source was maintained at 250 °C. Data acquisition was performed using ChromaTOF software version 5.32 (LECO Corporation).

RESULTS

Volatile fingerprint of A549 cells

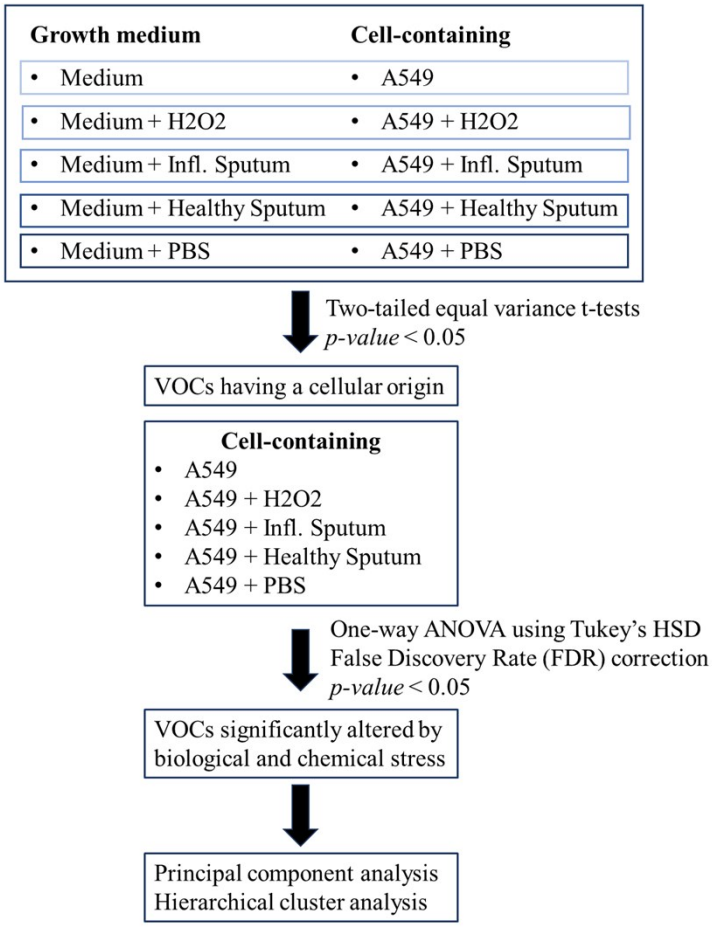

Figure E1. Statistical workflow followed to analyze and identify specific VOCs to the chemical and biological stress.

Effect of H_2O_2 on endogenous VOCs

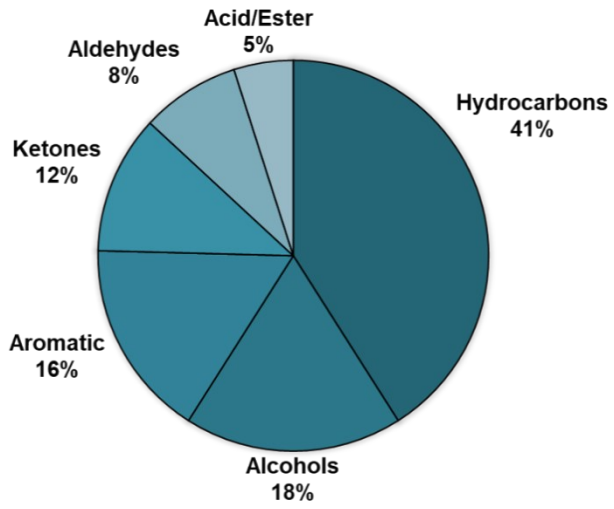

Figure E2. Affected chemical classes after the 0.1 mM H_2O_2 challenge on A549 volatile emissions.

Effect of inflammatory sputum supernatant on cell viability

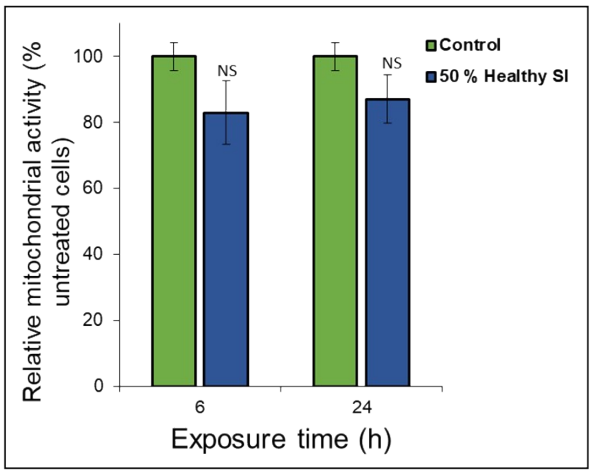

Figure E3. Mitochondrial activity of A549 cells treated with 50% (*v/v*) of healthy sputum supernatant; (NS) non-significant. (Control: mitochondrial activity of non-treated A549 cells).

Exposure of A549 with sputum increased the volatile production

Table E2. Tentative identification of the 97 altered VOCs following the chemical (H_2O_2) and the biological (inflammatory sputum, SI) challenge.

VOC#	Putative Name	Formula	Exp. LRI	Lib. LRI	Match Factor	MSI level	VOC alteration
1	Alkene	/	996	/	/	3	↓SI
2	2,4-Dimethyl-1-heptene	C_9H_{18}	840	842	873	2	↓SI
3	1-Nonene	C_9H_{18}	853	880	789	2	↓SI
4	1-Heptene, 4-methyl-	C_8H_{16}	761	755	880	2	↑H_2O_2
5	Octanal	$C_8H_{16}O$	1024	1004	801	2	↑SI, ↑H_2O_2
6	Cyclohexanol	$C_6H_{12}O$	890	869	800	2	↑H_2O_2
7	Diisopentyl oxalate	/	972	NR*	751	3	↑SI, ↑H_2O_2
8	Glutaric acid, butyl isobutyl ester	$C_{13}H_{24}O_4$	1657	1647	871	2	↑SI, ↑H_2O_2
9	Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	1554	1587	751	3	↑SI, ↑H_2O_2
10	Decanal	$C_{10}H_{20}O$	1206	1200	806	2	↑SI, ↑H_2O_2
11	1-Hexanol, 2-ethyl	$C_8H_{18}O$	1029	1029	894	2	↑H_2O_2
12	8-Hydroxy-2-octanone	$C_8H_{16}O_2$	930	NR*	793	3	↑SI, ↑H_2O_2
13	2-Butoxyethanol	$C_6H_{14}O_2$	910	893	764	2	↑SI, ↑H_2O_2
14	1,7-Octanediol, 3,7-dimethyl-	$C_{10}H_{22}O_2$	983	NR*	750	3	↑H_2O_2
15	2-Heptanone, 4-methyl-	$C_8H_{16}O$	939	936	758	2	↑H_2O_2
16	Hexene, 4-ethyl	C_8H_{16}	751	NR*	751	3	↑H_2O_2
17	Alkane	/	902	/	/	2	↑H_2O_2
18	Decane	$C_{10}H_{22}$	991	1000	853	2	↑SI, ↑H_2O_2
19	Terpene	$C_{10}H_{16}$	935	/	/	3	↑H_2O_2
20	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	1208	1181	859	2	↑H_2O_2
21	Hexanal	$C_6H_{12}O$	800	801	858	2	↑SI, ↑H_2O_2
22	Ester	/	957	/	/	3	↑SI, ↑H_2O_2
23	Hexane, 3-ethyl-	C_8H_{18}	804	775	767	2	↑SI, ↓H_2O_2
24	5-Hepten-2-one, 6-methyl	$C_8H_{14}O$	980	988	700	3	↑SI, ↓H_2O_2
25	Tetradecane	$C_{14}H_{30}$	1400	1400	824	2	↑SI, ↓H_2O_2
26	Alkane	/	970	/	/	3	↑H_2O_2
27	1-Hexanol	$C_6H_{14}O$	868	872	876	2	↑SI
28	2-Hexanone	$C_6H_{12}O$	796	790	823	2	↑SI
29	Benzaldehyde, 4-ethyl-	$C_9H_{10}O$	1164	1180	859	2	↑SI
30	Hydrazide derivatives	/	957	/	/	3	↑SI
31	Alkane	/	1481	/	/	3	↑SI

32	Decane, 2,3,7-trimethyl	$C_{13}H_{28}$	1463	1466	801	2	↑SI, ↑H_2O_2
33	2(5H)-Furanone	$C_4H_4O_2$	914	916	739	3	↑SI
34	2-Hexyl-1-octanol	$C_{14}H_{30}O$	1554	NR*	844	3	↑SI
35	Alcohol	/	1525	/	/	3	↑SI
36	1-Decanol	$C_{10}H_{22}O$	1258	1259	762	2	↑SI
37	Alkane	/	1264	/	/	3	↑SI
38	Alkane	/	1354	/	/	3	↑SI
39	Alkane	/	1252	/	/	3	↑SI
40	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	1007	1019	984	2	↑SI
41	Alkane	$C_{14}H_{30}$	1275	/	/	3	↑SI
42	1-Nonanol	$C_9H_{20}O$	1133	1151	773	2	↑SI, ↓H_2O_2
43	Nonanal	$C_9H_{18}O$	1120	1002	854	2	↑SI, ↓H_2O_2
44	Alkane	/	1058	/	/	3	↑SI
45	Alkane	/	1064	/	/	3	↑SI
46	Tridecene	$C_{13}H_{26}$	1307	1295	818	2	↑SI
47	Alcohol	/	1324	/	/	3	↑SI
48	Alcohol	/	1314	/	/	3	↑SI
49	Alkane	/	1337	/	/	3	↑SI
50	Alkane	/	1246	/	/	3	↑SI
51	Alkane	/	1281	/	/	3	↑SI
52	Ester	/	1381	/	/	3	↑SI
53	2,3-dimethyldecane	$C_{12}H_{26}$	1103	1122	827	2	↑SI, ↑H_2O_2
54	Alkane	/	1346	/	/	3	↑SI
55	Sulfur containing	/	1089	/	/	3	↑SI
56	Alkane	/	1237	/	/	3	↑SI
57	Undecane	$C_{11}H_{24}$	1096	1100	750	2	↑SI
58	5,9-Undecadien-2-one, 6,10-dimethyl-	$C_{13}H_{22}O$	1453	1435	882	2	↑SI
59	Aldehyde	/	1116	/	/	3	↑SI
60	Ketone	/	1469	/	/	3	↑SI
61	Alcohol	/	1665	/	/	3	↑SI, ↑H_2O_2
62	Ester	/	1258	/	/	3	↑SI, ↑H_2O_2
63	1-(2,3-Dimethylphenyl)ethanone	$C_{10}H_{12}O_2$	1265	NR*	822	3	↑SI
64	2-Heptanone	$C_7H_{14}O$	880	891	827	2	↑SI, ↑H_2O_2
65	Ethanone, 1,1'-(1,3-phenylene)bis-	$C_{10}H_{10}O_2$	1441	NR*	882	3	↑SI
66	Alkane	/	1215	/	/	3	↑SI
67	Furan Derivatives	/	1214	/	/	3	↑SI, ↑H_2O_2
68	Hexadecane	$C_{16}H_{34}$	1600	1600	744	3	↑H_2O_2
69	Aromatic	/	1643	/	/	3	↑SI
70	Cyclohexanone	$C_6H_{10}O$	895	896	820	2	↑SI, ↑H_2O_2

71	Ethanone, 1-(4-ethylphenyl)-	$C_{10}H_{12}O$	1290	1277	854	2	↑SI
72	Ester	/	1307	/	/	3	↑SI, ↑H_2O_2
73	Ethanone, 1,1'-(1,4-phenylene)bis-	$C_{10}H_{10}O_2$	1452	1461	910	2	↑SI
74	Aldehyde	/	957	/	/	3	↑SI
75	Dodecane	$C_{12}H_{26}$	1200	1200	806	2	↑SI, ↓H_2O_2
76	Tridecane	$C_{13}H_{28}$	1300	1300	727	3	↑SI
77	Alkene	/	1324	/	/	3	↑SI
78	Alcohol	/	1174	/	/	3	↑SI
79	Alkane	/	1171	/	/	3	↑SI
80	1-Dodecene	$C_{12}H_{24}$	1173	1189	750	2	↑SI, ↑H_2O_2
81	Ketone	/	1511	/	/	3	↑SI
82	Acetophenone	C_8H_8O	1066	1070	873	2	↑SI
83	Aromatic	/	1086	/	/	3	↑SI
84	Acetophenone, 2'-hydroxy-	$C_8H_8O_2$	1165	1163	863	2	↑SI
85	2,5-Hexanediol, 2,5-dimethyl	$C_8H_{18}O_2$	940	916	766	2	↑SI, ↑H_2O_2
86	Ketone	/	1480	/	/	3	↑SI
87	Alkane	/	1571	/	/	3	↑SI
88	Aromatic	/	1504	/	/	3	↑SI
89	Alkane	/	1454	/	/	3	↑SI
90	Ester	/	891	/	/	3	↑SI, ↑H_2O_2
91	Aldehyde	/	1273	/	/	3	↑SI
92	1-Octanol, 6-methyl-	$C_9H_{20}O$	1143	1154	778	2	↑SI
93	Alcohol	/	1039	/		3	↑SI
94	Alkane	/	1165	/	/	3	↑SI
95	Alcohol	/	1168	/	/	3	↑SI
96	Alkene	/	1100	/	/	3	↑SI, ↑H_2O_2
97	Butanoic acid, 4-hydroxy-	$C_4H_8O_3$	903	933	793	2	↑SI

*NR: Not reported in the library