Evaluating Genotoxicity of E-Cigarettes with an Automated 3-D Printed Array

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SUPPORTING INFORMATION

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Chemicals & Reagents

Safety note: Benzo[a]pyrene (B[a]P), 4-[methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone (NNK) and N'-Nitroso-2-(3-pyridyl)pyrolidine (NNN) and their metabolites are potential carcinogens. Handling of these chemicals was done taking protective measures including wearing gloves, safety glasses and working in a hood.

B[a]P (MW 252.31), NNK (MW 207.23), NNN (MW 177.20), poly(diallyldimethylammonium chloride) (PDDA, avg. MW= 100,000-200,000), poly(acrylic acid) (PAA, avg. MW= 1800), calf thymus DNA (Type I), and other chemicals were from Sigma Aldrich. Pooled male human liver microsomes were from BD Gentest. $[Ru(bpy)_2(PVP)_{10}]^{2+}$ {Ru^{II}PVP; (bpy=2,2-bipyridyl; PVP=poly(4-vinylpyridine)} was synthesized and characterized as described previously.[1] Pyrolytic graphite (PG) sheets are from Panasonic PGS-P13689-ND 70 µm thick.

3D printing specifications.

Resin: Formlabs Clear Photopolymer resin FLGPCL02

Printer: Form1+

Resolution: 0.05 mm

Steps

1. Convert 123 design file to printer preform slicer program file format (.preform).
2. Apply orientation and supports to the object.
3. Support specifications: Density 1.5 point size 1.0 mm, no internal supports.
4. Upload the design file to printer and start the print.
5. Post printing
    a. Remove the printed object from the platform and cut the supports to free the object.
    b. Flush the channels and the sample chambers with isopropanol and water three times.
    c. Sanding performed on the devices where the supports were initially present to smooth the surface.
    d. Dry and spray coat with Krylon colormaster acrylic crystal clear coat and allow to dry for several hours.

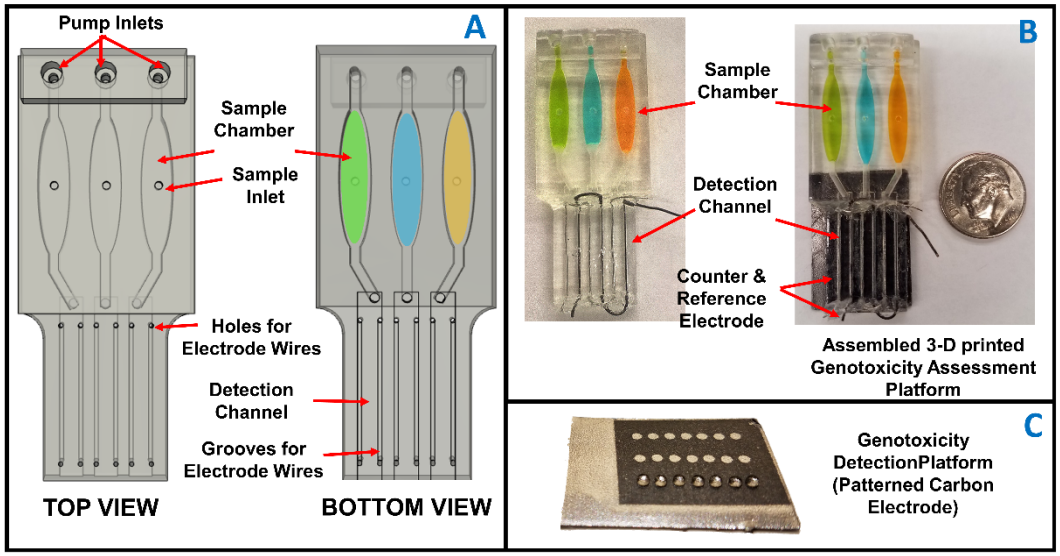

Figure S1. 3-D Printed genotoxicity array: (A) CAD design showing top and bottom view of 3-D printed arrays with pump inlets, sample chambers, detection channels and grooves for counter and reference electrodes; (B) Printed, assembled devices showing sample chamber containing dye solutions and electrodes wires inserted; (C) microwell patterned PG detection sheet showing droplet surrounded by hydrophobic boundary.

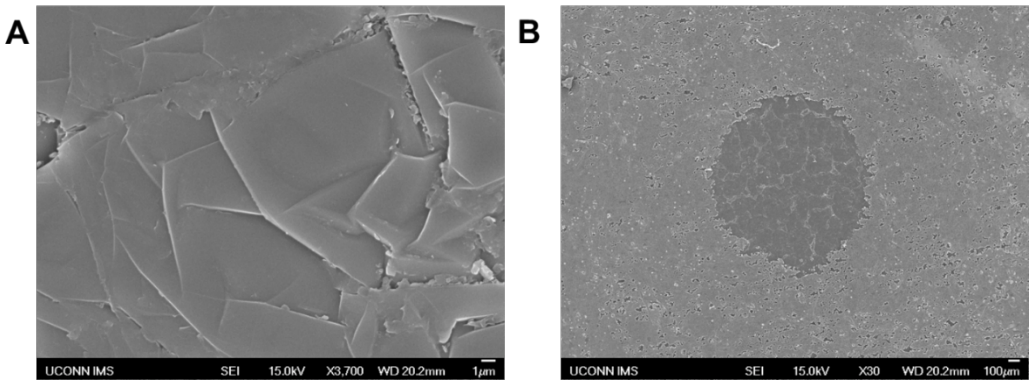

Figure S2. SEM images for A) bare pyrolytic graphite (PG) sheets and B) A Microwell on the PG sheet that holds tiny volumes of reagent required to complete layer-by-layer film assembly of enzymes, DNA and RuPVP.

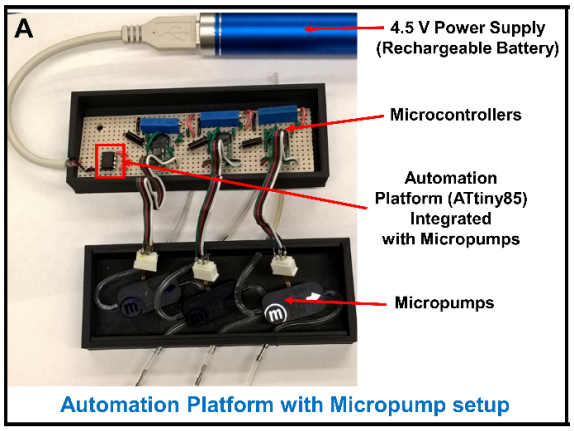

Figure S3. Assembled automated array: (A) Automated platform for micropumps showing ATtiny 85 integrated with 4.5 V battery and microcontrollers connected to micropumps that feed the array for the genotoxicity screening assay.

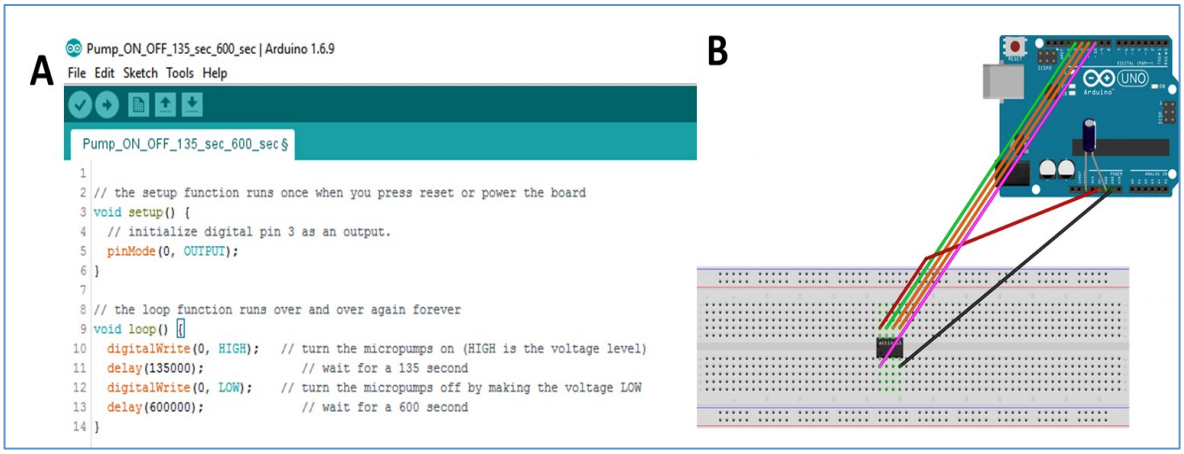

Figure S4. A) Arduino program for pumps automation B) Schematic upload of Arduino program to ATtiny85 chip.

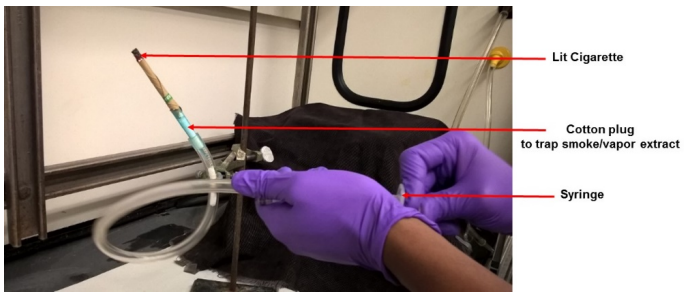

Figure S5. Artificial inhalation setup to extract smoke/vapor from cigarettes on to a cotton plug.

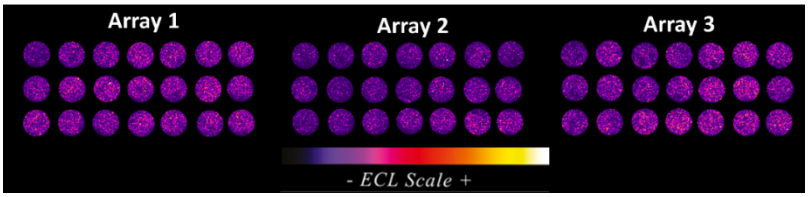

Figure S6. Recolorized ECL images from PDDA/PAA/$(Ru/DNA)_2$/Ru/Enzyme/DNA films in microwells captured by CCD camera in 10mM phosphate buffer, pH 7.4 upon application of 1.25 V against Ag/AgCl reference electrode for 180 s.

In order to assess the reproducibility of ECL generated between spot to spot and array to array, ECL captured from PDDA/PAA/$(Ru/DNA)_2$/Ru/Enzyme/DNA films in 3 different arrays were analyzed upon treatment with 10 mM phosphate buffer and 45s electrolysis followed by 180 s ECL capture. Spot to spot variability of ~ 6 % (n=21 spots) and array to array variability ~7% (n=3) was observed, Figure 3. ECL obtained from 3 different arrays were analyzed by one way analysis of variance, ANOVA and they did not differ statistically at 95 % confidence interval. ($p > 0.05$)

Table S1. Genotoxic reactivity of cigarette sample assessed in terms of known carcinogen concentration.

Sample	% ECL	NNK		NNN		B[a]P	
		[Conc.]	STDEV	[Conc.]	STDEV	[Conc.]	STDEV
1 Tob. Cig	23.32	45.74	2.58	26.40	1.49	45.54	2.56
3 Tob. Cig	33.58	85.52	8.50	49.74	4.94	77.84	7.74
5 Tob. Cig	40.46	117.76	3.36	68.77	1.96	102.37	2.92
1 nf- Tob. Cig	24.35	49.25	3.43	28.45	1.98	48.52	3.38
3 nf- Tob. Cig	39.58	113.41	10.10	66.20	5.90	99.12	8.83
5 nf- Tob. Cig	72.57	320.99	23.45	189.80	13.86	241.63	17.65
20 puff e-cig	33.17	83.74	3.26	48.70	1.89	76.45	2.97
60 puffs e- cig	58.11	219.19	21.79	128.99	12.82	174.28	17.33
100 puffs e-cig	84.24	414.64	35.43	245.96	21.01	300.87	25.71
20 puffs nn e-cig	27.48	60.61	4.04	35.11	2.34	57.96	3.87
60 puffs nn e-cig	39.47	112.84	3.17	65.86	1.85	98.70	2.78
100 buffs nn e-cig	42.71	129.25	6.47	75.56	3.78	110.86	5.55
1 e-cig cartridge	98.53	542.60	44.94	322.95	26.75	378.81	31.37
3 e-cig cartridges	166.15	1330.50	169.94	800.78	102.28	816.67	104.31
5 e-cig cartridges	218.43	2127.83	209.67	1288.16	126.93	1220.95	120.31

Abbrev.: Tob.=tobacco; nf=non-filtered; nn= non-nicotine

Reference

[1] L. Dennany, R. J. Forster, J. F. Rusling, *J. Am. Chem. Soc.*, **2003**, *125*, 5213-5218.