Electronic Supplementary Material (ESI) for Lab on a Chip.

Integration of a microfluidic multicellular coculture array with machine learning analysis to predict adverse cutaneous drug reactions

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Supplementary Data

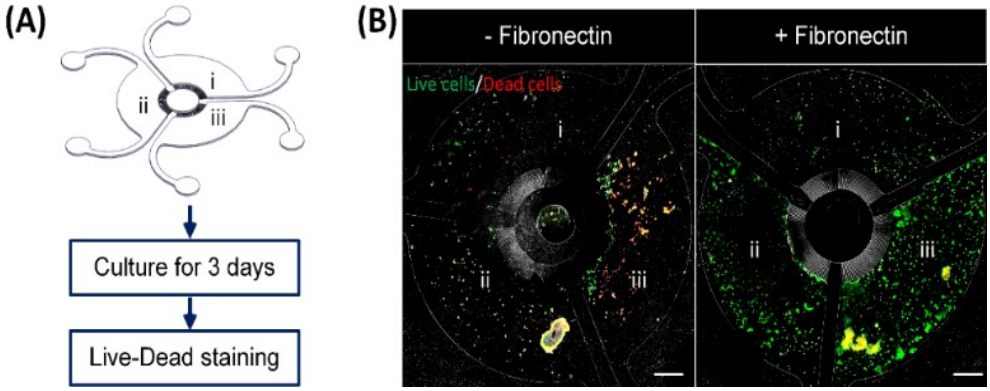
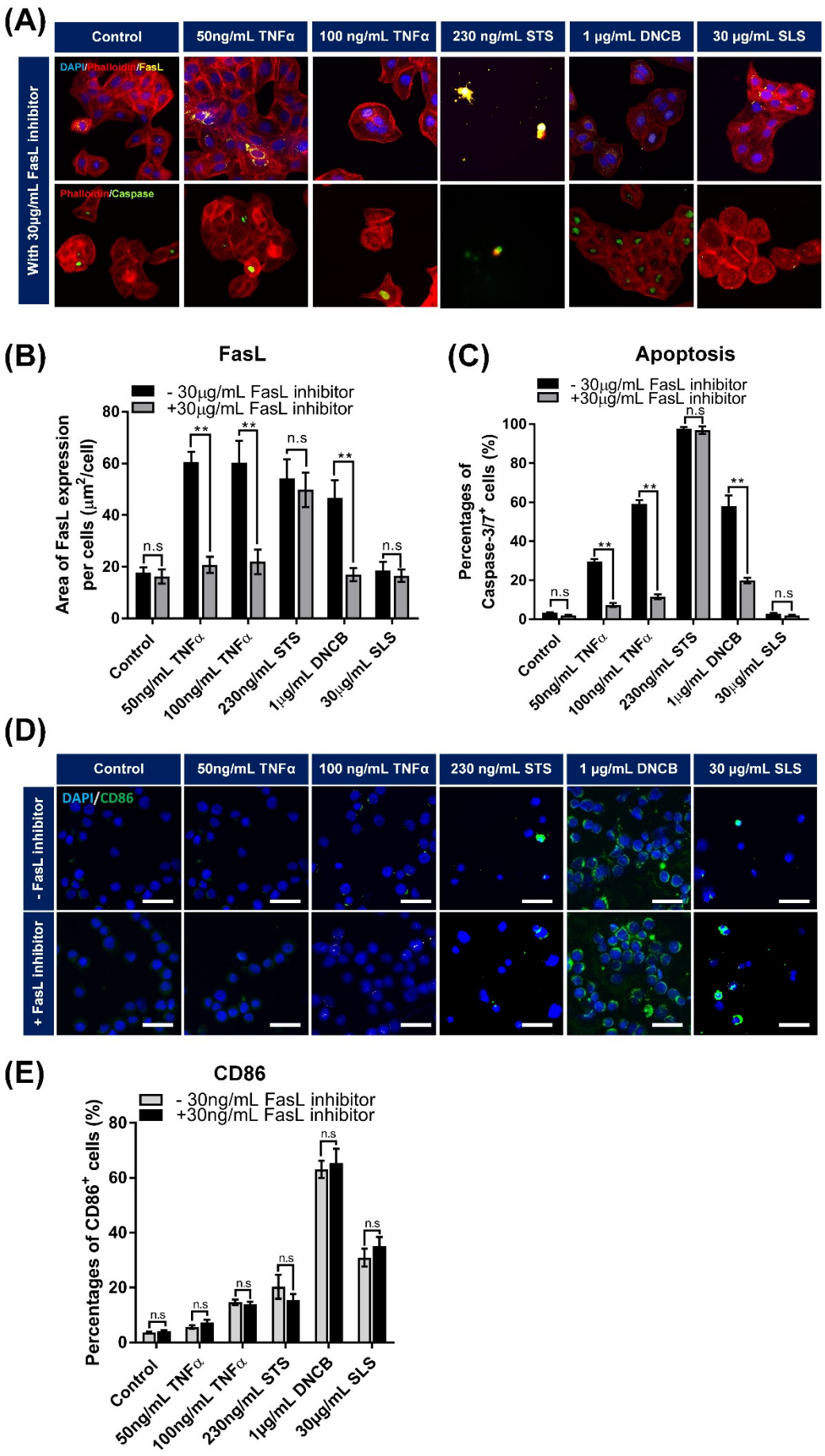


Figure S1. Optimization of cell seeding protocol in MCA. (A) Illustration of the process to optimize the seeding protocol using HaCaT in MCA. Cell suspensions at different cell densities including (i) 0.06 million/mL, (ii) 0.15 million/mL and (iii) 0.3 million/mL of HaCaT were seeded into different compartments of MCA respectively. (B) Fluorescent images of the overall cell seeding in MCA. Live cells are stained with green while dead cells are stained with red. The fibronectin coating (right) is found to promote the cell attachment in the device.


(A)
Control
50ng/mL TNFα
100 ng/mL TNFα
230 ng/mL STS
1 μg/mL DNCB
30 μg/mL SLS
With 30μg/mL FasL inhibitor
DAPI/Phalloidin/FasL
Phalloidin/Caspase
(B)
FasL
- 30μg/mL FasL inhibitor
+30μg/mL FasL inhibitor
Area of FasL expression per cells (μm²/cell)
n.s
**
Control
50ng/mL TNFα
100ng/mL TNFα
230ng/mL STS
1μg/mL DNCB
30μg/mL SLS
(C)
Apoptosis
- 30μg/mL FasL inhibitor
+30μg/mL FasL inhibitor
Percentages of Caspase-3/7⁺ cells (%)
(D)
Control
50ng/mL TNFα
100 ng/mL TNFα
230 ng/mL STS
1 μg/mL DNCB
30 μg/mL SLS
- FasL inhibitor
+ FasL inhibitor
DAPI/CD86
(E)
CD86
- 30ng/mL FasL inhibitor
+30ng/mL FasL inhibitor
Percentages of CD86⁺ cells (%)

Revised Figure S2 Effect of FasL inhibition to the cellular response of drug-treated HaCaT and U937. (A) Immunostaining of FasL (yellow), DAPI (blue) and phalloidin (red) in HaCaT with a quantification graph showing the total area of FasL expression per cell. (B) Immunostaining of caspase 3/7 (green) and phalloidin (red) in HaCaT with a quantification graph showing the percentages of caspase 3/7 positive cells after treatment. The red bars denote the HaCaT's culture without 30 ng/mL FasL inhibitor while the pink bars denote the addition of 30ng/mL FasL inhibitor. Data are average ± SEM of 3 independent experiments. Asterisks denote the statistical significant differences (Student's t-test, *$p < 0.05$; **$p < 0.01$; n.s = no significant differences). Scale bar = 50 μm.

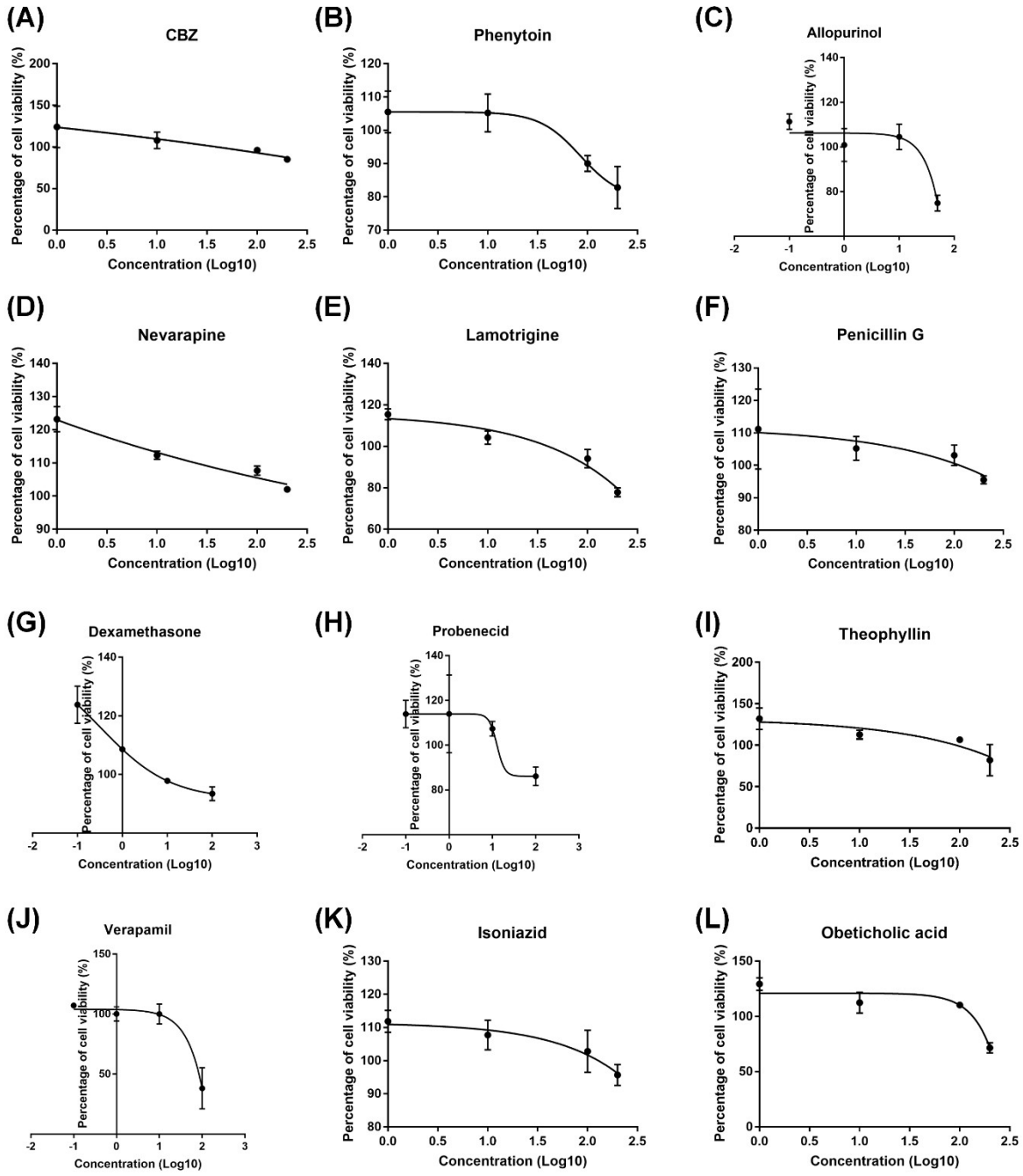
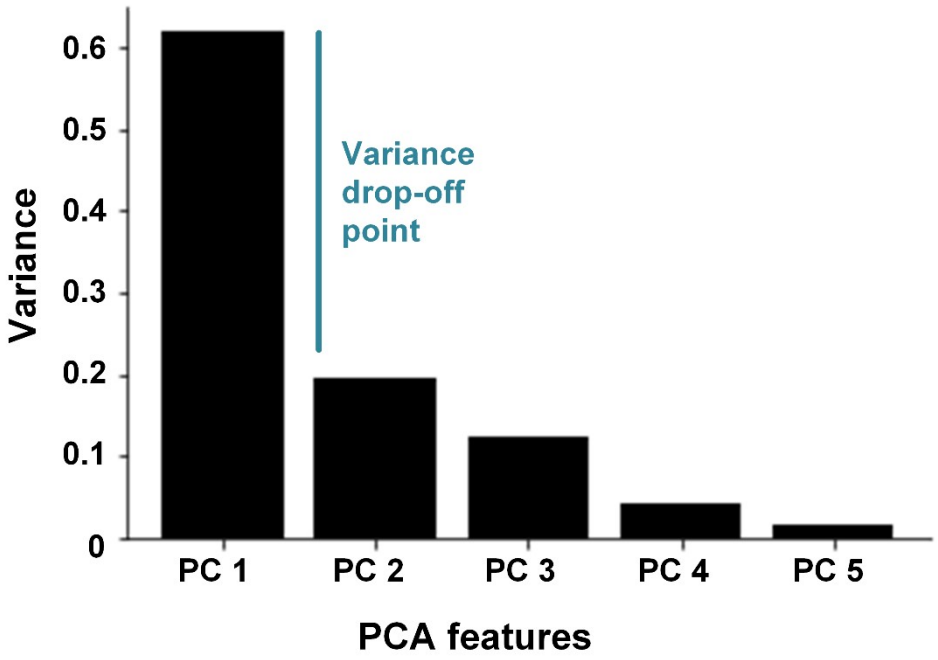


Figure S3 Dose-response curve for the paradigm drugs, including (A) Carbamazepine (CBZ), (B) Phenytoin, (C) Allopurinol, (D) Neverapin, (E) Lamotrigine, (F) Penicillin G, (G) Dexamethasone, (H) Probenecid, (I) Theophyllin, (J) Verapamil, (K) Isoniazid, and (L) Obeticholic acid. Data are average ± SEM of 3 independent experiments.



Revised Figure S4 Screen plot showing variance drop-off after the first principal component (PC1).

Drugs	Drug treatment in bulk culture and MCA
Tumor necrosis factor α (TNFα)	50 ng/mL and 100 ng/mL
2,4-dinitrochlorobenzene (DNCB)	1 µg/mL
Sodium lauryl sulphate (SLS)	30 µg/mL
Staurosporine (STS)	230 ng/mL

Table S1 Summary of the drug concentrations for FasL mediated apoptosis assay validation.

Drugs	Stock concentration (mM)	IC_{25} -Heparg-Hepatocytes Spheroid (µM)	Drug treatment in MCA (µM)
Carbamazepine (CBZ)	100	n.d.	100
Phenytoin (PHT)	100	200	100
Allopurinol	20	41.5	20
Nevirapine	80	n.d	80
Lamotrigine	40	n.d	40
Penicillin G	200	n.d	200
Verapamil	20	76.87	20
Theophyllin	20	n.d	20
Isoniazid	100	n.d	100
Probenecid	100	100	100
Dexamethasone	100	100	100

Table S2. 11 drugs with FDA classification on adverse cutaneous reactions were used in a pilot screen to create a data set to train the SVM classification algorithm. Table shows a summary of the drug stock concentrations, drug concentrations causing 25% of cell death (IC_{25}) in HHS after 48 hours, and drug concentration administered to the MCA during pilot drug screen. n.d. indicates not determined.

Cell Types	Markers	Primary Antibodies	Secondary Antibodies
HHS	CYP3A4	rabbit anti-CYP3A4(1:100) (Abcam, Cambridge, UK)	Alexa Fluor® 555 donkey anti-rabbit IgG (1:1000) (Life Technologies, Singapore)
U937	CD86+	Rabbit anti-CD86 antibody (Abcam, Cambridge, UK)(1:100)	Goat anti rabbit IgG (Alexa Fluor® 488) (1:1000) (Life Technologies, Singapore)
	DAPI		4,6-diamidino-2-phenylindole (1:1000) (Life Technologies, Singapore)
Hacat/human dermal fibroblast	FasL	Rabbit Anti-Fas Ligand antibody (1:200) (Abcam, Cambridge, UK)	Alexa Fluor® 647 donkey anti-rabbit IgG (1:1000) (Life Technologies, Singapore)
	Phalloidin		Alexa Fluor™ 568 Phalloidin (Life Technologies, Singapore)
	DAPI		4,6-diamidino-2-phenylindole (1:1000) (Life Technologies, Singapore)

Table S3. List of primary and secondary antibodies used in this study.