US EPA Transform Tox Testing Challenge: 384-Pillar plate for assessing metabolism-induced toxicity with 3D printed cells

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Abstract

Drugs are metabolized primarily in the liver by a variety of oxidative and conjugative enzymes including cytochromes P450 (CYP450s) and UDP-glucuronosyltransferases (UGTs) among others. These enzymes are involved in the initial clearance of drugs from the body and generate drug metabolites, some of which are unstable and toxic. Thus, understanding the role of these drug-metabolizing enzymes (DMEs) in drug metabolism and related toxicity is an important area of research for safer drug development. However, incorporating physiological levels of chemical metabolism into high-throughput screening (HTS) of drug candidates is still challenging. In response to EPA's Transform Tox Testing Challenge (TTTC) project to retrofit or "to incorporate physiological levels of chemical metabolism" into its existing high-throughput screening (HTS) assays, the 384-pillar plate containing 3D-cultured human embryonic kidney 293 (HEK293) cells was designed as a complement to conventional 384-well plates for rapid assessment of metabolism-induced toxicity. The toxicity of test compounds and their metabolites generated *in situ* by the DMEs in the 384-well plate were assessed by staining HEK293 cells encapsulated in alginate-Matrigel on the 384-pillar plate with calcein AM and CellTiter-Glo® luminescent cell viability kit and analyzing fluorescence and luminescence intensities from the cells. This straightforward approach generated the critical IC_{50} information necessary for evaluating metabolism-mediated toxicity in a high-throughput manner.

The 384-pillar/well plate for 3D cell culture and metabolism-induced toxicity assays

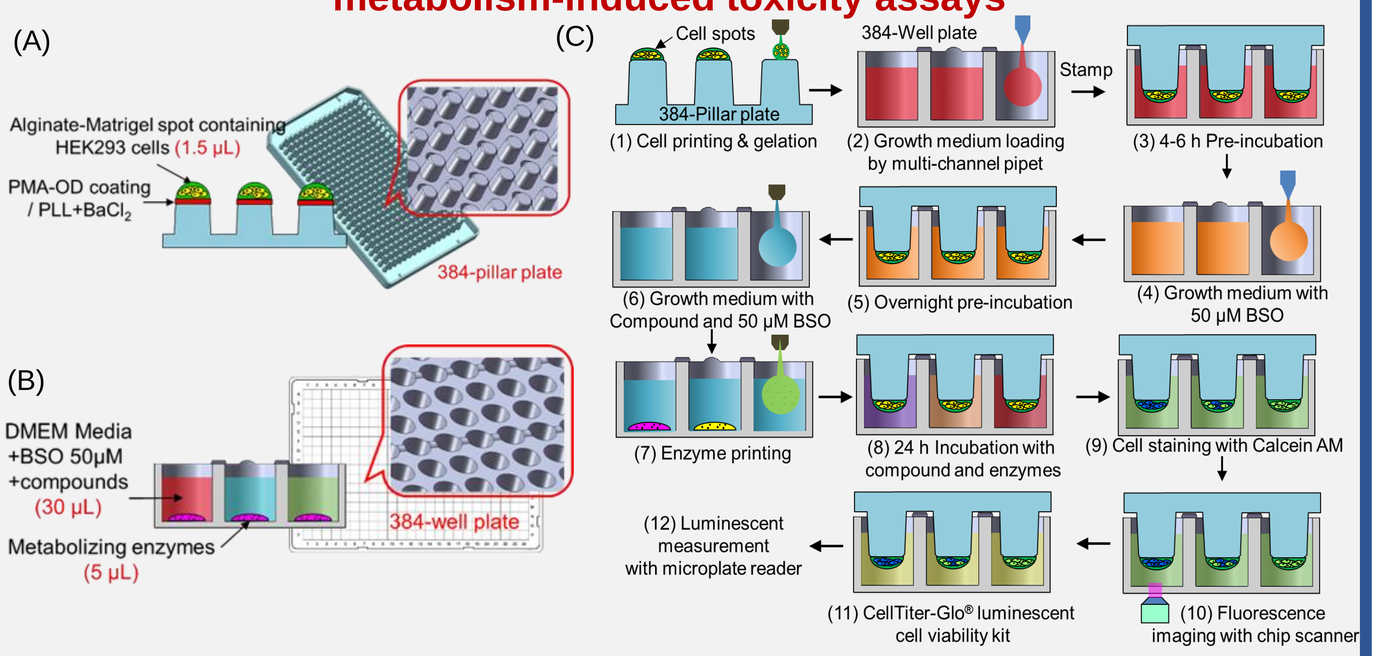
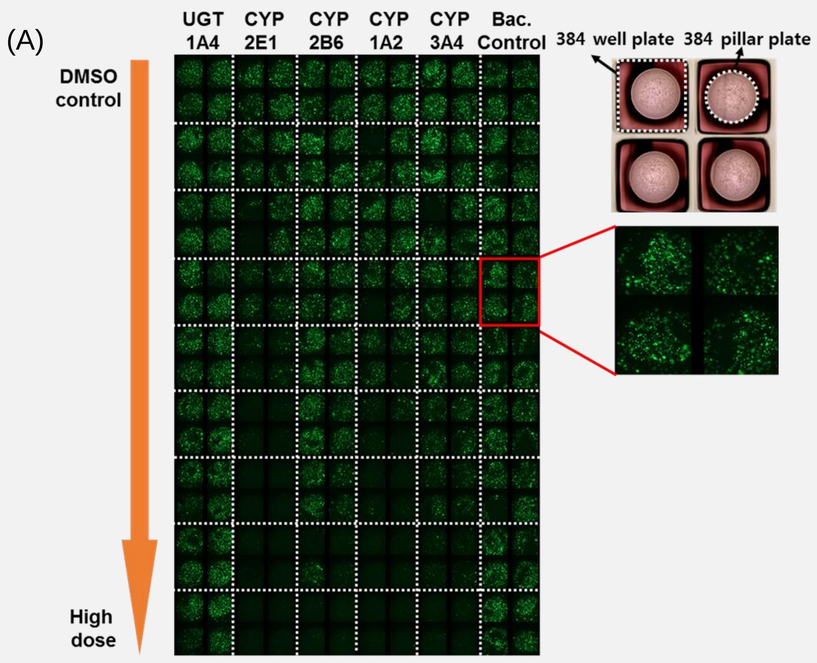


Figure 1. (A) Schematic of a 384-pillar plate with HEK293 cells cultured in 3D. (B) Schematic of a 384-well plate containing 5 drug metabolizing enzymes (DMEs) and a baculosome control. (C) Experimental procedures for metabolism-induced toxicity assays with HEK293 cells on the 384-pillar plate and compounds and DMEs in the 384-well plate.

Metabolism-induced toxicity assays on 384-pillar plates with HEK293 cells and in 384-well plates with DMEs and compounds



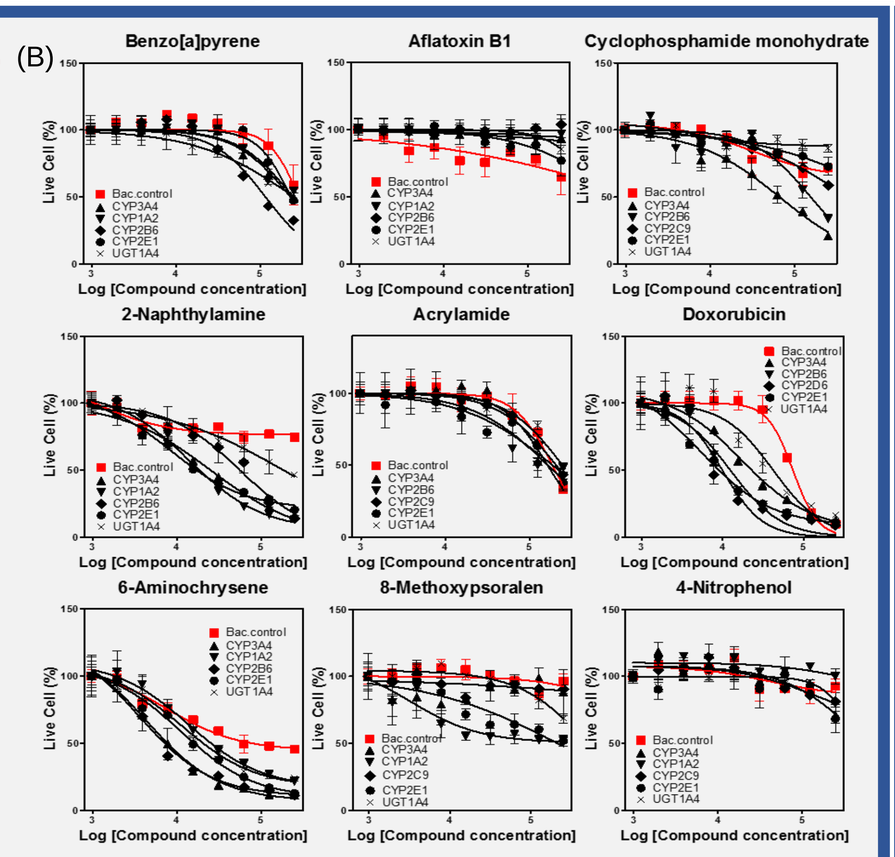
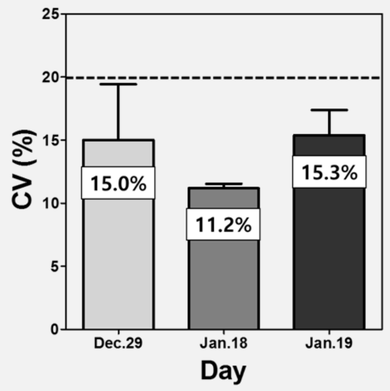


Figure 2. (A) Scanning images of HEK293 cells on the 384-pillar. The cells were stained with calcein AM after 24-hour incubation with the compound and DMEs on the 384-pillar/well plate. (B) Dose response curves and IC_{50} values for 9 TTTC compounds. The CellTiter-Glo® assay was performed with 9 compounds at 9 dosages (1.95 μM – 250 μM) and 6 DMEs in 4 replicates.

Robustness of the assays



Test conditions	R^2	Hill coefficient	Log IC_{50} + Log SE (nM)	Z' factor	IC_{50} values (μM)
Control baculosome	0.86	-1.30	2.10 ± 0.07	0.75	125 ± 1.2
CYP1A2	0.96	-1.56	1.89 ± 0.03	0.63	79 ± 1.1
CYP2B6	0.97	-2.04	1.85 ± 0.03	0.57	77 ± 1.1
CYP2C9	0.95	-0.98	1.82 ± 0.04	0.79	66 ± 1.1
CYP2E1	0.95	-2.78	1.79 ± 0.03	0.69	62 ± 1.1
CYP3A4	0.97	-1.51	1.95 ± 0.03	0.93	89 ± 1.1

Figure 3. The Z' factors and the coefficient of variation (CV) obtain from the CellTiter-Glo® assay on the 384-pillar plate to calculate the robustness and variability of the assay.

IC_{50} values of 9 compounds tested with the CellTiter-Glo® assay

Compounds	IC_{50} values (μM)							
	No enzyme	CYP 3A4	CYP 1A2	CYP 2B6	CYP 2C9	CYP 2D6	CYP 2E1	UGT 1A4
Benzo[a]pyrene	>250 ± 1.1	229 ± 1.2	245 ± 1.4	116 ± 1.1	-	-	233 ± 1.0	>250 ± 1.4
Aflatoxin B1	>250 ± 4.0	>250 ± 8.8	>250 ± 1.9	>250 ± 29	-	-	>250 ± 2.1	>250 ± 2.0
Cyclophosphamide	>250 ± 1.7	66 ± 1.1	-	155 ± 1.2	>250 ± 1.1		>250 ± 2.4	>250 ± 1.0
2-Naphthlyamine	>250 ± 4.4	25 ± 1.1	20 ± 1.1	56 ± 1.1	-	-	24 ± 1.1	179 ± 1.3
Acrylamide	186 ± 1.1	164 ± 1.2	-	165 ± 1.1	208 ± 1.2	-	188 ± 1.1	246 ± 1.1
Doxorubicin HCl	74 ± 1.1	25 ± 1.1	-	12 ± 1.2	-	10 ± 1.2	11 ± 1.4	42 ± 1.1
6-Aminochrysene	93 ± 1.1	9 ± 1.1	32 ± 1.1	9 ± 1.2	-	-	19 ± 1.1	26 ± 1.2
8-Methoxypsoralen	>250 ± 56	>250 ± 1.0	126 ± 1.8	-	>250 ± 4.6	-	213 ± 1.7	>250 ± 1.2
4-Nitrophenol	>250 ± 17	>250 ± 1.4	>250 ± 1.0	-	>250 ± 2.6	-	>250 ± 1.0	>250 ± 1.5

Conclusions

- We successfully detected metabolism-induced toxicity, as evidenced by augmented toxicity and detoxification of TTTC compounds on specific DMEs.
- Of the 9 compounds tested, 7 compounds were either activated by CYP450s/UGT1A4 or detoxified by UGT1A4, and 2 compounds were non-responsive to all DMEs used (Overall predictivity of 78%).
- The overall CV and the Z' factors were 13.8% and 0.57 – 0.93, which indicate that the CellTiter-Glo® assay performed on the 384-pillar plate is reliable and robust.
- We established high-throughput, high-content, metabolism-induced toxicity assays that can be used to assess the toxicity of parent compounds as well as their metabolites.

Acknowledgement

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