Assessment of a 3D Neural Spheroid Model to Detect Pharmaceutical-Induced Neurotoxicity

Supplementary Data

Fig. S1 (video): 3D-perspective video of immunostaining of spheroid with GFAP (green fluorescence) and MAP2 (red fluorescence) in Fig. 1A recorded by Nikon Eclipse Ti2 microscope (see doi:10.14573/altex.2112221s3)

Fig. S2: Exemplary graphs of dose-response curves of the 6 individual endpoints obtained from calcium oscillation assay for (A) doxorubicin, (B) carboplatin, (C) MPTP, (D) gabapentin, (E) AMPA, (F) acetylcholine, (G) 4-aminopyridine, (H) FPL64176, (I) acetaminophen, and from cellular ATP assessment for these compounds (J)

Dash lines are the clinical reported t\textsubscript{Pmax} for that compound if approved by regulatory agency. Experiments were conducted in triplicate on three individual neural spheroids for each compound.

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Carboplatin

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
MPTP

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Width (10% Amp)
D

**Gabapentin**

**Normalized Peak Count**

**Normalized Peak Amplitude**

**Normalized Peak Rise Time**

**Normalized Peak Decay Time**

**Normalized Peak Spacing**

**Normalized Peak Width (10% Amp)**
Acetylcholine

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
4-Aminopyridine

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
Acetaminophen

Normalized Peak Count

Normalized Peak Amplitude

Normalized Peak Rise Time

Normalized Peak Decay Time

Normalized Peak Spacing

Normalized Peak Width (10% Amp)
Fig. S3: Assay variability for calcium oscillation. Inter-plate variability of different measurements for the vehicle control samples in all the plates, data not normalized. Mean (black squares) is shown for each phenotype overlaid on top of gray circles representing individual well response. Coefficients of variation (%CV) are shown for each phenotype.
Fig. S4: Neural spheroids were treated with pharmaceuticals clinically associated with neurodegeneration (doxorubicin (A), paclitaxel (B), colchicine (C)) or control (ibuprophen (D)) for 24, 72 or 144 hours.
Neural spheroids treated with neurodegenerative pharmaceuticals were associated with a decrease in total cellular ATP in a time and dose-dependent manner, but ibuprophen-treated neural spheroids were not.