High-Throughput Brain Organoid Screening Platform for Discovery of Traumatic Brain Injury Therapeutics

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INTRODUCTION

Traumatic brain injury (TBI) is a major global health challenge, causing over **70,000 deaths annually.**[1] TBI lacks effective disease-modifying therapies due to poor translation from preclinical models to clinical success.[2]

- There are no FDA approved therapeutics for acute TBI

We developed a human-relevant, high-throughput TBI screening platform that integrates human iPSC-derived neural organoids, with pressure-induced TBI events, and functional and viability assays.

- The system can study both acute and chronic TBI symptoms

The platform quantitatively measures TBI-induced dysfunction, demonstrating that pressure injury disrupts synchronized neuronal calcium signaling and reduces cell viability, while known neuroactive compounds can restore neuronal function.

This integrated platform provides a predictive, scalable system for therapeutic discovery, enabling the evaluation and prioritization of candidate compounds for TBI and other neurological diseases, with the potential to improve clinical translation and drug development success.

INSTRUMENTATION

The Pu·MA System EC and 3D Flowchip features:[4]

- Automated media exchanges occur with cells in protected chamber
- Supernatants can be collected to monitor cell secretion
- Spheroids can be imaged in the flowchip, or samples removed for immunoassay or metabolomics analysis
- Assay protocols can be edited via the Pu·MA System Software

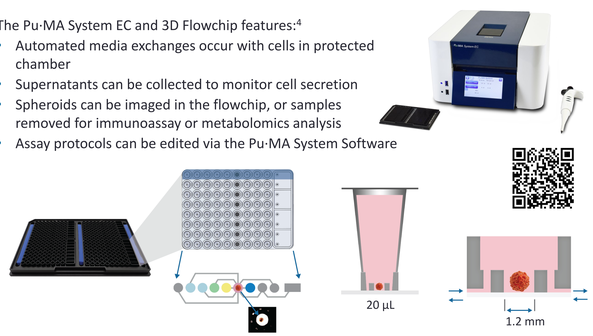


Figure 1. Pu·MA System flowchip layout and sample chamber dimensions.

The CellXpress.ai System Features:

- Scale up complex cell culture workflows with pre-configured protocols for organoid feeding, monitoring, and passaging on up to 40 plates.
- Automated imaging to identify outliers at the well, plate, or experiment level to help detect variability.
- Improve productivity and optimize hands-on time with image-based, deep-learning decision-making.
- Real-time feedback to alert users to milestones or events and can generate automated tasks to resolve them.
- Develop traceable and reproducible cell cultures specific to desired assay endpoints with a software environment using data-driven visualization tools.

References:
[1]Dewan, M.C. et al, Estimating the Global Incidence of Traumatic Brain Injury, J Neurosurg, **130**, 2019.
[2]Ng, S.Y., et al, Traumatic Brain Injuries: Pathophysiology and Potential Therapeutic Targets, Front Cell Neurosci, **13**, 2019.
[3]Sirenko, O. et al, Functional and Mechanistic Neurotoxicity Profiling using Human iPSC-Derived Neural 3D Cultures, Toxicol Sci, **167**, 2019
[4]Cromwell, E.F. et al, Disease Modeling with 3D Cell-Bases Assays using a Novel Flowchip System and High-Content Imaging, SLAS Tech, **26**, 2021
[5]Kelsen, J. et al, Copenhagen Head Injury Cyclosporin Study, J Neurotrauma, **36**, 2019.

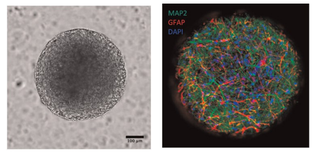
This material is based upon work supported by the US Army Medical Research Development Command (USAMRDC) under Contract No. HT942525P0066.

FUNCTIONAL BRAIN MODELS

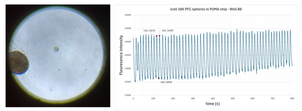
The two most common human brain models are Brain Organoids and Neurospheres.

Brain Organoids: Human progenitor stem cells (PSC) are differentiated into neurons and self-assemble into organoids over a 90-day period. They form brain-like structures and develop neuronal activity.

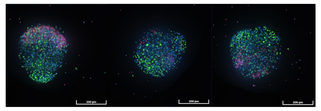
Neurospheres: Neuronal precursor cells and astrocytes are co-cultured over a 3-week period during which time they mature and form neurite outgrowth networks. They can exhibit synchronous neuronal firing activity.



Human iPSC-derived 3D neural culture spheroids shown with Transmitted Light (Left) and Fluorescence (Right) imaging. Spheroids are composed of active cortical neurons (MAP2, **Green**) and astrocytes (GFAP, **Red**). Nuclei are stained with Hoechst 33342 (**Blue**). Images were taken with ImageXpress Micro Confocal system with 20X water obj and are Maximum Projection of a confocal stack.[3]



Ca^{2+} flux measurements. Neuronal activity from individual neurospheres formed in PuMA flowchips was measured via Ca^{2+} flux on FLIPR system. Neurospheres were loaded with Calcium 6 dye for 2 hrs and read over a 10 min period at 2 Hz on FLIPR at 37 C.



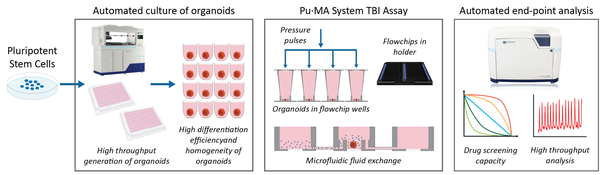
Viability staining of PFC iCell neurospheres. MIP projections depict three iCell PFC neurospheres. Cells are stained for nuclei (Hoechst, **Blue**), Live cells (CyQaunt, **Green**), and Dead cells (EthD-1, **Magenta**). Images were captured using ImageXpress 20X water objective with 16 Z slices spaced 5 µm apart.

TBI ASSAY WORKFLOW

The workflow for TBI assays consists of three critical parts:

- Forming large numbers of robust neural organoids
- Subjecting them to simulated TBI events
- Characterizing functional & phenotypic responses of the organoids.

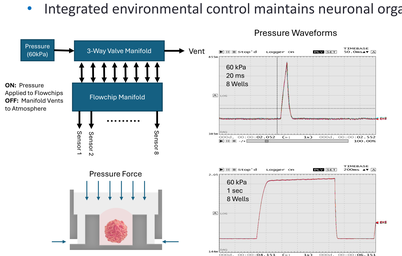
Use of automation for cell culture, pressure pulse generation, and assay readouts provides a scalable system that can screen for new TBI therapeutics at unprecedented rates.



Workflow for Screening of TBI Therapeutics using neural organoids

Simulated TBI events are generated with the Pu·MA System and can simultaneously deliver precise pressure pulses up to 384 sample wells.

- Acute TBI: Short pressure pulses up to 45 psi (3 atm) with <100 ms rise times
- Chronic TBI: Lower pressures (1 – 60 kPa) for > 30 days with programmed modulation
- Integrated environmental control maintains neuronal organoid viability

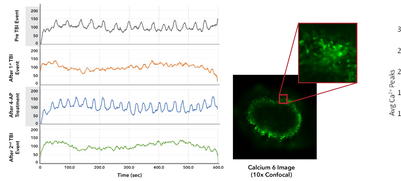


Pu·MA System pressure control system and pulse characterization. The Pu·MA System contains a microprocessor-controlled pressure manifold that can deliver a variety of pressure pulse waveforms to sample wells containing brain organoids. The manifold simultaneously seals up to 384 sample wells. Pressure forces are equalized to prevent fluid movement during a TBI event. The system can be configured for either flowchips (32 samples) or 384 well plates for HTS.

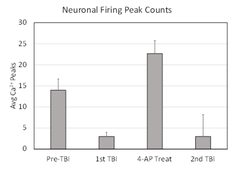
BRAIN MODEL RESPONSE TO TBI

- Neuronal activity of both neurospheres and brain organoids can be perturbed by applying pressure pulses that simulate TBI events.
- Synchronous neuronal firing activity is shown to be reduced or completely quenched by pressures from 10 psi to 30 psi.
- Higher pressures and repetitive pulses cause higher levels of perturbation.
- Brain organoids are able to recover activity post-TBI after a period of rest.

To quantify effects of TBI pressure pulses, brain organoids were incubated with Calcium 6 dye for 2 hours and then neuronal firing activity was measured on **the FLIPR Penta system**. Neuronal activity of brain organoids were measured on **the FLIPR Penta system** for baseline behavior (Pre-TBI) then they were subjected to TBI pressure pulses (30 psi, 500 ms, 5X) and re-measured (1st TBI). A clear decrease in synchronous neuronal firing was found in the organoids similar to effects previously observed with neurospheres (see Figure below). The organoids were then treated with 4-aminopyridine, a potassium ion channel agonist that has been shown to stimulate neuronal activity. Organoids were treated with 0.4 µM 4-AP, incubated for 15 minutes, and then measured again on FLIPR (4-AP Treat). The 4-AP compound was able to recover synchronous activity in all three organoids. The organoids were then subjected to a second round of TBI pressure pulses (2nd TBI) which once again disrupted the neuronal firing activity. The neuronal firing peaks from three organoids were manually counted and the average and standard deviation of these results are shown in the Figure below. This experiment validates the ability of the TBI-event system to affect ex vivo brain models in a physiologically relevant manner and assay effects of known therapeutics for neurological disorders.



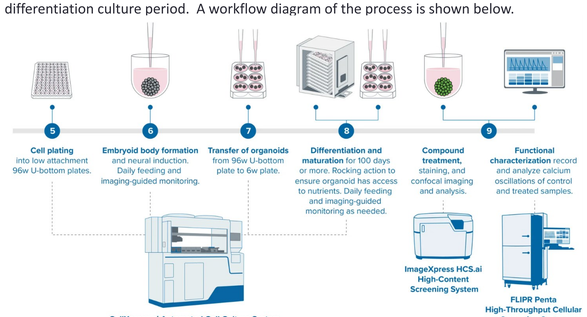
Fluorescence images of brain organoids incubated with Calcium 6 and the corresponding FLIPR Ca^{2+} flux measurements. FLIPR measurements were done 4 times over a ~1 hour period under different conditions as described in the text. The FLIPR Penta system data was smoothed & flattened, and traces were offset for display.



Analysis results showing average and standard deviation of neuronal firing peaks. Peaks were manually counted from the FLIPR Penta system traces.

NEURAL ORGANOID FORMATION

Dorsal forebrain organoids were developed from iPSC cells (ATCC ACS-1023 A*) using the STEMdiff Dorsal Forebrain Organoid Kit Catalog #08620 from STEMCELL Technologies Inc. The process contains several development steps: iPSC maintenance, seeding cells into Aggrewell V bottom plates, formation of spheroids (days 0-6), then transfer spheroids into 6-well plates and continuous culture with periodic automated media exchanges during the expansion period (days 6-25), differentiation period (days 26-43), then maintenance period (days 43-60+). After 60 days in culture, organoids were maintained in BrainPhys media. We used the CellXpress.ai system for automation of media exchanges and monitoring organoids starting from differentiation culture period. A workflow diagram of the process is shown below.

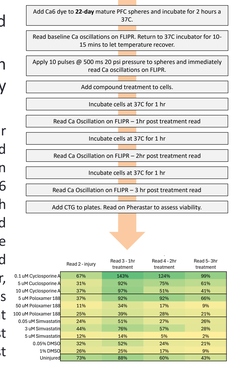


Workflow of neural organoid generation. Induced pluripotent stem cells (iPSC) are plated into 96-well ULA plates. AI-guided passaging enables hands-off automated brain organoid workflows.

TBI ASSAY DRUG RESPONSE

- Known neuroactive compounds were observed to help recover neuronal activity post-TBI.
- Concentration dependence was observed with higher amounts & time reducing % activity recovery.

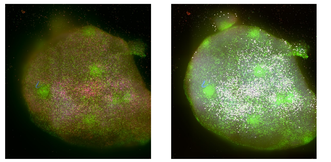
Neurospheres were incubated with compounds and their effect on neuronal firing activity was measured before and after TBI events. The workflow for this assay is shown on the Right. Neurospheres were incubated with Calcium 6 to allow measurements of baseline Ca^{2+} oscillations with FLIPR. Samples were then subjected to a TBI event and treated with compound. Compounds were added to the different sample wells using a 50% media exchange and incubated for 1 hr. The samples were then measured 1 hr, 2 hr, and 3 hr post treatment. Response was calculated as percent change in average peak counts. The TBI event significantly dropped baseline activity. The most improved recovery was observed with the lowest concentration of Cyclosporine A, a compound shown to decrease neuroinflammation.[5]

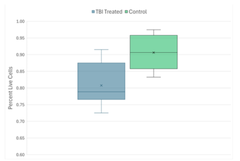


	Read 2 - Injury	Read 3 - 1hr treatment	Read 4 - 2hr treatment	Read 5- 3hr treatment
0.1 uM Cyclosporine A	67%	143%	134%	89%
5 uM Cyclosporine A	31%	92%	75%	61%
10 uM Cyclosporine A	37%	97%	91%	45%
5 uM Ptilosterin 188	37%	90%	90%	48%
10 uM Ptilosterin 188	11%	24%	17%	9%
100 uM Ptilosterin 188	25%	39%	28%	21%
0.05 uM Simostatin	24%	51%	27%	28%
1 uM Simostatin	44%	78%	57%	28%
5 uM Simostatin	12%	14%	5%	2%
0.05% DMSO	32%	92%	24%	21%
1% DMSO	20%	25%	17%	9%
Untreated	73%	88%	90%	43%

Neuronal Firing Peak Count Change as Percent of Baseline Activity

Viability response to TBI was done using neural organoid models. Neural organoids (n=8) were subjected to TBI event and then incubated with and without cyclosporine A for 48 hr. A control set of organoids (n=8) were incubated without being subjected to TBI events. After 48 hr the organoids were stained with viability dyes (CyQuant Green and EthD-1) and then imaged on a confocal fluorescence microscope (ImageXpress HCS.ai System, Molecular Devices). The images were analyzed for total Live cells (CyQuant Green Positive) and Dead Cells (EthD-1 Positive) using AI-based IN Carta® Image Analysis Software, and then the percent Live cells was calculated according to Area(Live)/[Area(Live) + Area(Dead)]. A significant difference in viability was found between TBI treated (0.808) and control organoids (0.906).





Left: Representative image (20X, MIP) of a neural organoid (Left) and Live/Dead segmentation results (Right). **Right:** Percent Live average and distributions for neural organoids subjected to TBI events. Organoids (n=8 per group) were imaged at 20X and analyzed for Live and Dead cells. P-value < 0.01

CONCLUSIONS

- We have demonstrated capabilities of a novel TBI assay system that performs complex protocols with neural organoids. This technology has the potential to accelerate therapeutic discovery and improve outcomes for those affected by brain injury.
- Neurospheres or Neural organoids are formed using automated cell culture, are subjected to TBI events using a modified Pu·MA System and measured with functional and viability assays.
- Response of neural organoids to TBI events mimics expected behavior with disruption of neural activity and increase in cell death.
- Next Steps:
  - Extend assays to longer term to study chronic TBI
  - Analyze for TBI biomarkers including GFAP, NfL, and Tau

MOLECULAR DEVICES
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