Neuroimmune activation and off-target toxicity testing of cell therapies using a novel brain-on-a-chip system

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INTRODUCTION

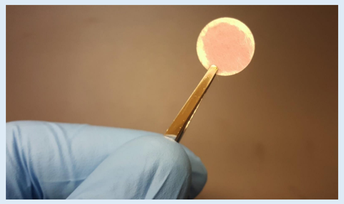
Adoptive cellular therapies such as chimeric antigen receptor T (CAR-T) cell therapy have become a promising avenue for cancer treatment. However, unexpected off-target effects of treatment resulting in brain edema and neurotoxicity have raised concern as to how the safety of these therapies can be adequately assessed. Animal models have been ineffective for predicting such events as they require humanized immunodeficient mice for testing. In vitro models are insufficient in that they are often too short-lived and lack the complexity required to accurately recreate the human neural environment. Lena Biosciences has created a novel brain-on-a-chip system comprising human neurons, astrocytes, and microglia in a three-dimensional (3D) scaffold that models neuroinflammation. iPSC-derived neurons and astrocytes were co-cultured with cells from the human microglia cell line HMC3 in SeedEZ scaffolds. Cultures in SeedEZ scaffolds are further integrated into a novel perfusion system that delivers superior oxygenation to explore the effects on the inflammation response.

METHODS

Cell culture

HepG2 cells and HMC3 cells (ATCC) were maintained in EMEM supplemented with 10% fetal bovine serum in a humidified incubator at 37 ºC. NoSpin HepaRG™ cells (TRL/Lonza), iCell® Hepatocytes 2.0 (Cellular Dynamics International), and Liverpool™ Cryoplateable primary human hepatocytes (BioreclamationIVT, 10-donor, mixed gender) were all cultured in the manufacturer's recommended media. Rat cortical cell cultures were generated by combining cells from two cortical tissue dissociations (BrainBits®, postnatal day 0 and embryonic day 18) and were cultured in Neurobasal medium supplemented with B-27 Supplement and GlutaMAX. Human cortical cultures comprising iPSC-derived neurons (Wen lab, BrainXell) and astrocytes (Wen Lab), and HMC3 microglia cells were plated and maintained in iPSC neuronal medium or BrainXell's Cortical Neuron Complete Medium. Jurkat T cells (kindly provided by Nate Dwarshuis from the Roy lab) were maintained according to ATCC specifications. Primary human T cells (Astarte) were cultured and activated according to protocols from the Roy lab.

3D cultures were generated using Lena Biosciences' patented SeedEZ™ scaffolds:



Hepatic cells and rat cortical cells were seeded in Matrigel® (8 mg/mL) at densities ranging from 100,000-500,000 cells per scaffold (depending on scaffold size and cell type). Cultures were allowed to gel at 37 ºC for 15 minutes and subsequently transferred to Lena Biosciences' PerfusionPal system or to a multi-well plate ("Control"). Cultures in PerfusionPal were either maintained without perfusion ("Static") or subjected to perfusion at a rate of 16 culture volume changes per 24 hour period ("Perfused") for the duration of the study. 2D control cultures consisted of the same number of cells plated in 24-well plates.

Human cortical cultures were plated at a in medium into SeedEZ™ scaffolds that had been coated with poly-D-lysine (PDL) (100 µg/mL) (Sigma-Aldrich). The total cell density was 1,000,000 cells/scaffold with an overall cellular ratio of 2:2:1 neurons:astrocytes:microglia.

CYP activity measurement

CYP1A activity was measured using the ethyxoyresorufin-O-deethylase (EROD) assay. Cells were exposed to 7-ethoxyresorufin (10µM) and salicylamide (inhibitor of phase II metabolism of resorufin, 1.5mM) for times ranging from 2 hours (HepaRG™) to 24 hours (Hep G2) at 37 ºC. After the incubation, 200 µL of medium from each sample was transferred to a 96-well plate and read on a Biotek Synergy 4 plate reader for fluorescence (ex/em 560/584 nm).

Cell viability assessment

After specified times, cultures were removed from the PerfusionPal system and were exposed to Calcein AM (in DPBS) for a period of 30 minutes at room temperature followed by multiple rinses with DPBS. Imaging was carried out using Neurolucida software controlling a Nikon Eclipse 80i upright microscope equipped with an Optronics MicroFIRE camera.

Pro-inflammatory stimulation

Cells were treated with lipopolysaccharide (LPS) (Sigma-Aldrich) and/or interferon gamma (IFNγ) (VWR) at concentrations of 100 ng/mL and 10 ng/mL, respectively for 24 hours.

Jurkat and human T cell activation

Cells were treated with antibodies against CD3 and CD28 in either soluble form (Jurkats) or immobilized on a multi-well plate (human T cells). Jurkat conditioned medium was collected 24-hours after stimulation. Human T cell conditioned medium was collected from activated cells on day 14 after 24 hours in fresh medium.

Gene expression

Gene expression in the HMC3 cell lines was quantified by qPCR by the Wen lab at Emory University.

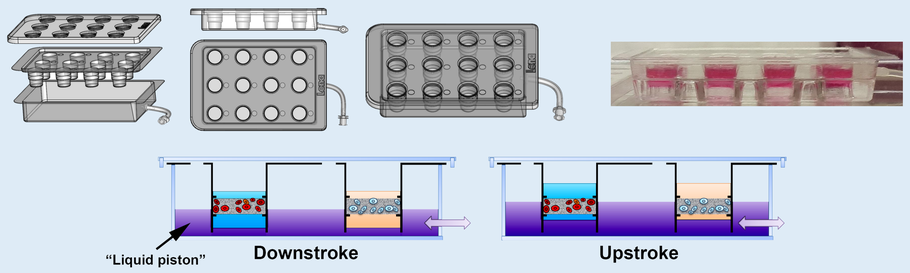
ELISA

Levels of cytokines in the medium were quantified using ELISAs specific for human TNFα and human CXCL10 (R&D Systems).

Statistical analysis

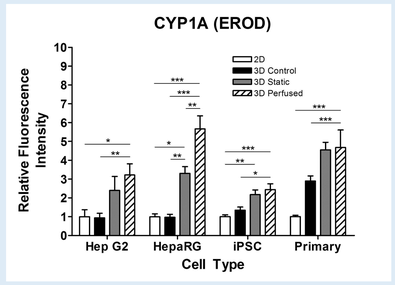
Statistical significance was determined by Student's t-test, One-way ANOVA and Tukey's post-hoc test, or Two-way ANOVA.

LENABIOSCIENCES PerfusionPal System



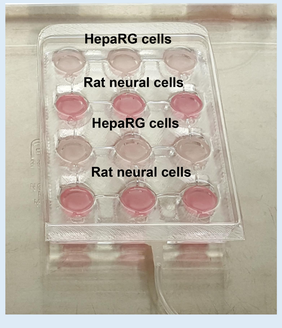
Cells seeded in 3D hydrogels supported by a rigid scaffold were maintained in statistically independent wells in their own medium which floats atop a high-density blood-substitute in which medium is immiscible. The blood-substitute readily dissolves atmospheric gases at levels which are orders of magnitude higher than for aqueous medium. Infusion and withdrawal of this blood-substitute acts as a "liquid piston" and introduces perfusion into the system without the need for separate pumps and tubing for each culture condition.

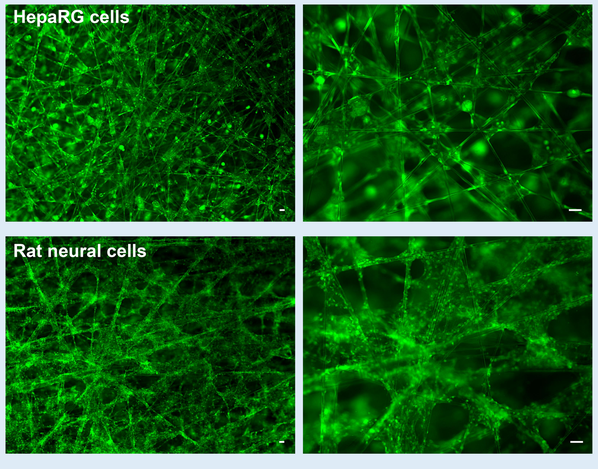
Culturing in PerfusionPal potentiates hepatic CYP activity for drug toxicity testing



Culturing human hepatocytes in the PerfusionPal system consistently enhances the baseline activity of the key drug metabolizing enzyme, CYP1A. HepG2 cells were maintained in culture over a 7-day period (n=3 for all conditions). It should be noted that Hep G2 cells have very low baseline CYP activity and that the cultures were incubated with 7-ethoxyresorufin and salicylamide for 24 hours. HepaRG™ cells were maintained culture over a 5-day period (n=6 for all 3D conditions and n=3 for 2D). HepaRG™ cells maintain higher levels of baseline CYP activity, resulting in an assay time of only 2 hours. "iPSC"-derived Hepatocytes 2.0 (Cellular Dynamics) were maintained in culture over a 5-day period (n=6 for all conditions). "Primary" hepatocytes (BioreclamationIVT Cryoplateable Liverpool™) were maintained in culture over a 5-day period (n=6 for all conditions). *p<0.05, **p<0.01, ***p<0.001

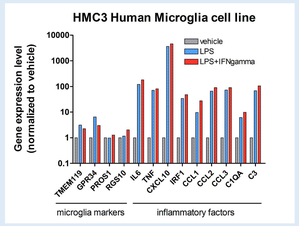
PerfusionPal supports simultaneous, long-term culture of a rat cortical model and a human liver model

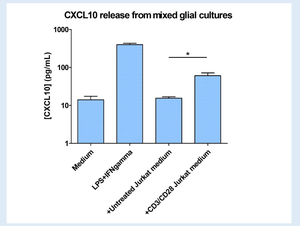


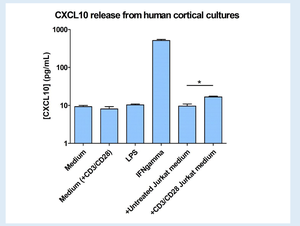


Multiple cell types can be maintained simultaneously in a single PerfusionPal system for a 4 weeks. Primary rat cortical cultures consisting of prenatally-derived neurons and postnatally-derived glial cells were cultured alongside HepaRG™ in a PerfusionPal system (6-wells each) for 28 days. Calcein AM images from this time show dense clusters of healthy cells at the nodes where fibers of the SeedEZ scaffold meet. The open structure of the scaffold permits perfused medium to flow between these clusters to deliver oxygen and nutrients more efficiently than can be achieved with diffusion alone. Scale bars: 50µm

Human cortical cultures show increased inflammatory gene and protein expression in response to pro-inflammatory stimuli



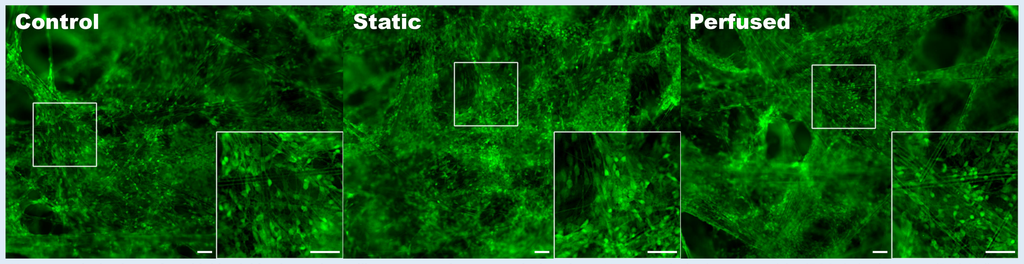




The human microglia cell line, HMC3, responds to LPS and interferon gamma stimulation with the upregulation of inflammatory factors. HMC3 cells cultured in 2D and exposed to vehicle, LPS alone, or LPS in combination with IFNγ, for 24 hours showed substantial increases in mRNA levels of key inflammatory factors, including IL-6, TNF, and CXCL10. Expression of microglia markers confirms the identity of these cells as microglia.

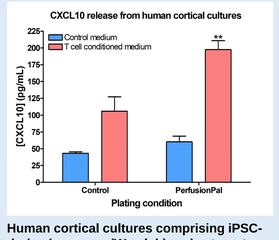
Mixed glial cultures comprising iPSC-derived astrocytes and HMC3 microglia (left) and human cortical cultures comprising iPSC-derived neurons and astrocytes and HMC3 microglia release CXCL10 in response to pro-inflammatory stimulation. iPSC-derived astrocytes and HMC3 microglia were cultured in 3D using scaffolds coated with PDL. Cultures were treated with medium alone, medium containing LPS (100 ng/mL) and IFNγ (10 ng/mL), or conditioned medium from Jurkat cells or Jurkat cells activated with CD3/CD28 antibodies for 24 hours. Complete human cortical cultures (containing in-house generated iPSC neurons) were treated with similar conditions as indicated. LPS alone does not results in significant CXCL10 release. In both experiments, medium from activated Jurkat cells significantly increased CXCL10 release. *p<0.05

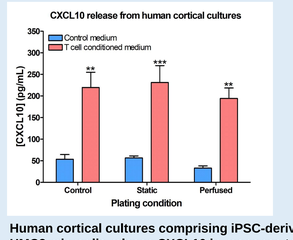
Human cortical cultures form viable, 3D tissues in SeedEZ™ and PerfusionPal™

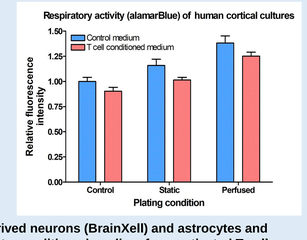


Human cortical cultures form highly viable, dense tissue-like structure after two weeks in culture. Human cortical cultures comprising iPSC-derived neurons (BrainXell, 2:1 ratio of Glutamatergic and GABAergic), astrocytes (Wen lab), and the HMC3 microglia cell line were seeded into PDL-coated SeedEZ scaffolds at a cellular ratio of 2:2:1 total neurons:astrocytes:microglia. Scaffolds were placed into a multi-well plate ("Control"), an unperfused PerfusionPal system ("Static"), or a perfused PerfusionPal system ("Perfused"). After 15 days in culture, cells were exposed to Calcein AM to assess cell viability. Without the presence of any exogenous extracellular matrix or hydrogel, the cells formed dense, 3D structures that expanded well beyond the nucleation sites where fibers crossed. Scale bars: 50µm

Culturing in PerfusionPal™ may produce more robust cortical cultures







Human cortical cultures comprising iPSC-derived neurons (Wen lab) and astrocytes and HMC3 microglia release CXCL10 in response to conditioned medium from activated T cells. CXCL10 release was increased in response to conditioned medium from human T cells (13 days in culture; 24 hour treatment). This effect was enhanced when the cultures were maintained in PerfusionPal. **p<0.01

Human cortical cultures comprising iPSC-derived neurons (BrainXell) and astrocytes and HMC3 microglia release CXCL10 in response to conditioned medium from activated T cells. CXCL10 release was increased in response to conditioned medium from human T cells (13 days in culture; 24 hour treatment). This effect was not enhanced with the addition of perfusion. Conversion of alamarBlue when cultures were maintained in PerfusionPal without or with perfusion indicates a trend toward increased cellular respiration. Both plating condition and treatment condition were determined to be significant by Two-way ANOVA. **p<0.01, ***p<0.001

CONCLUSIONS/FUTURE DIRECTIONS

- The unique human cortical cell culture model shows promise in terms of cell viability and response of individual cellular components to inflammatory stimuli.
- Secreted factors from activated T cells administered to the neural cultures elicit a measurable inflammatory response.
- A more detailed analysis of cytokine and chemokine release will be carried out.
- T cells and human cortical cell cultures will be grown simultaneously in PerfusionPal to model T cell-brain interactions.
- iPSC-derived microglia are being generated in-house to produce a more physiologically relevant response.

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