**INTRODUCTION**

Human cortical tissue can be markedly difficult to model in vitro. Issues of cellular curvature and supporting all 3D tissue elements, often yielding rapid viability and controllability. Prior cultures have been the standard method of growing with cultures. But to capture the complexities of brain microenvironments and the interactions between cells and their microenvironment is necessary. A three-dimensional (3D) culture of human neurons and astrocytes to recapitulate the complexity of the brain tissue microenvironment. Several 3D culture methods have been developed that use cell culture techniques. However, the use of primary cells in these methods requires extensive preculture, limiting their scalability and applicability.

**METHODS**

Cell cultures

Human cortical tissue derived neurons (HepG2) were maintained in glucose-supplemented DMEM containing 10% FBS, 1% penicillin-streptomycin, and 1% glutamine. Human derived astrocytes (BrainBits, J.T. Shoemaker, Shenandoah, VA) were cultured in DMEM/F-12 with 10% FBS. Human-derived microglia (HMC3) were cultured in RPMI 1640 with 10% FBS. Human-derived microglia (HMC3) were cultured in RPMI 1640 with 10% FBS. Tissue culture plates were coated with Matrigel® (BD Bio sciences). Cells were plated at a density of 10^5 cells per well in 24-well plates and allowed to adhere overnight.

**Cultures grown in PerfusionPal have increased metabolic function (respiration)**

Culture of HepG2 cells in the PerfusionPal system consistently resulted in increased metabolic activity as measured by respiration. Cultures grown in the PerfusionPal system showed 50% more alamarBlue signal than static cultures (p<0.01). Cultures grown in the PerfusionPal system showed 50% more alamarBlue signal than static cultures (p<0.01). LDH release was also significantly increased in perfused cultures (p<0.01) compared with control cultures, indicating improved cell viability presumably due to increased oxygenation.

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

**Cell source and matrix optimization for human cortical cultures**

**CONCLUSIONS/FUTURE DIRECTIONS**

- We have created a 3D model of human cortical tissue that shows high viability and tissue-like structure after two weeks in vitro.
- We have shown that the cultures are capable of responding to pro-inflammatory stimuli.
- The data suggest that the addition of blood substitute and perfusion may produce cultures with superior respiration and higher viability, though this will need to be substantiated with more rigorous assays and longer studies.
- The individual cellular components of the model will be characterized.
- The inflammatory response will be further characterized by measuring the release of other cytokines and chemokines.

**Figure Captions**

- **Figure 1:** The inflammatory response will be further characterized by measuring the release of other cytokines and chemokines.
- **Figure 2:** The individual cellular components of the model will be characterized.
- **Figure 3:** Human cortical cultures comprising PSC-derived neurons and astrocytes as well as microglia in a flexible and scalable model that models the complex microenvironment of the brain. Neurons were maintained in a perfusion system that facilitates long-term survival of 3D cultures and significantly improves metabolic function (respiration).

**Acknowledgments**

This research was supported by funds from the Marcus Foundation, The Georgia Research Alliance, and the Georgia Tech Foundation through their support of the Marcus Center for Therapeutic Cell Characterization and Manufacturing (MC3M) at Georgia Tech. This work was also funded by an NIH Phase I SBIR award (1R21NS092528-01A1), an NIH Phase II SBIR award (2R21NS092528-02) and an NSF Phase I SBIR award (1534023).