INTRODUCTION

As pharmaceutical therapies for diseases such as cancer become increasingly humanized in the development of personalized medicine, the traditional methods of preclinical testing become less relevant. Current methods of testing the safety of such compounds require the use of patient-derived xenografts in either humanized or immunocompromised animals. The former is quite costly and the latter suppresses the detection of off-target effects of the candidate drug. In vitro methods of preclinical testing offer an attractive alternative to animal use. Screening using primary cells derived from human donor tissue has the advantage of maintaining in vivo tissue characteristics, but the relatively short-term de-differentiation of these cells limits their efficacy in testing slow-clearing, large-molecule compounds. The use of cells in monolayer cultures has been effective in assessing the safety of small molecule drugs, but the disparity between cell lines and in vivo tissue biology proves too substantial for current potential therapies. Three-dimensional (3D) cultures have been shown to be more physiologically relevant models of in vivo tissue. However, even they fail to mimic living tissue and suffer from the lack of active oxygen and nutrient transport. Lena Biosciences has developed a groundbreaking in vitro system that facilitates long-term survival of 3D cultures and significantly improves cellular metabolic function (respiration).

PURPOSE

• Improve viability of 3D cell cultures
• Maintain hepatocyte function in a more in vivo-like state for improved drug metabolism
• Increase longevity of 3D cultures for use in screening slow-clearing drugs

METHODS

Cell Culture

HepG2 cells were maintained in EMEM supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C. 3D cultures were generated using Lena Biosciences’ patented SeedEZ™ scaffolds.

Cells were seeded in Matrigel® (8 mg/mL) at a density of 333,000 cells per scaffold. Cultures were allowed to gel at 37 °C for 15 minutes and subsequently transferred to Lena Biosciences’ PerfusionPal system or to a multidwell 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.

Cell Viability and Morphology Assessment

After 10 days in vitro (DIV), cultures were removed from the PerfusionPal system and were exposed to Calcein AM (in DPBS) and Hoechst 33342 nuclear counterstain 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 Optopan MicroIRF camera.

alamarBlue® Assay for Metabolic Function

After 10 DIV, alamarBlue® reagent was added to the cultures to a volume equal to 1/10 of the volume of culture medium. The cultures were returned to the incubator for a period of 2 hours after which a sample of the medium from each condition was transferred to a 96-well plate and read on a Biotek Synergy 4 plate reader for fluorescence (ex/em 545/590 nm). Data were normalized to cell number as determined by total LDH of in vivo tissue. However, even they fail to mimic living tissue and suffer from the lack of active oxygen and nutrient transport. Lena Biosciences has developed a groundbreaking in vitro system that facilitates long-term survival of 3D cultures and significantly improves cellular metabolic function (respiration).

CONCLUSIONS/FUTURE DIRECTIONS

• We have demonstrated significant improvements in cell morphology, metabolic function, and viability for 3D cultures grown in the PerfusionPal system
• Assessment of CYP activity of HepG2 cells in this system is underway
• The system will be tested using primary human hepatocytes, HepaRG cells, as well as other non-hepatic tissue types

This work was funded by an NSF Phase I SBIR award (1549126)