A Perfused 3D Cell Culture Platform for Preclinical Drug Screening

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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* characteristics, but the relatively short-term de-differentiation of these cells limits their efficacy in testing slow-clearing, large-molecule compounds. The use of cell lines 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 fully 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 ^{0}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 ºC for 15 minutes and subsequently transferred to Lena Biosciences' PerfusionPal system or to a multiwell 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 Optronics MicroFIRE camera.

alamarBlue Assay for Metabolic Function

After 10 DIV, alamarBlue reagent was added to the cultures at 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 a set of cell density standards described below.

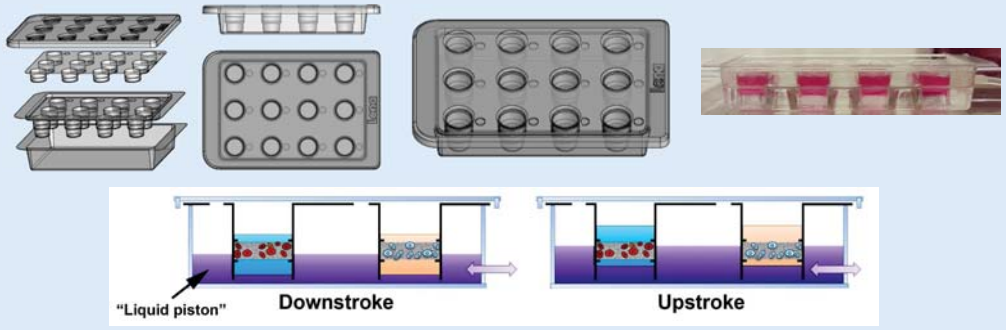
LDH Assay for Total and Released LDH

Prior to the addition of alamarBlue reagent, a sample of medium was taken to assess LDH release. After the alamarBlue assay, the cultures were lysed with Triton X-100 (1%) for 1 hour at 37 ºC for determination of total LDH. In addition, a freshly plated set of HepG2 3D cultures with densities ranging from 200,000 – 4,000,000 cells/scaffold were lysed and used to generate a standard curve to determine cell number in each culture. A sample of medium from each condition was transferred to a 96-well plate for a colorimetric LDH assay. LDH release data were normalized to cell number.

Statistical Analysis

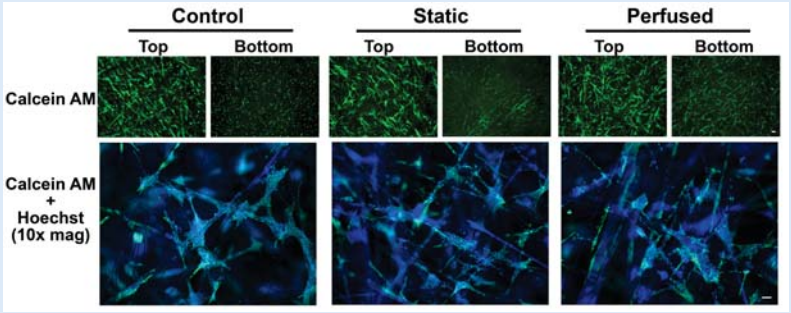
Statistical significance was determined by One-way ANOVA and Tukey's post-hoc test.

Lena Biosciences' PerfusionPal System



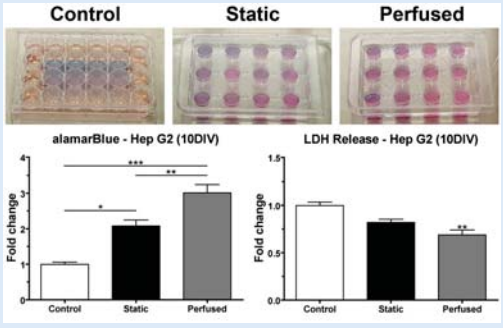
Cells seeded in 3D hydrogels supported by a rigid scaffold were maintained in statistically independent wells in their own medium which floats atop a high-density blood-substitute in which medium is immiscible. The blood-substitute readily dissolves atmospheric gases at levels which are orders of magnitude 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.

Cultures grown in PerfusionPal show improved cell morphology



At 10 days, the cells in the perfused cultures showed intense Calcein AM labeling on par with the static and control cells. Both the perfused and static cultures were differentiated from the control cultures in that cell morphology though the thickness of the 3D culture was more consistent. In a conventional culture system, SeedEZ scaffolds rest on a flat and rigid surface. The cells that come in contact with that surface, as in any Petri dish, were more spindle like. Our system (with and without perfusion) restored the cell shape to its nominal morphology and did so consistently through the culture thickness resulting in micro-engineered tissues and organs that are morphologically and anatomically more faithful surrogates of their *in vivo* counterparts. Representative fluorescence images of cultures labeled with Calcein AM and Hoechst 33342 nuclear stain show cell density and morphology after 10 DIV. Images from control (n=4), static (n=12), and perfused (n=12) cultures imaged from the top and bottom of the scaffold are shown. Scale bars: 50 µm.

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 alamarBlue assay. HepG2 cells maintained in a 12-well system over a 10-day period showed a two-fold improvement in metabolic activity over cultures maintained in a standard multiwell plate when the blood-substitute was introduced ($p<0.05$), and a three-fold improvement when the cultures were perfused ($p<0.001$). Perfused cultures showed 50% more alamarBlue signal than static cultures ($p<0.01$).

LDH release was also significantly decreased in perfused cultures ($p<0.01$) compared with control cultures, indicating improved cell viability presumably due to increased oxygenation.

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)