Validation of a 3D human liver model for predictive toxicology

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Introduction

The purpose of this project is to validate an *in vitro* human three-dimensional (3D) liver model using known hepatotoxic (liver-damaging) compounds. It was hypothesized that by using confocal, fluorescent microscopy with this 3D model, hepatotoxicity would be demonstrated by utilizing compounds known to be toxic. Until recent times, the majority of toxicological assessments were performed *in vivo*, such as animal testing. With technological advances, these newer cell models make *in vitro* toxicity testing a more viable option. Additionally, one goal of the Edgewood Chemical Biological Center is to reduce the number of animals used in toxicity testing. The development of improved *in vitro* toxicology methods contributes to this goal. Traditional cell culture methods use two-dimensional models for toxicity testing; however, new, innovative technology and the development of 3D cell culture models, such as human organoids, provide researchers with viable models for testing human toxicity (Mueller, Koetemann, & Noor, 2011). These types of methodologies could help reduce the number of animals used in traditional toxicology studies and experimental data using this *in vitro* model will be used to more accurately predict the human response to toxic compounds.

Materials and Methods

Cryopreserved HepG2 cells were thawed at 37 °C and transferred to a sterile 75 cm^2 flask in complete medium (EMEM + 10% fetal bovine serum) according to ATCC protocol. The cells were then placed into a humidified incubator at 37 °C and 5% CO_2 and subjected to medium renewal 2–3 times per week. To prepare for spheroid (3D cell colony) production, cells were trypsinized at room temperature for 10 minutes and counted using a Vi-Cell™ XR Cell Viability Cell Counter. A cell suspension at 7,000 cell/mL was then prepared in complete medium. Each well of a Corning® 96 well ultra-low attachment spheroid plate received 100 µL of this cell suspension (700 cells/well). After 4–5 days of growth (as shown in Figure 1) the spheroids were exposed to acetaminophen (APAP) at concentrations ranging from 1.25 mM to 50.00 mM for 24 hours along with a media (EMEM + 10% fetal bovine serum), positive (Digitonin), and vehicle (DMSO) control. The spheroids were stained with MitoTracker® Orange (mitochondria), Toto®-3 (dead cell), and Hoechst 33342 (nucleus). High-resolution images and quantitative data were obtained with a Thermo Cellomics Arrayscan® VTI HCS Reader using high content analysis.

Materials and Methods (cont.)

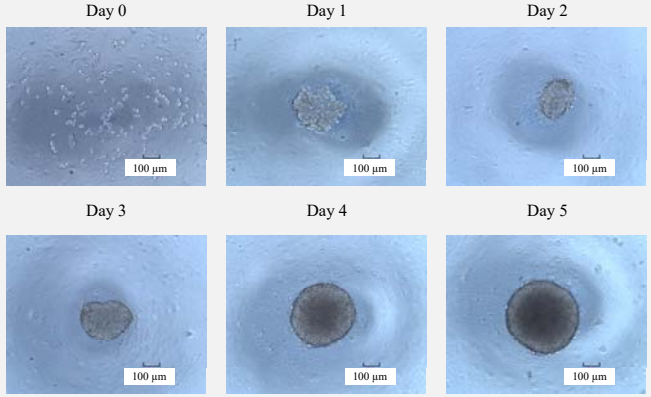


Figure 1: Progression of spheroid growth after 5 days in culture. Images were obtained with the ZEISS Axio Vert.A1 Inverted Microscope® at 10X objective.

Results

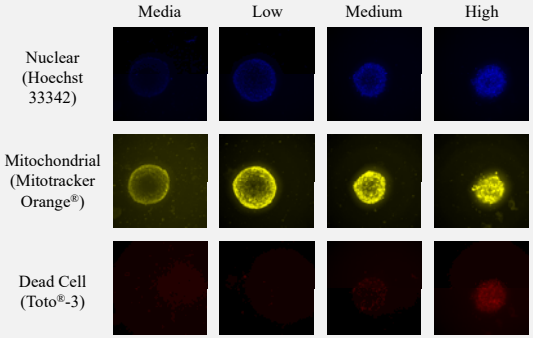
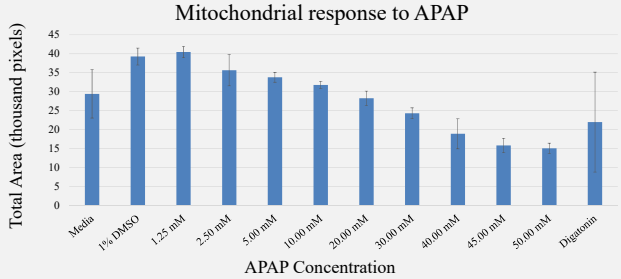


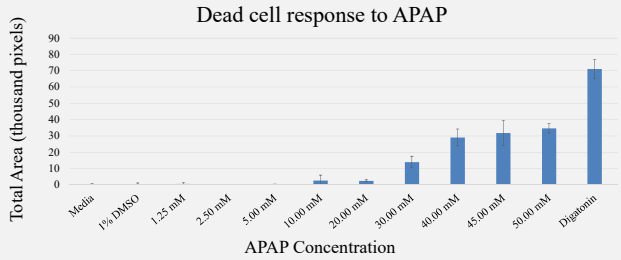
Figure 2 (left): Image montage of spheroids with nuclear, mitochondrial, and dead staining after exposure to APAP. This includes a media control and low (1.25 mM), medium (30.00 mM), and high (45.00 mM) concentrations of APAP. The average spheroid size ranged from 200–300 µm.

Exposed liver spheroids showed a dose response to increasing concentrations of the known hepatotoxic compound, APAP, after 24 hours. At high concentrations, the spheroid images were reduced in size as compared to the media control and the lower doses of APAP (as shown in Figure 2). Quantitation of these images shows a significant decrease in the total mitochondrial area as the concentration of APAP increased (as shown in Graph 1). These quantitations also show a significant increase in total dead cells as the concentration of APAP increased (as shown in Graph 2). The decrease in mitochondria and the increase in dead cells indicate that APAP is toxic to the human liver model.

Results (cont.)



Graph 1 (above): Total mitochondrial area in the images of four wells with increasing APAP concentrations. As the concentration of APAP increased, the total mitochondrial area decreased. The error bars on this graph represent one standard deviation.



Graph 2 (above): Total area of dead cells in the images of four wells with increasing APAP concentrations. As the concentration of APAP increased, the total area of dead cells increased. The error bars on this graph represent one standard deviation.

Conclusion

The results from this project demonstrate a dose response curve to APAP, a known hepatotoxic compound, using HepG2 spheroids and high content analysis. These findings suggest that this 3D human liver spheroid model, combined with high-content imaging and quantitative analysis may be a useful tool for determining human chemical toxicity. This human liver cell model may also be considered as an alternative to animal testing. Additionally, the staining and imaging techniques can be used for other 3D models.

References

Mueller, D., Koetemann, A., & Noor, F. (2011). Organotypic cultures of Hepg2 cells for in vitro toxicity studies. *Journal of Bioengineering & Biomedical Science*, *1*, S2.