

| Introduction |

Drug-induced liver injury (DILI) or injury to the liver caused by prescription or non-prescription medications continues to be a growing public health problem and a challenge for drug development. Effects can be acute or chronic and are compounded not only by the number of new drug substances but also by the growing market for herbal and other non-traditional remedies. Most DILI is the result of unexpected reactions to a particular medication that was used for an indication that was not associated with any known adverse effects. However, with the use of state-of-the-art microscopes and novel probes, DILI is detectable even when it occurs at very low doses. In vitro systems using primary hepatocytes are less costly and can be used to model potential drug-induced toxicity. Nevertheless, limitations such as high inter-individual variability, finite batch sizes and changes in cell morphology, as well as liver-specific functions during long-term culture, are challenges in this model. Human induced-pluripotent stem cells (iPSC)-derived hepatocytes, by comparison, are an promising in vitro alternative to in vivo models by demonstrating primary tissue-like phenotypes, high levels of consistency and unlimited availability.

When performing toxicity studies, hepatocytes are repeatedly dosed with varying concentrations of a potential drug over multiple days to assess any cumulative effects. This poses particular challenges when incorporating two-dimensional (2D) cell culture models due to the fact that the cells rapidly differentiate and lose metabolic activity when cultured in this manner. Three-dimensional (3D) cell culture models exist that allow cells to aggregate and retain the functionality and communication networks found in vivo. The favorable environment created by the 3D culture model then allows long-term dosing experiments to be performed that accurately assess the potential drug’s cumulative effects.

We demonstrate the suitability of 3D cultured human iPSC-derived hepatocytes for use in hepatotoxicity studies. Hepatocyte spheroids were exposed to multiple concentrations of the DILI category I and III drugs tolcapone, acetaminophen, and mitomycin C. Direct image-based assessment of hepatocyte mitochondrial health, after short-term and long-term exposure to the drugs, was performed. Comparisons were also made to iPSC-derived hepatocytes cultured in 2D.

| 2D Hepatotoxicity Testing |

When assessing the potential of a drug or its metabolites to cause DILI, it is common not only to examine the ability to induce overt cell death, but also to determine the extent of the observed hepatotoxicity. Two commonly measured mechanisms include induction of oxidative stress and superoxide formation, in addition to mitochondrial membrane potential and apoptosis. The capacity of acetaminophen, mitomycin C and tolcapone to induce oxidative stress and apoptosis, leading to downstream necrosis, following short-term and long-term treatment of 2D plated Cell Hepatocytes was determined using fluorescent microscopy-based probes (Figure 2).

Consistent with previously reported mechanisms of acetaminophen toxicity, these multiplexed fluorescent assays enabled detection of drug-induced hepatotoxicity effects following exposure to high doses of acetaminophen. Increasing acetaminophen concentrations and repetitive dosing resulted in detection of superoxide expression following a prolonged fourteen day mitomycin C treatment (Figure 5C).

| 2D Hepatocyte Image Analysis |

Quantification of superoxide expression, and induction of apoptotic and necrotic activity, was performed for all compound treatments and incubation periods using the cellular analysis features of the Gen5 software. Primary masks are placed around nuclei using the stained nuclei (Figure 3A), then secondary masks are added if the stained cell signal from the target probe exceeds the threshold values established from control well values. Finally, subpopulation criteria are set to identify cells statistically responding to compound treatments. The fraction of responding to total cells, expressed as a percentage, indicates the effect each compound treatment has on the hepatocytes in the well.

3D Hepatocytotoxicity Testing

In the same manner, the toxic effects of acetaminophen, mitomycin C and tolcapone were also examined in 3D cultured iPSC-derived hepatocytes (Figure 4). Superoxide activities, and apoptotic and necrotic activities were assessed following 1, 7, and 14 days to each compound.