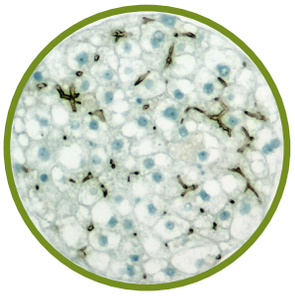
inSphero
Biomimetic Microtissue Technology



Bile canaliculi network expressing bile salt export pump (BSEP) protein (brown) and hepatocyte nuclei (blue)

3D InSight™ Service

14 Day Hepatotoxicity Testing

Take advantage of long-term repeat-dose toxicity testing in a physiologically relevant model performed by InSphero 3D experts. Our 3D InSight™ 14 Day Hepatotoxicity Testing service assesses the effect of compounds of interest on cellular viability (ATP content) in 3D InSight™ Human Liver Microtissues developed from primary human hepatocytes (PHH) in co-culture with Kupffer cells (KCs) and liver endothelial cells (LECs)

- **Predict DILI in an extensively validated model system** that achieves 2-fold higher sensitivity than 2D primary hepatocyte culture
- **Multiplex different biochemical endpoints** for more mechanistic insights
- **Increase confident decision making** with standardized, highly reproducible models and assays

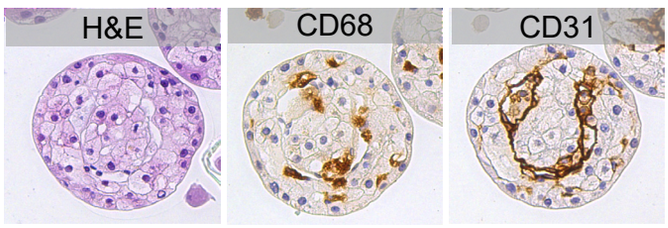


Figure 1. *Histology sections of 3D InSight™ Human Liver Microtissues (Multi-donor hepatocytes in co-culture with Kupffer cells (CD68+) and liver endothelial cells (CD31+).*

IC_{50} dose-response curves are generated following a 14-day, repeat dose exposure to compounds using cell viability (Promega CellTiter-Glo®) as an endpoint (Figure 2). This service is a valuable tool for *in vitro* DILI prediction and the evaluation of drug effects in 3D liver microtissues.

Figure 2: *Dosing protocol and workflow for 3D InSight™ 14 Day Hepatotoxicity Testing Service.*

Validated, predictive DILI Classification

With this service, we apply the same human liver model and protocol validated in a recent study conducted by AstraZeneca and Genentech (Proctor et al, 2017, Arch Toxiol). This paper confirms that 3D microtissues are two-fold more sensitive than 2D PHH culture (Figure 3) in discriminating between known hepatotoxicants and clinically safe drugs.

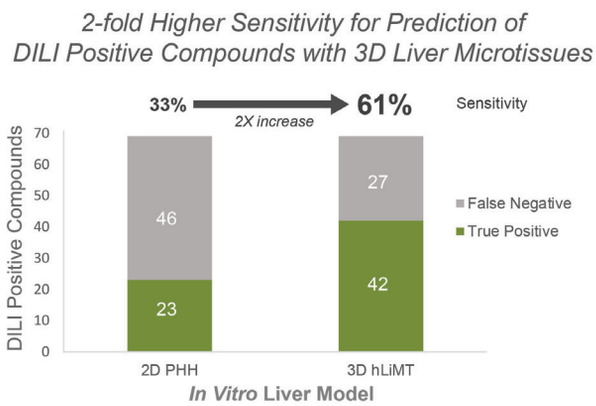


Figure 3: Procter et al. study results confirm 3D Liver Microtissues outperform 2D PHH in culture.

Multiplexed assessment of cell viability and cytotoxicity

We routinely assess cell viability assessment by intracellular ATP-content measurement after long-term compound exposures (Figure 4) as a predictive endpoint for evaluating DILI risk. Further insights into potential mode of action can be gained by multiplexing additional measurements, such as release of lactate dehydrogenase (LDH), indicating membrane leakage caused by necrotic and/or apoptotic activity. In addition, non-invasive assessment of toxic response kinetics helps distinguish acute toxicity from long-term effects. This is important because drugs showing a delayed mode of toxicity have a high risk of being missed by traditional short-term, acute toxicity tests, potentially delaying hepatotoxic liability identification.

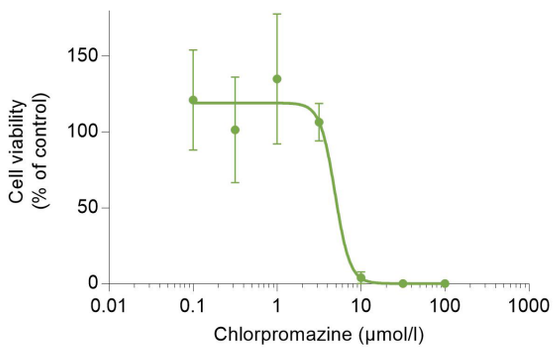


Figure 4. *Cell viability assessment after 14 days Chlorpromazine exposure with CellTiter-Glo® ATP assay (IC_{50} 4.91µL).*

Utilizing a highly sensitive membrane leakage marker assay, we can monitor supernatants of single 3D InSight™ Human Liver Microtissues for liver enzyme leakage over the exposure period. For example, the drug Chlorpromazine exhibited an IC_{50} value of 4.91 µM determined by ATP assay and showed a delayed mode of toxicity, and was toxic at 10 µM only after 5 days of exposure (Figure 4), confirming clinically observed latency periods of 1-2 weeks for liver enzyme elevations after Chlorpromazine administration.

Key Benefits

- Assess kinetics of toxic response
- Discriminate between acute and chronic toxicants
- Distinguish between metabolic versus necrotic hepatocellular injury

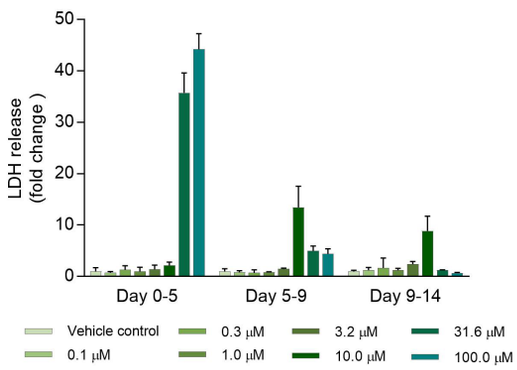


Figure 5. *Cytotoxicity monitoring of liver enzyme leakage over 14 days Chlorpromazine exposure.*

3D InSight™ 14 Day Hepatotoxicity Testing

Catalog number
SP-02-122-01

Model system
3D InSight™ Human Liver Microtissues (multi-donor hepatocytes, co-culture with Kupffer cells and liver endothelial cells)

Standard experimental setup
14 day drug exposure with cell viability endpoint at day 14

Number of dosings
3 (Days 0, 5, and 9)

Tested compound concentration
7-point dose-response curve

Positive control compound
Chlorpromazine

Endpoints

- ATP content (CellTiter-Glo®, Promega Corp.)
- Optional: LDH secretion (LDH-Glo®, Promega Corp.)
- Optional: Albumin secretion (ELISA)

Data analysis

- Cell viability dose-response curves with IC_{50} calculation and Hill slope
- Standard deviations of vehicle controls, minimum and maximum viability
- Report including material and methods, compound information, graphs, and results summary

Service Turnaround Time
3-4 weeks

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InSphero is ISO 9001:2015 certified