Use of 3D InSight™ Liver Microtissues for drug safety assessment from early hepatotoxicity screening to mechanistic investigations

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Introduction

Primary hepatocytes in 2D culture (PHM) are widely used for detection of drug-induced toxicity (DILI), however their predictivity is limited due to rapid cell differentiation enabling only testing of acute toxicity. Here we provide an overview on the application of 3D InSight™ Liver Microtissues (hLiMT) for use in DILI prediction at different stages in the drug development cycle.

The 3D hLiMT exhibited stable ATP-content, albumin secretion, formed bile canaliculi, expressed the bile salt transporters and have preserved basal and inducible cytochrome P450 (CYP) activity (CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) activity over 5 weeks in culture. We show that the hLiMT culture consists of hepatocytes and Kupffer cells in GravittyTRAP™ 96 tissue platform is a suitable model for a long-term toxicity studies at the late stage of drug development.

The toxicity of 110 marketed drugs with known DILI potential has been tested using ATP as a cell viability marker in 2D PHM and 3D hLiMT. The hLiMT culture predicts known clinical DILI drugs with more than 90% specificity and 64% sensitivity. Furthermore, the 3D hLiMT co-culture is suitable model to further investigate the molecular mechanisms involved in drug-induced toxicity. For example 3D hLiMT was used for classification of mitochondrial liability of compounds by comparing the cell viability with the Spare Respiratory Capacity (SRC) as described by Shaefer Agilent XPlnS-Flux analysis. 3D hLiMT detected the toxicity of Amiodarone, a drug known to exhibit mitochondrial liabilities 48h after treatment contrary to the non-toxic drug Ximelagatran.

Additionally we demonstrated that with three different lots of Kupffer cells, the 3D hLiMT co-culture release cytokines upon treatment with inflammatory stimuli such as LPS. Furthermore these co-cultures were able to detect the rare idiosyncratic toxicity of Influnza in the presence of PHM. Importantly, this toxicity effect was less pronounced in the absence of inflammatory stimuli.

Our data demonstrated that 3D hLiMT is a suitable model for prediction of DILI in early and late drug safety testing including mechanistic toxicological investigations.

Overview on DILI prediction paradigm and assays

Tier 1: Short-term, high-throughput toxicity testing

GravityTRAP™ 384-well tissue platform has:
- SBS standard format, which provides compatibility with advanced imaging and automated liquid handling systems
- Continuous flat glass base plate, which enables cortical and content imaging
- Black walled culture chamber, which eliminates fluorescent cross talk.
- High stable ultra-low attachment (ULA) treated growth chamber, which faciliates non-adherent MT culture for more than 2 months
- Defined 1 mm observation chamber, which simplifies MT toxicity observation and region of interest definition during microscopy applications
- Compatibility with any robotic liquid handling systems

Tier 2: Long-term, repeated dose toxicity testing

GravityTRAP™ 96-well microtissue platform

Figure 1: Application of the 3D hLiMT in early safety (384-well) and long-term, repeated dose safety testing for prediction of DILI. Mechanistic assays are performed in Tier 3, for which several model systems can be employed as well as a range of suitable assays (see table below).

Overview of the different assays to identify the mechanisms involved in DILI

Tier 3: Mechanistic toxicity assays

Example 1: Mitochondrial toxicity

Figure 2: Long-term maintenance of microtissue cell vitality, leakage and ATP activity in an extracellular matrix (ECM)-free environment (hLiMT). A) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. B) 3D hLiMT maintain high ATP activity in media containing the hepatocellular penetration blocker, C) Confluent hLiMT maintain high ATP activity in media containing the hepatocellular penetration blocker. hLiMT were treated with (black square) or without (white square) LPS (100 ng/mL) 3 days after treatment.

Figure 3: Measurement of drug-induced mitochondrial toxicity using Shaefer Agilent XPieron Analysis. A) InSight™ liver microtissue xenopus analyses shows the accumulation of intracellular ATP which was released after treatment with nefazodone (1 ng/mL). B) Long-term hLiMT co-culture model demonstrates the ability to maintain high cell viability for at least 20 days. C) 2D PHM demonstrated the ability to maintain high cell viability for at least 28 days. D) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. E) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. F) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. G) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. H) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. I) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. J) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. K) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. L) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. M) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. N) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. O) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. P) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. Q) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. R) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. S) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. T) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. U) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. V) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. W) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. X) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. Y) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. Z) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. AA) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. BB) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. CC) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. DD) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. EE) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. FF) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. GG) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. HH) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. II) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. JJ) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. KK) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. LL) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. MM) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. NN) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. OO) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. PP) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. QQ) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. RR) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. SS) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. TT) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days.UU) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days.VV) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. WW) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. XX) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. YY) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days. ZZ) 3D hLiMT demonstrated the ability to maintain high cell viability for at least 28 days.

Summary and Conclusions

- Multi-donor hLiMT exhibit stable morphology with expression of bile-canalicular transporters and presence of CBB-positive cells. The tissues have preserved basal and inducible CYP450 activity in 3D in vitro conditions comparable to 3D in vivo conditions.
- 3D hLiMT of hepatocytes in GravittyTRAP™ 384-well plate can be used for identification of DILI in early safety testing using HTS and simple experimental paradigm.
- 3D hLiMT in vitro culture containing hepatocytes and Kupffer cells in GravittyTRAP™ 96 plate platform is appropriate model to study short- and long-term drug induced toxicity. Long-term toxicity testing for 14 days enables prediction of known clinical DILI drugs with more than 90% specificity and 64% sensitivity (manifestation in preclinical).
- 3D hLiMT platform combining hepatocytes and Kupffer cells can be used to study the molecular mechanisms involved in drug-induced toxicity such as inflammation-mediated toxicity and mitochondrial toxicity.

In summary, hLiMT represent an advanced, organotypic model suited for prediction of DILI at early and late drug safety testing including mechanistic toxicology.

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