TruVivo™

All-Human 2D+ Hepatic System

Introduction

Predicting therapeutic safety and efficacy is an important part of preclinical drug development, but many of the current preclinical models have serious limitations. Animal-based testing often fails to identify drug efficacy and risk due to its inability to accurately mimic human hepatic clearance mechanisms.[1] Current *in vitro* hepatic model systems, such as traditional monoculture or sandwich cultures, lack phenotypic stability and longevity in culture, leading to loss of key hepatocellular function and architectural integrity over time.[2] Co-culture[1] or triculture[3] platforms have been introduced in which primary human hepatocytes (PHHs) are seeded with feeder cells onto fixed-sized, micropatterned plates. Though these systems may enable longer culture times, acquiring micropatterned plates with qualified hepatocyte lots can be a complex and time-consuming process, and non-human feeder cells can alter human hepatocyte functions and limit their application due to the innate background contribution to metabolism.[1,3] LifeNet Health® LifeSciences has developed TruVivo, a new, All-Human 2D+ Hepatic System for 96- and 24-well plates to help address these challenges.

TruVivo mimics the microarchitecture and basic functionality of the human liver, allowing for greater relevance and reliability. This triculture system is comprised entirely of primary human cells, including endothelial and stromal feeder cells with primary hepatocytes. The primary human hepatocytes self-assemble into multicellular colonies along with a mixed feeder cell population (Figure 1) – offering extended viability and architectural integrity, physiologically relevant albumin and urea production, and stable transporter and metabolic capacity for at least two weeks. This longevity and human relevance in culture, as well as high-throughput capability, makes TruVivo an ideal platform for drug discovery and development applications that require long-term and repeated chemical exposures.

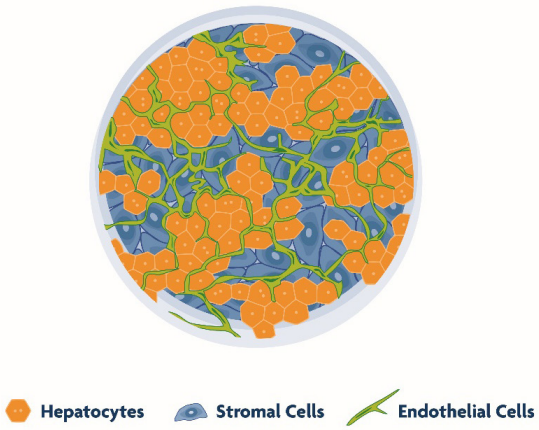

Figure 1. This schematic representation of TruVivo shows a top view of the self-assembled, multicellular hepatocyte colonies integrated among the mixed feeder cell layer.

TruVivo Mimics the Human Liver

Primary human hepatocytes cultured together with human endothelial and stromal cells in LifeNet Health's TruVivo system retain their native cuboidal morphology and self-assemble into distinct multicellular colonies with well-defined borders through two weeks (Figure 2).

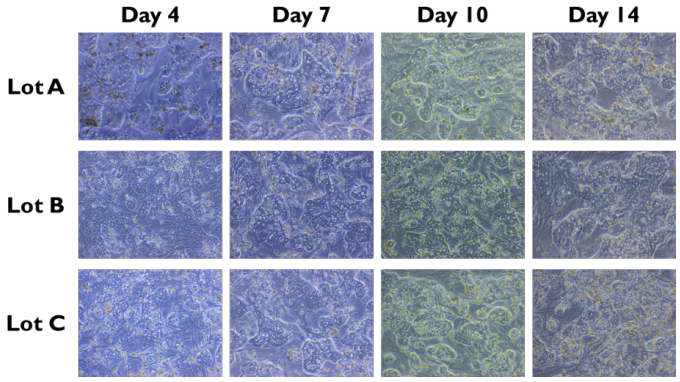

Figure 2. Representative images of PHHs cultured in TruVivo showing the retention of characteristic cuboidal morphology and self-assembly into multicellular colonies through 14 days in culture across three different lots, mimicking native hepatic organization. Magnification 10x.

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Within these hepatocyte colonies, the PHHs form cell-cell interactions and junctional complexes, as indicated by positive staining for CX-32 and ZO-1, which are markers for gap junctions and tight junctions, respectively (Figure 3). Extensive functional bile canalicular networks form between the PHHs, the extent of which increases over time through 14 days in culture (Figure 4). Collectively, these results demonstrate that PHHs in TruVivo self-assemble and mimic native human hepatocyte properties.

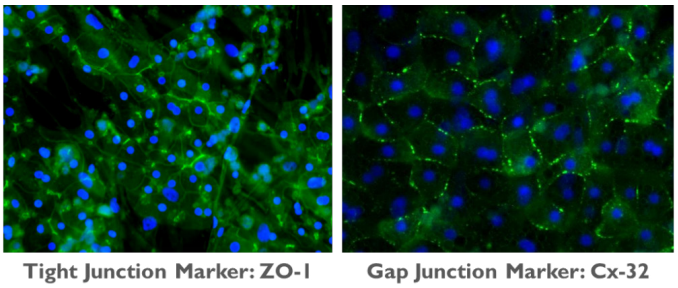

Figure 3. Representative images of PHHs forming native cell-cell interactions in TruVivo, as indicated by tight junction (ZO-1) and gap junction (CX-32) staining, supporting the retention of native hepatocyte polarity. Staining with DAPI (blue) shows cell nuclei. Magnification 10x.

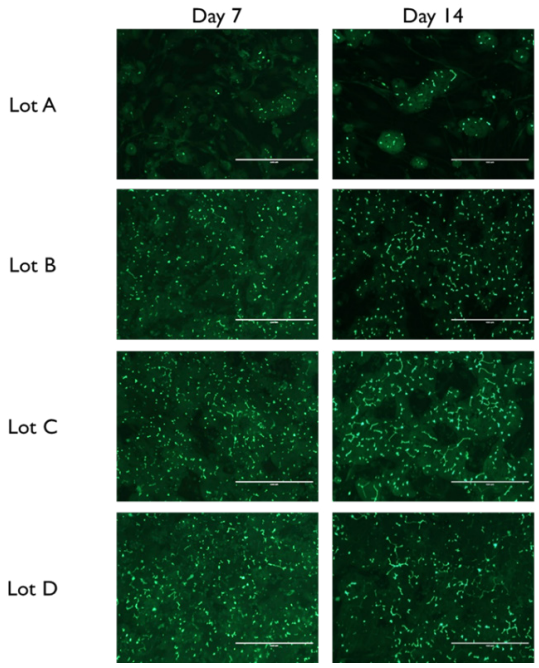

Figure 4. PHHs cultured in TruVivo form functional bile canaliculi between the PHHs, as evidenced by CDFDA staining (green), which increased through 14 days in culture. These results support the retention of physiologic hepatocyte polarity and function. Staining with DAPI (blue) shows cell nuclei. Magnification 10x.

Further, robust expression of key liver-specific proteins was detected in PHHs cultured in TruVivo through at least 14 days, including albumin and CYP1A2 (Figure 5). Additionally, CK-18, an epithelial cytoskeletal marker protein, was likewise detected through 14 days, and highlights the clearly defined borders of the hepatocyte colonies. Collectively, these results demonstrate the retention of native hepatic protein expression in PHHs through 14 days of culture in the triculture system.

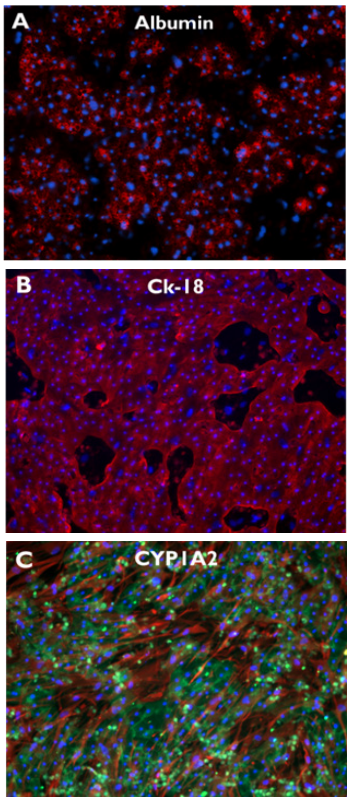

Figure 5. PHHs in TruVivo show robust expression of liver-specific proteins through at least 14 days in culture, including albumin (red) (A), CK-18 (red), an essential hepatic cytoskeletal protein (B), and CYP1A2 (green) (C), supporting retention of the hepatic phenotype. Red staining in panel C shows vimentin expression in the FCs. Staining with DAPI (blue) shows cell nuclei in all panels. Magnification 10x.

TruVivo Maintains Human Hepatic Functionality and Culture Longevity

As a further measure of hepatocyte phenotype, native hepatocyte function of PHHs in the TruVivo system was evaluated by measuring urea and albumin secretion levels, which were shown to be within physiologic levels of 56-159 and 37-105 µg/day/10^6 hepatocytes, respectively, as reported previously[4] (Figure 6). Notably, these physiologically relevant levels were stable and consistent through a minimum of 14 days in culture and across multiple lots.

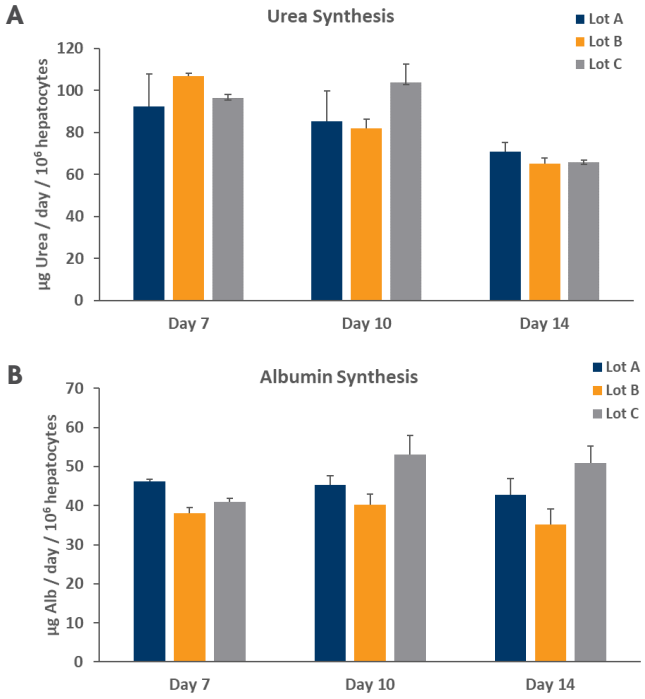

Figure 6. Urea (A) and albumin (B) synthesis from three different lots of PHHs cultured in TruVivo consistently remain within physiologic levels of 56-159 and 37-105 µg/day/10^6 hepatocytes, respectively, as reported previously,[4] through 14 days in culture. These results indicate retention of native hepatocyte function over time with low variability. Mean ± SD.

Members of the Cytochrome P450 (CYP450) enzyme family are responsible for the liver's metabolism of the majority of known therapeutic drugs.[5] Drug-drug interactions due to receptor-mediated increases in CYP expression can be an important consequence of exposure to receptor agonists. Therefore, the stability of the induction capacity of PHHs cultured in the TruVivo system versus traditional sandwich cultures was determined by evaluating the induced activity of key CYP450 enzymes. For PHHs cultured in the TruVivo system, there was a significant increase in induced levels of CYP1A2, CYP2B6, and CYP3A4 compared to uninduced levels, with no detectable background signal from feeder cells, and in induced CYP1A2 and CYP3A4 levels in PHHs in sandwich cultures (Figure 7). Notably, the induced levels of CYP3A4 were similar in both culture models, whereas the induced levels of CYP1A2 and CYP2B6 were significantly higher in TruVivo compared to those in the sandwich culture. The induced CYP3A4 activity in PHHs in TruVivo was very stable and reproducible across multiple lots through 16 days in culture. Therefore, PHHs cultured in TruVivo maintain their metabolic capacity and response to inducers that is higher and more stable over time compared traditional sandwich cultures.

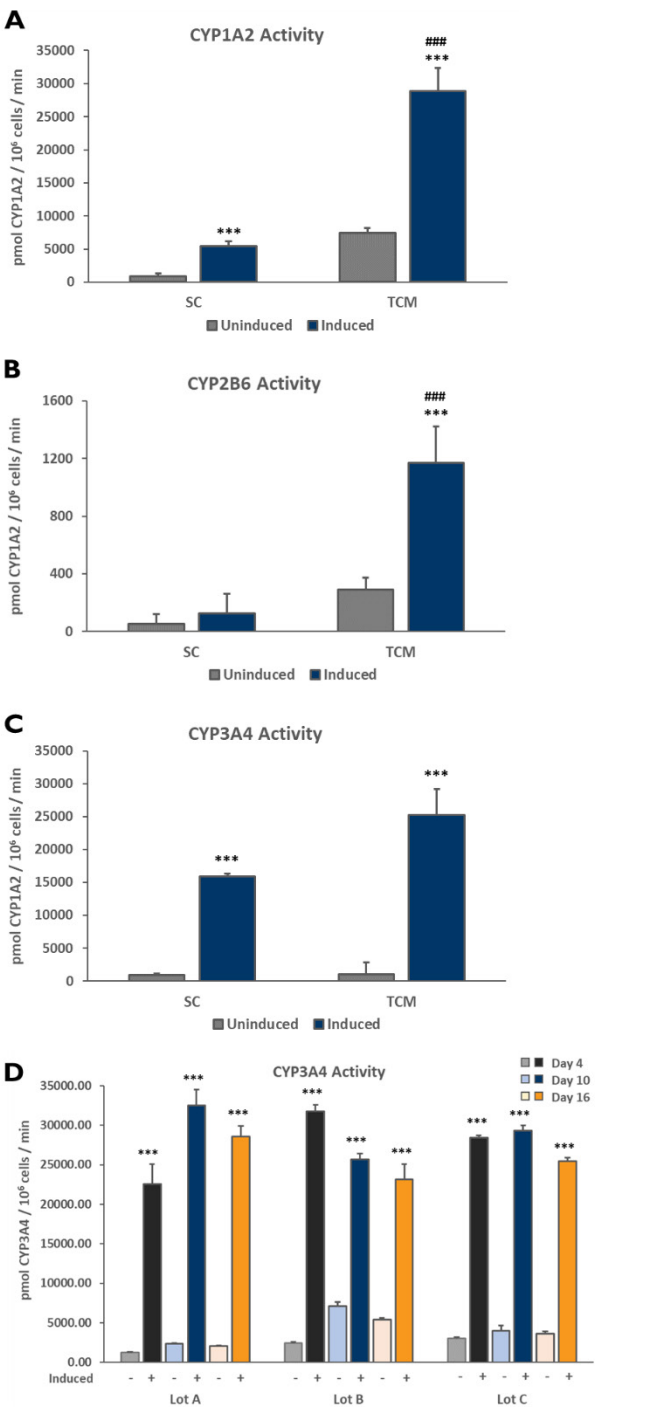

Figure 7. Induced activity levels of CYP1A2 (A), CYP2B6 (B), and CYP3A4 (C) in PHHs cultured in either TruVivo (TCM) or sandwich cultures (SC) on day 4. In each model, PHHs were treated with either 100µM omeprazole, 100nM CITCO, or 25µM rifampicin to induce CYP1A2, CYP2B6, or CYP3A4, respectively, for 48 hours prior to enzyme detection. *** p≤0.001 versus uninduced samples in the same model; ### p≤0.001 versus induced values in the SC. D) Three different lots of PHHs were cultured in TruVivo and uninduced (light shaded bars) and induced (dark shaded bars) CYP3A4 activity levels were measured on days 4, 10, and 16. *** p≤0.001 versus uninduced samples at same time point. Mean ± SD. These results indicate retention of metabolic activity in PHHs cultured in TruVivo that is stable over time and significantly greater than in sandwich cultures.

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Conclusion:

LifeNet Health's all-human TruVivo system mimics the microarchitecture and functionality of the human liver, offering a more physiologically relevant *in vitro* liver model versus alternative *in vitro* and *in vivo* models. By combining primary human hepatocytes with human endothelial and stromal cells, TruVivo effectively maintains viability and hepatic functionality over long-term culture. LifeNet Health's all-human TruVivo system represents a promising new analytical tool for chemical screening under more physiologic conditions.

Features and benefits include:

- Maintains physiologic hepatocellular morphology and architecture, and self-assembles into hepatocyte colonies, mimicking native hepatocytes
- Maintains viability and physiologically-relevant hepatic protein expression
- Maintains native hepatocyte functionality, metabolic capacity, and integrity long-term
- Allows for experiments evaluating metabolic clearance of low-turnover compounds and potential drug/metabolite hepatotoxicity
- Enables prolonged chemical exposures or re-exposures, as necessary, through extended culture
- Combines the simplicity and flexibility of a 2D model, with the relevance, longevity, architectural integrity, and robustness of a 3D model

References

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68-40-339.01

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