Micropatterned Primary Hepatocyte Co-Cultures for Drug Metabolism and Toxicity Studies

Amada Moore, Chitra Kanchagar, Stacy Krzyzewski, Jeanemarie Gaffney, Julienne Shi, Jack McGeehan and Salman R. Khetani
Hepregen Corporation, Medford MA

Abstract

Primary hepatocytes display a precipitous decline in phenotypic functions when cultured in a sandwich of extracellular matrix proteins (i.e. collagen, Matrigel). We describe a human liver model, Hepregen™, with precise microscale architecture and optimal cell/substrate interactions (micropatterned co-cultures) that display stable functions for several weeks in vitro. Micropatterned co-cultures were coupled with micromanipulation strategies (i.e. 24- and 96-well format) and optimized for the screening of genotype-specific and clinically relevant drug disposition. CYPs are also assessed here. We have investigated the toxicity of several hepatotoxic drugs (i.e. Thalidomide, Indomethacin) and observed some drug toxicity on cell viability, cell morphology, and gene expression. Furthermore, drug-induced changes in gene expression have also been detected and these micropatterned co-cultures are able to recapitulate clinical outcomes. In the future, micropatterned micromanipulated co-cultures may find utility in the development of novel compounds (drugs, biologics), in evaluating the potential of environmental toxicants, in fundamental investigations of liver physiology, and in personalized medicine for liver disease.

Introduction

Drug induced liver injury (DILI) is a leading cause of pre-launch and post-market attrition of pharmaceutical compounds (3–4). The gold standard for toxicological evaluation of new drug candidates, however, presents species varieties between rodents and humans that can be significant, especially in liver-specific metabolic pathways (i.e. CYP450s). This severely limits the utility of animal models for predicting human-specific responses (5). Isolated primary human hepatocytes in adherent culture are widely considered to be the most suitable for in vitro testing. They are relatively simple to use and maintain an intact cellular architecture with complete, undamaged cytoplasmic and nuclear compartments. Cultured model systems utilized for industrial safety/toxicology screening require hepatocytes in human-derived Matrigel™ and/or collagen (sandwich culture). When utilized with near confluent monolayers, these models allow better retention of hepatocyte cytoarchitecture and ultrastructure (6). However, sandwich cultures are inherently limited in their metabolic functions and their short-term functionality does not allow for chronic drug metabolism and toxicity to be measured. Indeed, the current sensitivity of sandwich cultures, even with highly sensitive high content imaging readouts, is estimated to be approximately 10-60% (7). Furthermore, sandwich cultures are notoriously difficult to scale down to 24- and 96-well formats, well-suited for medium-to-high throughput screening. This is due to instability of the overlying gel, and heterogeneity in monolayer confluence and cellular viability across the well bottom, especially noticeable at low cell densities. Accordingly, there is a need for better in vitro models of primary human hepatocyte viability and functionality. In order to address this need, we have developed Hepregen™, a cell-based platform that can provide a more predictive approach to preclinical drug screening. Hepregen™ hepatocytes retain their in vivo-like morphology, express liver, metabolic, and microvascular functions active in vivo, and demonstrate drug metabolism and transport, as well as drug interaction with liver cells in vivo (Figures 2, 3). Furthermore, Hepregen™ allows for culture of multiple cell types with respect to magnitude and longevity of liver-specific functions (11). Hepregen™ is therefore a versatile tool and useful model for studying human drug metabolism in vivo, and complex drug interaction studies.

The HepaPac™ Platform

Randomized Distributed Pure Human Hepatic Cells

Minicellular Hepatocytes

Hepatic Micropattern

Efficiency

Safety

Efficacy

Cellular Imaging

Drug Discovery

Inflammation

Genetics

Membrane Transport

Nanomaterials

Pharmacology

Proteomics

Metabolites

Microarrays

Hierarchical Clustering

Gene Pathways

Results

1. HepaPac™ demonstrates two short- and long-term enzyme induction. HepaPac’s ability to be induced with clinically-relevant results, along with its longevity and stable enzymatic functions, make it a potentially useful tool for CYP studies.

2. Drug metabolism is observed in a microtiter plate format.

3. The HepaPac™ model outperforms in vivo bile production and can be used for drug metabolism and transport studies.

4. Drug metabolism is observed in vivo in a microtiter plate format.

5. The HepaPac™ model outperforms in vivo bile production and can be used for drug metabolism and transport studies.

6. The HepaPac™ model outperforms in vivo bile production and can be used for drug metabolism and transport studies.

Conclusions

• Transporter-Mediated Drug Uptake and Biliary Efflux: HepaPac™ increases the accuracy and predictive power of in vivo uptake and efflux studies compared to traditional in vitro assays, thereby reducing the number of compounds needed through the development pipeline. The clinical accuracy of HepaPac™ is further demonstrated by its ability to predict drug interaction outcomes.

• Metabolite Identification: Long-term incubations in HepaPac™ have shown the production of 75-80% of clinically-relevant metabolites as opposed to less than 5% in traditional model systems, including suspension hepatocytes, 96-well plates, and isolated human liver biopsies. The HepaPac™ model has been used to predict drug interactions, thereby increasing the confidence of candidate selection moving forward in the drug development process.

• Effortless Assay: HepaPac™ has been used and endorsed for the prediction of potential drug candidates that target the liver for diseases such as Hepatitis C and diabetes. The unique ability of HepaPac™ to interpret other factors of drug metabolism is currently under investigation.

References


Figure 1. The HepaPac™ platform mimicked as an industry-standard multiwell format (96-well format shown here).

Figure 2. Long-term functionality of human HepaPac™. Enzyme activities are retained in HepaPac™ over several weeks of culture for two separate hepatocyte donors. HepaPac™ displays high levels of albumin secretion and urea synthesis over several weeks of culture.

Figure 3. HepaPac™ transports/drug-mediated hepatic uptake and biliary efflux. Radioactive compounds [85]-Guanethidine and [14C]Tetracaine were incubated with either HepaPac™ or sandwich cultures. The cells were lysed and the amount of radioactive compound in the cell was measured using scintillation counters. HepaPac™ shows higher transport and no uptake, biliary excretion and efflux than sandwich cultures. A robust bile acid output from HepaPac™ in shown using a fluorescent dye excreted via MRP2 into the bile canaliculi.

Figure 4. HepaPac™ predicts clearance rates of high, medium, and low turnover compounds. HepaPac™ from a single donor, was incubated with 13 drugs of varying in vivo clearance rates for up to 7 days without a media change. The calculated clearance rates were plotted against observed clinical clearance velocities in the literature. Susceptible hepatocytes showed little to no turnover of the medium and low turnover compounds in a standard 6-24 h clearance assay. HepaPac™’s longevity, physiologically-relevant metabolism, and transporter function allow for a more accurate prediction of in vivo clearance.

Figure 5. HepaPac™ produces more clinically relevant major metabolic systems than traditional model systems. HepaPac™ was incubated with 27 drugs for up to 7 days without a media change. The incubation time was limited to 4h in the traditional model systems due to their inherently short lifetime. Primary and secondary reactions were generated in both systems and identified with labeled CYPs/MS/MS analysis. The longevity and stable enzymatic function of primary human hepatocytes in HepaPac™ allow for the identification of clinically-relevant liver metabolites that are missed in traditional systems.

Figure 6. HepaPac™ demonstrates short- and long-term enzyme induction. HepaPac’s ability to be induced with clinically-relevant results, along with its longevity and stable enzymatic functions, make it a potentially useful tool for CYP studies.

Figure 7. Application of bulk viability assays to HepaPac™. (A) Improved sensitivity of bulk viability assay compared to sandwich culture. (B) Improved specificity. Primary hepatocytes from a single donor were incubated with either HepaPac™ or sandwich cultures over 5 to 5 days. HepaPac™ outperforms sandwich cultures in terms of cell viability and functionality whereas sandwich cultures show standard bulk assays (ATP levels, total glutathione, etc.) and cellular activity.

Figure 8. HepaPac™ is compatible with high-content imaging readouts. These high-content imaging readouts can be quantified with automated scanners. Both fixed and live cell cultures can be used to assess the potential of HepaPac™ and HepaPac™ High Content imaging.