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

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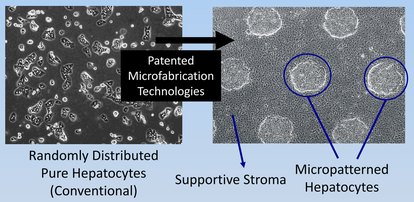
## Abstract

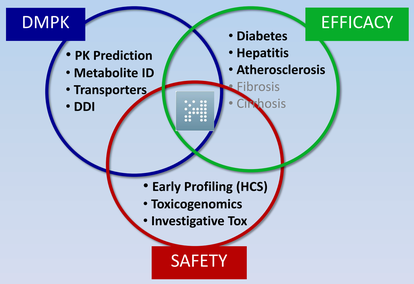
Primary hepatocytes display a precipitous decline in phenotypic functions when cultured in a sandwich of extracellular matrix proteins (i.e. collagen, Matrigel). We describe a human liver model, HepatoPac ™, with precise microscale cyto-architecture and optimal stromal interactions (micropatterned co-cultures) that displays stable functions for several weeks *in vitro*. Micropatterned co-cultures were coupled with miniaturization strategies (i.e. 24- and 96-well format) and optimized for the screening of genotype-specific and clinically-relevant drug disposition. DILI can also be assessed using standard end-points (i.e. ATP depletion, mitochondrial activity). We have investigated the toxicity of several hepatotoxins (i.e. Trazodone, Isoniazid, Imipramine etc. ) in our model under both acute and chronic dosing regimens and show concordance with preclinical and clinical findings. Since metabolism is an important determinant to the overall disposition of drugs and the profile of metabolites can have an impact on efficacy and safety, the utility of micropatterned co-cultures for prediction of compound clearance and generation of human metabolites was evaluated. Micropatterned co-cultures classifies compounds based on rates of clearance and generated metabolites arising from both phase 1 and 2 reactions (single and sequential). Transporter assays (uptake and efflux) could be performed on micropatterned co-cultures towards simultaneous assessment of the interplay of drug transport, metabolism, and toxicity. Long-term drug-drug interaction studies (i.e. enzyme induction and inhibition) have also been explored and results indicate that micropatterned co-cultures are able to recapitulate clinical outcomes. In the future, miniaturized micropatterned co-cultures may find utility in the development of several classes of therapeutic compounds (drugs, biologics), in evaluating the injury 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 substances are whole rodent models; however, species-specific variations between rodents and humans can be significant, especially in liver-specific metabolic pathways (i.e. CYP450). 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, undisrupted enzymes and cofactors [6]. Conventional culture models utilized for industrial ADME/Tox screening expose hepatocytes to tumor-derived Matrigel and/or collagen-I gels (sandwich cultures). When utilized with near confluent monolayers, these models allow better retention of hepatocyte cyto-architecture and activities of specific CYP450s for 3-5 days [6]. However, sandwich cultures are inherently unstable in their phenotypic 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 50-60% [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 overlaying gels, and heterogeneity in monolayer confluence and cellular viability across the culture well, becoming especially noticeable at well edges. Accordingly, there is a need for better *in vitro* models of primary human liver tissue that are more predictive of clinical outcomes and can be used with existing industrial automation for high-throughput screening in industry-standard multi-well formats. We have utilized microtechnology tools to both optimize and miniaturize in a multi-well format (up to 96-well) an *in vitro* model of the human liver called HepatoPac [1] **(Figure 1).** Specifically, human hepatocytes are organized into colonies of prescribed, empirically-optimized dimensions and subsequently surrounded by supportive non-parenchymal cells. Hepatocytes in HepatoPac retain their *in vivo*-like morphology, express liver genes, metabolize compounds using active Phase I/II drug metabolism enzymes, secrete diverse liver-specific products, and display functional bile canaliculi for 4-6 weeks *in vitro* **(Figures 2,3,5).** Furthermore, HepatoPac outperforms conventional culture models with respect to magnitude and longevity of liver-specific functions [1]. Animal versions (rat, mouse and monkey) of HepatoPac are under development for refining and reducing live animal testing. The data generated so far indicates that the platform is compatible with both human and animal hepatocytes commonly used in preclinical testing.

### The HepatoPac™ Platform




### Miniaturized HepatoPac™

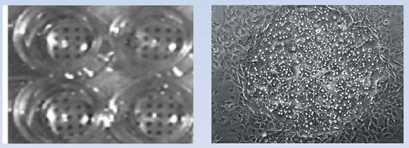
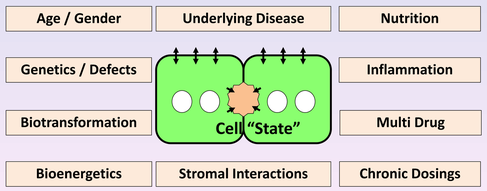


**Figure 1.** The HepatoPac platform miniaturized into an industry-standard multi-well format (96-well format shown here).




**HepatoPac Allows for Modeling of Chronic Microenvironmental Factors.** Stable liver models may allow for modeling of environmental and genetic factors *In Vitro* to understand mechanisms underlying acute and chronic toxicity.

## Results

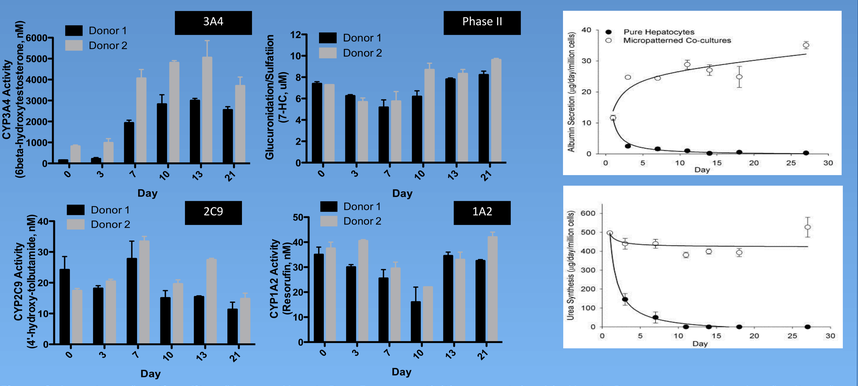
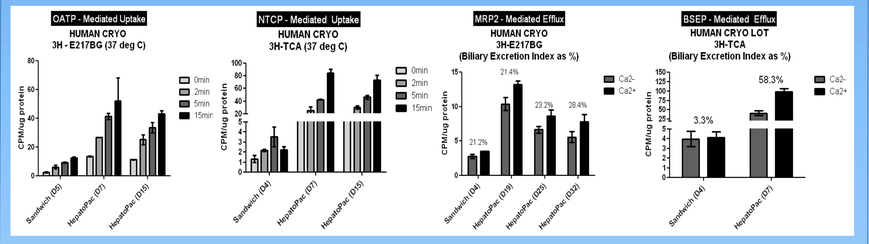
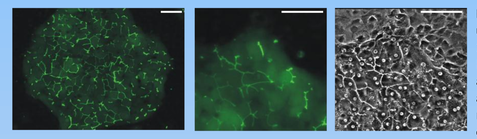



**Figure 2. Long-term functionality of human HepatoPac.** Enzyme activities are retained in HepatoPac over several weeks of culture for two separate hepatocyte donors. HepatoPac displays high levels of albumin secretion and urea synthesis over several weeks of culture.



CDF (5-and-6-carboxy-2',7'-dichlorofluorescein diacetate)

**Figure 3. HepatoPac demonstrates transporter-mediated drug uptake and biliary efflux.** Radiolabeled compounds (Estradiol 17β-Glucuronide and Taurocholic Acid) were incubated with either HepatoPac or sandwich cultures. The cells were lysed and the amount of radioactive compound in the cells was measured using a scintillation counter. HepatoPac shows higher transporter-mediated uptake and efflux than sandwich cultures. A robust bile canaliculi network forms in HepatoPac as shown using a florescent dye exported via MRP-2 into the bile canaliculi.

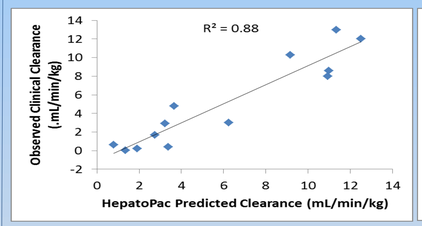



**Figure 4. HepatoPac predicts clearance rates of high, medium, and low turnover compounds.** HepatoPac from a single donor, was incubated with 13 drugs of varying *in vivo* clearance rates for up to 7 days without a media change. The calculated clearance rates were plotted against observed clinical clearance values found in the literature. Suspension hepatocytes showed little to no turnover of the medium and low turnover compounds in a standard 4-8hr clearance assay. HepatoPac's longevity, physiologically-relevant metabolism, and transport function allow for a more accurate prediction of *in vivo* clearance.

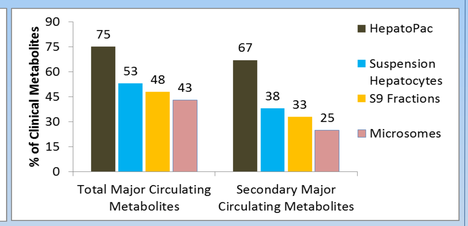



**Figure 5. HepatoPac produces more clinically relevant major metabolites than traditional model systems.** HepatoPac was incubated with 27 drugs for up to 7 days without a media change. The incubation time was limited to 4hrs in the traditional model systems due to their inherently short lifetime. Primary and slowly generated secondary metabolites were identified in the collected supernatant by LC-MS/MS analysis. The longevity and stable enzymatic function of primary human hepatocytes in HepatoPac allow for the identification of clinically-relevant liver metabolites that are missed in traditional systems.

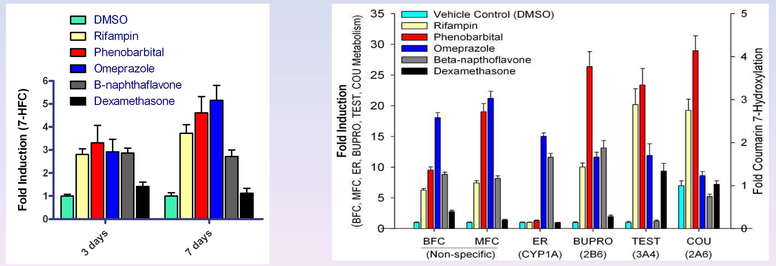
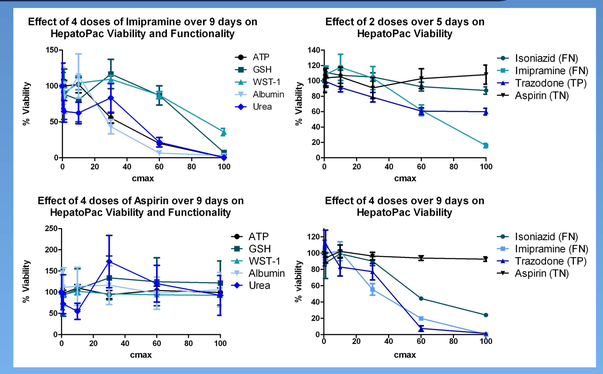



**Figure 6. HepatoPac demonstrates both short- and long-term enzyme induction.** HepatoPac's ability to be induced or inhibited with clinically–relevant results, along with it's longevity and stable enzymatic functions, makes it a potentially useful model for DDI studies.

**Figure 7. Application of bulk viability assays to HepatoPac.** HepatoPac improves the sensitivity of toxicity screening as compared to sandwich cultures while maintaining high specificity. Primary human hepatocytes from a single donor were subjected to 2 to 4 doses over 5 to 9 days in HepatoPac or 2 doses over 3 days in sandwich cultures. Hepatocyte functionality and viability were assessed using standard bulk assays (ATP levels, total glutathione content, metabolic activity, albumin and urea levels). Sensitivity was calculated as the ratio of TP (true-positives with respect to clinical observations) and the sum of FN (false negatives) and TP, while specificity was calculated as the ratio of TN (true-negatives) and the sum of FP (false positives) and TN.



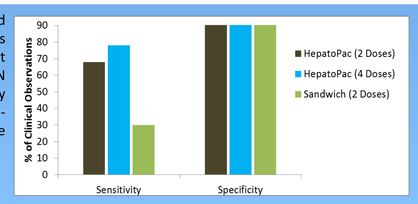
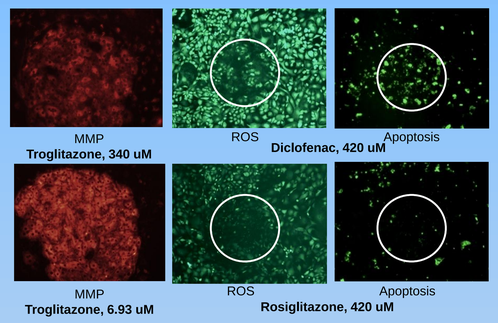




**Figure 8. HepatoPac is compatible with High Content Imaging.** High content imaging readouts can be quantified with automated scanners. Both fixed and live cultures can be imaged. Specific algorithms are used to gate out the stromal cells. High content imaging may provide higher sensitivity than "conventional" assays. Please see Abstract# 2511 and Poster# 103 for more information on HepatoPac and High Content Imaging.




## Conclusions

• **Transporter-Mediated Drug Uptake and Biliary Efflux:** HepatoPac increases the accuracy and predictive power of *in vitro* uptake and efflux studies compared to traditional systems due to the presence of transporters on primary hepatocytes in HepatoPac combined with an extensive bile canalicular network **(Figure 3)**. Furthermore, retention of both metabolic capacity and transporter functions over several weeks allows assessment of drug as well as metabolite transport, whereas in sandwich cultures metabolic capacity declines asynchronously with transporter functions. Greater than 90% of cryopreserved human hepatocyte lots show robust transporter-mediated uptake and efflux in HepatoPac as compared to less than 50% in sandwich cultures.

• **Clearance Predictions**: HepatoPac allows for more accurate determination of drug dosing regimens *in vivo*, even for low turnover compounds, thereby reducing risk as compounds move through the development pipeline **(Figure 4).**

• **Metabolite Identification**: Long-term incubations in HepatoPac have been shown to produce 75-80% of clinically-relevant metabolites as opposed to less than 55% in traditional model systems, including suspension hepatocytes, S9 fractions and microsomes [8] **(Figure 5).**

• **Drug-Drug Interactions:** Both short- (1-3 days) and long-term (4-14 days) enzyme induction and inhibition studies can be performed with HepatoPac with clinically-relevant results **(Figure 6).** Furthermore, a clinical DDI study can be potentially modeled with HepatoPac towards determining effects on clearance and toxicity of co-administered compounds. Thus, assessing metabolism and toxicity in the same model.

• **Preclinical Toxicity Screening and Mechanistic/Investigative Toxicology:** Through increased sensitivity relative to traditional methods (75-80% versus 50-60%), HepatoPac decreases the false negative rate and increases the true positive rate of toxic compound identification *in vitro*, thereby increasing the confidence of candidate selection moving forward in the drug development process **(Figure 7)**.

•**Efficacy Assessment:** HepatoPac has been used in efficacy assessment of potential drug candidates that target the liver for diseases such as Hepatitis C and diabetes [10-11]. Utility of HepatoPac for other types of diseases is currently under investigation.

## References

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