

# IN VITRO MODELING OF CYTOKINE- DRUG INTERACTIONS USING MICROPATTERNED CO-CULTURES OF PRIMARY HEPATOCYTES AND KUPFFER MACROPHAGES



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## INTRODUCTION

Drug induced liver injury (DILI) is a major health problem in the United States and accounts for the majority of clinical holds and postmarketing use restrictions by the FDA. The majority of adverse liver reactions are idiosyncratic and their underlying mechanisms are still not well understood. Better predictive models for iDILI would enable the preclinical elimination of drug candidates with hepatotoxic liabilities.

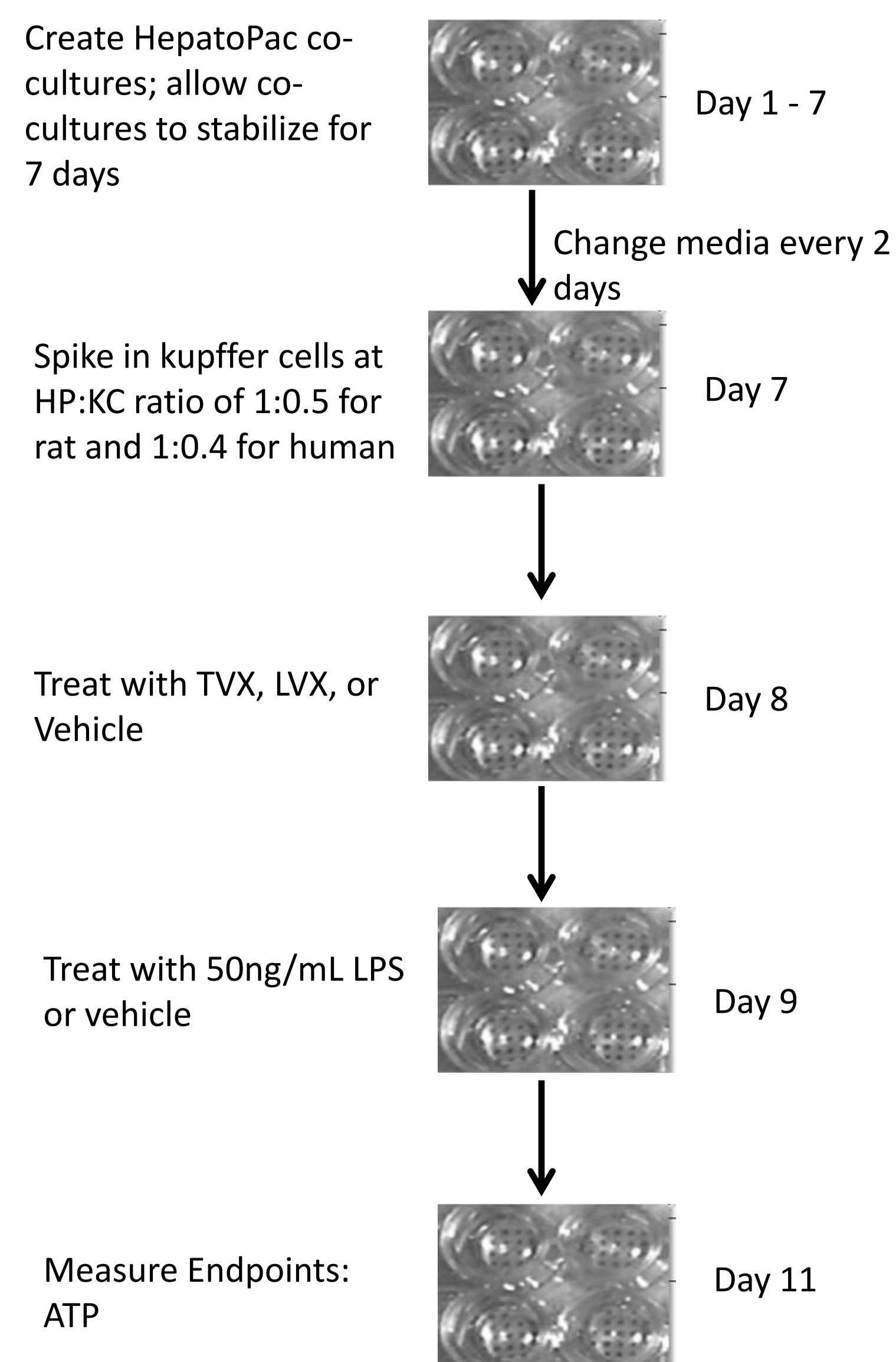
We have previously developed a model in which primary hepatocytes (rat, human, dog, or monkey) are seeded onto ECM-coated domains of optimized dimensions and subsequently co-cultivated with murine embryonic fibroblasts (HepatoPac™). Hepatocytes in HepatoPac retain their *in vivo*-like morphology, express a complete complement of liver-specific genes, metabolize compounds using active Phase I/II drug metabolism enzymes, secrete diverse liver-specific products, and display functional bile canaliculi for several weeks *in vitro*.

Here, we supplement the HepatoPac co-cultures with primary Kupffer macrophages for use in evaluating cytokine- drug interactions. Drug- induced stresses may interact with cytokine signalling to cause hepatotoxicity. Previous work using an *in vivo* mouse model demonstrated that LPS-induced inflammatory stress rendered trovafloxacin (TVX), but not its non-toxic analog, levofloxacin (LVX), hepatotoxic by a TNF- $\alpha$ -mediated mechanism. Using TVX as a prototype compound, we evaluated the ability of our HepatoPac- Kupffer cell co-culture model to detect drug/ cytokine hepatic toxicity synergies.

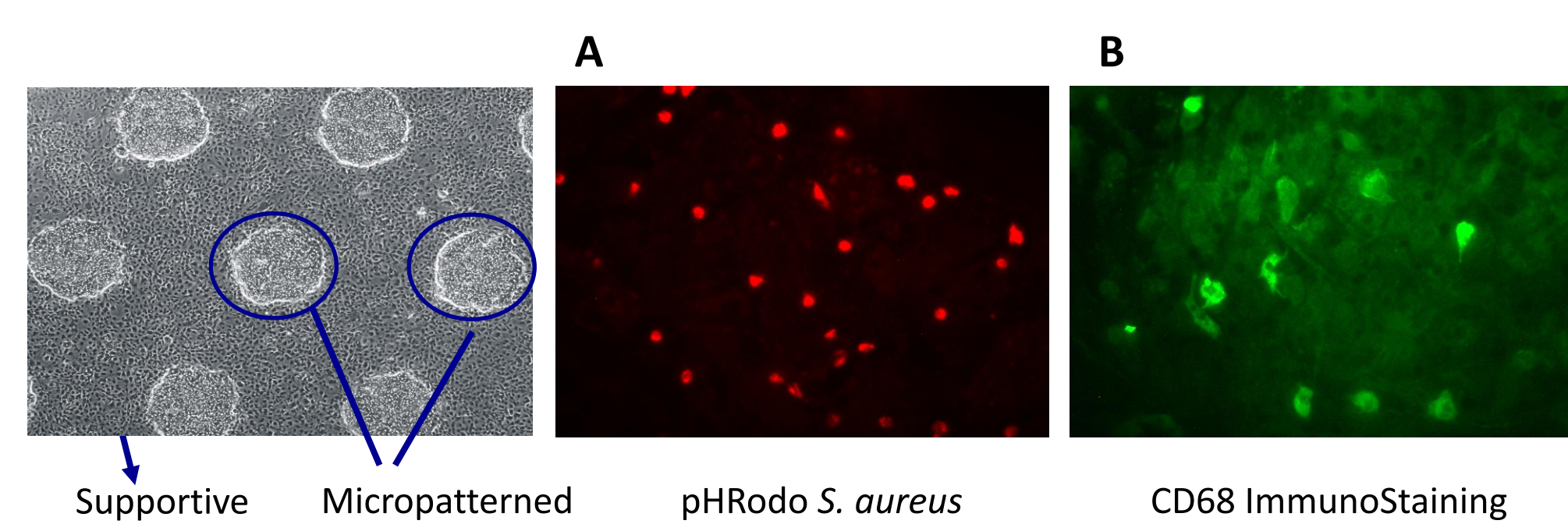
## MATERIALS & METHODS

Cryopreserved human hepatocytes were obtained from Celsis In Vitro Technologies. Cryopreserved rat hepatocytes, rat kupffer cells, and human kupffer cells were obtained from Life Technologies. Cytokine ELISA kits were purchased from BD Biosciences while pHrodo™ *S.aureus* Bioparticles were obtained from Life Technologies. Trovafloxacin, Levofloxacin, Pentoxifylline, and Lipopolysaccharide were obtained from Sigma-Aldrich. Cell-titer Glo (ATP) and CYP3A4-Glo Kits were obtained from Promega.

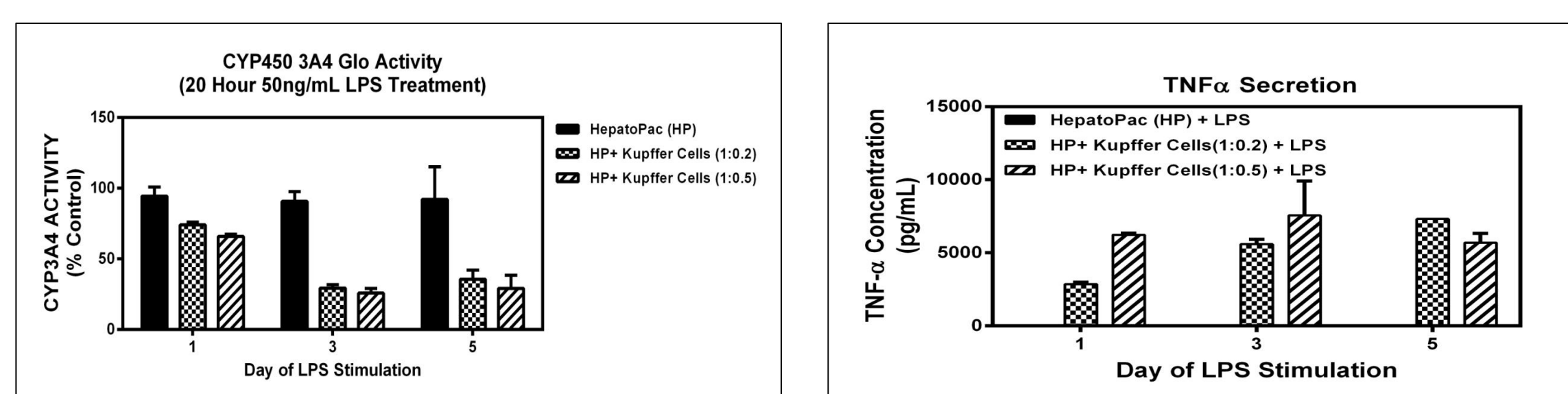
Rat or human HepatoPac co-cultures (HP) were created using cryopreserved hepatocytes and 3T3-J2 murine embryonic fibroblasts following Hepregen's proprietary protocols and quality controls. HepatoPac co-cultures were stabilized in serum-supplemented media for 7 days prior to addition of Kupffer cells (KC) at hepatocyte:Kupffer cell ratios of 1:0.5 for the rat platform and hepatocyte:Kupffer cell ratios of 1:0.4 for the human platform. HepatoPac-Kupffer cell co-cultures were treated with 50 ng/mL Lipopolysaccharide (LPS) for 20 hours and cell supernatants were analyzed for cytokine secretion.



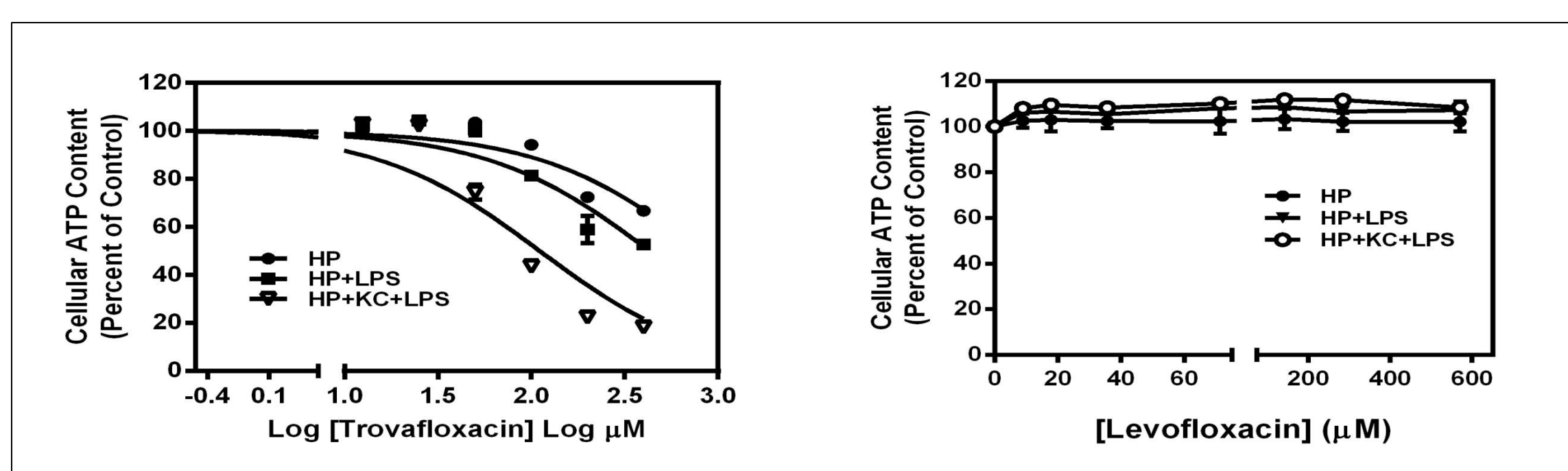
## RESULTS



**Figure 1. The HepatoPac-Kupffer cell co-culture.** HepatoPac co-cultures were created using patented microfabrication tools and consists of primary hepatocytes arranged in optimized domains and surrounded by stromal fibroblasts. Cryopreserved primary Kupffer cells were added on day 7 of HepatoPac culture. **A & B.** pHrodo dye and CD68 staining illustrates the presence of functional Kupffer cells 10 days after kupffer cell addition (i.e. 17 days of HepatoPac culture).

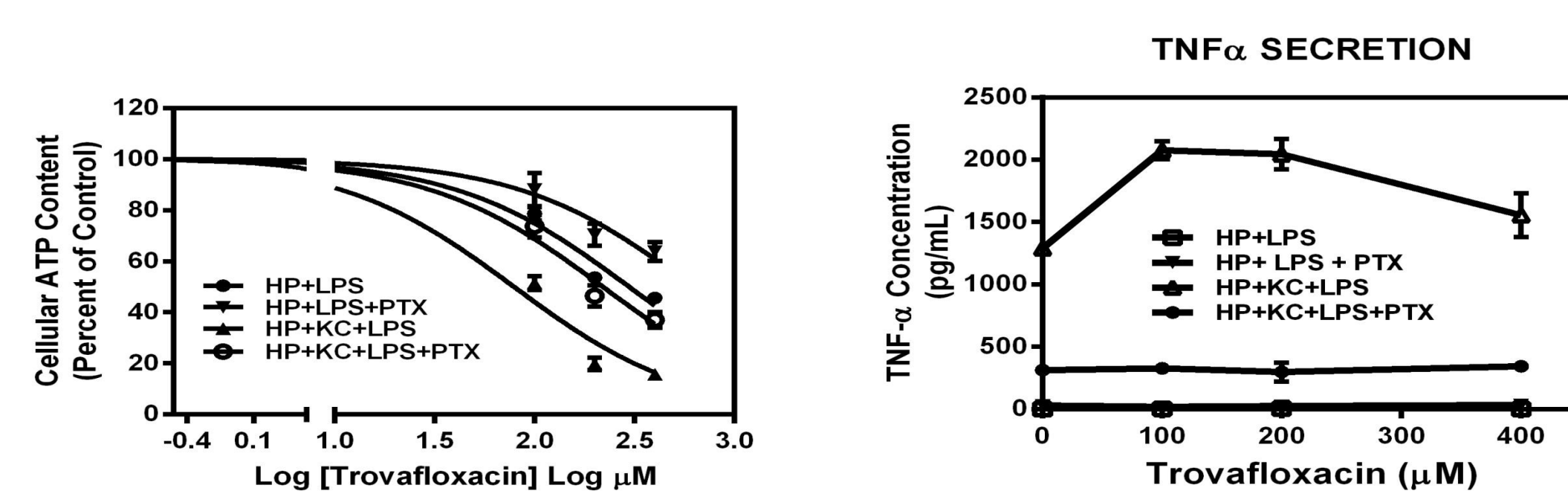


**Figure 2. Characterization of Rat HepatoPac-Kupffer cell co-cultures.** Stimulation of rat HepatoPac-Kupffer cell co-cultures at different time-points with 50ng/mL LPS for 20 hours caused robust TNF $\alpha$  secretion and down-regulation of CYP3A4 activity.



Trovafloxacin TC50 ( $\mu$ M)		
Rat	- LPS	+ LPS
HepatoPac (HP)	>400	>400
HP + Kupffer cell (1:0.5)	356.0	113.3

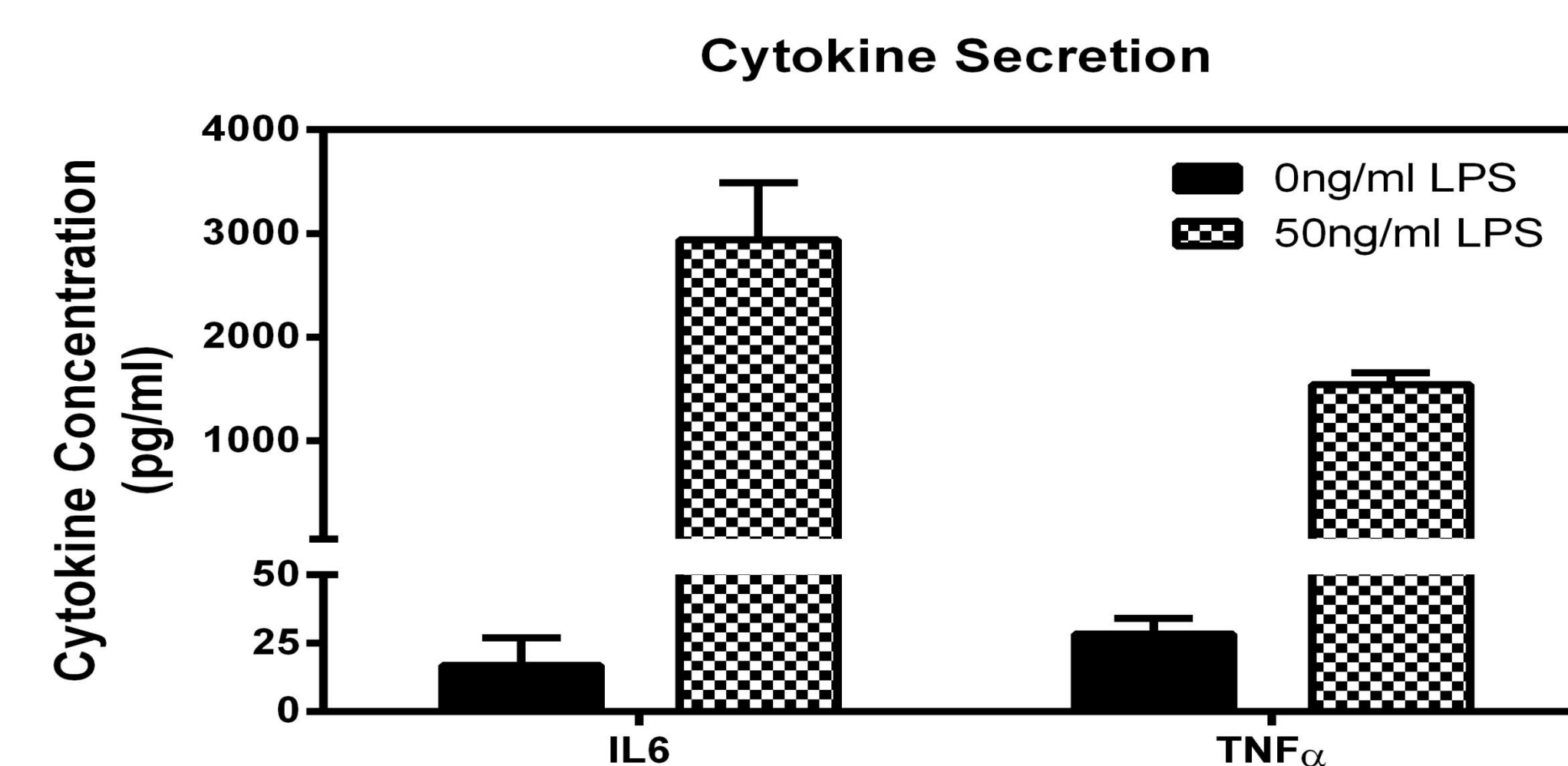
**Figure 3. Trovafloxacin (TVX) toxicity is potentiated in LPS- treated rat HepatoPac-Kupffer cell co-cultures.** TVX showed characteristic dose-dependent cytotoxicity when added to rat HepatoPac- Kupffer cell co-cultures. Stimulation of the cultures with LPS exacerbated TVX-induced toxicity as seen above where there's a leftward shift (lower TC50 values) in the concentration- response curves for ATP content. Levofloxacin (right panel) was not toxic to rat HepatoPac-Kupffer cell cultures even when stimulated with LPS.



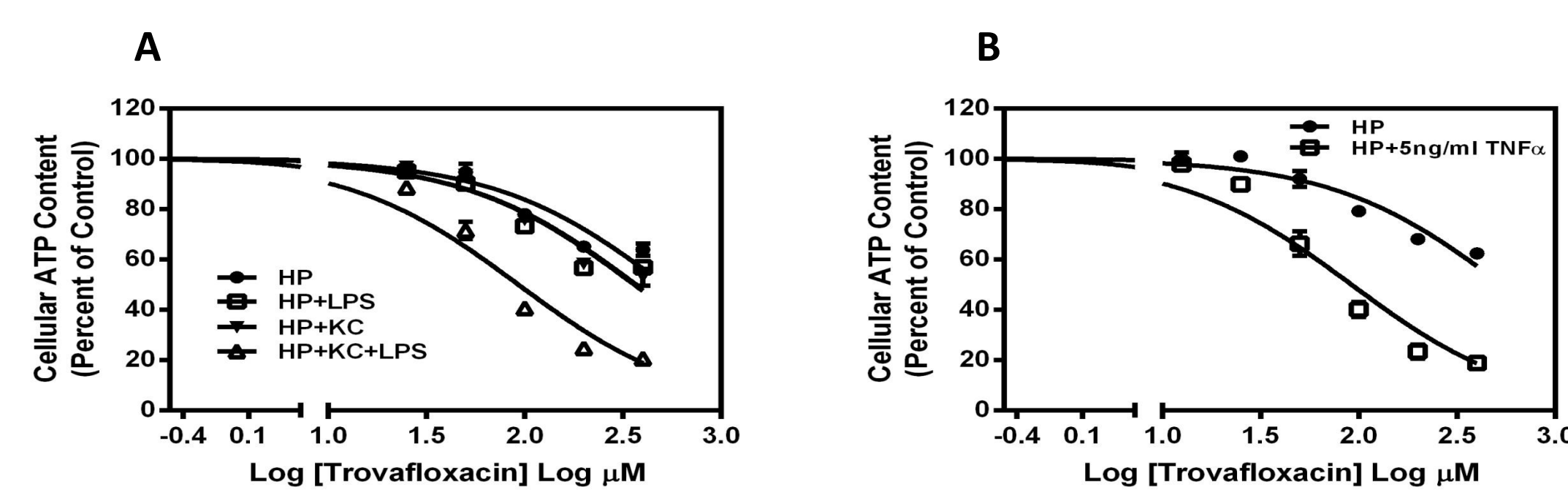
Trovafloxacin TC50 ( $\mu$ M)		
Rat	+ LPS	+ LPS + PTX
HepatoPac	303.1	>400
HP + Kupffer cell (1:0.5)	80.5	311.4

**Figure 4. Treatment with pentoxifylline (PTX), an inhibitor of TNF- $\alpha$  transcription, significantly decreased TVX/LPS-induced Hepatotoxicity and TNF $\alpha$  secretion in rat HepatoPac- Kupffer cell co-cultures.** Cultures were treated with Trovafloxacin and 5mM Pentoxifylline for 72 hours. After 24 hours of dosing, Kupffer cells were activated with 50ng/ml of LPS.

## RESULTS



**Figure 5. LPS stimulation of human HepatoPac- Kupffer cell co-cultures causes robust secretion of TNF $\alpha$  and IL6.**



**Figure 6. Trovafloxacin (TVX) toxicity is potentiated in LPS- treated and TNF $\alpha$ -treated human HepatoPac-Kupffer cell co-cultures.** (A) Stimulation of HepatoPac-Kupffer cell co-cultures with LPS exacerbated TVX-induced toxicity as seen above where there's a leftward shift (lower TC50 values) in the concentration- response curves for ATP content. (B) Simultaneous treatment of human HepatoPac cultures with TNF $\alpha$  and TVX exacerbates TVX toxicity in a manner similar to that observed in LPS-TVX treatment of human HepatoPac-Kupffer cell co-cultures.

## CONCLUSIONS

- HepatoPac™ is a multi-well (i.e. 96-well plates) platform that consists of primary hepatocytes organized in empirically optimized clusters and subsequently surrounded by 3T3-J2 murine embryonic fibroblasts. Here, both human and rat HepatoPac were supplemented with primary human or rat Kupffer cells for utility in assessment of cytokine-drug interactions.
- Kupffer cells in human HepatoPac-Kupffer co-cultures remained viable and functional for up to 10 days post addition to the wells as assessed via positive phagocytosis of pH-sensitive *S.aureus* bioparticles and CD68 staining.
- Trovafloxacin toxicity was potentiated in LPS-treated HepatoPac-Kupffer cell cultures in both rat and human cultures.
- LPS-induced potentiation of trovafloxacin toxicity in the rat HepatoPac-Kupffer cell cultures was reversed by treatment of the cultures with pentoxifylline (an inhibitor of TNF $\alpha$  transcription), while TNF $\alpha$  treatment of the human cultures potentiated Trovafloxacin toxicity.
- The HepatoPac-Kupffer cell co-culture platform could be a good model for evaluation of cytokine-mediated toxicities.
- Future studies on the HepatoPac-Kupffer cell co-culture will seek to evaluate the toxicity profiles of compounds known to cause immune-mediated liver toxicities.

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