# Demonstration of Hepatocyte-targeted siRNA Transfection and Gene Silencing in the Micro-patterned Hepatocyte Co-culture System (HEPATOPAC)

**POSTER # 1276**

**Miranda Yang, Jeannemarie Gaffney, and Onyi Irrechukwu**

BioIVT (formerly Ascendance Biotechnology and Hepregen Corporation), Medford, MA

## Abstract

The HEPATOPAC® model, an *in vitro* bioengineered co-culture of primary hepatocytes and fibroblasts, has demonstrated invaluable utility for liver-based safety, metabolism, and efficacy evaluation for small molecule drug candidates, due to its longevity and close resemblance to the *in vivo* liver[1-3]. Here, we identify a method to specifically deliver small-interfering RNAs (siRNA) into the hepatocytes in the HEPATOPAC co-cultures by using a commercially available, non-liposomal transfection reagent that targets hepatocytes (PromoFectin-Hepatocyte). Upon the transfection of a fluorescent control siRNA, fluorescent signal was detected mainly in the hepatocyte islands, but not in the surrounding stromal cells. When siRNA targeting a cytochrome P450 enzyme was transfected in HEPATOPAC cultures, a time-dependent reduction in the CYP activity following transfection was observed. The results provide a proof of concept that the HEPATOPAC platform is amenable to hepatocyte-specific siRNA transfection and siRNA-mediated gene knockdown, which can be useful in elucidating the hepatocellular mechanisms in various research areas, aiding in reaction phenotyping assessment, as well as *in vitro* safety and efficacy studies for novel RNA therapeutics.

## Methods

**HEPATOPAC preparation**

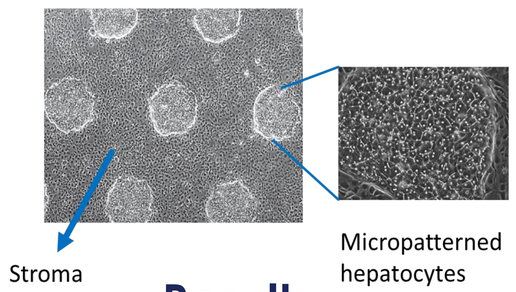
Human micropatterned co-cultures (HEPATOPAC) were created using patented microfabrication tools and consist of primary hepatocytes arranged in optimized domains and surrounded by 3T3 J2 murine fibroblasts. In this configuration (Fig 1), the HEPATOPAC co-cultures retain *in vivo* functionality *in vitro*. The co-cultures were first allowed to stabilize functionally in serum-supplemented medium for 7~14 days prior to siRNA transfection or IL-6 treatment.

**Evaluation of siRNA transfection reagents in HEPATOPAC cultures**

Transfection reagents tested for delivering siRNA into HEPATOPAC cultures included: Lipofectamine® 3000, Lipofectamine® RNAiMAX (both from Thermo Fisher), METAFECTENE® PRO, METAFECTENE® SI+ (both from Biontex), and PromoFectin-Hepatocyte (PromoCell). A fluorescent labeled control siRNA (BLOCK-iT™, Thermo Fisher) was used to monitor siRNA transfection efficiency and localization. Transfection efficiency and siRNA localization was manually observed using fluorescent microscopy.

**Transfection of CYP3A4-targeting siRNA and evaluation of functional knockdown**

On day 7 or 14 post seeding, siRNA targeting CYP3A4 (ON-TARGET*plus* SMARTpool, Dharmacon) or control siRNA (ON-TARGET*plus* Non-targeting Pool, Dharmacon) were transfected into HEPATOPAC co-cultures, using different transfection reagents following users manuals. Transfections were conducted in proprietary serum-free application medium. Four hours after transfection, media were replaced by serum-containing HEPATOPAC maintenance medium. CYP3A4 activity was measured 24hrs, 48hrs, 72hrs and 96hrs post transfection, using Promega P450-Glo™ CYP3A4 Assay Kit. IL-6 treatment (10,000pg/ml) served as a positive control for CYP3A4 down-regulation[4].




## Results

**Figure 1. HEPATOPAC Platform.** HEPATOPAC micropatterned co-culture platform is created using patented microfabrication tools and consists of primary hepatocytes arranged in optimized domains and surrounded by stromal fibroblasts, which support the high level of performance observed in the HEPATOPAC. HEPATOPAC cultures retain long-term functionality for several weeks *in vitro*.

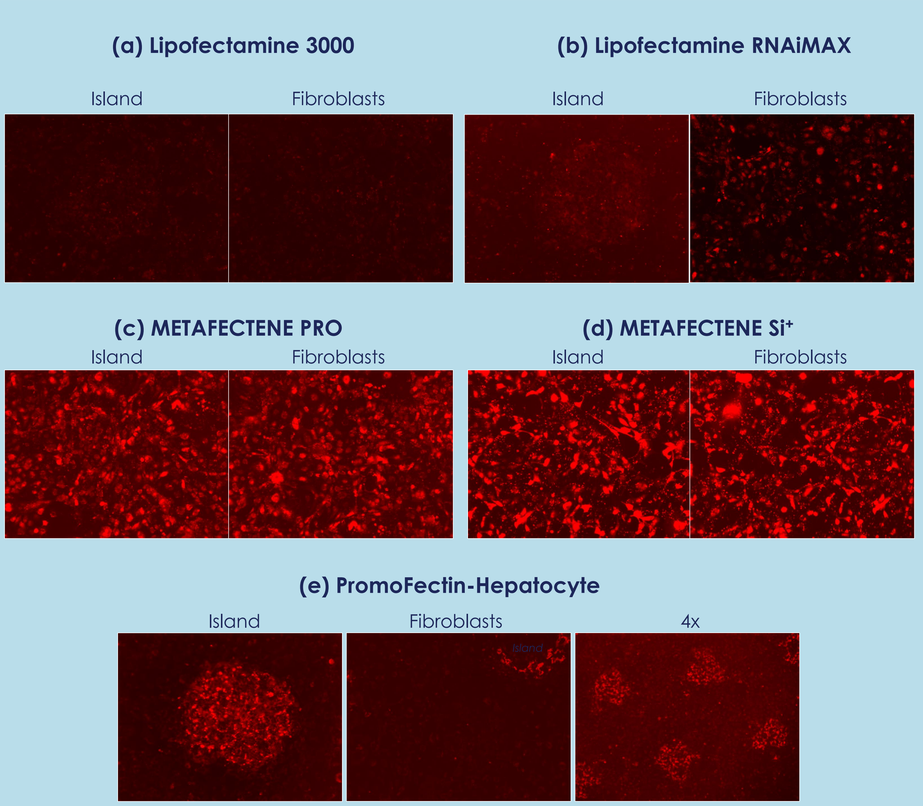
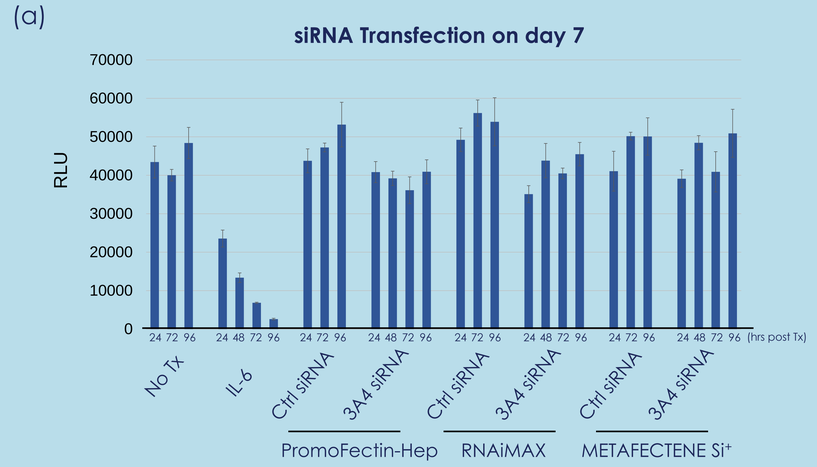



**Figure 2. Evaluation of siRNA transfection reagents in human HEPATOPAC co-cultures.** Five transfection reagents were evaluated for the delivery of the fluorescent control siRNA (BLOCK-iT) into human HEPATOPAC co-cultures. Twenty-four hours after transfection, images were taken to illustrate whether siRNA was delivered into the hepatocytes in the co-cultures. (a) Transfection with Lipofectamine 3000 didn't result in detectable siRNA in HEPATOPAC cultures. (b) Lipofectamine RNAiMAX delivered low levels of siRNA into both hepatocyte islands and fibroblasts. (c and d) METAFECTENE PRO and METAFECTENE Si+ introduced high amounts of siRNA into HEPATOPAC cultures. However, siRNA colocalized with fibroblasts and no siRNA was observed in hepatocytes. (e) PromoFectin-Hepatocyte preferentially delivered siRNA into hepatocyte islands in the co-cultures.



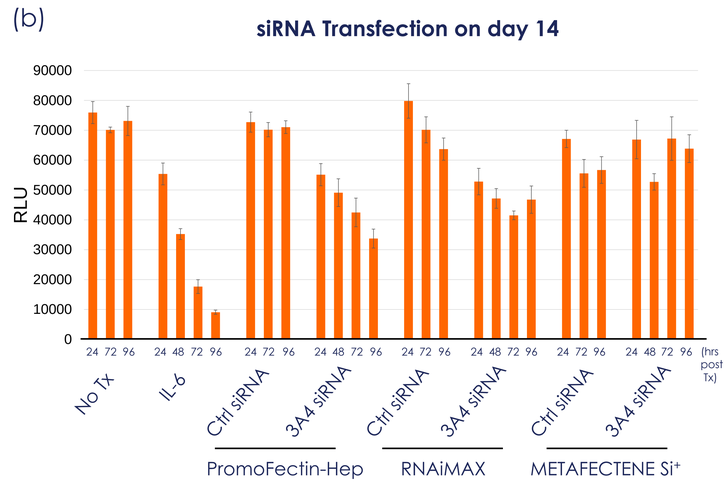




**Figure 3. CYP3A4 functional knockdown after siRNA transfection.** CYP3A4-targeting siRNA or control siRNA were transfected on (a) day 7 or (b) day 14 post seeding, using PromoFectin-Hepatocyte, Lipofectamine RNAiMAX, or METAFECTENE Si+. Transfection using either PromoFectin-Hepatocyte or Lipofectamine RNAiMAX resulted in a reduction in CYP3A4 activity, while transfection using METAFECTENE Si+ didn't. The functional knockdown was more pronounced when siRNA was transfected on day 14 (up to 53%, by PromoFectin-Hepatocyte at 96hrs) than on day 7 (~20%). IL-6-treated (10,000pg/ml) cultures were used as a positive control for CYP3A4 down-regulation.

## Conclusion

1. Five transfection reagents were evaluated for the delivery of siRNA into HEPATOPAC co-cultures.
2. PromoFectin-Hepatocyte reagent specifically delivered siRNA into hepatocytes in HEPATOPAC co-cultures (human and rat).
3. Lipofectamine RNAiMAX delivered siRNA into both hepatocytes and fibroblasts in HEPATOPAC co-cultures.
4. Transfection of CYP3A4-targeting siRNA using either PromoFectin-Hepatocyte or Lipofectamine RNAiMAX in HEPATOPAC co-cultures resulted in functional knockdown of CYP3A4. Transfection at a later time (day 14) generated greater knockdown (up to 53%).
5. The results provide a proof of concept that the HEPATOPAC system is amenable to siRNA transfection and siRNA-mediated gene knockdown, by using commercially available transfection reagents. Furthermore, hepatocyte-specific siRNA delivery can be achieved by using PromoFectin-Hepatocyte reagent.
6. This application can be useful in elucidating the hepatocellular mechanisms in various research areas, aiding in reaction phenotyping assessment, as well as *in vitro* safety and efficacy studies for novel RNA therapeutics.

## References

1. Khetani SR, Will Y, et al. Use of micropatterned cocultures to detect compounds that cause drug-induced liver injury in humans. *Toxicol Sci.* (2013) 132:107-17.
2. Kratochwil NA, Fowler S, et al. Simultaneous Assessment of Clearance, Metabolism, Induction, and Drug-Drug Interaction Potential Using a Long-Term *In Vitro* Liver Model for a Novel Hepatitis B Virus Inhibitor. *J Pharmacol Exp Ther.* (2018) 365:237-248.
3. Ware BR, Khetani S, et al. Exploring Chronic Drug Effects on Microengineered Human Liver Cultures Using Global Gene Expression Profiling. *Toxicol Sci.* (2017) 157:387-398.
4. Ramsden D., Zhou J., Tweedie DJ. Determination of a Degradation Constant for CYP3A4 by Direct Suppression of mRNA in a Novel Human Hepatocyte Model, HepatoPac. *Drug Metab Dispos.* (2015) 43: 1307-1315.

**Contact Information:**

**Miranda Yang, Ph.D.**
**Senior Scientist, Product Development**
**myang@bioivt.com**
**781.285.7429**

**Jeannemarie Gaffney**
**Senior Manager, Manufacturing and Process Development**
**jgaffney@bioivt.com**
**781-391-0205 ext.1504**

**BioIVT.com**



*SOT, Baltimore MD, March 10-14, 2019*