Improved IC-50 Prediction using Quasi Vivo®

Kelly S. Davidge, Jennifer Bishop, Maureen Bunger, J. Malcolm Wilkinson,
Kirkstall Ltd, 3 Aspen Way, Centurion Business Park, Rotherham, South Yorkshire, S60 1FB

Quasi Vivo® is an interconnected cell culture system which incorporates flow. This has been used to improve the gene expression of multiple cell types, including hepatocytes, making expression more comparable to primary human hepatocytes and also the IC50 prediction of diclofenac and other compounds more comparable to clinical data.

QV500

QV600

QV900

QV500 allows cell culture in a submerged chamber and is compatible with scaffolds, gels and coverslips
QV600 is designed for growth at an air-liquid or liquid-liquid interface
QV900 provides a range of configuration options within the footprint of a standard well-plate

1. Gene Expression Changes:

An environment with flow causes widespread changes in gene expression, including key detox genes and transporters. This has been shown in multiple cell types, including primary human hepatocytes (Figure 1).

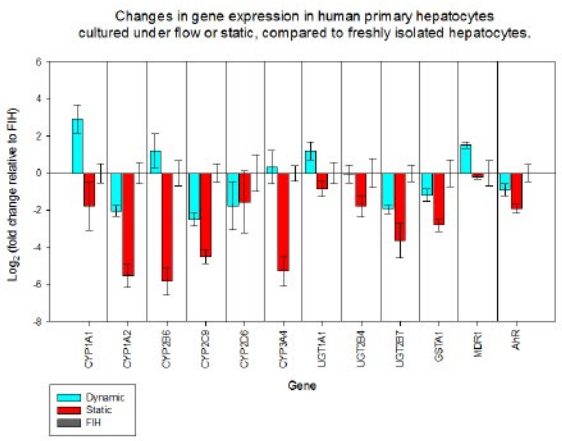

Figure 1. Graph shows a significant downregulation of gene expression in static cultures compared to freshly isolated primary human hepatocytes, and a significant increase in gene expression in primary human hepatocytes cultured with flow compared to those cultured in static conditions. Vinci *et al* 2011, *Biotechnol. J.* 6:554-564

2. IC50 Prediction:

This upregulation of metabolic genes is reflected in the metabolism of certain tested drugs. In line with the mRNA data, CYP2D6 (dextromethorphan) was not influenced by flow, but both phase I and II metabolism of Midazolam (CYP3A4 and UGT) were strongly upregulated (Figure 2).

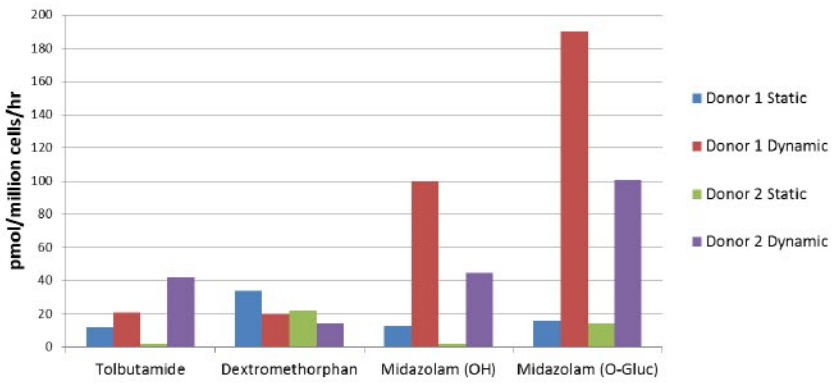

Figure 2. Graph showing upregulation metabolism of different drug molecules after 8 days of growth in Quasi Vivo and then a 24 hour curve. Vinci et al 2011, Biotechnol. J. 6:554-564

3. Focus on CYP3A4:

Primary human hepatocytes were cultured for seven days in static culture and then either transferred to flow conditions or kept in static for the following 20 days. Flow conditions restored functionality and greatly increased CYP3A4 expression levels, to a level comparable with freshly isolated human hepatocytes within five days, and expression remained at this level for 20 days.

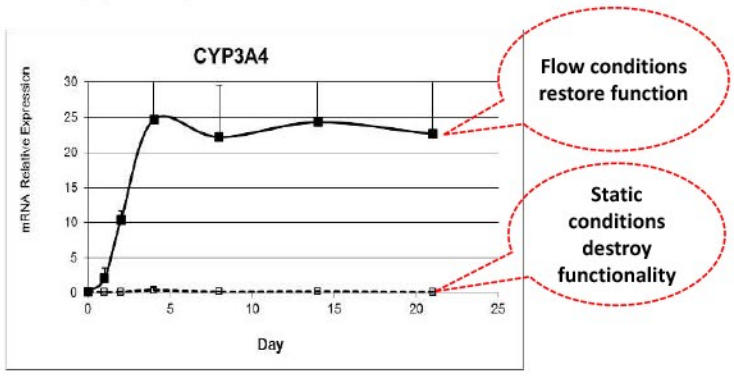

Figure 2. This graph demonstrates that flow results in more human-like expression of the key detox gene, CYP 3A4. Vinci *et al* 2011, *Biotechnol. J.* 6:554-564

4. Diclofenac Assay:

When challenged by diclofenac for up to 24 hours, primary rat hepatocytes give a more realistic assessment of toxicity than classical methods, predicting an IC50 of around 50 µM. This is more accurate than static conditions, which predicted 500 µM, as the clinical toxicity has been measured at 4.2 µM.

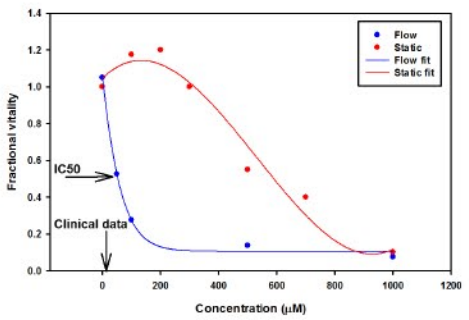

Figure 4. Graph showing the IC50 predictions of diclofenac using a static *in vitro* model and a flow *in vitro* model compared with clinical data. Vozzi & Ahluwalia, University of Pisa

We have also shown that the Quasi Vivo® system is 50% more accurate in predicting the IC50 for APAP and cyclophosphamide. Primary rat hepatocytes cultured under flow are more sensitive to these drugs than those cultured under static conditions. (Data not shown)

5. Conclusion:

It has been demonstrated that Quasi Vivo® can be used to create liver models that more closely mimic the human *in vivo* environment compared to static cultures or animal models. A liver model in Quasi Vivo® is better for research into disease and toxicity than these alternative options.

References:

Vinchi *et al* 2011, *Biotechnol. J.* 6:554-564
Sbrana & Ahluwalia 2011, *New Technologies for Toxicity Testing* 138–153