Improved *in vitro* culture of primary hepatocyte using alvetex®
**in vitro** drug screening

Drug discovery process is long and expensive

- At present only 1:10,000 new chemical entities become a new drug.
- It costs in excess of $800 Million to develop a new drug.
- One of the main causes of drug failure is toxicity resulting from drug metabolism by the liver.
- *in vitro* toxicity screens are routinely used as a tool for screening potentially harmful compounds.

However many of the liver models used for predicting the potential toxicity and/or biotransformations of new compounds have limitations.
In vitro toxicity models

Limitations of current models for in vitro liver toxicity screens

HepG2 – Cancer derived cell line
HepRG - Mixed cell population
Rat primary cells – Species differences
Human Primary cells – High batch-batch variability

Primary hepatocytes are considered the gold standard for in vitro toxicity testing but are known to undergo changes during cell culture.
Primary Hepatocytes

Changes over the first 48 – 72 hours of culture

- Cells undergo morphological changes
- Have reduced viability (~60%)
- Have lower expression of liver markers compared to *in vivo*
- Loose the inducible expression of phase-1 metabolism markers

Primary hepatocytes in the liver are in a 3D environment.

Can the changes exhibited by primary hepatocytes be overcome through the use of 3D culture using alvetex® scaffolds?
alvetex® scaffolds

alvetex® is made from the same polystyrene material as normal cell culture plastic but is structurally different.
Experimental overview

- alvetex® scaffolds were coated with 1mg/ml collagen

- Primary rat hepatocytes were seeded onto scaffolds (or 24 well plate normal cell culture controls) and maintained in culture for up to 72 hours

- The cells were examined for
  - Morphology (confocal microscopy)
  - Viability
  - Phase I enzyme expression
  - Cytotoxicity (to model toxicant – Acetominophen)
  - Gene expression
Cells grown in 3D alvetex® scaffolds retain a more in-vivo like cell architecture compared to cells grown in standard culture.

- **alvetex® scaffolds**: Retained 3D morphology
- **Standard cell culture**: Flattened morphology
Cells grown in 3D alvetex® scaffolds retain a higher viability over a range of different cell seeding densities.
Hepatocytes in 3D alvetex® cell culture express approximately 5 times higher levels of cytochrome P450/1A2, 2B1 and 3A4.

CYP Activity (nmol/min/mg protein)

CYP – 1A2

CYP – 2B1

CYP – 3A4
Cytotoxicity measurements

Cells grown in alvetex® scaffolds show increased sensitivity to the model compound Acetaminophen.
Gene Expression

Gene targets

Apoptosis
- BAX
- CD95
- Bcl-X
- Mcl-1

Liver markers
- HNF-4
- Albumin
- CEPB-a
- CEPB-b
- TCF-1
- Fox-A2

Acetaminophen
- 0.4mM
- 4.0mM

Phase I metabolism
- Cyp 1 A1
- Cyp 1 A2
- Cyp 2 B6
- Cyp 2 D6

Phases II metabolism
- GST A1/2
- GST M1
- UGT 1 A1
- CAR

Phase III transporters
- P-gp
- BSEP
- OATP-C
Principal component analysis shows that cells grown in alvetex® scaffolds have differential gene expression patterns (red circle) compared to normal culture (blue circle). In response to acetaminophen treatment (green circle) gene expression patterns are similar in both culture systems.
Conclusions

- Primary hepatocytes in the alvetex® 3D scaffold retain a more *in vivo* like morphology

- 3D culture leads to higher cell viability and a significant increase in the expression of a range of CYP enzymes

- Higher CYP enzyme expression makes the hepatocytes in the 3D system more sensitive to toxic effects of APAP

- Hepatocytes in the 3D scaffold have different gene expression patterns and could potentially be used in co-culture systems.
Human hepatocytes CYP expression

Diclofenac → Cyp 2C9
Midazolam → Cyp 3A4/5
Paracetamol → Cyp 1A2

Cyp activity (pmol/min/mg protein)

Cyp2C9

Cyp3A4/5

Cyp1A2

2D controls  Alvetex

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