Engineering liver model using CELLBLOKS®: a novel approach to *in-vitro* pre-clinical drug screening

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Introduction

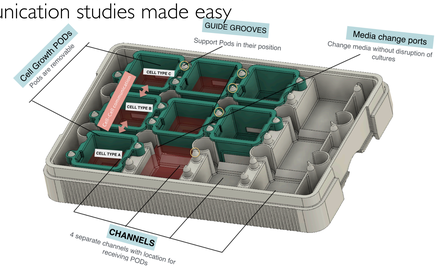
Drug induced liver injury (DILI) continues to be the leading cause of attrition during drug development in all phases of clinical trials as well as number one cause of post-market drug withdrawal, accounting for 20-40 % of all cases (1).
Current preclinical *in-vivo* animal models often fail to predict drug adverse reactions in humans (hepatotoxicity by 50--60%) due to substantial differences of drug metabolising enzymes between animals and humans, and existing *in-vitro* cell culture models do not represent complex organotypic conditions (2,3).

Typically cells are grown in 2-D surfaces and in isolation containing only one cell type. In these environments cells become flattered, lack cell-cell communication vital for replicating biological relevance and behave in aberrant fashion

The study demonstrate how a novel platform, CELLBLOKS® aims to improve research in drug discovery and reduce animal testing.

CELLBLOKS® introduction

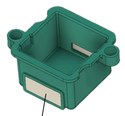
A first of its kind modular "plug and play" cell culture platform: cell-cell communication studies made easy



Achieve better representation of different Organtype communications

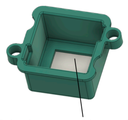
- Circulatory Pods can be used to simulate organs in systematic circulation
- Barrier Pods organs representing biological barriers
- Blank Pods are also available for isolating cell cultures during experimentation.

CIRCULATORY PODS



Selective permeable membranes
Media circulation through the side membranes

BARRIER PODS



Selective permeable membranes
Allows cells to form barriers with tight-junctions

BLANK PODS

Closed POD
Prevents cells communicating with other Pods.

Modelling multiple cell-type liver archetype

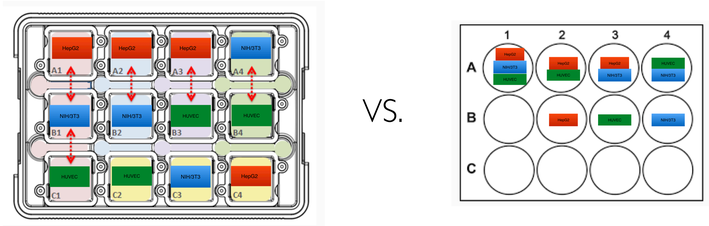


Figure 1. Liver model set up on CELLBLOKS® platform in non-contact vs. contact co-culture standard format.

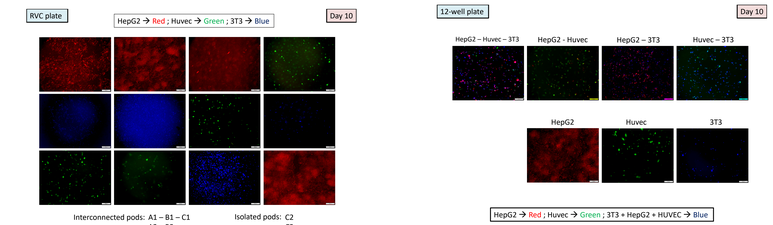
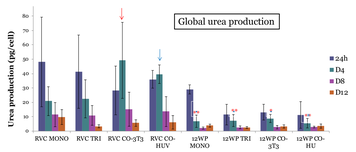


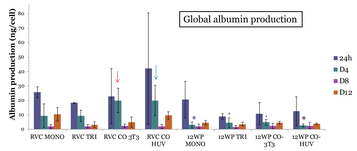
Figure 2. Images of cells in CELLBLOKS® and 12-well plate format

Results

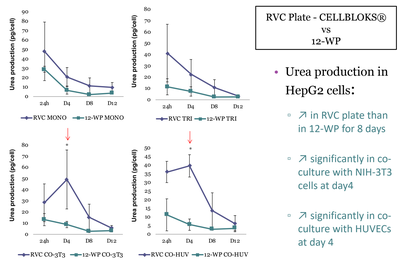
Hepatic function comparing contact cell culture in CELLBLOKS® compared non-contact co-culture in standard well plate



- Urea production in HepG2 cells:
  - in RVC plate and ↘ in 12-WP for 8 days of culture
  - significantly in co-culture with NIH-3T3 cells in RVC plate compared to the one in 12-WP
  - significantly in co-culture with HUVECs in RVC plate compared to the one in 12-WP

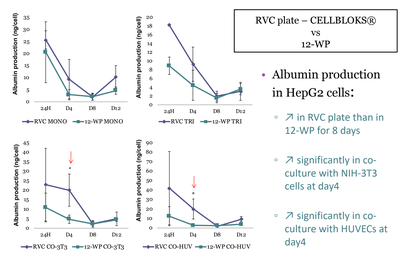


- Albumin production in HepG2 cells:
  - in RVC plate and ↘ in 12-WP for 4 days of culture
  - significantly in co-culture with NIH-3T3 cells in RVC plate compared to the one in 12-WP
  - significantly in co-culture with HUVECs in RVC plate compared to the one in 12-WP



RVC Plate - CELLBLOKS® vs 12-WP

- Urea production in HepG2 cells:
  - ↗ in RVC plate than in 12-WP for 8 days
  - ↗ significantly in co-culture with NIH-3T3 cells at day4
  - ↗ significantly in co-culture with HUVECs at day 4



RVC plate – CELLBLOKS® vs 12-WP

- Albumin production in HepG2 cells:
  - ↗ in RVC plate than in 12-WP for 8 days
  - ↗ significantly in co-culture with NIH-3T3 cells at day4
  - ↗ significantly in co-culture with HUVECs at day4

Results

- Overall Albumin and Urea production measured in HepG2 cells was significantly higher in non-contact CELLBLOKS platform compared to contact cell cultures in standard 12 Well plate leading to up to three times increase in production.
- Both Urea and Albumin production remained higher in CELLBLOKS® compared to well plate format over the duration of 12 days where protein production plateaued in both platforms
- In general Albumin and Unreal production was higher when HepG2 cells were in co-cultures compared monocultures in CELLBLOKS®. However, in contact 12-WP format when HepG2 cells were cultured with either HUVEC and NIH/3T3 cells hepatic function either remained unchanged or was inhibited.

Discussion

- CELLBLOKS® is a novel cell culture platform where cell-cell *in-vitro* interaction studies can be easily set up and analysed as the platform is compatible with current imaging and analysis techniques.
- The data from this study shows that hepatic function is significantly improved in non-contact co-cultures compared to mono-cultures indicating the need incorporation of co-cultures in DILI drug screening.
- CELLBLOKS® aims to increase the success rate of drug screening by prioritising candidates that are likely to succeed and eliminates those likely to fail in human clinical trials, particularly in Phase I and at the same time reducing the reliance on animal testing.

References

1. Almario E.E., Borlak J., Suzuki A., Chen M. Drug-induced liver injury. BioMed Res. Int. 2017;2461694 (2000).
2. Ison H et al., Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol. 2000;32.
3. L Kuna et al., Models of Drug Induced Liver Injury (DILI) - Current Issues and Future Prospectives. Current Drug Metabolism. 2018:19:10.

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