

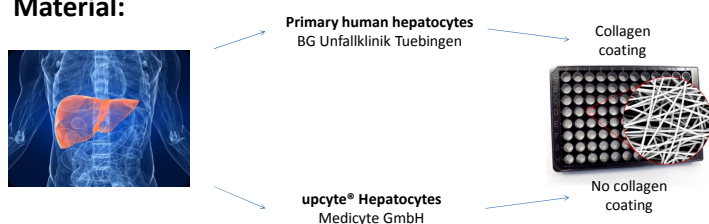
Mimetix[®] electrospun scaffolds for 3D culture of upcyte[®] and primary hepatocytes

E. Heister, R. McKean – **The Electrospinning Company Ltd, Harwell Oxford, UK** • S. D. Ramachandran, B. Müntz – **Medicyte GmbH, Heidelberg, Germany**
B. Burkhardt, V. Häussling, A. Nüssler – **Berufsgenossenschaftliche Unfallklinik Tübingen & University of Tübingen, Germany**

Background:

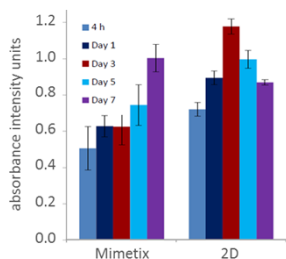
Liver toxicity is a major cause of drug discovery failure in clinical trials and of adverse events leading to regulatory action on approved drugs. It is therefore critical to use *in vitro* models that are predictive of *in vivo* liver toxicity in the drug discovery cascade. There are a number of *in vitro* liver toxicity models using immortalized cell lines and isolated primary hepatocytes, but these are limited by throughput, loss of viability and decrease in liver-specific functionality and gene expression. In order to recreate the complex cellular microenvironment of the liver and to extend primary hepatocyte culture longevity and functionality, three-dimensional culture systems are being developed. These are designed to provide more realistic data in drug discovery testing that is predictive of behaviour in the clinic.

Material:



Primary human hepatocytes as well as upcyte[®] Hepatocytes from Medicyte GmbH were cultured in the Mimetix 3D electrospun scaffold to assess its value for cell-based assays compared to standard 2D. upcyte[®] Hepatocytes are genetically engineered human strains, derived from single donor primary cells which are driven into proliferation without inducing immortalisation, uncontrolled cell growth or changing the typical phenotype.

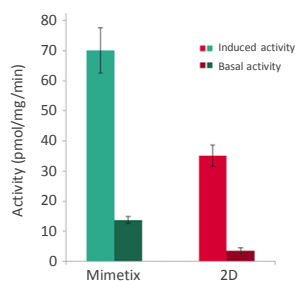
Cell viability of upcyte[®] Hepatocytes over 7 days



The proliferation of upcyte[®] Hepatocytes (50,000 cells per well) was studied over a period of 7 days within a Mimetix 96-well plate and a standard 2D 96-well plate by means of an MTS assay.

The Mimetix scaffold provides a tissue-like environment, which supports the growth of upcyte[®] Hepatocytes. Cell proliferation in 3D in the Mimetix scaffold is initially slower than in 2D but continues beyond the point at which cell numbers start to decrease in 2D.

Basal and induced CYP3A4 activity in upcyte[®] Hepatocytes



The CYP3A4 enzyme activity of upcyte[®] Hepatocytes (50,000 cells per well) was studied after 7 days of culture and subsequent induction with rifampicin for 3 days within a Mimetix 96-well plate and a standard 2D 96-well plate.

Basal CYP3A4 levels were at about 3 and 14 pmol/min/mg in 2D vs. 3D, whereas induced levels were at 35 and 70 pmol/mg/min in 2D vs. 3D for this donor (donor 151).

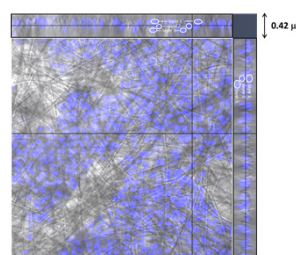
Both basal and induced activities of CYP3A4 were higher in Mimetix[®] than when cultured in a 2D monolayer.

Conclusions:

In this study we investigated the growth and performance of different types of hepatocytes in the Mimetix scaffold. upcyte[®] Hepatocytes were shown to grow and migrate into the scaffolds up to a depth of 40 µm and exhibited higher induced CYP3A4 activity in the scaffold than in a 2D monolayer. Isolated primary hepatocytes of human origin exhibited typical hepatocyte function, such as basal urea production, which was found equal in Mimetix vs. a 2D monolayer culture medium-term (4-8 days), but higher in Mimetix for a longer-term experiment (15 days). Similarly, upcyte[®] Hepatocytes exhibited higher basal and induced activities in Mimetix than in 2D. This shows that the Mimetix scaffold, designed to mimic the extracellular matrix, is a valuable tool for 3D cell-based assays, which holds great promise to reduce the number of costly drug failures in clinical trials.

This study investigates the Mimetix[®] electrospun scaffold, which consists of highly uniform electrospun PLLA microfibres supporting the three-dimensional growth of cells, as a cell-based assay system for upcyte[®] and primary hepatocytes. Migration into the scaffold, cell viability, as well as urea production and basal and induced CYP3A4 enzyme activity as typical hepatocyte functions were analysed. Both cell types were able to grow in the scaffolds for longer periods than in 2D. Primary hepatocytes also showed increased urea production in long-term cultures (2 weeks), while upcyte[®] Hepatocytes were responsive to Rifampicin, a proto-typical inducer of CYP 3A4 and showed higher basal, as well as induced levels of CYP activity than when grown in a 2D monolayer.

Cell penetration of upcyte[®] Hepatocytes into the scaffold

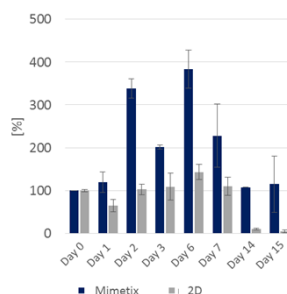


upcyte[®] Hepatocytes proliferating in the Mimetix scaffold 5 days after seeding

upcyte[®] Hepatocytes have been shown to migrate into the Mimetix scaffold with 4 µm fibre diameter over time. Cells were found up to 40 µm deep (= 3 cell layers), although most cells remained close to the surface even after culturing periods of up to 14 days. This suggests that upcyte[®] Hepatocytes exhibit contact inhibition once they fill the upper layers of the scaffold and do not proliferate further.

A scaffold thickness of 50 µm was hence considered optimal for upcyte[®] hepatocyte culture within Mimetix.

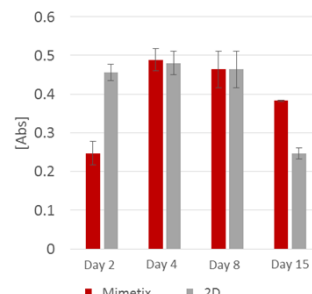
Cell viability of primary hepatocytes over 14 days



The viability and metabolic activity of primary human hepatocytes (25,000 cells per well) was studied over a period of 14 days within a Mimetix 96-well plate and a standard 2D 96-well plate by means of an Alamar Blue assay. Both Mimetix and the 2D plate were coated with 0.1 mg/mL rat tail collagen.

Metabolic activity was significantly higher at any time point after cell seeding in the Mimetix scaffold than in 2D (about 3 times higher at days 2 and 6). After 2 weeks, hardly any metabolic activity was observed in the 2D culture, whereas the activity in Mimetix dropped to the same level as on day 0.

Urea production of primary hepatocytes over 15 days



Basal urea production of primary human hepatocytes (25,000 cells per well) was measured over 15 days in Mimetix vs. 2D (both coated with 0.1 mg/mL rat tail collagen) to assess their metabolic function.

While cells showed higher urea production in 2D than in Mimetix on day 2, similar levels were measured for medium-term experiments (day 4 and 8). However, urea production significantly dropped in 2D after 2 weeks but much less so in Mimetix, indicating that providing a 3D environment has a long-term benefit on primary hepatocyte function.