As a result of the increased prevalence of diabetes, obesity and metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) is now the most common chronic liver disease in developed countries (1). NAFLD is a spectrum of pathologies ranging from benign hepatic steatosis through to non-alcoholic steatohepatitis (NASH), which can ultimately lead to cirrhosis (1). NASH is a serious condition, defined as a combination of hepatic steatosis, inflammation, hepatic damage and periporal fibrosis. There are currently no FDA approved drugs for the treatment of NAFLD/NASH and there is a clear requirement for better models to understand this disease.

Currently available preclinical models, be it in vivo or in vitro, have a range of limitations and do not fully represent the key aspects of the human disease state. In particular, many preclinical rodent models have been developed, but no individual model fully recapitulates the human disease and significant differences exist in the transcriptomic profile of the liver tissue, the manner in which triglycerides accumulate within the liver, and the level of hepatic fibrosis (2). Staging NASH sampled human in vitro models allows the offer performance to study cells at the cellular level allowing for mechanistic barometers to be elucidated and the genetic drivers of the disease to be specifically explored. We have investigated here a human in vitro NASH model as a potential tool to improve understanding. Understanding the co-culture of a model in a culture of primary human hepatocytes (PHH), Hepatic (HK) and hepatic stellate cells (HSC), cultured in conditions including >5% excess saturated or exogenous TGFF. These co-cultures are maintained in 3D microcultures in an organ-on-chip (OOC) microphysiological system (MPS), which uses microfluidics to perfuse tissue culture medium across the microchips.

AIM
To explore the transcriptive, soluble biomarker and fibrosis phenotype of a fully human in vitro organ-on-chip NASH model and compare it to profiles from current preclinical models and patient murine models. We have also validated the model for use in NASH drug discovery to assess drug efficacy, efficacy of Elafibranor or disease endpoints, whilst ensuring that dosing of compounds used are physiologically relevant and translational.

MATERIALS & METHODS
Cryopreserved primary human hepatocytes (PHH), human hepatocellular (HK) and human hepatic stellate cells (HSC) were obtained from Life Technologies (USA). OOC x 10x hepatocytes were seeded into each well on the Physiomer™ OOC LC-12 plate (Cair Bio Innovations Ltd) along with 0.56x 10² HepG2 and 0.6x 10² HSC (Steadfast conditions contained 10⁻⁵ less HK and HSC). Cells were cultured in HEP-Laun or HEP-Fat medium which contains physiologically relevant concentrations of glucose, insulin, and high concentrations of saturated and unsaturated free fatty acids. Cells were cultured in the Physiomer™ platform for up to 15 days.

Fat accumulation was measured by Oil Red O staining of fixed microcultures, with images acquired on a Nikon Eclipse Ti-E inverse fluorescence microscope. Staining was quantified by ImageJ software and fat content percentage was measured by BCA assay (ThermoFischer). Production of 8,15-LOX, TIMP-1, Fibronectin and α-SMA were all quantified by ELISA. Adiponectin levels were measured by human adiponectin ELISA kit (Biovendor). The expression of inflammatory markers, cytokines and chemokines was measured by cytokine Array kit (Zymark). The number of cells was quantified by counting 5 fields per well under a microscope. Images were captured by a Leica DMi8 confocal microscope.

RESULTS

Figure 1 – Human in vitro OOC NASH model set up

A) OOC NASH model showing the key components of the model. B) Cultures on day 7 for the OOC NASH model set up. The key components of this model include a co-culture of primary human hepatocytes (PHH), Hepatic (HK) and hepatic stellate cells (HSC) cultured in co-culture model. The cell types were cultured together for 15 days in 24-well plate or 10 days in 96-well plate, alongside steatosis controls. Cells were harvested for 15 days for further analysis.

Table 1 – Determining scaled dosing strategy for studies with Obeticholic acid and Elafibranor in OOC NASH model

<table>
<thead>
<tr>
<th>Compound</th>
<th>NASH model set up</th>
<th>OOC NASH model set up</th>
<th>Data collected</th>
<th>Dosing strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obeticholic acid</td>
<td>10 µg</td>
<td>10 µg</td>
<td>10 day</td>
<td>5 µg per day</td>
</tr>
<tr>
<td>Elafibranor</td>
<td>10 µg</td>
<td>10 µg</td>
<td>10 day</td>
<td>5 µg per day</td>
</tr>
</tbody>
</table>

Figure 2 – Co-culture microcultures loaded with fat

A) Fat staining in co-culture microcultures. B) Fat staining was measured and quantified by Oil Red O staining. C) Adiponectin biomarkers were measured in cell culture medium. D) Inflammatory biomarkers were measured in cell culture medium. E) Fibronectin and α-SMA were all measured in cell culture medium. F) Adiponectin and Fibronectin were measured and quantified by high content confocal microscopy. Microcultures were imaged (representative of at least 5 fields each shown) and (f) images quantified. All scale bars 300 µm. Data are mean ± SD, n = 3, *P<0.05.

Figure 3 – Soluble biomarkers expressed in OOC NASH model

A) Soluble biomarkers expressed in OOC NASH model on day 15. B) Elafibranor and Obeticholic acid increased the expression of inflammatory cytokines.

Figure 4 – Transepithelial property of OOC model

A) Transepithelial permeability of OOC model to FITC-dextran. B) Transepithelial permeability of OOC model to calcein. C) Transepithelial permeability of OOC model to DAPI. D) Transepithelial permeability of OOC model to Trypan Blue. E) Transepithelial permeability of OOC model to Lucifer yellow.

Figure 5 – Obeticholic acid and Elafibranor reduce NASH phenotype in co-culture microcultures

A) Control and Obeticholic acid treated samples showing a significant reduction in fibrosis. B) Control and Elafibranor treated samples showing a significant reduction in fibrosis.

CONCLUSIONS
Using the organ-on-chip Physiomer™ platform we have generated a fully human in vitro model of NASH. Co-cultures of PHH, HK and HSC were cultured in fat containing medium which induced key features of clinical disease including fat loading, inflammation and fibrosis. In particular, extra-cellular fibrin-like proteins were quantified using an automated unbiased confocal microscopy approach. To further explore the phenotype of the model the soluble biomarker profile of the model was assessed when the model was exposed to range of different biological cues. The use of LPS, fructose and TGFF were all observed to drive an enhanced inflammatory profile in the model, but TGFF also enhanced the fibrotic phenotype by enhancing TIMP1 and Fibronectin expression. We then compared the transcriptional profile of the model following the addition of various biological cues and compared the expression of key genes associated with human NAFLD/NASH development as previously identified (4,5). Under particular conditions the model was shown to have a high correlation of the expression of the disease associated genes (45%), this could either be induced through a dietary model (just fat) or through the use of TGFF. When explored against the human gene atlas the fat only NASH model was also found to correlate with an expression profile that matches human NASH. This data demonstrates that the OOC model presents a highly similar phenotype and transcriptional profile to human NASH and this phenotype can be modulated through the use of different biological cues, in particular TGFF which will induce an enhanced fibrotic phenotype. We determined a scaled dosing strategy for OCF and ELF in the OOC model to closely mimic clinical dosing of these compounds. Both dosed into the OOC NASH model and demonstrated efficacy similar to what is observed in vivo. Fibrotic disease was observed to reduce a range of inflammatory markers and added useful expression of the key genes of key fibrin markers in a dose dependent manner. This work demonstrates that the OOC in vitro NASH model is ideally suited to exploring the molecular mechanisms that underlie the development of human NASH and is a useful tool for analysing the efficacy of novel anti-NASH therapeutics across a range of different target pathways.

REFERENCES

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