NASH Drug Discovery Using Human Organ-On-Chips

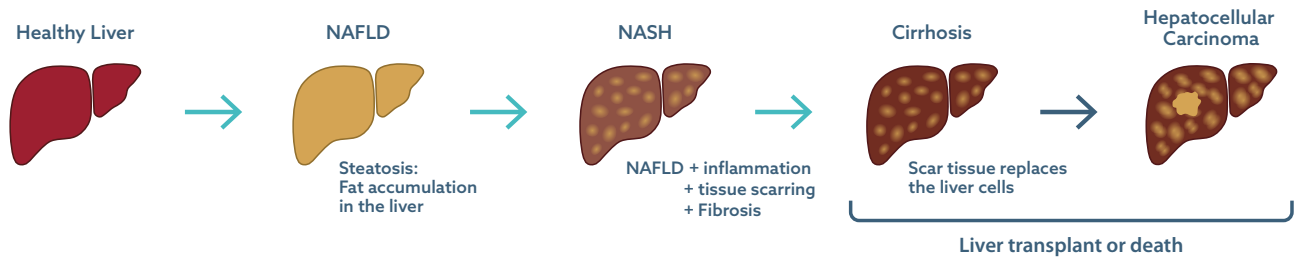
CN Bio Innovations have developed an innovative NAFLD/NASH assay using our 3D perfused Organ-On-Chip (OOC) PhysioMimix™ platform. PhysioMimix™ OOC enables advanced long term *in vitro* liver cultures and modelling of different stages of NAFLD/NASH disease in a range of culture conditions. This physiologically relevant assay is designed to help fast-track the investigation of new therapies for this chronic liver disease.

About NAFLD/NASH

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. It affects approximately 20 – 30 % of adults in Western countries. NAFLD is a spectrum of metabolic diseases in which fat builds up in the liver, due to diet and lifestyle, in the absence of excess alcohol consumption.

Approximately 20% of people diagnosed with NAFLD go on to develop non-alcoholic steatohepatitis (NASH), which is associated with steatosis, inflammation, presence of immune cells in the liver, and insulin resistance (Fig 1). Left untreated, NASH may progress further into cirrhosis and/or liver cancer, for which a liver transplant is the only possible therapeutic intervention (Fig 1).

Figure 1: Schematic of NAFLD/NASH in a human liver



Mimicking NAFLD/NASH disease physiology

There are currently no approved therapies for NAFLD/NASH. Clinical trials for both conditions are resource intensive, requiring large cohorts and years to fully assess a drug's efficacy and toxicity in patients.

Our understanding of the molecular pathways underlying NAFLD/NASH progression has relied primarily on *in vivo* mouse models. Yet, none of the current mouse models displays the full spectrum of NASH pathophysiology. The culture of primary human liver cells *ex vivo* has traditionally been challenging, with cells losing phenotype and function after just a few days.

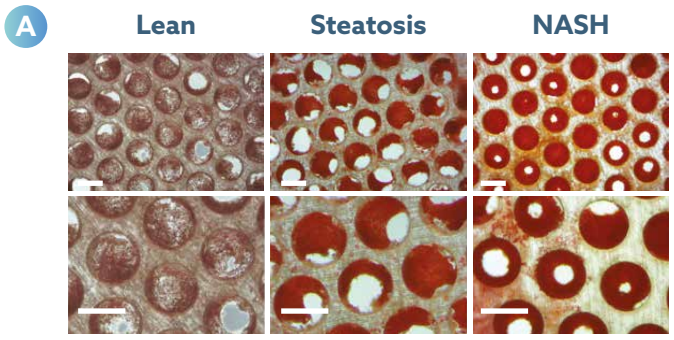
This has limited *in vitro* studies of NAFLD/NASH to short term experiments, generally using immortalised hepatic cell lines.

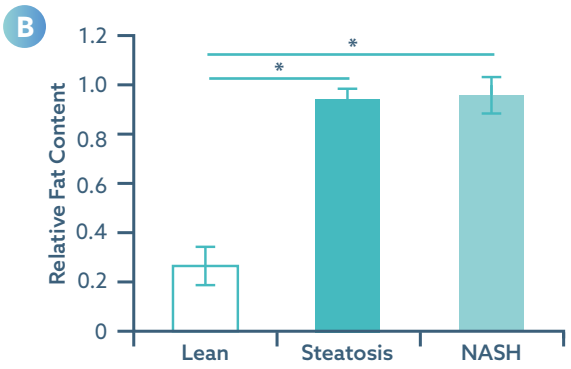
The **CN Bio NAFLD/NASH model** is one the most advanced *in vitro* models currently available. It utilizes CN Bio's proprietary liver-on-chip technology which enables longer term culture (>1 month) of primary liver cells in 3D microtissue structures. The NASH model captures all key aspects of the human disease: intracellular fat accumulation (Fig 2A-B), inflammation (Fig 2C) and fibrosis. The transcriptomic profile of CN Bio's model closely matches the profiles for human disease tissue (Fig 2D).

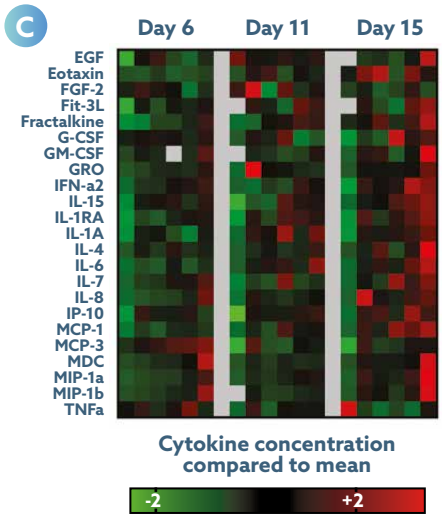
The model demonstrates a quantifiable fibrosis phenotype with the presence of extracellular matrix proteins present through the liver microtissues (Fig 2E and 2F). Disease and cell health markers can be analysed throughout experiments from the cell culture perfusate or by removing the engineered scaffolds used to support the 3D liver tissue.

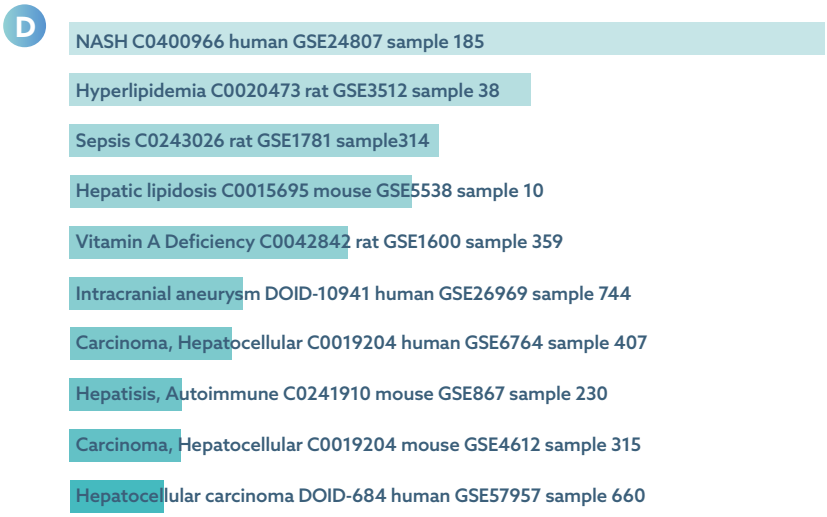
Figure 2: CN Bio NASH model mimics clinical NASH phenotype

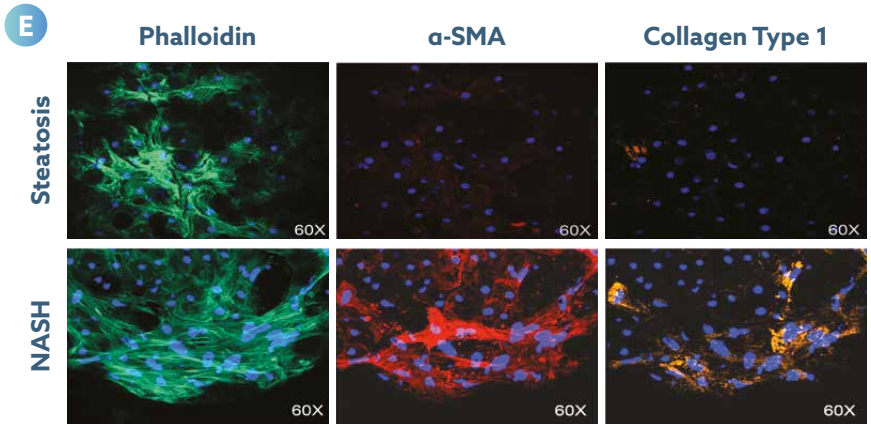
Cultures of hepatocytes, Kupffer and stellate cells can be cultured for up to 4 weeks under lean and high fat dietary conditions.

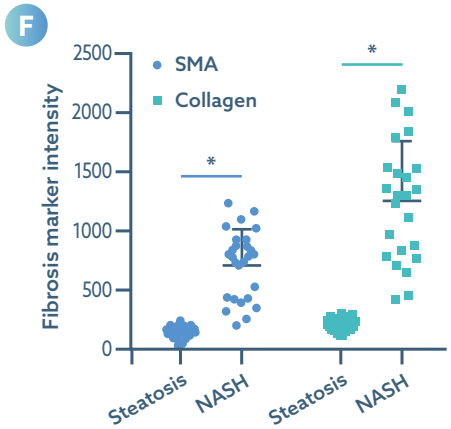












Fat loading

2A. Fat loading, under lean and fat condition, is measured by Oil Red O staining of microtissues, which are also observed by microscopy.

2B. Fat loading is then quantified by absorbance at 510 nm and normalised to total cellular protein to give a relative fat content.

Inflammation

2C. The expression of various inflammatory biomarkers can be measured throughout the experiment from samples of the cell culture perfusate.

Transcriptomic profile

2D. The transcriptomic profile of our NASH models was compared to the human gene atlas to evaluate the expression profile.

Fibrosis

2E. Confocal microscopy of fibrotic markers in microtissues, cultures were stained with phalloidin, α-SMA and collagen type 1. Representative images shown for NAFLD (steatosis) and NASH phenotypes.

2F. Quantification of confocal fibrosis imaging. Photo credit: Dr Samantha Peel, Discovery Sciences IMED Biotech Unit, AstraZeneca.

CN Bio NAFLD/NASH model: a human-relevant preclinical model for Drug Development

CN Bio's NAFLD/NASH *in vitro* model can be used to screen and investigate mechanisms of action for compounds against this complex human metabolic disease in an advanced and physiologically relevant model at a reduced cost. Progress of the disease can be monitored using a range of -omics assays as well as microscopy and biomarker assays.

Dose-response treatments of our model with clinically relevant doses of Obeticholic acid (OCA) and Elafibranor (ELF), two of the most advanced molecules in NASH development, cause similar responses to those observed in clinical trials (Fig 3).

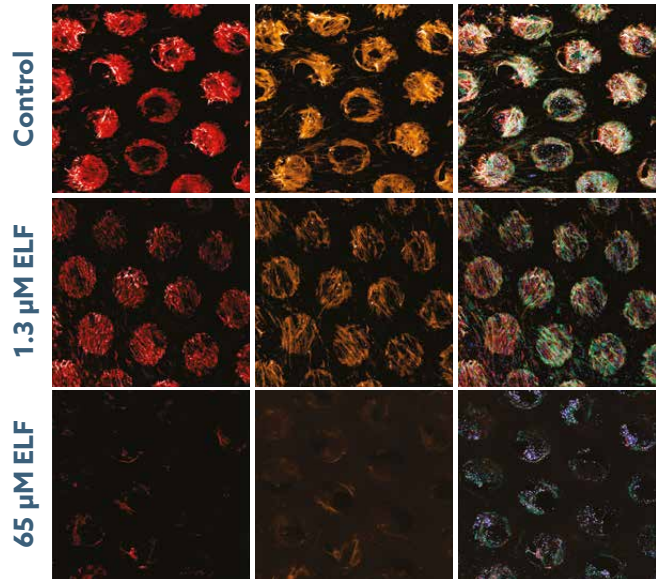
Figure 3: Elafibranor reduces inflammation and fibrosis in CN Bio NASH model

Cultures of hepatocytes, Kupffer and stellate cells under high fat dietary conditions were exposed to various concentration of Elafibranor for 10 days (re-dosed every 24 h).

A

Sample	Time point	IL-1RA	IL-4	IL-6	IL-8	IP-10	MCP-1	MIP-1a	MIP1b	VEGF
Control	Day 8									
	Day 14									
ELF 1.3 µM	Day 8									
	Day 14									
ELF 13 µM	Day 8									
	Day 14									
ELF 65 µM	Day 8									
	Day 14									

B



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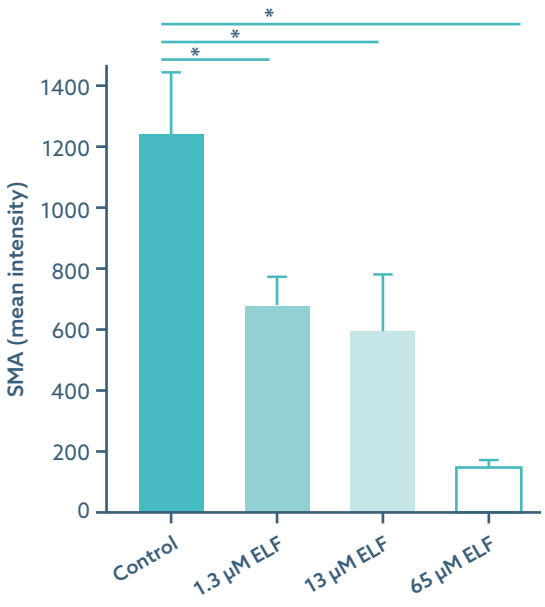


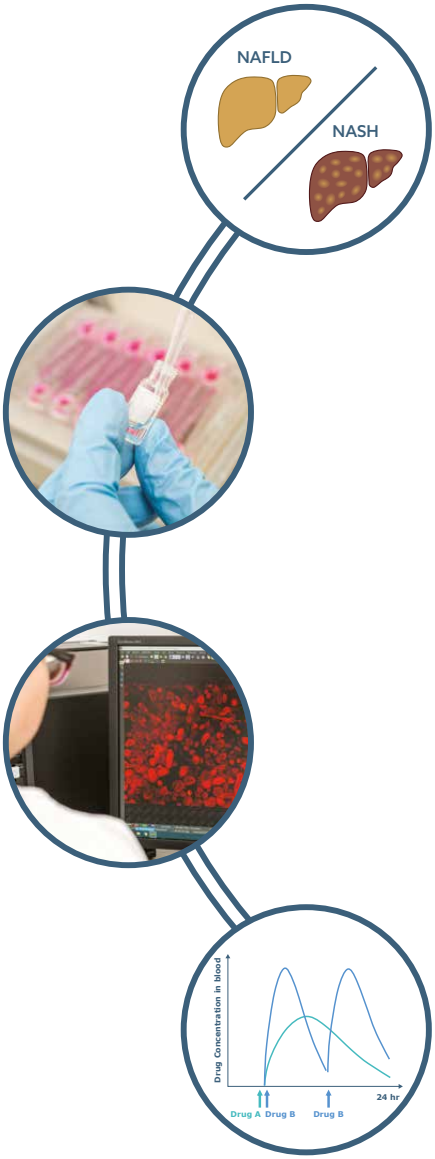
3A. The expression of various cytokines decreases over time during Elafibranor treatment in NASH *in vitro* model.

3B. and 3C. Expression of fibrosis markers, such as alpha-SMA, are reduced in a dose-dependent manner by Elafibranor over time in the NASH *in vitro* model. Quantified using confocal microscopy images of 3D microtissues.

Accessing our NAFLD/NASH in vitro model

We work with you to design the study, acquire compounds and source cells from commercial vendors with appropriate ethical approval to fit to your research needs.

All studies are performed and analysed by CN Bio Innovations, using our PhysioMimix™ OOC platform, and reported at the completion of study.



Select Model of Interest

- NAFLD model: Primary Human Hepatocytes (PHH) under fat dietary conditions
- NASH model: PHH + Kupffer cells (KC) + Human Stellate Cells (HSC) under fat dietary conditions
- Advanced fibrosis NASH: PHH + KC + HSC under fat dietary conditions and TGFß dosing to further increase fibrosis

Compound Dosing

- Media only control
- Vehicle Control: e.g. 0.1% DMSO up to 0.5% if required
- Single dose of Elafibranor (positive control)
- Six doses of compounds of interests (24 wells)

End Point Analysis

- Steatosis: Oil Red O Quantification
- Inflammation: Secretion of key cytokines/chemokines (IL-6, IL-8, IL-1RA, IP-10, MCP-1)
- Fibrosis: Secretion of biomarkers (TIMP-1 and Fibronectin) and imaging of ECM proteins and quantification
- Cell health: Albumin, LDH release, microtissue size

Additional Pharmacology Analysis

- Optimisation of dosing regime
- Refinement of dosing strategy: every other day, daily (standard approach), twice daily, or more
- Bioanalysis to determine compound's behaviour in OOC platform and its clearance in liver microtissues

PhysioMimix™ OOC
A CN BIO INNOVATION

We'd be happy to tell you more about our NAFLD/NASH model. Please contact us by phone or email and we will get in touch as soon as possible.

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