A Pressing Need for Accurate Preclinical Models of Metabolic Disease

How using organ-on-a-chip (OOC) can more accurately recapitulate the physiology and functions of the human body by culturing under flow perfusion – to mimic the blood – in 3D

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Metabolic disease is a broad term to describe pathologies that disrupt normal metabolism and often centre around obesity and insulin resistance. Lifestyle choices, such as poor diets and inactivity, have largely been blamed for the increasing prevalence of obesity, high blood sugar, and diabetes, but genetic factors can also promote these diseases. Whatever the cause, there is no denying metabolic disorders are a modern epidemic. One example is non-alcoholic fatty liver disease (NAFLD), which occurs when there is excessive deposition of fat in the liver. NAFLD is currently the most common chronic liver disease worldwide, affecting around 25% of the general populace and over 50% of diabetics (1). Eventually, the increasing fat content becomes toxic, leading to liver inflammation, hepatic cell death, and potentially a build-up of scar tissue within the liver (cirrhosis). Advanced NAFLD, referred to as non-alcoholic steatohepatitis (NASH), can quickly lead to organ failure or cancer and is the second leading indication for liver transplants.

Despite its prevalence and severity, there is a lack of FDA-approved treatments. Over the past couple of years, a 'first wave' of at least ten different NASH pharmaceuticals, such as obeticholic acid, elafibranor, and selonsertib, hit clinical trials. While initially promising, all failed to meet efficacy targets and none made it to market. Such results are not uncommon; in general, nine in ten preclinical drug candidates are likely to fail. These failures occur for a myriad reasons, but within the field of metabolic disorders, more predictive preclinical models are required to improve the translatability of data between the laboratory and the clinic.

The Limitations of Conventional Methods

The standard *in vitro* liver model is the 2D monoculture of primary human hepatocytes. While these cells are human-relevant and maintain some *in vivo* metabolic activity, they are prone to dedifferentiation and remain viable for only a period of days in 2D. Unfortunately, this limits their use to short-term effect studies. Including non-parenchymal cells (NPCs), like hepatic stellate cells and/or Kupffer cells, improves these models, as they enable the maintenance of the differentiated status of hepatocytes for a longer period. Adding NPCs is also essential to reproducing diseased liver states. While NAFLD can be induced in 2D monoculture via the addition of free fatty acids (FFA), co-culture is required to recapitulate the inflammation associated with advanced NASH progression.

As is often the case, hepatocyte morphology and behaviour in 2D is substantially different compared to those cultured in a more physiologically-relevant 3D format (2). Hepatic spheroids are the most common 3D culture model and, while cell viability and functional

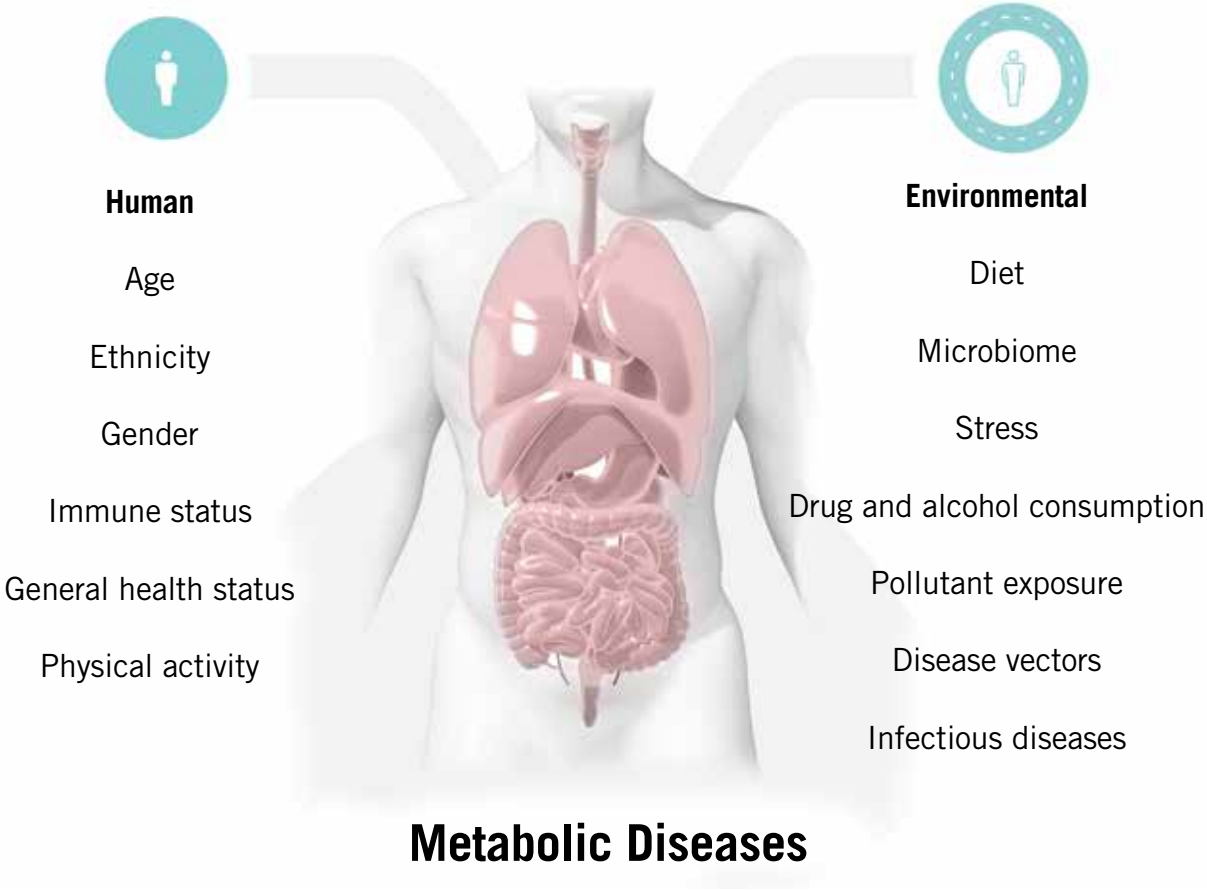

Figure 1: Metabolic diseases are multi-factorial

readouts are improved, this approach struggles to accurately recapitulate the organisation and architecture of *in vivo* tissue (i.e., cell polarisation and tight junction formation). Another key aspect to consider when building *in vitro* liver models is the nutrient demands of the resident cells. Metabolically active hepatocytes consume oxygen at anywhere from 10- to 100-fold the rate of most other cell types. Providing adequate oxygen delivery becomes particularly challenging in 3D, where a thickness of only five cells is sufficient to reduce oxygen levels from normoxic to hypoxic.

On the other side of the preclinical spectrum, *in vivo* animal models present higher-order cell organisation and architecture, while also accounting for organ-organ interactions and off-target effects on the animal as a whole. Human metabolism, however, is considerably different to that of commonly used *in vivo* animal model species (i.e., mice). Indeed, animal models utilise pathways that aren't necessarily human-relevant, and, often, gene expression profiles don't mirror the human disease (3). Because of this, it can prove challenging to replicate metabolic disarray in these systems. Such is the case for NASH, where animal models fail to recreate the extent of fibrosis seen in human patients (4). Furthermore, controlling studies at the cellular level and investigating molecular mechanisms and/or genetic drivers via *in vivo* models is difficult, so results can become blurred by other, unavoidable compensatory factors.

Organ-on-a-Chip Mimics Human Physiology

The need to combine human-relevant biology and reductionist control of *in vitro* systems with the higher-level organisation and complex crosstalk of *in vivo* models has prompted growing interest in organ-on-a-chip technologies. Here, microfluidic components are incorporated into 3D cell cultures to recapitulate the mechanical forces, tissue-tissue interfaces, and spatiotemporal chemical gradients of the cellular microenvironment present in human tissue.

For metabolic disorders and, specifically, the development of *in vitro* liver models, the addition of active perfusion ensures adequate levels of nutrients are supplied to the active hepatocytes. Flow also establishes gradients of autocrine and paracrine signalling, a critical factor in cell polarisation and organisation. This not only results in improved hepatocyte functionality (measured via albumin secretion, CYP expression, etc.), but also functionality that is maintained for significantly extended periods (up to

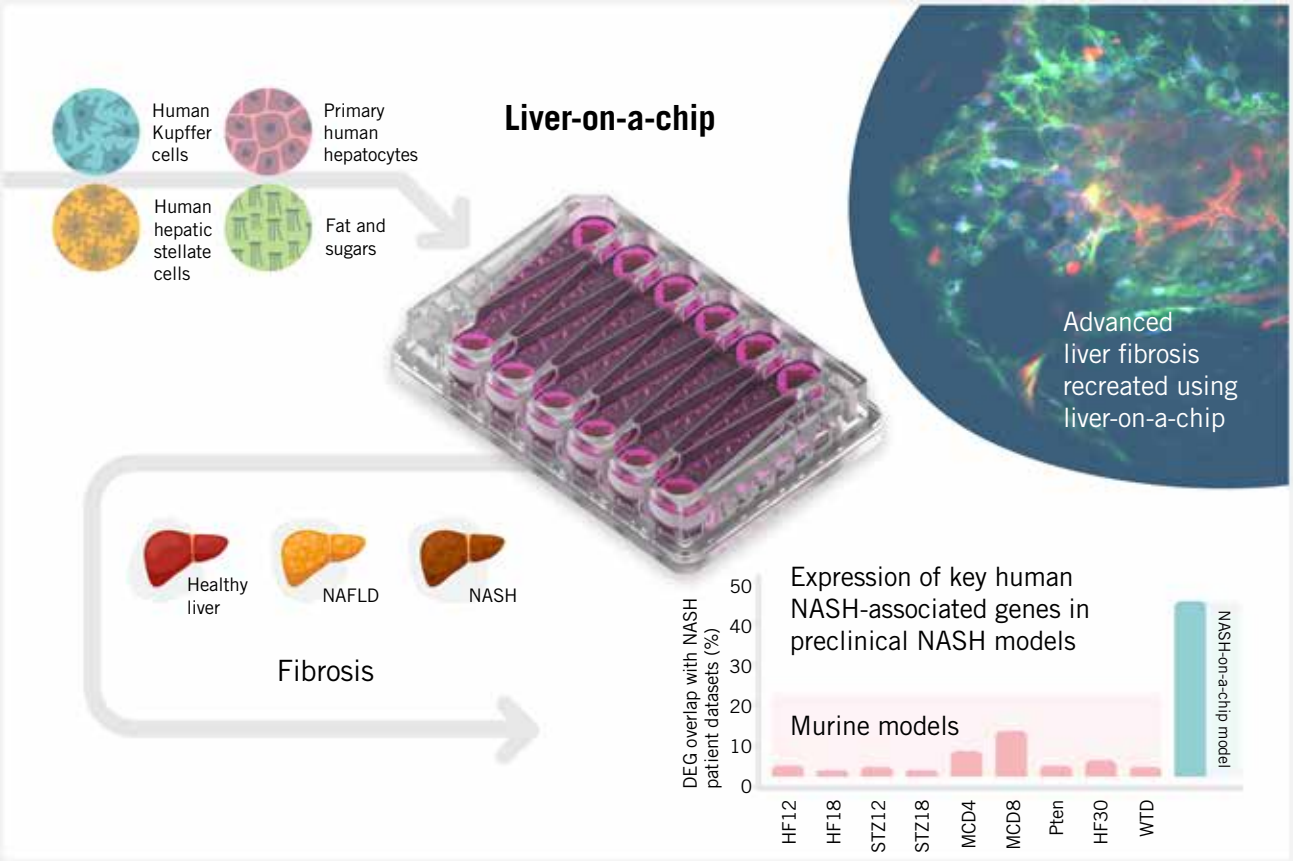

Figure 2: Liver-on-a-chip models recapitulate human metabolic disease phenotypes. Liver tissues grown on 3D scaffolds, under perfusion, to recreate the liver microarchitecture and NASH phenotypes. Image credit: Dr Gareth Guenigault, CN Bio

40 days). As with static cultures, the introduction of non-parenchymal cells increases the physiological relevance of the model. Indeed, perfused 3D liver tri-cultures grown in excess FFAs exhibit advanced markers of inflammation and a more robust fibrotic phenotype when compared to conventional preclinical NASH models (5).

These liver-on-a-chip systems excel at recapitulating the human liver microenvironment, but multifactorial diseases like metabolic dysfunction can also be influenced by immunological responses, the interplay between the gut and the liver, and, subsequently, the impact of the microbiome on the gut. To account for this higher-level biology, interconnected OOC platforms facilitate flow between different *in vitro* organ models. For example, when considering the gut-liver axis, inflammatory bowel and liver diseases are closely linked – patients with one are often at an increased risk of developing the other (6). Advanced *in vitro* multi-organ models have been developed to investigate this correlation. For example, a gut endothelial cell barrier on a Transwell® can be connected by flow to a 3D scaffold of hepatocytes and NPCs in another section of the plate (7). While subtle, there are significant changes in the metabolic properties and gene expression of both the gut and liver models, ultimately yielding more *in vivo*-like behaviour. Furthermore, by incorporating circulating immune cells, the sequence of events connecting gut inflammation to cytokine production and, eventually, liver injury can be studied.

The influence of the gut can also be modulated by its microbiota, a critical component of digestion, nutrient absorption, and immune regulation. Modelling this interaction has proved challenging in the past since gut microbes are extremely sensitive to oxygen and cannot survive in static co-cultures with human cells. Once again, OOCs with microfluidics can address this barrier. By establishing steep oxygen gradients to provide anaerobic and normoxic conditions within a single culture, the anti-inflammatory effects (i.e., histone deacetylation, NF-kB pathway activation) of gut microbes on a primary human mucosal barrier was uncovered, demonstrating how the interactions between the human gut and a variety of microbiota, pathogens, and bacteriotherapeutics can now be explored *in vitro* (8).

The Future of Organ-on-a-Chip Technology

While OOC has the potential to dramatically increase understanding of metabolic disease and revolutionise

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the development of targeted biotherapeutics, there are challenges that must be overcome first. The most pressing is the accessibility barrier these new technologies present. As innovative as they are, OOC setups can appear overly cumbersome, complicated, and difficult to handle without technical expertise. They are also a new technology, so an effort must be made to accept them in standard regulator processes alongside established and traditional platforms (e.g., 2D). Finally, as the immune response is such a large aspect of metabolic dysfunction, it must be feasible to incorporate immune components. These collective issues may dissuade biologists who are interested in the greater physiological relevance OOCs offer but do not have a background in 3D platforms or microfluidics.

In answer to this challenge, start-ups and established companies alike are advancing OOC systems beyond their original academic prototypes, which generally involved yards of intricately set up tubing and a degree in bioengineering to run. Such commercial solutions are firmly designed for the cell biologist's hands. Due to this focus on delivering robust, reliable, and industry-proven products that any cell culture scientist can utilise, OOCs are becoming an accessible path to producing high-impact work. Furthermore, due in part to the lower barrier of entry, regulatory agencies, like the FDA, are exploring OOC results and comparing them to traditional methods (9).

With the rate of metabolic disorders rising, the demand for relevant models will grow. Traditional approaches utilising animal and 2D *in vitro* platforms have serious limitations when it comes to modelling the human metabolism that OOC platforms can overcome. The development of easy-to-use commercial OOC products has reduced the technical access barriers associated with these systems and the adoption of said technology is on the rise. With the development and commercialisation of single and multi-organ OOC models that allow for circulating immune cells, it will certainly be exciting to see where exactly the boundaries of OOC reside.

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