Applications of Microphysiological Systems to Disease Models in the Biopharmaceutical Industry: Opportunities and Challenges

Onyi Irrechukwu¹, Ronnie Yeager², Rhiannon David³, Jason Ekert⁴, Anitha Saravanakumar⁵ and Colin K. Choi⁶

¹Preclinical Sciences and Translational Safety, Janssen Research and Development, LLC, Spring House, PA, USA; ²Preclinical Safety, AbbVie, North Chicago, IL, USA; ³Safety Innovation, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Cambridge, UK; ⁴US Discovery, Early Solutions, UCB, Cambridge, MA, USA; ⁵Drug Metabolism and Pharmacokinetics, Takeda Pharmaceutical Company Ltd, Cambridge, MA, USA; ⁶Preclinical Safety, Biogen, Cambridge, MA, USA

Abstract
Disease models enable researchers to investigate, test, and identify therapeutic targets that would alter the patients’ disease condition and improve quality of life. Advances in genetic alteration and analytical techniques have enabled rapid development of disease models using preclinical animals and cell cultures. However, success rates of drug development remain low due to limited recapitulation of clinical pathophysiology by these models. To resolve this challenge, the pharmaceutical industry has explored microphysiological system (MPS) disease models, which are complex in vitro systems that include but are not limited to organ-on-a-chip, organoids, spheroids, and 3D bioengineered tissues (e.g., 3D printing, hydrogels). Capable of integrating key in vivo properties, such as disease-relevant human cells, multi-cellularity/dimensionality of organs, and/or well-controlled physical and molecular cues, MPS disease models are being developed for a variety of indications. With on-going qualifications or validations for wide adoption within the pharmaceutical industry, MPS disease models hold exciting potential to enable in-depth investigation of in vivo pathophysiology and enhance drug discovery and development processes. To introduce the present status of MPS disease models, this paper describes notable examples in six disease areas: cancer, liver/kidney diseases, respiratory diseases/COVID-19, neurodegenerative diseases, gastrointestinal diseases, and select rare diseases. Additionally, we describe current technical limitations and provide recommendations for future development that would expand application opportunities within the pharmaceutical industry.

1 Introduction

Having experimental capabilities to probe and elucidate human pathophysiology, pharmacology, and toxicology is key to successful drug discovery and development. Despite unprecedented investment in cutting-edge analytical tools and standardized establishment of animal studies, drug attrition remains high in the pharmaceutical industry. Only about 10% of Phase 1 candidates are approved by the U.S. Food and Drug Administration (FDA), which leads to delay of life-saving or disease-modifying therapies as well as increases in the cost of research and development (R&D) (over 1 billion U.S. dollars per marketed drug) (Blomme and Will, 2016; Munos, 2009). The high prevalence of drug development failures is caused primarily by insufficient efficacy or safety demonstrated in clinical trials (Arrowsmith and Miller, 2013; Hwang et al., 2016). Moreover, current industry and regulatory paradigms rely largely on animal studies that are hampered by poor preclinical to clinical translation (Bailey et al., 2015; Clark and Steger-Hartmann, 2018; Monticello et al., 2017; Shanks et al., 2009).

Pharmaceutical research entails identifying the underlying molecular mechanisms by which human diseases are regulated and identifying the druggable targets for therapy. For drug discovery, various animal disease models have been developed and utilized (e.g., transgenic mice with targeted mutations). However, due to the differences in physiology and disease manifestations be-
between animals and humans, these models often do not recapitu-
late human disease phenotypes or induce expected responses to
investigational treatments (Seok et al., 2013; Vitek et al., 2020).
This imperfect concordance between preclinical animal models
and patients is one of the main reasons why promising drug can-
didates often fail to meet efficacy or safety endpoints in clinical
trials (Mak et al., 2014).

Incorporation of human cells in drug discovery assays is signif-
icant for bridging the gap between preclinical and clinical R&D.
Primary or immortalized human cells are cultured routinely us-
ing two-dimensional (2D) plastic dishes, providing a relatively
simple workflow at low cost to support a range of throughput
specifications (Tab. 1). These models typically sit at an intersec-
tion between screening disease biology pathways with isolated
proteins or enzymes and movement into whole animal systems.
Disease-associated mutations are readily introduced in vitro via
gene editing tools such as CRISPR-Cas9, and high-throughput
multi-wells with human cells are widely employed for rapid
screening of hundreds or thousands of compounds via automat-
ed high-content screening (HCS) systems. Unfortunately, human
and other animal cells grown on relatively rigid 2D surfaces in
static culture settings (i.e., lack of fluid flow/exchange) often
do not express (or lose during culture) normal protein expres-
sions and phenotypes that mediate the molecular, cellular, or tis-
ssue-level processes of human diseases, thus limiting their utility.
For proper in vitro functions, cells in vitro need to experience a
specific combination of chemical, molecular, and physical cues
that are present in a target tissue or organ. Pharmaceutical com-
panies are exploring microphysiological systems (MPS) to de-
velop disease models to better mimic pathophysiology for drug
discovery and development (Marx et al., 2020).

This perspective paper is part of a series organized by the IQ
MPS Affiliate within the International Consortium for Innovation
and Quality in Pharmaceutical Development (IQ Consortium), a
not-for-profit organization comprised of leading pharmaceutical
and biotechnology companies aligned to foster key scientific and
regulatory aspects of drug discovery and development. The pa-
per aims to provide a glance at MPS disease models and describe
their current position and potential in the pharmaceutical industry.
MPS described in this paper are defined by the IQ MPS Affili-
te relatively broadly to capture their varied natures and include
one or more of the following elements: multicellular environment
within biopolymer or tissue-derived matrix, 3D structures, me-
chanical factors such as stretch or perfusion, incorporation of pri-
mary or stem cell-derived cells, and/or immune components (Fab-
ne et al., 2020). Currently, MPS disease models are either academ-
ic proof-of-concept devices or relatively specialized products that
integrate multicellularity, multi-dimensionality, fluid flow, and/
or mechanical forces. Nevertheless, with its reductionist design
approach of building only the key functional units of living or-
gans, MPS offer cutting-edge yet pragmatic means to recapitulate
various aspects of different human diseases (Benam et al., 2015;
Ma et al., 2021; Watson et al., 2017). The advancement of hu-
man induced pluripotent stem cells (iPSCs) or adult stem cell-de-
terminated organoids enables generating an unlimited supply of cell
types specific to any tissues/organs that may also exhibit targeted
disease phenotypes via patient-derived sourcing or gene editing
(Kim et al., 2020). By bringing together in-depth understanding of
pathophysiology, stem cell technologies, and innovative bio-
engineering, MPS disease models offer unique opportunities to
screen compounds in a range of disease contexts of interest (Ing-
ger, 2022). Additionally, such disease models enable mechanistic
investigations of drugs to enhance target identification as well as
efficacy and toxicity predictions for improved preclinical to clin-
ical translation. Six disease areas of on-going R&D activities and
relevant MPS disease model examples are described in the paper:
cancer, liver/kidney diseases, respiratory diseases/COVID-19,
neurodegenerative diseases, gastrointestinal diseases, and select
rare diseases. Furthermore, we provide perspectives on the lim-
itations and the areas for improvement for MPS disease models to
become qualified assays in the pharmaceutical industry.

### 2 Major MPS disease models

#### 2.1 Cancer

Despite the high incidence of cancer and substantial investment in
cancer research, oncology drug development continues to
have a high level of attrition (Wong et al., 2019). While there has
been progress in the development of cancer drugs, highly inva-
sive cancers, such as pancreatic cancer, remain difficult to treat,
with metastasis as the predominant cause of cancer-related death
(Fidler and Kripke, 2015; Puls et al., 2018). Tumor heterogeneity
presents challenges due to changes in genetics, transcriptomics,
epigenetics, and phenotype. Intra-tumor heterogeneity drives the
evolution in cancers, resulting in cells with different molecular
signatures that show varying levels of drug resistance (Dago-
go-Jack and Shaw, 2018).

Additionally, there is a lack of predictive, physiologically rele-
vant preclinical tumor models (Puls et al., 2018). Animal models

---

**Tab. 1: Throughput specifications**

<table>
<thead>
<tr>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>96, 384, and 1536 wells/devices</td>
<td>12, 24, and 48 wells/devices</td>
<td>Single plate/device, 6 wells</td>
</tr>
<tr>
<td>Supports fast, large-scale compound</td>
<td>Regularly used for mechanistic studies</td>
<td>Applicable for academic proof-of-concept</td>
</tr>
<tr>
<td>screening. Primarily with 2D well-plates.</td>
<td>involving relatively complex assay conditions.</td>
<td>prototypes or highly complex designs.</td>
</tr>
<tr>
<td>Automation increases cell culture and</td>
<td>Current preferred standard for commercial MPS.</td>
<td>Lacks scale-up manufacturing to increase</td>
</tr>
<tr>
<td>assay capacity. Negligible availability</td>
<td></td>
<td>throughput or quality.</td>
</tr>
<tr>
<td>for MPS.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
play a crucial role in drug development; however, they are poor models of human cancer as they lack features of the tissue-specific microenvironment (Gould et al., 2015). There has been a move towards patient-derived xenograft (PDX) and patient-derived orthotopic xenograft (PDOX) models, which enable patient tumor tissue to be examined in vivo in a physiologically relevant environment, as histological characteristics, some genetic heterogeneity, and stromal architecture are retained (Bleijs et al., 2019; Gould et al., 2015; Veeranki et al., 2019). These models have been shown to have improved sensitivity to drug treatment over cell line xenograft models and to mimic tumor growth and metastasis (Veeranki et al., 2019). Both PDX and PDOX retain key features of parental tumors (e.g., genetic and phenotypic heterogeneity). However, human stroma is replaced with mouse stroma in these models; thus, they do not fully recreate the human tumor microenvironment (TME) (Veeranki et al., 2019). Also, they are time-consuming and labor-intensive and not amenable to high-throughput drug screening (Fan et al., 2019).

Traditional 2D cell culture is used to evaluate toxicity and/or efficacy of drugs preclinically; however, cells in 2D culture have differential gene expression compared to the in vivo tumor and lack the stroma, both of which are important for tumor development and phenotype (Birgersdotter et al., 2005). Metastasis involves tumor cell engagement, remodeling, and invasion of the surrounding extracellular matrix (ECM), which cannot be accurately recreated in 2D (Makale, 2007; Spill et al., 2016). Solid tumors grow in a 3D environment with heterogeneous exposure to oxygen, nutrients, and drugs, and they consist of multiple cell types and ECM (Trujillo-de Santiago et al., 2019). Traditional 2D culture does not recapitulate these complex environmental signals nor the cell-cell interactions and processes that characterize a tumor and its environment (Kim et al., 2019; Shang et al., 2019). Liquid tumors (hematological cancers) present an additional challenge because they show profound heterogeneity, complexity, and dynamics as well as contain cancer stem cells and reprogrammed stromal cells (Chowdury et al., 2019).

To improve oncology drug development, testing, and clinical success, more physiologically relevant 3D models are required. There is literature evidence demonstrating that these multicellular and multidimensional models recapitulate in vivo cancer biology better than 2D culture (Berg et al., 2021; Rae et al., 2021). The level of complexity (spheroids, scaffold-based models, or MPS) and cellular origin (cancer cell lines, biopsies/patient-derived cells, or primary cells) of 3D tumor models can vary, each with their own pros and cons. Examples of each type of model are discussed and summarized in Table 2.

**Tab. 2: A glance at MPS cancer models**

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>Spheroids</td>
<td>– Cell proliferation and migration</td>
<td>Ekert et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Microfluidic device</td>
<td>– Real-time monitoring (pH, TEER), toxicity, evaluation of drugs (IC50 values), confocal imaging for live/dead assay</td>
<td>Khalid et al., 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Human NCI-H1437 non-small cell lung cancer cell line</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Organoids</td>
<td>– Histopathology, hormone receptor status, HER2 status, DNA copy number variations and sequence changes, gene expression</td>
<td>Sachs et al., 2018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– &gt; 100 primary and metastatic human breast cancer organoid lines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microfluidic device</td>
<td>– Real-time monitoring of nanoparticle transport, accumulation, and cytotoxicity</td>
<td>Kwak et al., 2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Human MCF-7 breast cancer cell line in type 1 collagen and human primary microvascular endothelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Collagen remodeling, invasive epithelial cell phenotype with a 3D fibroblast co-culture compartment</td>
<td>Sung et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Human mammary fibroblasts (HMFs) with human mammary epithelial cells (MCF10-DCIS)</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer (CRC)</td>
<td>Organoids</td>
<td>– Single cell transcriptomics</td>
<td>Yan et al., 2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Early onset CRC organoid biobank from 20 individual patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microfluidic device</td>
<td>– 3D formation of endothelial sprouts, changes in cellular organization; gene expression analysis and immunocytochemistry on chip</td>
<td>Carvalho et al., 2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– CRC cell line HCT-116 in Matrigel, primary human colonic microvascular endothelial cells (HCoMECs)</td>
<td></td>
</tr>
</tbody>
</table>
SKOV-3 (ovarian cancer cell line) proliferation was inhibited by trastuzumab in 3D compared to 2D due to HER2 activation in the MCTS (Pickl and Ries, 2009). Other aspects of tumor biology have improved with spheroid culture: Ewing tumor MCTS showed a similar proliferative index, cell morphology, cell-cell junctions, and pathway activation (ERK1/2 MAPK and PI3K/AKT) to patient tumors (Lawlor et al., 2002). Spheroids also offer an advantage over other complex models: They are amenable to automated or high-throughput screening due to their consistent size, shape, and easy/rapid handling (Weiswald et al., 2015). Limitations of spheroids include lack of shear stress and interstitial flow (present in vivo and can affect metastasis processes), and

### 2.1.1 Spheroids

Multicellular tumor spheroid (MCTS) models are 3D cancer cell aggregates generated using cancer cell lines or primary cells, and they can be combined with TME cells, including fibroblasts, endothelial cells, or immune cells (Li and Kumacheva, 2018). MCTS have many features of solid tumors, including strong cell-cell junctions and greater cell-cell contacts than 2D culture (Kim et al., 2019), gradients in nutrient and oxygen concentration, and ECM deposition (Li and Kumacheva, 2018). Furthermore, MCTS have been reported to more accurately mimic drug sensitivity/resistance of cancer cells in solid tumors compared to 2D culture. For example, SKBR3 (breast cancer cell line) and SKOV-3 (ovarian cancer cell line) proliferation was inhibited by trastuzumab in 3D compared to 2D due to HER2 activation in the MCTS (Pickl and Ries, 2009). Other aspects of tumor biology have improved with spheroid culture: Ewing tumor MCTS showed a similar proliferative index, cell morphology, cell-cell junctions, and pathway activation (ERK1/2 MAPK and PI3K/AKT) to patient tumors (Lawlor et al., 2002). Spheroids also offer an advantage over other complex models: They are amenable to automated or high-throughput screening due to their consistent size, shape, and easy/rapid handling (Weiswald et al., 2015). Limitations of spheroids include lack of shear stress and interstitial flow (present in vivo and can affect metastasis processes), and
the influence of spheroid size on functionality. Small spheroids are amenable to screening and recapitulate cell-cell and cell-matrix interactions but do not contain hypoxic regions or oxygen gradients. The method of spheroid formation can impose certain restrictions, such as the lack of uniformity in suspension culture or reduced throughput with the hanging drop method (Carvalho et al., 2016; Katt et al., 2016). High-resolution imaging of spheroids can be challenging due to light scattering, potential loss of 3D information from preparation techniques (e.g., sectioning, staining), or limitation to the outer cell layers. This can be overcome by using tissue clearing techniques and light sheet microscopy (Edwards et al., 2020).

2.1.2 Patient-derived human organoids
Organoids can be derived from patient biopsies, providing an avenue for a personalized approach. They differ from spheroids in that they are propagated from stem cells, and organoids derived from patients’ biopsies have been demonstrated to be predictive of patient response. Patient-derived organoids have been shown to have a more relevant gene expression profile compared to 2D cultures. For example, organoids derived from endometrial tumors better reflected the genetic profile of the tumor (Berg et al., 2021). Cancer patient-derived organoids may be used to evaluate treatment options and response of gastrointestinal cancers, as biopsy specimens from metastatic gastrointestinal cancer patients were evaluated across various factors including response rates, refractory, and lines of therapy/standard of care (Vlachogiannis et al., 2018). Additionally, organoids derived from patients with gastrointestinal metastatic cancers that were treated with common oncology drugs showed molecular profiles similar to those from the parent tumors (Nagle et al., 2018), indicating that understanding patient outcomes in response to therapies provides the context from which the derived organoids can be tested to evaluate molecular mechanisms contributing to tumor sensitivity against various therapeutics.

Patient-derived organoids have been utilized to test 240 kinase inhibitors for personalized treatment recommendations (Phan et al., 2019), and more recently, pan-cancer tumor organoids using cultures from over 1,000 patients were developed for molecular profiling and high-throughput screening of 351 compounds (Larsen et al., 2021). Such applications are suggested to enhance preclinical drug response projections, especially for challenging cancers including esophageal and pancreatic cancer. Patient-derived organoids also allow for the determination of inter-patient variability in cancer stem cell populations, which could be used to inform on sensitivity differences within a cohort (Nagle et al., 2018). For example, selective inhibition by some EGFR inhibitors against pancreatic tumor stem cells was demonstrated in pancreatic tumor organoids (Kaushik et al., 2021). Organoids from tumor and matched normal patient tissues can also be used to screen drugs for their ability to specifically target the tumor (Rauth et al., 2021).

However, it is worth noting that challenges exist in using patient-derived organoids, including the low success rate of generating organoids from biopsies due to the inability of the cells to adapt to the in vitro environment or small amounts of starting material (Foo et al., 2022). Patient-derived organoids are also more expensive and take more time to establish than 2D culture or 3D spheroids, but this can be outweighed by the improved relevance (Rae et al., 2021). Ethical challenges in developing these models must also be considered, such as informed consent of cell donors, which have been discussed in depth (Mollaki, 2021).

2.1.3 3D hydrogel cultures
3D tumor models have also been developed using multiple types of scaffolds, including paper, hydrogels, or bioprinted scaffolds. Hydrogels are networks of chemically or physically cross-linked polymer molecules inflated with an aqueous medium. They can be designed to have a range of biophysical properties, biological functions, and compositions (Li and Kumacheva, 2018), can be easily controlled, and have high reproducibility (Kim et al., 2019). Hydrogels have been shown to be useful for studying metastasis as the stiffness of the hydrogels can be modulated, enabling the mechanical properties of the TME and specific tumor types to be recapitulated (Beck et al., 2013; Pradhan et al., 2017). Furthermore, the encapsulation of cancer cells in hydrogels provides more precise control of size and shape, making them suitable for high-throughput platforms (Kim et al., 2019).

Potential limitations of hydrogels include limited nutrient and oxygen diffusion (and transport), creating hypoxic conditions and necrotic cores (Kim et al., 2019). However, these can be overcome by forming microchannel arrays in the hydrogel, enhancing nutrient delivery and cell viability (Miller et al., 2012). Additionally, the limited oxygen transport can be advantageous where hypoxic environments are required, and hydrogel dimensions can be modulated to enable controlled hypoxic conditions (Kim et al., 2019; Szot et al., 2011). For example, by using hypoxia-inducible hydrogels, tubule and lumen formation by endothelial colony forming cells was observed, which did not occur in non-hypoxic conditions (Park and Gerecht, 2014).

Scaffolds such as hydrogels can also be produced by bioprinting, which enables fabrication of in vivo-like TMEs with arbitrary geometry and material properties, such as stiffness, to control the spatial distribution of cells (Kim et al., 2019). Via this approach, cancer cell-stromal cell interactions during metastasis can be investigated, such as breast cancer cell-osteoblast interaction on 3D-printed nano-bone matrices (Zhu et al., 2016). Challenges to bioprinting include the technique and type of bioink used as well as the inclusion of cells (i.e., viability of iPSCs and stem cells could decrease owing to high temperature and/or shear forces of direct 3D printing) that demand specialized equipment and/or expensive materials that still limit the broad application of this technique (Placone et al., 2020).

2.1.4 Microfluidics-based devices
Microfluidics-based MPS have emerged as potentially powerful tools in cancer research. The inclusion of microfluidics provides another dimension of physiological mimicry by allowing a continuous feed of nutrients (and pharmaceutical compounds) (Trujillo-de Santiago et al., 2019). MPS also control cell and tissue
perfusion and enable long-term maintenance of stable concentration gradients, which have been shown to be important for investigating the acquisition of drug resistance (Kim et al., 2019). For example, treatment with doxorubicin initially induced cytotoxicity in three quarters of the microchambers containing U87 glioblastoma cells, but cell repopulation was observed two days later, indicating that the surviving cells were resistant to doxorubicin treatment and migrated to areas without cells. These cells showed increased efflux activity compared to wild type cells, suggesting acquisition of resistance during doxorubicin treatment (Hane et al., 2016).

Fluid shear stress and hydrostatic pressure that exist in the TME can also be recapitulated in microfluidics-based MPS (Bregenzer et al., 2019). Shear stress in these devices has been shown to increase proliferation and viability of cancer cells (Fan et al., 2019), while interstitial pressure was shown as an important factor controlling cell migration (Polacheck et al., 2014). Multi-organ “metastasis-on-a-chip” platforms have been developed to examine cell migration from one tissue/organ site to another and thus detect circulating tumor cells (Zhao et al., 2019). Colon cancer cells were shown to metastasize from a colon cancer organoid and engraft in a liver organoid within the same recirculating microfluidic chip (Skardal et al., 2016). In another device, the extravasation of breast cancer cells through an endothelial cell layer into bone was observed (Bersini et al., 2014). While fluidic shear stress provided by MPS is important for recapitulating the structure and function of tumor, blood vessel, and lymphatic vessels (Kim et al., 2019), there is growing evidence of important secondary effects of exposing stromal cells to shear stress. These include contraction of cancer-associated fibroblasts, which remodels the surrounding matrix (Ng et al., 2005; Winer et al., 2009), and interstitial flow, which can lead to leaky blood and lymph vessels, both of which can exacerbate tumor progression. As such, for a more accurate representation of the TME and thereby cancer, these secondary effects should be addressed in MPS (Kim et al., 2019).

2.2 Liver/kidney (metabolic)

2.2.1 Liver

The liver plays a crucial role in all metabolic processes in the body. Apart from xenobiotic metabolism and transport, the liver maintains glucose homeostasis via glycogenesis, glycogenolysis, and gluconeogenesis. Its roles in lipid and protein metabolism include oxidation of triglycerides for energy, removal of ammonia through production of urea and lipid synthesis (e.g., cholesterol and phospholipids), conjugation of bilirubin, and production of plasma proteins (e.g., albumin and clotting factors). Thus, hepatic diseases impact many critical physiological functions, and in vitro platforms capable of replicating this milieu will be invaluable to the drug development process. Although most hepatic diseases directly affect and are initiated in hepatocytes, disease progression often depends on the contribution and interaction of the non-parenchymal cells (NPCs) – Kupffer cells, stellate cells, liver sinusoidal endothelial cells (LSECs) – with the hepatocytes.

The ideal liver platform should have a multicellular architecture to reflect the liver in vivo environment. Furthermore, the platform should demonstrate stability and longevity in culture (2 weeks or more) with robust expression of phase I/II drug metabolizing enzymes, transporters, and relevant receptors. Long-term cultures allow for formation of metabolites from low-turnover compounds and detection of delayed toxicities. We define liver MPS models as those that go beyond the traditional 2D hepatocyte sandwich culture and include micropatterned models, hepatocyte-derived spheroids, 3D-bioprinted liver, liver-on-chip, and stem cell-derived liver organoids (Baudy et al., 2020). Also included in this section are models consisting of hepatic cell lines cultured in 3D hydrogels and spheroids derived from hepatic cell lines and iPSC-derived hepatocytes. The importance and commitment to reducing liver disease are exemplified by the number of therapeutics currently in the clinic, clinical trials, and in various preclinical stages of drug discovery/development. A few of these disease models are summarized below and in Table 3.

Tab. 3: A glance at MPS liver and kidney disease models

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver – NASH/NAFLD</td>
<td>Microfluidic device (liver-on-a-chip)</td>
<td>- Lipid accumulation, increased expression of fibrotic and ECM markers (α-SMA, Col I, Col III, Col IV), production of pro-inflammatory cytokines (TNF-α, MCP-1, IP-10)</td>
<td>a Jang et al., 2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Quad-culture of PHH, Kupffer cells, stellate cells, and LSECs; 3D hepatocyte microtissues</td>
<td>b Kostrzewski et al., 2017</td>
</tr>
<tr>
<td></td>
<td>Spheroids</td>
<td>- Lipid accumulation, lipogenic genes (PCK1, PDK4)</td>
<td>c Kozyra et al., 2018</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Micropatterned co-cultures</td>
<td>- Glucose production, glycogen, insulin secretion; response to glucagon and insulin</td>
<td>Davidson et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- PHH and mouse 3T3 fibroblasts</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>Micropatterned co-cultures</td>
<td>- Viral load (DNA and protein): HbsAg, HbeAg, cccDNA, HBV DNA</td>
<td>a Shlomai et al., 2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-cultures of PHH + 3T3 mouse fibroblasts – required JAK3 or PEG supplementation</td>
<td>b Kratochwil et al., 2018</td>
</tr>
<tr>
<td>Disease model</td>
<td>Platform specification</td>
<td>Key readouts and cell types</td>
<td>References</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>Microfluidic device</strong></td>
<td></td>
<td>- Viral load (DNA and protein): HbsAg(^a), HbeAg(^b), HbcAg(^a,b), HBV DNA(^a,b)</td>
<td>(^a) Kang et al., 2017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-cultures of PHH + immortalized BAEC(^a), 3D PHH microtissues (collagen-coated scaffold)(^b)</td>
<td>(^b) Ortega-Prieto et al., 2018</td>
</tr>
<tr>
<td><strong>3D hydrogel cultures</strong></td>
<td></td>
<td>- Viral load: HBV DNA (^b)</td>
<td>(^c) Petropolis et al., 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-cultures of Huh7-NTCP + LSECs(^c)</td>
<td></td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td>Spheroids</td>
<td>- Viral load: HCV RNA, expression of viral entry factors – CD81, SCARB1, occludin, claudin(^a,b)</td>
<td>(^a) Ananthanarayanan et al., 2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Huh7, PHH (cultured in cellulose sponges)(^a)</td>
<td>(^b) Molina-Jimenez et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Viral load (RNA and protein): HCV RNA, HCcAg(^b)</td>
<td>(^c) Cho et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Huh7 (Matrige(^b), PEG hydrogel(^b))</td>
<td></td>
</tr>
<tr>
<td><strong>Autosomal dominant polycystic kidney disease (ADPKD)</strong></td>
<td>3D hydrogels</td>
<td>- Proliferation, cyst diameter/volume, number of cysts, intracellular cAMP, fluid secretion</td>
<td>(^a) Grantham et al., 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Mono-cultures of MDCK(^b), primary human ADPKD epithelial cells(^b), human proximal tubule cell line (LLC-PK1) (Matrigel and collagen)(^a), MCD cells(^b)</td>
<td>(^b) Wallace et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Proliferation, number of cysts, ECM compaction</td>
<td>(^c) Kuo et al., 2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Induced/human pluripotent stem cells (deletion of PKD1 and PKD2 genes)</td>
<td>(^d) Montesano et al., 2009</td>
</tr>
<tr>
<td><strong>Kidney organoids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Drug-induced nephrotoxicity</strong></td>
<td>3D hydrogels</td>
<td>- Expression of proximal tubule markers (AQP1, Na-K/ATPase, albumin uptake), kidney injury biomarkers (KIM-1, NAGL, clusterin); secretion of inflammatory proteins (TNF-(\alpha), MCP-1, IL-6, IL-1(\beta), MIP-(\alpha), and RANTES)</td>
<td>(^a) Astashkina et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Excised mouse proximal tubules(^a), immortalized human proximal tubule epithelial cells (PTECs)(^b)</td>
<td>(^b) DesRochers et al., 2013</td>
</tr>
<tr>
<td><strong>Microfluidic device (kidney-on-a-chip)</strong></td>
<td></td>
<td>- Expression of PT markers – AQP1, Na-K/ATPase, albumin uptake, kidney injury biomarkers – KIM-1, NAGL, clusterin</td>
<td>(^a) Jang et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-culture of PTECs and renal microvascular endothelial cells(^a), MDCK cells(^b,c)</td>
<td>(^b) Choucha-Snouber et al., 2012</td>
</tr>
<tr>
<td><strong>Renal fibrosis</strong></td>
<td>3D hydrogels</td>
<td>- Expression of fibrotic genes (e.g., CTGF, TGF-b1, Col1a1, TIMP-2), collagen deposition</td>
<td>(^c) Choucha-Snouber et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-cultures of PTECs and dermal fibroblasts</td>
<td></td>
</tr>
<tr>
<td><strong>Renal cell carcinoma</strong></td>
<td>Microfluidic device (kidney-on-a-chip)</td>
<td>- Expression of angiogenic genes (CA9, VEGFA, PGF, ANGPTL4); angiogenic/endothelial sprouting</td>
<td>Miller et al., 2018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Co-cultures of ccRCC cells and HUVEC-2 cells in 3D collagen gel</td>
<td></td>
</tr>
</tbody>
</table>

AQP1, aquaporin 1; \(\alpha\)-SMA, alpha smooth muscle actin; BAEC, bovine aortic endothelial cells; CA9, carboxic anhydrase 9; ccRCC, clear cell renal cell carcinoma; CTGF, connective tissue growth factor; Col1a1, collagen type 1; Col III, collagen type III; Col IV, collagen type IV; cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; HbsAg, hepatitis B surface antigen; HbcAg, hepatitis B e antigen; HCV, hepatitis C virus; HCcAg, hepatitis C core antigen; HUVEC-2, human umbilical vein endothelial cell 2; IP-10, interferon gamma-induced protein 10; IL-1\(\beta\), interleukin-1beta; IL-6, interleukin-6; JAK, Janus kinase inhibitor; KIM-1, kidney injury marker 1; LSEC, liver sinusoidal endothelial cells; MIP-1\(\alpha\), macrophage inflammatory protein-1 alpha; MCP-1, monocyte chemoattractant protein 1; MDCK, Madin-Darby Canine Kidney; MCD, mouse collecting duct; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; NAGL, neutrophil gelatinase-associated lipocalin; PEG, polyethylene glycol; PGF, placentonal growth factor; PHH, primary human hepatocytes; PT, proximal tubule; PTEC, proximal tubule epithelial cells; RANTES, regulated upon activation; normal T cell expressed and presumably secreted; TGF-b1, transforming growth factor beta 1; TNF-\(\alpha\), tumor necrosis factor alpha; VEGFA, vascular endothelial growth factor A
2.2.1.1 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is an all-inclusive term for conditions where excess fat accumulates in the liver. The spectrum of liver disorders encompassed by NAFLD starts from lipid accumulation (simple steatosis) to steatosis with inflammation and varying degrees of fibrosis (non-alcoholic steatohepatitis (NASH)) to cirrhosis and eventually liver failure and/or hepatocellular carcinoma (Müller and Sturla, 2019). Key initiating events include increases in hepatic de novo lipogenesis and inhibition of lipolysis of adipose tissue. All this, together with increased free fatty acid (FFA) levels in the serum, results in FFA accumulation in the liver and thereby lipid accumulation. Excess lipid accumulation causes endoplasmic reticulum (ER) stress, oxidative stress, and lipotoxicity, which will in turn trigger hepatocyte injury. The ensuing activation of the inflammatory Kupffer cells and stellate cells drives steatosis to NASH (Chen et al., 2020; Ibrahim et al., 2011). LSECs have also been implicated in the progression of NAFLD.

Pharmaceutical intervention has been hampered by lack of translational and reliable disease models. Three main MPS models have been used for NAFLD: microfluidic liver-on-chip (2D), 3D hepatic microtissues with perfusion, and 3D-spheroids. In these models, the cells were exposed to higher concentrations of fatty acids (oleic and palmitic acids), resulting in lipid accumulation in the hepatocytes (Jang et al., 2019; Kostrzewski et al., 2017; Kozyra et al., 2018). For the 3D static spheroids—a mono-culture of primary human hepatocytes—the cells were also exposed to high fructose medium. In all models, lipid accumulation, the primary endpoint, was recapitulated. Other endpoints included smooth muscle actin (α-SMA) as a marker of stellate cell activation, ECM markers to assess collagen deposition, pro-inflammatory cytokines in the quad-culture chip, and induction of lipogenic genes in the 3D microtissues. In both 3D models, treatment of the lipidic cultures with the antisteatotic compound metformin caused reduction in cellular lipid content, showing the potential relevance of these models for the discovery of novel drug candidates (Kostrzewski et al., 2017; Kozyra et al., 2018).

A major drawback of a mono-culture spheroid model is the absence of NPCs, which are necessary for progression of steatosis to NASH, precluding its use for either modeling NASH and/or screening of drugs targeting the inflammation axis. Notwithstanding, there is mounting evidence that decreasing lipid accumulation may be an effective treatment against NAFLD. Therapeutics aimed solely at decreasing lipid accumulation could therefore be tested using simpler hepatocyte mono-culture MPS models.

2.2.1.2 Diabetes mellitus

Type 2 diabetes mellitus (T2DM) is characterized by progres- sive insulin resistance, beta cell dysfunction, and chronic inflammation, the sum of which results in glucose dysregulation. Although T2DM is multifactorial, a key initiating event is the loss of insulin sensitivity in glucose target tissues. The liver is one of the first organs to become insulin-resistant. Consequently, hepatic glucose production is increased. A major feature of T2DM is increased hepatic de novo lipogenesis (and hyperlipidemia), which can be both an initiating event and a consequence of increased glucose production. Chronic elevation of blood glucose leads to a myriad of complications in T2DM, which include diabetic retinopathy, nephropathy, and neuropathy (Rogal et al., 2019).

Although there are no stand-alone diabetes models using the MPS platforms, hepatocytes in the micropatterned co-cultures (MPCC) platform (a co-culture of primary hepatocytes and 3T3 murine fibroblasts) have been used to show responsiveness to insulin and glucagon. Both glycogen storage and lysis were shown to be modulated when cells were exposed to the hormones (Davidson et al., 2015). In addition, exposure of the hepatocytes to high-glucose medium (≥ 25mM) resulted in intracellular accumulation of lipids, simulating increased lipogenesis and hyperlipidemia observed in T2DM. Furthermore, treatment of the cells with metformin, a T2DM drug, inhibited gluconeogenesis, a key endpoint analyzed in the study (Davidson et al., 2015).

2.2.1.3 Hepatitis B virus and hepatitis C virus

Over 350 million people worldwide are infected with hepatitis B virus (HBV) and hepatitis C virus (HCV), the main causes of liver cirrhosis and hepatocellular carcinoma (Gural et al., 2018). One of the major challenges to developing in vitro models of HBV or HCV has been identifying cells permissive to infection. Viruses are known to interact with several host factors to initiate entry into cells. Despite the contributions of hepatoma-derived cell lines (HepG2, Huh7, HepaRG) to the elucidation of HBV and HCV biology and host-virus interactions, there are still limitations: Infections are often short-lived, and these cell lines have altered intracellular pathways, biochemical mechanisms, and functions compared to in vivo hepatocytes (Gural et al., 2018; Tong et al., 2005; Witt-Kehati et al., 2016). Nonetheless, cell lines cultured as spheroids or in 3D hydrogels display a more differentiated phenotype and exhibit better hepatic function than in 2D formats. Although primary hepatocytes are considered the gold standard for modeling HBV and HCV infections, routine use of these primary cells is hampered by donor-to-donor variability, which often makes infection less efficient. Moreover, primary hepatocytes quickly lose their phenotype and permissiveness to infection following culture in traditional 2D configuration (Witt-Kehati et al., 2016).

For HBV, the liver bile acid transporter sodium taurocholate co-transporting peptide (NTCP) has been found to be necessary but not sufficient for effective viral infection (Herrschler et al., 2020). Additional host factors required for susceptibility to HBV infection have also been identified: epidermal growth factor receptor (EGFR) and E-cadherin (Gural et al., 2018). Infection is achieved either by using infected patients’ sera or HBV isolated from cell culture supernatants of HBV-producing cell lines (e.g., HepAD38). Culture media are supplemented with DMSO and PEG to support efficient infection (Kratochvil et al., 2018). After viral entry into the cells, the HBV virus is directed to the nucleus, where its relaxed circular DNA is converted to covalently closed circular DNA (cccDNA), serving as a template for viral RNA production.
The major MPS platforms used for modeling both HBV and HCV infections are MPCC, 3D spheroids, and 3D hydrogel cultures (Gural et al., 2018; Kratochwil et al., 2018). HBV infection has also been modeled using microfluidic devices: a liver sinusoid-on-a-chip consisting of primary human hepatocytes and bovine aortic endothelial cells cultured on opposite sides of a porous membrane under flow conditions (Kang et al., 2017) and 3D hepatocyte microtissues that required lower viral MOI (multiplicity of infection) to infect hepatocytes and demonstrated long-term HBV infectivity. The main endpoints measured in HBV disease models include cccDNA and HBV antigens (HBsAg and HBeAg), which are secreted into the culture media. It is noteworthy that chronic HBV often results in the infection of 5-40% of the hepatocytes in the liver, and varying infection rates may result in differing progression rates and response to treatment (Kratochwil et al., 2018). These should be accounted for in the disease models. Moreover, treatment of the 3D primary human hepatocyte (PHH) cultures with interferon-alpha (an immunotherapy for HBV/HCV) resulted in decreased secretion of HBV DNA and HBsAg, although cccDNA levels were unaffected (Ortega-Prieto et al., 2018).

For HCV, host factors required for viral entry include LDL receptor, CD81, and scavenger receptor class B member 1 (SRB1). The additional host factors claudin 1 and occludin have also been identified as being requisite for efficient cell infection (Chang et al., 2017; Gural et al., 2018). Release of genomic RNA into the cytosol upon viral entry into the cell initiates synthesis of the HCV polyprotein. HCV infection was modeled using Huh7 cells in 3D spheroids and 3D hydrogel cultures. In addition, PHH cultured as 3D spheroids were also used to model HCV infection. The main endpoints analyzed in these models include viral RNA and HCcAg (HC core antigen). Treatment of the infected PHH and the Huh7 spheroids with JS-81, an antibody against CD81, resulted in a dose-dependent decrease in HCV pseudo-particle entry (Ananthanarayanan et al., 2014).

2.2.2 Kidney

The kidney's primary functions include excretion of waste and toxins (via urine formation), maintenance of blood composition, pH and osmolality, and regulation of blood pressure. Most renal diseases would, if untreated, result in end-stage renal failure, which would necessitate dialysis and/or transplantation. The nephron, the main structural and functional unit of the kidney, is made of many different cell types. It consists of a renal corpuscle (made up of glomerulus and Bowman’s capsule) and renal tubule (proximal and distal tubules). Recapitulating fluid flow through the multicellular architecture, interactions, and organization of the nephron is challenging, and as such, many in vitro models focus on a single cell type. Historically, scientists overcame these issues using suspensions of isolated tubule segments or approaches using in situ or ex vivo isolated perfused tubule segments.

Current kidney MPS models have focused on the proximal tubule since it is a major target of drug-induced nephrotoxicity (Phillips et al., 2020). Other models such as glomerular MPS models have also been developed, but the complex physiology and function of the glomerulus have not been sufficiently replicated in any in vitro model. For proximal tubule (PT) models, AQPI (aquaporin 1) is measured as a PT marker; and for glomerular models, Wilm’s tumor-1 (WT-1) and nephrin are used as podocyte-specific markers. In addition, albumin uptake was used in these models as marker of kidney function (Astashkina et al., 2012; Jang et al., 2013). Below we describe some renal diseases that have been modeled using MPS platforms.

2.2.2.1 Autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder caused by mutations in one of two genes, Pkd1 and Pkd2, that encode the polycystin proteins. It is characterized by progressive formation of cysts in tubular segments of nephrons. Cystogenesis involves several key components including loss of polycystin protein, changes in apical and basolateral organization, disruption of cell-matrix interactions, and increases in proliferation and fluid secretion (and/or accumulation) (Dixon and Woodward, 2018). Recent findings indicate that increased intracellular cyclic AMP results in cyst epithelial cell proliferation and fluid secretion. Expansion of cysts and the associated extensive interstitial fibrosis eventually leads to kidney failure. MPS platforms used to model ADPKD include 3D hydrogels/scaffolds (DesRochers et al., 2013) and kidney organoids (Cruz et al., 2017). A main advantage of the kidney organoids for ADPKD is the capacity of the stem cells to differentiate into various kidney cell types (and structures): podocytes, tubules, and the associated endothelium and mesenchyme. ADPKD disease modeling was achieved using organoids derived from ADPKD patients’ cells that formed cysts spontaneously in vitro (Dixon and Woodward, 2018). Cell types used in the 3D models include mouse embryonic kidney (MEK) cells, Madin-Darby canine kidney (MDCK) cells, mouse collecting duct (MCD) cells, HK-2 cells (human proximal tubule cell line), LLC-PK1 (pig proximal tubule cell line), all of which can form tubular structures in vitro. The 3D models include co-culture of MEK cells and fibroblasts in collagen/Matrigel infused in porous silk scaffold and MDCK cells, MCD cells, HK-2 cells, and LLC-PK1 cells, as mono-cultures in collagen hydrogels. However, with the animal cell lines and immortalized human cell line, questions remain regarding the relevance of these cells to human physiology and a need to select sublines or clones that exhibit the cyst phenotype (Dixon and Woodward, 2018). In addition, proximal tubule cells from ADPKD patients have also been used to model the disease in vitro. Unlike normal kidney cells, these diseased cells do not form tubular structures but are able to form cysts (DesRochers et al., 2013). It is noteworthy that none of these models incorporate flow, a major limitation to the translatability of the results. The main endpoints analyzed in these ADPKD models include cyst formation (number, volume, and diameter of cysts), cell proliferation, fluid secretion, and intracellular cAMP signaling. Furthermore, for a few of these mod-
2.2.2.2 Drug-induced nephrotoxicity

Drug-induced nephrotoxicity (DIN) is renal damage and/or loss of function caused by exposure of the kidneys to xenobiotics. It often occurs via pathogenic mechanisms, which include tubular cell toxicity, inflammation, altered intraglomerular hemodynamics, and crystal nephropathy (caused by insoluble drugs or drug metabolites) (Schetz et al., 2005; Zager, 1997). It is estimated that 30-50% of all cases of severe acute renal failure in patients are due to drug-induced nephrotoxicity. Traditional methods used for modeling DIN in vitro have been 2D culture of animal cells and kidney organ slices. The MPS platforms used to model DIN include 3D hydrogels and microfluidic kidney-on-chip. Cell types used in the 3D hydrogel models include excised mouse proximal tubules (Astashkina et al., 2012) and immortalized human proximal tubule epithelial cells (DesRochers et al., 2013). These models were kept in culture for more than 2 weeks. The microfluidic kidney-on-chip platforms have used MDCK cells as well as a co-culture of proximal tubule epithelial cells and renal microvascular endothelial cells. MDCK cells are widely used in tissue-engineered kidney models as they recapitulate the cellular polarity seen in vivo. However, being of canine origin, they do not have the same phenotype as human proximal tubule cells (DesRochers et al., 2013). Nonetheless, exposure of the proximal tubular cells to flow enhanced phenotype and function via increased cilia formation, transporter function, albumin transport, and glucose reabsorption (Jang et al., 2013). Indeed, the increased glucose reabsorption in the human proximal tubule epithelial cells (PTEC) kidney chip was, in part, attributed to increased protein expression of sodium-glucose transporter (SGLT2). Furthermore, the authors demonstrated enhanced function for organic cation transporter (OCT)-2 and P-glycoprotein (P-gp) transporters with incorporation of flow. The main endpoints analyzed in these models included cytotoxicity markers such as lactate dehydrogenase (LDH) and markers of kidney injury such as kidney injury marker 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NAGL), clusterin, and albumin uptake. Additional endpoints included proinflammatory markers such as TNF-α, MCP-1, IL-6, IL-1β, MIP-1α, and RANTES. Treating the excised mouse PT model (in a 3D hydrogel) with nephrotoxic compounds, cisplatin, doxorubicin, 4-aminophenol, and colchicine resulted in significant upregulation of IL-6 and MCP-1 with all drug exposures (Astashkina et al., 2012).

2.2.2.3 Renal fibrosis

Renal fibrosis involves excessive accumulation of ECM (primarily collagen) in the kidney, often because of dysregulation of the normal wound-healing process leading to loss of renal function. Injury to tubular epithelial cells may result in activation of fibroblasts, a key event in the pathogenesis of fibrosis. Renal fibrosis has been modeled using 3D hydrogels (DesRochers et al., 2013). Dermal fibroblasts were encapsulated in a collagen type I gel with human PTEC grown in a monolayer on top of the matrix. The model was able to recapitulate injury-induced renal fibrosis as injury to the epithelial cells resulted in a change in phenotype of the fibroblasts (Moll et al., 2013). Typical endpoints analyzed in fibrosis models include the expression of fibrotic genes (e.g., connective tissue growth factor (CTGF), transforming growth factor (TGF)-beta 1, collagen type 1 (Col1) a1, and tissue inhibitor of matrix metalloproteinase (TIMP)-2).

2.2.2.4 Renal cell carcinoma

Renal cell carcinoma (RCC) is derived from proximal tubule epithelium, and many cases are asymptomatic until the cancer is advanced with only 10% of RCC patients presenting with the classic triad of hematuria, pain, and flank mass. A third of RCC patients do not survive, and individuals with metastatic RCC have a 5-year survival rate of < 10%. A microfluidic device-based RCC model was developed with RCC cells isolated from clear cell RCC (ccRCC) tissues and co-cultured with HUVEC-2 cells in a 3D collagen gel. Endpoints analyzed included expression of angiogenic genes (CA9, VEGFA, PGF, ANGPTL4) and angiogenic/endothelial sprouting via immunohistochemistry and confocal microscopy (Miller et al., 2018).

2.3 Infectious respiratory diseases

The first line of defense against a respiratory viral infection is the respiratory epithelium along with infiltrating innate immune cells and resident immune cells. Homeostasis of both ions and fluids is disrupted when a pulmonary infection occurs, which leads to changes in barrier integrity for the thin aqueous layer in the upper and the lower respiratory epithelium and edema formation in the alveoli. Acute lung injury and acute respiratory distress syndrome (ARDS) caused by either viral or bacterial pathogens occur in the presence of extensive inflammation within the lungs, fluid accumulation in the alveolar airspace, and reduced gas exchange, resulting in respiratory failure and mortality rates of 40-58% (Benam et al., 2016, 2019). In the case of SARS-CoV-2 infection, patients can experience profound disruption of the normal lung structure: the transformation of respiratory tissue into fibrotic material, extensive alveolar damage, and thrombosis of the lung micro- and macro-vasculature. During chronic disease, viral RNA can be found in pneumocytes and endothelial cells with infected cell syncytia (Bussani et al., 2020).

With the recent SARS-CoV-2 pandemic there has been an added emphasis on finding suitable preclinical efficacy models to study infection and resolution of the disease. Most reported animal models that are being used in SARS-CoV-2 infection involve protective immunity and resolving pathology (Cleary et al., 2020). Moreover, studies in animal infection models for SARS-CoV-2 do not replicate some key aspects of severe COVID-19 in patients. The ideal preclinical model for viral infection would enable the study of potential therapeutic intervention(s) to prevent infection and/or promote resolution of severe viral infection using clinically relevant endpoints. Importantly, these models should enable drug interventions to be administered at realistic time points, thus requiring models with extended longevity in culture. Select examples of respiratory MPS disease models are discussed in this section and summarized in Table 4.
2.3.1 Static air-liquid interphase models
The most common lung in vitro models are air-liquid interphase (ALI) models in a transwell format, in which primary lung epithelial cells are plated on the apical side of the transwell. The epithelial cells recapitulate the mucociliary biology but subepithelial extracellular matrix is absent. Viral particles will typically be added directly to the apical side (epithelial side) of the transwell. Transwell cultures vary from 12- to 96-well, but it becomes increasingly difficult to create a robust epithelial layer in a 96-well format. A common endpoint used in AI culture is the measurement of transepithelial electrical resistance (TEER), which provides a convenient indicator of tight junction development and barrier function. Human airway epithelium grown under ALI conditions can be infected by different viral pathogens such as respiratory syncytial virus (RSV) (Persson et al., 2014). Recently, well-differentiated proximal airway cells were shown to be permissive to SARS-CoV-2 infection and subsequent viral replication (Mulay et al., 2020).

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
</table>
| Viral respiratory pathogens (influenza, HRV, RSV, SARS-CoV-2) | Air-liquid interphase cell culture (Static transwell with an apical and basal side)                                                                                                                                   | - Viral entry, viral load measured by plaque assay or RNA, barrier integrity by TEER or FITC-Dextran, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), bulk RNA-Seq, immune activity (cytokines), and viability - live/dead assay  
- Epithelial cells that recapitulate the mucociliated biology; typically absent in the subepithelial extracellular matrix  
- Viral entry, viral load measured by plaque assay or RNA, barrier integrity in transwell system by TEER or FITC-Dextran, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), bulk RNA-Seq, immune activity (cytokines), and viability - live/dead assay | a Persson et al., 2014  
b Mulay et al., 2020 |
| Alveolar organoids or adult-derived bronchial organoids derived from pluripotent stem cells (iPSC/E1S) | Viral entry, viral load measured by plaque assay or RNA, viral spread based on imaging, barrier integrity by FITC-Dextran, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), bulk RNA-Seq, immune activity (cytokines), and viability - live/dead assay  
- Alveolar epithelial progenitor cell that can replicate trachea, bronchi/bronchioles, alveoli, and multi-lineage structures  | Zacharias et al., 2018 |
| Organ-on-a-chip (with flow and/or stretch) | Viral entry, viral load measured by plaque assay or RNA, viral spread based on imaging, barrier integrity by FITC-Dextran, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), immune activity (cytokines), and viability - live/dead assay  
- (1) Co-culture of bronchiolar cells (ciliated cells, goblet cells, neuroendocrine cells, immune cells) and alveolar cells (type II pneumocytes, interstitial fibroblasts, pericytes, myofibroblasts, capillary endothelium, immune cells) in healthy, asthmatic or COPD chip with an air interface, (2) airway epithelium, (3) matrix-embedded fibroblasts, (4) pulmonary endothelium, and (5) a vascular compartment representing blood flow and immune cell containment  | a Benam et al., 2016  
b Nawroth et al., 2020 |
| 3D bioprinted tissue constructs/replicate tissue (microarchitectures of the human lung) | Viral entry, viral load measured by plaque assay or RNA, viral spread based on imaging, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), immune activity (cytokines), and viability - live/dead assay  
- Cell lines (e.g., BEAS-2b or A549), primary cell (e.g., bronchial epithelial or small epithelial cells) or lung organoids could be used with the bioprinter. Fibroblasts currently do not exist in these models  | a Horvath et al., 2015  
b Park et al., 2018b |
| Lung precision slice cultures (static with tissue in cell culture plates or transwells) | Viral entry, viral load measured by plaque assay or RNA, viral spread based on imaging, IHC (expression of key receptors for viral pathogens), histology (tissue differentiation), and viability - live/dead assay  
- Patient lung slices  | a Goris et al., 2009  
b Liu et al., 2019 |

COPD, chronic obstructive pulmonary disease; ES, embryonic stem cells; FITC, fluorescein isothiocyanate; HRV, human rhinoviruses; IHC, immunohistochemistry; iPSC, induced pluripotent stem cells; RSV, respiratory syncytial virus; TEER, transepithelial electrical resistance
An advantage of ALI models is the potential to use aerosol delivery to mimic an inhaled drug.

### 2.3.2 Organoids

Lung organoids are composed of multiple cell types from the lung and should be able to model physiological conditions of the lung. They can be generated from embryonic stem cells, iPSC-based cultures, or derived from adult lung tissue. Adult lung organoids have been generated from numerous segments of the respiratory tract. Tracheospheres have been formed from single basal cells from the tracheal pseudostratified epithelium with basal and ciliated luminal cells (Rock et al., 2009). 3D airway organoids have been generated through a mixed co-culture model combining human adult primary bronchial epithelial cells, lung fibroblasts, and lung microvascular endothelial cells that were able to self-organize into distinct epithelial and endothelial structures (Tan et al., 2017). Bronchospheres created from primary human airway basal cells showed functional multi-ciliated cells, mucin-producing secretory cells, and airway basal cells. Bronchospheres have the added advantage of being cultured in a 384-well assay format, allowing high throughput assays (Hild and Jaffe, 2016).

Several studies have been able to demonstrate the viral infectability of airway organoids. For example, airway organoids consisting of basal cells, functional multi-ciliated cells, mucus-producing secretory cells, and CC10-secreting club cells can be infected with influenza virus (Hui et al., 2018), enterovirus (Van der Sanden et al., 2018), and RSV (Sachs et al., 2019).

For SARS-CoV-2 studies, human bronchial organoids (hBO) have been generated from cryopreserved human bronchial epithelial cells that contain basal, club, ciliated, and goblet cells expressing both angiotensin-converting enzyme 2 (ACE2), which is a receptor for SARS-CoV-2, and transmembrane serine protease 2 (TMPRSS2), which is an essential serine protease for priming spike (S) protein of SARS-CoV-2. Interestingly, hBO infected with SARS-CoV-2 and treated with Camostat, an inhibitor of TMPRSS2, significantly inhibited viral copy number (Suzuki et al., 2022). Another study using 3D alveolar organoids (a co-culture system of distal lung epithelial cells and fibroblasts embedded in Matrigel) infected with SARS-CoV-2 demonstrated efficacy of a number of candidate COVID-19 drugs and confirmed that remdesivir strongly suppressed viral infection/replication in alveolar organoids (Mulay et al., 2020). Moreover, a study using a 3D culture of hAT2 cells derived from primary human lung tissue demonstrated viral replication and increased interferon-associated gene and proinflammatory gene expression; two features that mimic human physiological SARS-CoV-2 infection (Youk et al., 2020).

Numerous organoid culture techniques have differentiated human pluripotent stem cells into various lung cell lineages, including bronchi/bronchioles (Chen et al., 2017; Konishi et al., 2016; McCauley et al., 2017) and alveoli (Jacob et al., 2017; Zacharias et al., 2018). Among these, Chen and colleagues demonstrated that iPSC-derived human airway epithelium can be infected by RSV virus (Chen et al., 2017). Additionally, lung alveolar type II (ATII)-like cells derived from pluripotent stem cells are permissive to SARS-CoV-2 infection and show upregulation of chemokines (Han et al., 2020).

It is difficult to obtain fully differentiated lung cell types derived from iPSC. A major limitation of using organoids embedded in a matrix is the difficulty of accessing the lumen located inside the organoid since natural viral exposures would occur through the apical surface of the lungs.

### 2.3.3 Organ-on-a-chip

There are drawbacks to the use of static 3D cell models, such as ALI and organoids. Chief among these are the lack of perfusion and absence of mechanical forces induced by breathing. In vivo, perfusion is mediated by blood vessels. Incorporation of fluid flow in in vitro models and/or addition of a vascular channel/component that can be perfused would mitigate this issue. Most of the static models also fail to recapitulate normal functional coupling between epithelium and endothelium, and none enable analysis of the recruitment of circulating immune cells under active fluid flow (Benam et al., 2016). However, lung-on-a-chip models can mimic many of these attributes. The lung-on-a-chip should exhibit physiologically relevant cell polarity and respiratory mucosa. The device should have minimal adsorption of drugs to biopolymer or matrix components, allow cyclical stretch to mimic breathing, possess optical transparency to facilitate imaging, and enable cell processing for immunohistochemical or ultrastructural examination (Ainslie et al., 2019).

**Small airway**

Numerous microfluidic designs have endeavored to create a more physiologically relevant in vitro airway model that features a mucociliary airway epithelium and allows the trafficking of immune cells. A recent study utilizing an airway lung-on-a-chip design that contained mucociliary airway epithelium and hemodynamic perfusion was able to demonstrate key features of viral-induced exacerbation of asthma (Nawroth et al., 2020). This is important as asthma typically is aggravated during episodes of viral infection. Human rhinovirus (HRV) infection in the asthma-induced lung-on-a-chip was able to stably infect the human mucociliary airway epithelium causing loss of ciliated cells and goblet cell hyperplasia, driving epithelial inflammation and recruitment of immune cells, such as neutrophils, to the epithelium. All these events are hallmarks of rhinovirus-induced responses of the asthmatic airway. An advantage of this platform is that it is amenable to high-content imaging and microfluidic sampling. Another study using a similar lung-on-a-chip design was able to show influenza viral entry, replication, strain-dependent virulence, host cytokine production, and recruitment of circulating immune cells. The chip was further evaluated for its usefulness in SARS-CoV-2 studies where lung epithelial cells showed high levels of ACE2 and TMPRSS2. The study assessed the inhibitory effects of 8 clinically approved drugs (chloroquine, hydroxychloroquine, amodiaquine, toremifene, clomiphene, arbidol, verapamil, and amiodarone) on pseudo-SARS-CoV-2 virus infection in these chips. Interestingly, only 3 of the 8 drugs, amodiaquine, toremifene, and clomiphene, significantly inhibited entry of the pseudo-SARS-CoV-2 virus.
A recent chip design that includes passive medium exchange and physiologically relevant cyclic strain allowed the culturing of human primary ATI and ATII epithelial cells on the apical side and endothelial cells on the basal side of a stretchable membrane over multiple days (Stucki et al., 2018). This model could be used in SARS-CoV-2 lung infection studies to simulate alveolar breakdown and cytokine storm. A less sophisticated platform was used to test the infectability of SARS-CoV-2 cells in a microfluidic device that contains human alveolar epithelial cells and vascular endothelial cells separated by a thin, porous membrane under physiological fluid flow conditions. The interplay of immune cells exacerbating the SARS-CoV-2 viral response was studied by adding peripheral blood mononuclear cells (PBMCs) to the vascular compartment. Interestingly, the immune cells decreased cell confluence on the vascular side and increased proinflammatory cytokines, which was not observed with SARS-CoV-2 alone (Zhang et al., 2020).

2.3.4 3D-bioprinted lung tissue constructs
As discussed earlier, 3D bioprinting allows the reproducible layering of multiple cells and biomaterials in a spatially controlled manner, creating a construction of tissues with microarchitectures mimicking those of organs. There are a few examples of 3D bioprinting of lung tissue such as the air-blood barrier of lung alveoli in vitro (Horvath et al., 2015; Park et al., 2018b), and examples where non-cell laden hydrogel 3D-bioprinted models more closely mimic the air sac. To this point, there are no current published examples studying viral infection in 3D-bioprinted lungs (Grigoryan et al., 2019). Bioprinting enables mass production of culture systems with a wide range of applications, such as drug screening, gene editing, and disease modeling. However, the high-resolution nature of 3D printing and the ability to miniaturize potentially limits bioprinting’s application in drug discovery to secondary or tertiary screens and mechanistic studies.

2.3.5 Human lung tissue
Precision-cut lung slices (PCLS) are thinly sliced lung sections that can be cultured for up to 14 days. The lung sections allow the maintenance of the lung architecture, including the epithelia, respiratory parenchyma, smooth muscle, vasculature, and resident immune cells. PCLS have been utilized for studying inflammatory processes and viral infection in the lungs of patients with asthma to better understand which cells are being infected in the intact lung as compared with in vitro ALI cultures (Goris et al., 2009). PCLS have also been obtained from patients with COPD, asthma, and IPF. These can be utilized as a model system to study respiratory diseases and viral exacerbation (Liu et al., 2019).

Drawbacks of PCLS include technical difficulties associated with isolation and preparation of PCLS and a dearth of donor lung tissue resulting in small sample sizes for experimentation. Additionally, the lack of perfusion and interstitial fluid flow in PCLS models precludes the recruitment of immune cells. Moreover, use of PCLS is restricted to only direct compound administration to the tissue while its low throughput limits its use in drug discovery beyond evaluating lead compounds (Liu et al., 2019).
2.4 Neurodegenerative diseases

Neurodegenerative diseases are progressively debilitating and largely incurable, characterized by loss or dysfunction of cells in the central nervous system (CNS) and the peripheral nervous system (PNS). Globally, the number of individuals with neurodegenerative diseases is increasing, and the disorders have become the leading cause of years lost due to disability (disability-adjusted life years) and second leading cause of deaths (Feigin et al., 2019). The severe effects on the patients’ quality of life have intensified the burden on healthcare systems and the demand for effective treatments. Despite the critical need, drugs for the treatment of cognitive deterioration, such as Alzheimer’s disease (AD), locomotor deficiencies, including Parkinson’s disease (PD) and multiple sclerosis (MS), and seizures are either unavailable or limited due to incomplete understanding of biological mechanisms of the diseases.

Clariﬁying the cellular and molecular processes regulating the pathophysiology of neurodegeneration remains a major challenge in drug development due to a lack of suitable experimental systems. A variety of factors, including genetic predisposition, aging, and changes in the cellular microenvironment, can induce defective protein aggregation/folding, inﬂammatory responses, and disruption of neuronal network homeostasis (Gitler et al., 2017). To tease apart each component of neurodegenerative pathology and identify disease-modifying therapies, researchers have been utilizing animal models as in vivo surrogates (Dawson et al., 2018). For example, recapitulation of increased amyloid β (Aβ) plaque, a major hallmark of AD, is established with mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2), leading to axonal inﬂammation, microgliosis, and astroglisis found in AD (McGowan et al., 2006). Additionally, mice overexpressing the mutant microtubule-associated protein tau (MAPT) develop AD-like pathology of neurofibrillary tangles in neurons (taupathy) (Andorfer et al., 2005; Goedert et al., 2017; McGowan et al., 2006). PD onset and progression, characterized by loss of dopaminergic neurons in the substantia nigra and accumulation of α-synuclein in neurons (Lewy bodies), can be induced by several chemical agents. For 5-10% of genetically-driven cases, mutations in α-synuclein, leucine repeat kinase 2 (LRRK2), parkin, DJ1 (PARK7), or PTEN-induced putative kinase 1 (PINK1) enable the development of animal models (Blesa and Przedborski, 2014; Poewe et al., 2017). Other neurodegenerative diseases have been modeled using transgenic rodents as well, such as overexpression of TDP-43 or mutations of superoxide dismutase 1 (SOD1) or C9orf72 to elicit amyotrophic lateral sclerosis (ALS) phenotypes of motor neuron degeneration and cognitive deﬁcits (Philips and Rothstein, 2015; Picher-Martel et al., 2016). Despite these advancements, neurodegenerative disease drugs developed using animal models often fail in clinical trials due to insufficient recapitulation of disease phenotypes and poor translatability from animals to human patients (Beal, 2010; Jucker, 2010; Anderson et al., 2017; Dawson et al., 2018; Ransohoff, 2018).

Alternatively, traditional in vitro nervous system cultures involved isolating animal or human brain tissues and maintaining the explants for a period for examination. The first CNS organotypic culture was established using rat hypothysis, and subsequent iterations and improvements included specific cerebral regions that represent physiologically-relevant 3D models of the brain (Bousquet and Meunier, 1962; Croft et al., 2019; Walsh et al., 2005). However, brain organotypic cultures have limitations for probing neurodegenerative diseases, including diﬃcult and inefficient preparation, inherent variability between samples, and relatively poor predictability and translation (Croft et al., 2019; Pacitti et al., 2019; Walsh et al., 2005). 2D cultures offer simpler techniques compared to brain tissue explants, yet their animal sources and the lack of complex tissue organization prevent use as effective neurodegenerative disease models for drug development.

To overcome the limitations, a more effective strategy is emerging in the form of MPS that integrate key CNS/PNS parameters, including 1) patient-sourced iPSC-derived cells that exhibit in vivo neuro-pathophysiology, 2) interactions between multiple cell types (e.g., neurons, astrocytes, microglia, oligodendrocytes, immune cells, endothelial cells) as well as 3D matrix/tissue organizations to model various microenvironments of neurodegenerative diseases, and 3) functional blood-brain barrier (BBB) for examining disease-associated immune responses and transport of drugs, nutrients, and/or waste between the CNS and periphery (Nikolakopoulou et al., 2020; Slanzi et al., 2020). The in vitro engineering of the highly complex human nervous system and its pathophysiology is achieved primarily via a reductionist approach, in which one or few key in vivo functions are recapitulated, instead of building every aspect of the human brain. Table 5 and this section describe select examples of these novel tools and how critical insights into major neurodegenerative diseases are being investigated for the development of safe and eﬃcacious targeted therapies.

2.4.1 Alzheimer’s disease

In recent years, several 3D Alzheimer’s disease (AD) models have been utilized to investigate the role of Aβ in neurotoxicity and clarify the pathological mechanism regulating the disease. Uniform-sized spheroids with connected neural networks were generated in microwells using rat neural progenitor cells from cerebral cortical regions, and when co-cultured with Aβ, viability of the cells within the spheroids decreased (Choi et al., 2013b). Fluorescence confocal imaging and electron microscopy revealed reduced synapsin II and acetylcholine along with apoptotic cellular structures, indicating direct neurodegeneration by Aβ. In follow-up studies, microfluidic devices were developed to generate osmotic pressure recapitulating the interstitial flow in the brain, and the slow, diffusion-like transport of Aβ aggregates increased the neurotoxicity effect signiﬁcantly, indicating a major role of CNS fluid dynamics in regulating Aβ oligomeric assembly and transport to drive AD (Choi et al., 2013a; Park et al., 2015). Additionally, application of fluid flow enhanced neuronal differentiation and network connections between 3D neuro-spheroids (Park et al., 2015). To better examine vascular dysfunction in AD, a microfluidic model of the BBB was engineered (Shin et al., 2019). Deposition of Aβ plaques by human...
The significance of 3D ECM on the effects of Aβ and tauopathy was examined by culturing the ReN cells in 3D Matrigel (Choi et al., 2014). Compared to 2D, the human neural stem cells in 3D ECM underwent more robust differentiation into neuronal and glial cells. Additionally, 3D ECM promoted the accumulation of cell-secreted Aβ plaques, which in turn promoted hyperphosphorylation of tau aggregates and neurofibrillary tangles that produced the dystrophic neurite phenotype found in the brains of AD patients. The process of neuronal loss in AD involves mi-

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>Microfluidic device</td>
<td>– Interstitial flow-mediated Aβ fibril size/number and neuronal cell viability; Aβ and tauopathy neuroinflammation – microglial recruitment, secretion of TNF-α, IFN-γ, MCP-1, and viability/morphology changes of neurons and astrocytes via 3D tri-culture; BBB dysfunction measurements – Aβ deposition, permeability, adherens and tight junction protein expression, inflammatory cytokines, and neuronal cell viability with drug treatments; Rat neural progenitor cell-derived neurons; SV40 human microglia cells; human ReN cell-derived neurons and astrocytes; hCMEC/D3 cells</td>
<td></td>
</tr>
<tr>
<td>Parkinson’s disease (PD)</td>
<td>Microfluidic device</td>
<td>– 3D culture for dopaminergic neuron differentiation – morphology, polarization, and β-tubulin III and tyrosine hydroxylase and degeneration – mitochondrial morphometric measurement and microarray gene expression analysis; intracellular transport of α-synuclein fibrils; Human iPSC-derived neurons; iPSCs from PD patients with LRRK2-G2019S mutation-derived neurons; primary mouse neurons</td>
<td></td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis (ALS)</td>
<td>Spheroids + microfluidic device</td>
<td>– Development and characterization of 3D mid-brain PD models that express altered differentiation markers (e.g., tyrosin hydroxylase, FOXA2, LMX1A) and iPSCs from PD patients with LRRK2-G2019S mutation or idiopathic PD-derived neurons</td>
<td></td>
</tr>
<tr>
<td>Huntington’s disease (HD)/ blood-brain barrier (BBB)</td>
<td>Microfluidic device</td>
<td>– Development and characterization of disease-specific BBB models with laminar flow – junction-related gene and brain microvascular endothelial cell marker expressions, and permeability; HD patient iPSC-derived brain microvascular endothelial cells, astrocytes, and neurons</td>
<td></td>
</tr>
</tbody>
</table>

Aβ, amyloid β; BBB, blood-brain barrier; FOXA2, forkhead box protein A2; hCMEC/D3, human cerebral microvascular endothelial cells; IFN-γ, interferon gamma; LMX1A, LIM homeobox transcription factor 1 alpha; LRRK2, leucine repeat kinase 2; MCP-1, monocyte chemoattractant protein 1; TDP-43, TAR DNA-binding protein 43; TNF-α, tumor necrosis factor alpha
crogial recruitment to Aβ plaques and activation to trigger neu-
roinflammation. To recapitulate this key AD event and clarify
the interactions between microglia and neurons, a microfluidic
3D tri-culture of differentiated human neurons, astrocytes, and
microglia was developed (Park et al., 2018a). In response to Aβ
plaque formation and hyperphosphorylated tau aggregates, neu-ons released cytokines and chemokines (e.g., CCL2, CXCL10,
CX3CL1), which recruited microglia towards neurons and astro-
cytes via microfluidic channels. Activated microglia increased
proinflammatory factors, including TNF-α and interleukin (IL-)
8, and induced significant loss of colocalized neurons and astro-
cytes partly by IFNγ and toll-like receptor (TLR)4-dependent
mechanisms. It is worth noting that such synergistic mechanisms
of Aβ, tauopathy, and microglia neuroinflammation have not
been observed in 2D or most animal AD models, demonstrating
the distinct power of in vitro neurodegenerative disease models
with human stem cells and 3D microenvironment.

2.4.2 Parkinson’s disease

The need for an effective in vitro Parkinson’s disease (PD)
model is well recognized as it is the most common age-related
neurodegenerative disorder after AD, and the number of people
with PD is expected to double between 2005 and 2030 (Dors-
ey et al., 2007; Van Den Eeden et al., 2003). PD drug develop-
ment has shown only moderate successes in clinical trials due to
limited in vivo relevance and reproducibility of animal models
and cell culture systems, such as the SH-SY5Y neuroblastoma
cell line that has been used widely for its PD-associated dopa-
minergic phenotype (Slanzi et al., 2020; Xicoy et al., 2017).
To address these shortcomings, recent efforts have generated
PD-specific human iPSCs, which have been combined with mi-
crofluidics and 3D culture. For example, neurons derived from
PD patients carrying the LRRK2-G2019S mutation showed in-
creased dopaminergic neuron loss and degeneration compared
to cells derived from healthy individuals when differentiation
was conducted in a commercially available microfluidic plate
with 96 bioreactors with 3D Matrigel; no significant changes
were observed in 2D cultures (Bolognin et al., 2019). Although
the study did not include cell types other than neurons that may
be involved in PD pathology, such 3D systems could be utilized
to stratify PD patients for targeted drug treatment. Similarly, 3D
Matrigel in microfluidic devices facilitated the differentiation of
human iPSCs into dopaminergic neurons with proper mor-
phology and electrophysiological activity, demonstrating the
unique utility of 3D bioengineering technologies for PD modell-
ing (Moreno et al., 2015).

To investigate key molecular dynamics of PD, primary corti-

cal mouse neurons were cultured in a microfluidic device with
microgrooves that separate the soma from axonal projections,
and fluorescence live-cell imaging was applied to track the trans-
port of α-synuclein fibrils within cells (Freundt et al., 2012).
Measurements showed that α-synuclein can be internalized by
neurons and be mobile in axons, indicating that α-synuclein
aggregates may be transported between connected neurons and
accumulate (Lewy bodies) to initiate or promote PD. Because
the study employed rodent cells, follow-up investigations using
neurons derived from PD-patient iPSCs would provide relevant
translation to human pathophysiology. Additionally, if the α-
synuclein transport dynamics is a PD biomarker, a redesign of
the microfluidic device to adopt standard tissue culture plate formats
or increase the number of microgrooves would enable its use for
drug candidate screening. Another promising PD model is a 3D
human midbrain organoid consisting of midbrain dopaminergic
neurons derived from iPSCs (Chlebanowska et al., 2020; Smits
et al., 2019). The organoids, generated using neurons differenti-
ated from iPSCs of PD patients carrying the LRRK2-G2019S
mutation or from peripheral blood mononuclear cells of idiop-
athic PD patients, exhibited key PD phenotypes.

2.4.3 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Geh-
rig’s disease, involves loss of motor neurons causing progressive
muscle atrophy and paralysis, which in turn leads to death within
2 to 5 years of disease onset (Picher-Martel et al., 2016). Iden-
tification of several key mutations associated with the familial
form of the disease and related animal models have advanced the
current understanding of ALS pathophysiology. However, differ-
ences in preclinical models and humans as well as the relatively
minimal insight into sporadic ALS have prompted the develop-
ment of effective in vitro models for mechanistic investigation
and drug screening. Towards this aim, an elegant ALS-on-a-chip
was developed that consists of 3D skeletal muscle cell bundles
interfaced with iPSC-derived motor neuron spheroids that acti-
uate muscle contraction (Osaki et al., 2018). The muscle fiber
bundles were formed on flexible micropillars, and contraction
forces were quantified by measuring pillar displacement. Ex-
cess glutamic acid stimulated sustained motor neuron excitations
relevant to ALS and resulted in weaker muscle contraction and
decreased frequency. Prolonged excess glutamic acid treatment
caused neurite regression and muscle atrophy, demonstrating the
significance of physical interaction between motor neurons and
muscle cells in the context of excitotoxicity. In addition, motor
neuron spheroids generated using iPSCs from a patient with spo-
radic ALS and transfected with light-sensitive-channel rhodopsin
were stimulated by light, resulting in fewer muscle contractions
than the control group (iPSCs from a healthy volunteer). The
ALS condition showed increased TDP-43 mRNA expression, de-
creased nerve fibers and neuromuscular junctions, and increased
caspase-3/7-positive cells, indicating ALS-associated motor neu-
ron degradation and apoptosis. Interestingly, single treatment or
co-treatment with rapamycin and bosutinib prevented the reduc-
tion of muscle contractility observed with ALS motor neurons,
suggesting that such ALS-on-a-chip and optogenetics technol-
ogy could help clarify the pathogenesis of ALS and screen for
drug candidates.

2.4.4 Blood-brain barrier – Multiple sclerosis and Huntington’s disease

Multiple sclerosis (MS) is the most common non-traumatic neu-
rological disabling disease to affect young adults (Kobelt et al.,
2017). It is an autoimmune chronic inflammatory neurodegen-

erative disease involving complex interactions between multiple
cell types, including demyelinated axons, astrocytes, activated microglia, lymphocytes, macrophages, and oligodendrocytes (Compston and Coles, 2008; Stys and Tsutsumi, 2019; Wu and Alvarez, 2011). Traditionally, preclinical animal models and 3D organotypic brain models have been utilized widely with limited success, providing insights into drug-mediated remyelination (Tan et al., 2018; Zhang et al., 2011). Similar to advances made in AD and PD in vitro models, 3D cultures of differentiated cells involved in MS may provide a viable strategy to build an effective model, as human oligodendrocytes, astrocytes, and neurons have been differentiated using fibroblasts/iPSCs from MS patients (Douvaras et al., 2014; Song et al., 2012). A major challenge for in vitro MS models is a lack of lymphocytes (B cells and T cells), which play a critical role in triggering and maintaining MS. To address this, a functional BBB model needs to be engineered and integrated to recapitulate the physiological interactions between the neuronal network and cerebrovascular components.

The BBB regulates the permeability and transport of important nutrients and molecules from blood into the brain, and many neurodegenerative disorders involve BBB dysfunction (Agrawal et al., 2017; Spencer et al., 2018). Assessment of lipid-mediated transport of small and large molecules across the BBB is critical for neuroscience drug research, as many drugs, due to their lipid solubility and/or molecular weight, do not pass through the BBB. For this, several in vitro BBB models that incorporate fluid shear stress of blood flow have shown in vivo gene and protein expressions, endothelial adherens/tight junctions, and barrier functions (Ahn et al., 2020; Park et al., 2019; Wevers et al., 2018). Additionally, iPSCs are integrated with BBB microfluidic technologies to model neurodegenerative diseases. For example, Huntington’s disease (HD) is a rare genetic disorder characterized by progressive degeneration of nerve cells in the brain that leads to cognitive, emotional, and motor defects (McColgan and Tabbrizi, 2018). A BBB chip developed using HD patient iPSC-derived brain microvascular endothelial-like cells, astrocytes, and neurons showed a significant increase in permeability compared to control models with cells from healthy donors that exhibited physiologically relevant values (Vatine et al., 2019). Such an approach demonstrates the advantage of direct patient cell sourcing for developing BBB MPS that exhibit proper disease phenotypes and could help screen for personalized druggable targets.

2.5 Gastrointestinal diseases

The development, characterization, and application of disease models within the gastrointestinal (GI) system present significant opportunities to better understand and characterize fundamental biology, disease function and pathologies, and identify novel mechanisms and targets for therapeutic interventions. Comparable to other organ systems, the in vitro/ex vivo to in vivo translation for the GI tract is encumbered by numerous complexities to ultimately recapitulate the human anatomy and physiology. Therefore, there have been parallel as well as iterative approaches to reconstruct key aspects of the GI tract in vitro that serve as a composite representation of disease models across various platforms and modalities. Building upon the learnings across the industry, significant advancements have been made to provide more accurate architecture and biomechanical systems for GI research.

For several decades, the colorectal tumor-derived Caco-2 cell line has been widely used to study intestinal permeability and absorption. However, the Caco-2 cell system includes well-recognized limitations such as a lack of in vivo-like expression of drug metabolizing enzymes and drug transporter (Blutt et al., 2017). This has prompted the exploration and adoption of alternative model systems that better reflect human gut absorption, metabolism, and cell architecture. Primary advancements from the Caco-2 based system include, but are not limited to, the use of progenitor/stem cells, primary cells from normal or disease donor tissues, and more representative crypt and villi composition. Additionally, a more in vivo-like microenvironment to reflect both apical and basal interfaces as well as the interactions between GI and connected organs are engineered via 3D scaffolds and geometries, biomechanical forces, and multi-compartment systems.

Representative applications for GI disease models are in the areas of acute studies for cellular signaling, ADME (absorption, distribution, metabolism, and excretion), efficacy, and/or toxicity. Amongst the in vitro disease models, there may be readily available opportunities for further mechanistic evaluation and understanding in areas of metabolism (e.g., drug-drug interaction liability), infection, cell proliferation/regeneration or toxicity (e.g., inflammation), etc. The complexity of intestinal damage and toxicity arising from infections, radiation, chemical exposure, and therapeutics may be studied across GI compartments in disease models. Two areas of potentially significant opportunity for MPS GI disease models are in evaluating host-pathogen interactions and screening novel therapeutics (Blutt et al., 2017). Globally, pathogen-induced diarrhea poses a significant health issue. With in vivo models, understanding the root causes and exploring treatment options is challenging, and predictivity can be low. In comparison, intestinal organoids may demonstrate sensitivity to human pathogens and thus serve as a model system by which therapeutics can be evaluated for possible mitigation of associated toxicities (In et al., 2016b). With respect to novel therapeutics, systemic effects and lack of GI-specific targeting are shortcomings of more traditional in vivo toxicology models. In vitro platforms may provide the ability to better control for GI restricted assessment of target engagement and possible amelioration of toxicities, with potential applications of treating diseases or predicting potential GI-related adverse events.

The following attributes are recognized as desired for optimal development, implementation, and application of platform GI disease models: primary cells from representative epithelium (including mucosal layers) that maintain differentiation and cell balance; 3D structure with appropriate crypt/villi composition; representative motility, contact surfaces, and biomechanical forces; and incorporation of nervous system, immune system (including microbiota), and vasculature (Blutt et al., 2017; Costa and Ahluwalia, 2019). Table 6 summarizes select GI MPS disease models by platform type. This does not completely reflect all the potential disease model applications; rather the intention is to present a diverse spectrum of opportunities for characterization of GI biology and toxicity/disease states.
### 6: A glance at MPS gastrointestinal disease models

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
</table>
| Transport/ADME Enteric infections (host-pathogen interactions) | Organoids (mini-guts – enteroids, colonoids) | - Secretion and absorption (ion, nutrient, and water) and evaluation of molecular events during infections  
- Derived from LGR5+ intestinal stem cells isolated from the small intestine or colon (crypt-based or iPS cells) with further differentiation to relevant cell types of interest | a In et al., 2016b  
 b Arian et al., 2022 |
|                                                   | Microfluidic device (small intestine)         | - Analysis of fluid samples for nutrient digestion, mucus secretion, and barrier function (permeability) for examining metabolism, nutrition, infection, and pharmacokinetics  
- Epithelial cells from healthy regions of intestinal biopsies, forming villi-like projections lined by polarized epithelial cells that undergo multi-lineage differentiation | Kasendra et al., 2018 |
|                                                   | Microfluidic device (duodenum)                | - Study of transport (MDR1/P-gp), drug-drug interactions, and metabolism (CYP3A4); mechanical forces to recapitulate fluids and stress lead to formation of polarized cells and microvilli  
- Representative intestinal epithelial cell types (by ratio) and expression of transporters and cytochrome P450s | Kasendra et al., 2020 |
|                                                   | Microfluidic device (interconnected gut and liver MPS) | - Measurement of drug concentrations in multi-organ systems via a common mixing chamber with directed outputs to liver and gut compartments to reflect in vivo blood flow and model various dosing routes  
- Liver (hepatocytes to Kupffer cells) and gut (absorptive enterocytes to mucus-producing goblet cells) ratios to reflect in vivo (surface area to cell type ratio) | Tsamanoudaras et al., 2017 |
| Cytotoxicity, barrier functions                   | Bioprinted 3D model                            | - Demonstrates barrier function and selective permeability via polarized epithelium with tight junctions, inducible CYP450 enzymes, and histological and biochemical ADME and toxicity readouts  
- Human primary epithelial cells and myofibroblasts (2+ weeks viability) | Madden et al., 2018 |
|                                                   | Microfluidic device (instrumented MPS)        | - Use of TEER/impedance spectroscopy to evaluate barrier function; H&E and IHC staining also performed  
- Primary human epithelial cells and epithelial cell lines (e.g., Caco-2, A549) | a Soucy et al., 2019  
 b Costa and Ahluwalia, 2019 |
|                                                   | Human 3D GI microtissue                        | - Intestinal barrier function evaluated in GI microtissues using TEER  
- Cryopreserved fibroblasts, primary human small intestinal epithelial cells, and Caco-2 cells | Peters et al., 2019 |
| Tissue (mucosal) engineering, regeneration, cell growth, and differentiation | Microfabrication (organoids also applicable) | - Development of 3D tubular scaffold with intestinal topography for the growth and differentiation of intestinal progenitor cells – potential use as diseased intestine  
- Intestinal progenitor cells isolated from preclinical toxicology species or human intestine and ancillary cells – macrophages, myofibroblasts | Shaffiey et al., 2016 |
| Genetic-based disorders /digestive diseases       | Organoids                                      | - Application for understanding natural history of disease progression, disease modeling/screening, and sensitivity to therapeutics (personalized medicine)  
- Healthy or diseased tissue stem cell organoids coupled with the CRISPR-Cas9 technology | Fuji et al., 2019 |
2.5.1 Transport/ADME – enteric infections (host-pathogen interactions)

The development of mini-guts to enteroids (small intestine) and colonoids (large intestine) has paved the way to study drug transport and metabolism characteristics of compounds as well as enteric infections (host-pathogen interactions) (In et al., 2016b). These models can provide an established and time efficient system with some level of representative physiology in areas such as secretion and absorption. Derived from intestinal stem cells, the use of growth factors and ECM gels can figuratively help shape and organize these systems (e.g., organoids) for various research purposes. For example, the growth factor WNT3A helps support a non-differentiated proliferative environment for epithelial cell types. Subsequent removal of this growth factor can then drive a phenotype of differentiation, lack of proliferation, and secretory/surface cell lineages. Despite the inherent advantages over CaCo-2 or other transformed/immortalized cell-based systems, the organoid models have potential limitations due to primary reliance on epithelia, lack of luminal/blood flow, stroma, vascular compartment, and restricted (closed) luminal access requiring use of microinjections for exposure. However, techniques developed for monolayer plating can overcome challenges associated with restricted access for administering test compounds (VanDussen et al., 2015; In et al., 2016a). Thus, further expansion upon the organoid technologies via chip/microfluidics-based multicompartment systems, such as small intestine-on-a-chip and duodenum intestine-chip, presents a logical next step toward a more in vivo-like GI tract (In et al., 2018, 2020). The incorporation of individual patient-derived cells lends further application of the chip/microfluidic-based technology to the potential study of the influence of genetics, disease, age, sex, or diet on GI biology as well as ADME.

An area for expansion in the chip-microfluidic models is the exploration of multi-organ systems, an example being the integrated gut and liver MPS (Tsamandouras et al., 2017). As expected, the engineering controls and considerations are more complex. A common mixing chamber is used to supply input/flow to the gut and liver. The gut MPS receives input flow directly from the mixing chamber, mirroring that of systemic circulation exposure in vivo. And in parallel, the liver MPS receives partial input flow from the mixing chamber (mimicking hepatic artery blood flow) and the majority input flow from the outflow of the gut MPS compartment (mimicking portal vein blood flow). The location of initial exposure within this type of system could be used to reflect intravenous (start with mixing chamber, with flow to both liver and gut compartments) or oral (start with gut MPS, then flow to liver compartment) administration. A couple of possible advantages of this type of multi-organ system may be in the research areas of hepatic-GI interface for drug-drug interactions, general pharmacokinetics properties, and impacts of GI physiology or disease state (e.g., inflammation) on hepatic clearance. As such, key factors in the predictivity and relevance of such integrated models are representative cell-type composition and ratios between the various organ systems.

2.5.2 Cytotoxicity – barrier function

Building upon the incorporation of multi-cell models, such as microfluidic/chip-based, 3D bioprinting provides a system designed with laminar bilayer architecture that includes human intestinal epithelial cells supported by human intestinal myofibroblast interstitium (Madden et al., 2018). The tissue layers are in direct contact, and expressions of key cell-specific markers, such as E-cadherin, ZO-1, and villin, are evaluated to confirm appropriate tight junction formation and barrier function. Moreover, immuno-histochemical staining can be used to identify goblet cells and mucous secretion as well as Paneth cells that secrete lysozyme. The engineering and design of the 3D printed system allows for access to apical and basolateral surfaces, which may be limited in other types of models. Both gene expression and function of

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation damage</td>
<td>Microfluidic device (gut-on-a-chip)</td>
<td>- Assessment of cytotoxicity, apoptosis, and altered cell morphology (e.g., barrier integrity). Indication of microvascular endothelium being a key mediator of radiation damage. - Human intestinal epithelium interfaced with human vascular endothelium; differentiates to 3D villi when cultured in flow/peristalsis-like deformations</td>
<td>Jalili-Firoozinezhad et al., 2018</td>
</tr>
</tbody>
</table>
Phase 1 and 2 metabolizing enzymes as well as transporters can be assessed. A hallmark of this integrated system may be the ability to evaluate for composite signatures of affected gene expression/function, barrier integrity, and cytotoxicity (via histology). As evidenced by case examples using indomethacin and TNF-α, coupling assessments of ADME-related outputs such as CYP or transporter expression and function with advanced assessments of barrier function via TEER (Soucy et al., 2019) and cytotoxicity/immunohistochemical/histologic alterations may provide an in vivo-like comprehensive evaluation across endpoints in 3D-printed models. Furthermore, as demonstrated by the development of human 3D GI microtissue models, there is the potential to screen for diarrhea-inducing drugs, which may afford insights to exposure thresholds and/or altered dosing regimens to possibly mitigate GI-related effects in the clinic (Peters et al., 2019).

2.5.3 Tissue (mucosal) engineering and regeneration – Cell growth and differentiation

Similar in concept as other 3D models, the architecture of scaffolds and Matrigel-supported structures may be further modulated to provide in vivo-like architecture. Fabricated tubular scaffolds are examples that have been engineered to evaluate intestinal cell growth and differentiation, with application in non-clinical toxicology species such as mice and dogs as well as human cells (Shaffiey et al., 2016). In this model system, there may be opportunity to use diseased intestine as a cell source, with potential need for additional chemical stimulus to recapitulate the disease conditions. Primary intestinal progenitor cells, with co-culture of macrophages, myofibroblasts, and probiotic bacteria within a tube-shaped environment can be used to support tissue engineering for in vivo implantation studies of mucosal defect and regeneration. Functional aspects of the myofibroblasts and macrophages may activate growth factors and associated pathways, which can aide in cellular proliferation, differentiation, and migration. The potential for in vitro development of tubular scaffolds for in vivo implantation present unique applications for such model systems. In a model of mucosectomy, dogs implanted with the scaffold had colonoscopic imaging and histologic evidence of development of a normal mucosal layer with native epithelium within 1 to 2 months, with Alcian-blue positive cells in the colonic mucosa indicative of differentiation into goblet cells (Shaffiey et al., 2016). This type of platform may be used in research for tissue engineering and regeneration such as research on mucosal growth and differentiation.

2.5.4 Genetic-based disorders/digestive diseases

By encompassing several platforms, including organoids to microfluidic/chip-based systems, translation medicine approaches are evidenced by disease models with applications toward researching genetic-based disorders and digestive diseases as well as cancer and potential associated radiation treatment-related damage. Aspects of predictive and personalized medicine are reflected in the use of in vitro/ex vivo platform-based models that look to couple technologies. An interesting example of this includes potential for CRISPR-Cas9 modified organoids (Fuji et al., 2019). Sources healthy or diseased cells, the development of organoids with advanced gene editing technology may afford tools to better understand the underlying genetic anomalies leading to diseases, including cancer.

2.5.6 Radiation damage

Within the oncology therapeutic area, toxicities associated with radiation on a cellular level may be difficult to investigate in in vivo animal studies. Chip-based microfluidic platforms incorporating vascular endothelium have demonstrated the cascade of responses upon exposure to gamma radiation. Reactive oxygen species and lipid peroxidation leading to cell death within 24 hours of exposure in turn triggered the anticipated response within the epithelial cells. Such a model to evaluate the cellular response and relationship between intestinal endothelium and epithelium may provide research opportunities toward mitigation of radiation-associated or related toxicities (Jalili-Firoozinezhad et al., 2018).

2.6 Rare diseases

While the definition of “rare” differs between countries (i.e., defined as less than 200,000 US patients by the FDA; “not more than 5 in 10,000” by the European Union (EU)), less than 5% of the 7,000 identified rare diseases have effective treatments (Low and Tagle, 2016). The lack of therapies is in part due to the low prevalence of these disorders that limits research activities to clarify pathobiology and disease progression. Additionally, patient populations for each rare disease are geographically scattered and demographically diverse, which restricts healthcare providers in conducting reliable diagnosis, obtaining patient information, or conducting thorough case studies. Despite these limitations, there has been rapid growth in the rare disease market, partly due to favorable government policies that enable priority review for these drugs in development. For example, US Congress appropriated $24 million in 2009 for the development of new drugs for rare and neglected diseases (Austin, 2010).

To overcome the obstacles and develop wide-ranging rare disease programs, the pharmaceutical industry has been exploring MPS-based disease models. With the emergence of patient-derived cells and iPSCs, these versatile in vitro devices can be engineered to recapitulate the rare disease microenvironment and associated cellular responses with relatively low resource and development time compared to generating in vivo models. Such a strategy decreases the risks of high R&D cost and potentially low profit margins to encourage investment in rare diseases. Taken together, the recent development in rare disease MPS models affords the opportunity to address currently confounding mechanisms of pathogenesis, identify new disease biomarkers, and ultimately develop effective therapies to help patients across the globe. Several notable examples are included in Table 7 and are described in this section to illustrate the research advancements.

2.6.1 Barth syndrome

The cardiac abnormalities associated with Barth syndrome (BTHS), a monogenic cardiomyopathy, have been modeled using a microfluidic heart-on-a-chip (Wang et al., 2014). In this study, iPSCs were generated from two patients with Barth syndrome,
### Tab. 7: A glance at MPS rare disease models

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Platform specification</th>
<th>Key readouts and cell types</th>
<th>References</th>
</tr>
</thead>
</table>
| Barth’s syndrome                       | Microfluidic device (heart-on-a-chip)                       | – Assessment of cardiolipin depletion, mono-lysocardiolipin accumulation, mitochondrial function via oxygen consumption rate and electron microscopy, generation of ATP, immunostaining of α-actin, and diastolic and systolic stress assessment of cardiomyocytes (CM) by measuring contraction of muscular thin films  
– Patient iPSC-derived VCAM-1+ CM, normal iPSC lines generated by retroviral or modRNA, and human iPSC cells with TAZ mutation via CRISPR-Cas9 | Wang et al., 2014                                      |
| Hutchinson-Gilford progeria syndrome (HGPS) | Microfluidic devices (progeria-on-a-chip)                   | – Changes in diameter of engineered blood vessels and immunostaining of contractile proteins (e.g., f-actin, calponin), progerin, and H2A.X (DNA damage) under different conditions of strain; mRNA expression levels of progerin, CAV1 (injury marker), and inflammatory markers in response to phenylephrine and acetylcholine stimuli (e.g., IL6, IL1B, and JUN)  
– iPSCs generated from HGPS fibroblasts differentiated to vascular smooth muscle cells (SMCs) – normal (iSMCs) or HGPS iSMCs induced smooth muscle cells |  
  a Atchison et al., 2017  
  b Ribas et al., 2017 |
| Timothy syndrome                       | Spheroids                                                   | – Calcium signaling imaging following depolarization in cardiomyocytes, electrophysiological recordings of iPSC derived-neurons and iPSC-derived cardiomyocytes, single-cell transcriptomics to characterize migrating interneurons; immuno-staining of SST, GAD67, GABA, CR, CB on migrating interneurons, and treatments with nimodipine and roscovitine to rescue the phenotype  
– Cortical spheroids and subpallium spheroids from patient-derived iPSCs and control iPSCs |  
  a Yazawa and Dolmetsch, 2013  
  b Birey et al., 2017 |
| Pompe disease                          | 3D-tissue engineered myobundle                              | – RNA-Seq of healthy vs. disease model, GAA activity using 4-MU assay, glycogen storage content with calorimetric assay (at 560 nm) and immunostaining of lysosomes with lysotracker and nuclei with DAPI; measurement of tetanic force in healthy vs disease model  
– Myobundles generated using primary muscle cells from infantile-onset Pompe disease (IOPD) patients |  
  a Wang et al., 2021  
  b Rao et al., 2018 |
| Ewing sarcoma (ES)                     | Microfluidic devices (3D vascular system)                  | – Assessment of osteoclast activation with TRAP staining, measurement of osteoblast marker mRNA levels – OPN, BSP, OCN, and expression levels of EWS/FLI and NKX2.2  
– Patient MSC-derived mature osteoblasts in bovine bone scaffolds and mature osteoclast differentiated via co-culture with CD14+ monocytes | Villasante et al., 2014 |
| Hereditary hemorrhagic telangiectasia (HHT) |                                         | – Confocal imaging of endothelial adherence junction via PECAM-1; inhibition of TGF-beta signaling using neutralizing antibodies against TGF-beta to assess its role in vascular formation  
– Co-culture of human umbilical endothelial cells and human embryonic stem cell-derived pericytes in 3D microfluidic channels | van der Meer et al., 2013 |

**AP-1**, transcription factor subunit; **BSP**, bone sialoprotein; **CAV1**, caveolin-1; **CB**, calbindin; **CD14**, cluster of differentiation 14; **CM**, cardiomyocytes; **DAPI**, 4′,6- diamidino-2-phenylindole; **CR**, calretinin; **EWS/FLI1**, Ewing sarcoma breakpoint region 1/Friend leukemia integration 1 transcription factor oncogenic protein; **GAA**, alpha-glucosidase enzyme; **GABA**, gamma aminobutyric acid; **GAD**, glutamic acid decarboxylase 67; **H2A.X**, histone family member X; **IL1B**, interleukin 1 beta; **IL6**, interleukin 6; **JUN**, Jun proto-oncogene; **NKX2.2**, homeobox protein Nkx-2.2; **OCN**, osteocalcin; **OPN**, osteopontin; **PECAM-1**, platelet endothelial cell adhesion molecule-1; **SMCs**, smooth muscle cells; **SST**, somatostatin; **TAZ**, TAFazzin; **TRAP**, tartrate-resistant acid phosphatase; **VCAM-1**, vascular cell adhesion protein 1
which is caused by a mutation of the tafazzin (TAZ) gene to study the pathophysiology causing the cardiomyopathy. iPSCs differentiated to cardiomyocytes (CMs) were seeded onto thin elastomers micropatterned with fibronectin lines and supported by glass coverslips. Over a 5-day culture period, the iPSC-CMs self-organized into laminar, anisotropic myocardium, taking a form of muscular thin film (MTF). Following this, the MTF constructs were manually “peeled” from their glass coverslips and allowed to take on a curved shape. Stimulation of these films with electrodes caused contraction of the heart muscle cells. The degree of contractibility was measured based on the twitching of the films. The MTFs from Barth syndrome patients did not exhibit contractions as strong as those from control group. Finally, the investigators showed the rescue of cardiac-contractibility by restoring the TAZ function using CRISPR Cas-9 gene editing techniques. To evaluate potential therapies, the iPSC-CMs were treated with bromoenol lactone, an inhibitor of mitochondrial phospholipase A2 that catabolizes mature cardiolipin, linoleic acid (LA), an essential unsaturated fatty acid precursor of mature cardiolipin, or amino acids such as arginine and cysteine, which are commonly deficient in BTHS patients (Wang et al., 2014). Among these, LA was most effective in correcting the metabolic phenotype of BTHS cardiomyocytes, leading to partial correction of monolysocardiolipin to cardiolipin ratio, increased ATP as well as normalized basal and FIF0 ATP synthase oxygen consumption rate.

### 2.6.2 Hutchinson-Gilford progeria syndrome

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disease caused by a single point mutation in the lamin A/C (LMNA) gene (Pitrez et al., 2020). The disease is characterized by an accelerated aging disorder caused by nuclear accumulation of progerin, a truncated and farnesylated form of prelamin A. The farnesyl group in progerin binds strongly to the protein of the nuclear membrane, leading to the distortion of the nuclear membrane shape. This eventually leads to defects in DNA repair and proliferation as well as to accelerated cell senescence. HGPS affects mesenchymal cell lineage and primarily vascular smooth muscle cells. The primary cause of HGPS mortality is atherosclerosis or stroke that is driven partially by smooth muscle cell senescence and its loss in the cardiovascular system. Moreover, the vasculature of HGPS patients exhibits excessive calcification, lipid accumulation, and vessel wall thickening due to smooth muscle cell (SMC) malfunctions.

A 3D model of HGPS (progeria-on-the-chip) was developed from patient-derived iPSCs that were differentiated into smooth muscle cells (iSMCs), which express significantly high levels of progerin mRNA (Atchison et al., 2017). To recapitulate the disease model on the chip, a novel microfluidic device using a polydimethylsiloxane (PDMS) membrane was developed. The device reconstructed a tissue engineered blood vessel (TEBV) using induced pluripotent stem cell (iPSC)-derived SMCs from an HGPS patient. The human cord blood-derived iPSC-CMs were utilized in supporting the medial wall. TEBVs fabricated from HGPS iSMCs and hCB-EPCs showed reduced vasoactivity and increased medial wall thickness, calcification, and apoptosis relative to TEBVs fabricated from normal iSMCs. The study also demonstrated the potential therapeutic effects of the rapamycin analog everolimus, which increased HGPS TEBV vasoactivity and induced iSMC differentiation in the TEBVs.

### 2.6.3 Timothy syndrome

Timothy syndrome (TS) is a mutation in the CACNA1 gene, which encodes the voltage-dependent L-type Ca\(^{2+}\) channel (LTCC) CaV1.2 (Splawski et al., 2004). The mutation slows down channel inactivation significantly and prolongs cellular repolarization in cardiomyocytes. Resultant effects include QT prolongation, ventricular tachycardia, and development of neuropsychiatric diseases such as autism and epilepsy. Interestingly, a study demonstrated that cardiomyocytes differentiated from TS patients recapitulated abnormal ventricular contraction of cardiomyocytes (Yazawa and Dolmetsch, 2013). Additionally, roscovitine (Ros), a cycline-dependent kinase inhibitor that increases voltage-dependent inactivation of CaV1.2 channels was tested to understand its potential as a putative therapy for TS. Ros significantly reduced both the irregular timing and amplitude of Ca\(^{2+}\) transients, demonstrating beneficial effects on TS cardiomyocytes. Ros also rescued the electrophysiological phenotypes of TS cardiomyocytes as shown with whole-cell patch voltage clamping studies (Yazawa and Dolmetsch, 2013).

In the CNS, the mutation in LTCCs resulted in the loss of channel inactivation (CaV1.2) that induced increased Ca\(^{2+}\) influx and prolonged action potential, which in turn reduced saltation length and speed across the neurons. Moreover, Birey and colleagues (2017) demonstrated the leading cause for the neurocognitive decline is due to the mutation in LTCCs as seen in TS patients (Birey et al., 2017). The group developed a model using healthy/patient derived-iPSC cells that were differentiated to cortical and sub-pallium spheroids. Functional bridges were generated between the spheroids via interneuron micro-connectivity, and this disease-state model displayed impaired salutary migration of interneurons in comparison to healthy state. The study also demonstrated that the migration defects in interneurons carrying the TS gain-of-function mutation can be restored by reducing the activity of LTCCs using nimodipine.

### 2.6.4 Pompe disease

With an estimated frequency of 1 in every 40,000 births, Pompe disease is an autosomal-recessive disorder that affects the heart and skeletal muscles (Ausems et al., 1999). Pompe disease is also known as glycogen storage disease type II and is caused by a mutation in the GAA gene that leads to the expression of defective acid α-glucosidase enzyme (GAA) (Wang et al., 2021). Normally, GAA is used to break down lysosomal glycogen in the body. Defective GAA leads to an accumulation of glycogen in the lysosomal compartment of both striated and smooth muscles. This eventually results in cardiac complications, skeletal muscle atrophy, and respiratory failure. An in vitro 3D model for Pompe disease was developed from patient-derived primary muscle cells, which were obtained from muscle biopsies. The myogenic cell fraction was expanded and embedded into a fibrin-based gel matrix (Wang et al., 2021). The cells were further differentiated...
for two weeks to form 3D tissue-engineered myobundles. The Pompe model demonstrated myotubules with reduced tetanic force production, decreased fatigue resistance, and increased glycogen accumulation. The phenotype was partially rescued via AAV-mediated GAA expression in the disease-state myotubules. Additionally, this intervention reduced glycogen accumulation significantly; however, it did not rescue stress-induced functional deficits (Wang et al., 2021).

Furthermore, the clinical responses of clenbuterol have been mimicked in bioengineered human skeletal muscle (Madden et al., 2015). Clenbuterol is a β2-adrenergic agonist that shows concentration-dependent effects on muscle. At lower concentration, clenbuterol increases contractile force and hypertrophy, whereas at high concentrations it induces apoptosis and necrosis (Burniston et al., 2006). In the bioengineered muscle model, both acute and chronic effects of clenbuterol were recapitulated that showed a dose-dependent contractile force generation of the myobundles with a stronger contraction at 0.1 μM and diminished contraction at about 1 μM.

2.6.5 Ewing’s sarcoma

Ewing’s sarcoma (ES) is an aggressive and osteolytic pediatric bone tumor that affects 1-3 per one million children and young adults in the United States (Hesla et al., 2021). To model ES, a patient’s mesenchymal stem cells were differentiated to mature osteoclasts and osteoblasts within a 3D-engineered de-mineralized bone matrix, which calcified and compacted over time to form a human bone (Villasante et al., 2014). Incorporation of ES cell aggregates in the model reproduced the expected decrease in bone density, connectivity, and matrix deposition seen in patients. Moreover, the model with ES media extended the culture time (2-4 weeks) to recapitulate the initial steps of tumor generation that represent the induced glycolytic enzyme and hypoxic microenvironment in the core of tumor cell growth. Similar to the disease state, strong upregulation of cancer-related gene expression of hypoxic and glycolytic tumor phenotypes was observed, indicating that the model could be extremely effective for therapeutic testing. Indeed, treatment with zoledronic acid, an osteoporosis therapeutic reagent, inhibited bone resorption within the MPS model. This result was comparable to the drug’s effect in clinic with ES patients (Villasante et al., 2014). Moreover, effects of an anti-cancer drug, linsitinib, were examined in bioengineered human tissues of bone ES tumor and heart muscle cultured in isolation as well as in an integrated system (Chramiec et al., 2020). The integrated format showed a poor tumor response with no observed cardiotoxicity, which matched the responses from clinical trials from both target and non-target tissues.

2.6.6 Hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder that is characterized by abnormal (dilated) blood vessel formation (McDonald et al., 2011). HHT is caused by mutation in at least five genes, but about 85% of cases have mutations in two genes (ENG and ACVRL1/ALK1). The majority of patients with HHT suffer from epistaxis manifested during early childhood. The severity of epistaxis may vary between patients starting from occasional minor bleeding to life-threatening blood loss which may require repeated blood transfusions (McDonald et al., 2011). To model HHT, Van der Meer et al. (2013) developed an MPS system resembling a vascular tissue with microchannels. The model was engineered by injecting a mixture of human umbilical vein endothelial cells and human embryonic stem cell-derived pericytes onto a layer of rat tail collagen I incorporated into a PDMS microfluidic channel. Over the course of 12 hours, the cells organized themselves into a single long tube resembling a blood vessel that followed the contours of the channel. Confocal microscopy revealed a mature endothelial monolayer with PECAM-1 staining at cell-cell contacts with pericytes incorporated inside the tubular structures. In addition, by utilizing neutralizing antibodies against TGF-β signaling that effectively inhibited the downstream signal, the model disrupted tube formation consistent with the current understanding of vascular development. The TGF-β signaling pathway is essential for normal vascular development, and inhibition of TGF-β signaling resulted in tubes with smaller diameters and higher tortuosity, resembling the abnormal vessels observed in patients with HHT.

3 Challenges and opportunities for MPS disease models in the pharmaceutical industry

3.1 Current challenges

Disease modeling using MPS platforms has a distinct advantage over traditional 2D systems – incorporation of bioengineered features that emulate parts of human (in vivo) physiology, which enables disease research and drug screening with improved clinical predictivity and reduction of animal use. Growing interest from both the pharmaceutical industry and regulators is fostering MPS applications, and a range of MPS are being explored for platform testing and assay development within numerous companies for pharmaceutical R&D (e.g., target identification, toxicology, pharmacology, ADME). For example, an evaluation of 3D human liver spheroids for in vitro hepatotoxicity screening showed that the 3D model has higher sensitivity in identifying compounds with clinical drug-induced liver injury (DILI) effects compared to widely-utilized 2D primary human hepatocytes (Proctor et al., 2017). In another industry study, iPSC-derived 3D human neural spheroids exhibited improved specificity for screening compounds with known seizure and neurodegenerative clinical liabilities when compared to data from nonclinical animals, indicating the potential of the 3D assay for discovery-stage neurotoxicity de-risking (Wang et al., 2022). For disease model applications, a microfluidic device with engineered intestinal epithelium, ECM with macrophages (intestinal stroma), and circulating PBMCs was utilized to recapitate T cell infiltration into tissues with cancer-related damage analysis to enable mechanistic examination of how drugs alter immune responses in the TME (Lewin et al., 2022). Through such assessments, MPS technologies are slowly being implemented in early discovery screenings or investigative studies. Thus far, much progress has been made in toxicology, in which cytotoxicity measurement in response to drug treatment is a relatively simple but core readout (e.g., 3D
liver spheroids in 384-well plates). MPS application for other pharmaceutical R&D disciplines could also make significant impact; however, current activities in the pharmaceutical industry are largely exploratory, as more complex MPS platforms need further optimizations to overcome several common challenges to expand their use.

A major limitation of MPS disease models for preclinical safety and efficacy screening is that most platforms are low throughput. Unlike academic or governmental labs, where single-throughput devices and 6/12/24-well formats are readily used for proof-of-concept research, early stages of drug discovery entail rapid and automated characterization of hundreds to tens of thousands of compounds, which requires high-throughput in vitro screening (Tab. 1) to achieve "economies of scale". With the advent of 3D bioprinting and nano-printing, which aim to facilitate miniaturization and better device engineering, a trade-off between complexity and scale may be avoided. However, current MPS disease models with intricate microenvironmental factors, such as 3D multicellular architectures and/or flow, have limited throughput for broad use in the pharmaceutical industry.

As no in vitro model can fully recapitulate the in vivo environment, it is generally accepted that the models or platforms need to be qualified or characterized for each context(s) of use, which would consist of certain fundamental and distinct physiological features. Many MPS disease models are being developed with the aims of elucidating disease pathophysiology and discovering novel biomarkers of disease/toxicity among others. Towards this, incorporating the cell types (at the appropriate seeding densities and ratios) relevant for initiation as well as progression of disease is of paramount importance. However, recapitulating the homotypic and heterotypic interactions between cell types as well as the biochemical and mechanical signaling between the cells and their microenvironment is challenging. Moreover, the absence of certain cues in vitro results in the loss of in vivo features. For example, liver regeneration in response to injury is known to occur in vivo, but in vitro models with incomplete physiological factors are not yet able to recapitulate this key process. In another example, most MPS cancer models do not incorporate the tumor microenvironment (e.g., stroma and vasculature, including the ECM, fibroblasts, endothelial cells, etc.), immune components, and soluble signals including chemokines. All these components are essential for recapitulating cancer hallmarks, including invasive cell migration (metastasis), uncontrolled proliferation, drug resistance, and the interactions between non-malignant and malignant cell populations.

Another key challenge is incorporating proper biological barriers for the disease of interest. Many MPS have been utilizing synthetic interfaces, including permeable polymer membranes and gel-liquid interfaces, for the complex architecture and function of biological barriers. However, integration of tissue and disease-relevant cells with correct phenotype likely influences barrier integrity and its enzyme and transporter expression profiles and activity. The ideal attributes are complex; for example, optimal development for the GI disease model would include primary cells from representative epithelium, and incorporation of nervous system, immune system (including microbiota), and vasculature (Blutt et al., 2017; Costa and Ahluwalia, 2019). Readouts of disease and drug effects may be modulated by permeability of the biological barriers; thus, proper barriers modeled using the appropriate cell types are necessary to ensure physiologically relevant responses. Additionally, although scaffolds often used for MPS platforms are typically components of the in vivo ECM such as collagen, they can also consist of non-physiological materials, such as alginate or polyacrylamide gels, thereby having a composition that is inherently different from the native tissue microenvironment (Kim et al., 2019). This can alter cellular response, which in turn may lead to erroneous readouts from drug screening or mechanistic studies.

One key limitation of MPS disease models is their short life-span, which is common among in vitro platforms. In vivo disease onset and progression are often relatively slow processes that may take multiple days, months or even years. Thus, insights acquired from disease modeling induced by acute exposure to toxic compounds or blunt physical injury (i.e., wound) often do not provide meaningful information about specific steps of disease development with associated biochemical, cellular, and microenvironment changes. Compared to traditional 2D models, MPS disease models with more in vivo cellular and tissue organizations can support extended cellular viability, functions or disease phenotypes that enable longer assay duration (e.g., a few weeks). Nevertheless, further lifetime enhancement (e.g., several weeks to months) is warranted to monitor and examine a full range of pathophysiology, targeted drug efficacy, and toxicity, which would help in vitro-to-in vivo (preclinical and human) translation.

A roadblock to the adoption of MPS is the translatability of the in vitro findings to in vivo outcomes. Readouts from preclinical in vivo studies are typically standardized. These studies have large historical data sets based on which scientists can predict some in vivo outcomes. Efforts should be put into developing animal MPS models with high concordance with preclinical in vivo data to build confidence in human cell-based MPS models. This will require standardized testing using large numbers of drugs or chemicals with well annotated in vivo effects. Such an endeavor can be very costly and should be considered as a cost sharing program across industry to validate and bring these models into full scale production and use.

Lack of clarity in how regulatory agencies assess the data generated from MPS disease models is another major barrier for rapid adoption. Analytical methods utilized in pharmaceutical R&D are qualified or validated to ensure data quality and reproducibility for regulatory review. However, given the lack of standardization of MPS platforms (i.e., high variabilities in system design, fabrication methods, applications, and readouts), pharmaceutical companies fear delays in their regulatory submissions with inclusion of MPS data. Herein lies the dilemma: as of the writing of this manuscript, very little MPS-generated data have been submitted by industry. However, regulatory agencies need access to MPS data (including validation data) to learn the technology, applications, and quality in order to review properly and decide on validity, reliability, and relevance (Hargrove-Grimes et al., 2022; Baran et al., 2022). Ultimately, regulatory agencies’ confidence in MPS data will grow with increased familiarity and
experience with the models as well as the industry acceptance that requires strict standards in manufacturing, intended use, and assay performance by suppliers and end-users. Towards this, industry stakeholders, such as the IQ MPS Affiliate, and regulatory agencies have on-going strategic discussions to facilitate best MPS practice in pharmaceutical R&D, including a “safe harbor” for data submissions.

Overall, there will always be limitations and challenges associated with the use of reductionist in vitro models to predict in vivo responses. Despite these challenges, MPS disease models are poised to offer unprecedented opportunities to facilitate efficient and more successful pharmaceutical R&D. These are described in the next section.

3.2 Future opportunities

A major advantage of the microfluidic MPS is the incorporation of flow that mimics various physiological fluid dynamics such as blood flow. Controlled fluid flow over cells applies shear stress for mechano-transduction and enables continuous transport of cellular and microenvironmental cues as well as drugs, resulting in a more in vivo-like phenotype of cultured cells and tissues for drug discovery (Holton et al., 2017; Lanz et al., 2017). However, improvement opportunities remain.

As the interconnectivity of the organs and tissues in the body directly regulate the PK and PD of drugs, future MPS disease models should be designed to integrate multiple major organs (Edington et al., 2018). Drugs can have off-target toxicity on several organs; thus, an integrated MPS disease model system that addresses both on- and off-target effects would be beneficial (Van Ness et al., 2017). Multi-organ MPS disease models could provide more accurate ADME profiles of dosed drugs for efficacy and safety assessments. Healthy and diseased organs may be incorporated in the same chip, enabling the determination of safety margins based on healthy and diseased cells/tissue in the same model (Trapecar et al., 2021). Moreover, inclusion of interconnected compartments with organ/tissue-specific ECMs, media, and physiological barriers should be important features of MPS disease models, including cancer MPS that incorporate multiple factors, such as mechanical stimuli, neighboring non-tumor organs, and immune components. In addition to the six disease areas described in this paper, MPS of various organ types and diseases are being developed, including the heart, immune system, fat (obesity), bacterial infections/microbiome, and developmental/reproductive systems (e.g., uterus) (Ingber, 2022). To better mimic human biology and pathophysiology, continuous progress in a range of MPS is vital for the multi-organ approach and may help in bridging current gaps between in vitro and in vivo.

In vivo tissues are highly vascularized with organ-specific architectures and immune components; however, many MPS disease models that involve complex 3D structures, such as tumors, the liver, and the brain (e.g., BBB), lack organized blood vessels. Having functioning vasculature would support accurate drug and nutrient transport within relatively thick 3D cellular layers, which would lead to extended cellular viability and drug-induced responses for longer-duration assays (i.e., increased lifespan of the models). Moreover, incorporation of immune cells via the integrated vascular system would elucidate key functions in disease development, progression, or response to drug efficacy and/or toxicity in MPS disease models. For example, a study using a multi-organ device consisting of functioning cardiac and skeletal muscle, and liver modules with recirculating THP-1 monocytes indicated that damaged cardiomyocytes via amiodarone treatment released IL-6 and activated the immune cells (M2 phenotype) (Sasserath et al., 2020). In contrast, lipopolysaccharide (LPS) and IFN-gamma treatment induced defects in all three modules, upregulation of multiple proinflammatory cytokines, and distinct THP-1 cell activation (M1 phenotype), revealing different immune cell effects in response to drug treatment and changes in target tissues. Taken together, the findings demonstrated the importance of microfluidic vasculature and immune components in enhancing MPS disease models.

For many viral infections, no effective therapies exist. Animal models, as mentioned in earlier sections, are not always good surrogates for human models. Viral replication and drug efficacy studies are often conducted with immortalized cell lines, such as Vero cells, Caco-2, Calu-3, HEK293T, and Huh7 cells that do not represent normal tissue physiology. Given their robust maintenance of physiological cellular signatures, primary cells cultured in MPS disease models are likely to retain their in vivo permissiveness to infection and enable predictive in vitro viral screening and infection studies.

Human MPS offer an opportunity for more translatable pre-clinical testing of modalities which often have poor cross-reactivity in nonclinical species such as oligonucleotides or biologics, and/or when a longer duration of treatment is required or where responses are delayed. When considering disease MPS specifically, these offer an opportunity to investigate whether disease state influences the ADME or toxicity of new modalities. For example, a disease model of a kidney MPS could enable evaluation of the impact of disease state on oligonucleotide clearance, while disease MPS in general provide an opportunity to determine if this influences receptor-mediated oligonucleotide uptake (Ramsden et al., 2022). Patient-derived organoids also provide an opportunity for patient centricity, enabling evaluation of modalities such as antibody-drug conjugates (ADCs) in different patient-derived organoids to stratify patients based on efficacy (Lyons et al., 2021).

Finally, commercial MPS companies, academic researchers, pharmaceutical industry scientists, and regulatory agencies should work together to standardize MPS testing and use. As the MPS technology advances and its market grows, coordination among stakeholders with standardized criteria recognized by regulatory agencies would facilitate comparison across different models and assays for faster and wider industry adoption. As an example, to address the inconsistencies in the characterization of liver MPS models, Baudy and his co-authors put forth a guidance paper on best approaches for validating liver models, which included recommendations for thresholds for albumin and urea synthesis rates, drug metabolizing enzyme levels, and a set of pharmacologically relevant compounds to be tested in the systems (Baudy et al., 2020). Additionally, regulators support the principles of the 3Rs and advise sponsors to consult with re-
view divisions when considering a non-animal testing method, such as MPS models, for safety evaluation (Avila et al., 2020). Moreover, the TEX-VAL Tissue Chip Testing Consortium, in collaboration with the National Center for Advancing Translational Sciences (NCATS) and industry stakeholders, evaluated independent end-user experience and reproducibility of several MPS models (Rusyn et al., 2022). The findings indicate that for MPS to be considered as viable alternatives to less effective assays and animal testing, several key optimizations are recommended, including reliable cell/reagent sources to reduce biological variability, well-defined configurations (e.g., flow control, biomaterial for barrier function), and end users’ multidisciplinary technical skills that consist of bioengineering, cell biology, microscopy, and chemistry. Taken together, acceptance of these models will require continuous dialogue and practical actions among all partners (academic and commercial MPS developers, pharmaceutical companies, and regulatory agencies) as well as the intentional inclusion of MPS data as supporting material in regulatory submissions to increase exposure and help improve the review process.

### 3.3 The 3Rs

The 3Rs principle calls for replacement, reduction, and refinement of animal use to promote ethical and scientifically sound biomedical research (Russell and Burch, 1959). To this goal, current efforts in development and application of in vitro disease models demonstrate a major commitment. These innovative tools have significant potential to transform the dependency of animal models in pharmaceutical R&D. Integration of human disease-relevant cells, physiology, and pharmacology would help enable target identification, mechanistic investigation, and screening that enhance predictability and translation of small and large molecules and avoid unnecessary testing in animals. Complete replacement of animals in the near future may not be likely due to challenges in recapitulating certain complex in vivo processes; however, numerous companies as well as government and regulatory agencies, including the NCATS of the US National Institutes of Health (NIH), US Department of Defense, and the National Science Foundation (NSF), are working towards a wider adoption of MPS within the pharmaceutical and biotechnology industry (Low and Tagle, 2016; Zhang and Radisic, 2017). As MPS disease models continue to improve, their implementation in drug development will help companies to decrease animal studies and increase the efficiency of R&D pipelines.

### 4 Conclusion

Pharmaceutical drug discovery and development have been undergoing continuous optimization to improve efficiency, safety predictions/assessments, and preclinical-to-clinical translatability. Key processes such as target identification, compound screening, and proof-of-concept and toxicity studies cannot rely mainly on in vivo models but need in vitro approaches that provide representative disease biology with ease of use, high throughput, and accuracy as well as consideration of overall costs. Although the simplicity and accessibility of 2D models make them advantageous for exploratory studies, these models largely lack target-specific disease phenotypes. For later-stage drug discovery programs, MPS disease models that integrate patient and iPSC-derived cells in engineered 3D microenvironments pose to offer enhanced insights into in vivo pathophysiology for predictive efficacy and toxicity data, while minimizing preclinical animal studies. Taken together, MPS disease models are becoming a vital technology in pharmaceutical research, and they are providing unique and ethical opportunities to enhance human translation, leading to more effective and safer drugs for patients.

### References


Austin, C. P. (2010). NIH translational research for rare diseases
and orphan products: NC GC and TR ND. Presentation to the IOM Committee on Accelerating Rare Diseases Research and Orphan Product Development. Washington, DC.


Chlebanowska, P., Tejchman, A., Sulkowski, M. et al. (2020). Use of 3D organoids as a model to study idiopathic form of Parkin-


Fabre, K., Berridge, B., Proctor, W. R. et al. (2020). Introduction to a manuscript series on the characterization and use of microphysiological systems (MPS) in pharmaceutical safety and ADME applications. Lab Chip 20, 1049-1057. doi:10.1039/c9fc01168d


Freundt, E. C., Maynard, N., Clancy, E. K. et al. (2012). Neu-


Irrechukwu et al. ALTEX 40(3), 2023


516


Walsh, K., Megyesi, J. and Hammond, R. (2005). Human central nervous system tissue culture: A historical review and examina-


Conflict of interest
The authors declare no conflicts of interest.

Data availability
No original data was generated for this manuscript.

Acknowledgements
The authors thank constructive input from numerous colleagues to help improve the manuscript. This perspective paper was written as part of a manuscript series by members of the IQ MPS Affiliate with the support of the International Consortium for Innovation and Quality in Pharmaceutical Development (https://iqconsortium.org/). The IQ Consortium is a not-for-profit organization of pharmaceutical and biotechnology companies with a mission of advancing science and technology to augment the capability of member companies to develop transformational solutions that benefit patients, regulators, and the broader research and development community. The IQ MPS Affiliate was established by the IQ Consortium in 2018 to provide a venue for appropriate cross-pharma collaboration and data sharing to facilitate industry implementation and qualification of MPS models.