



Recent Advances in the Development and Application of Human-Specific Biomedical Research and Testing Methods

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Introduction

There is an increasing appreciation of the urgent need to take positive steps to ensure that biomedical research and testing is based on science of the very highest quality. Research and testing programs must comprise the very best, most reliable, productive, and relevant scientific methods. If they don't, they are failing those who fund, and who stand to benefit from, those endeavors. This is perhaps especially important right now, as investigative methods that could barely have been envisioned in recent history are not only being developed, but also are presenting genuine and exciting opportunities for scientists to push the envelope.

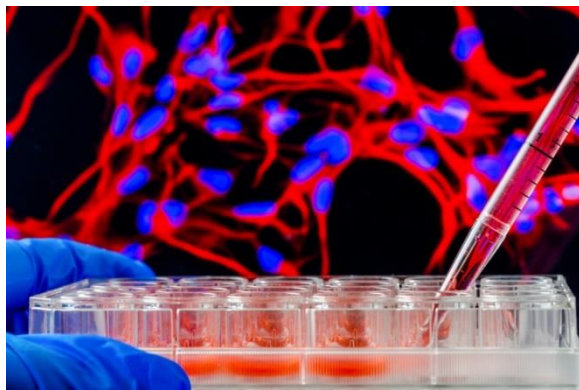
This overview is intended to provide a snapshot of some salient and exciting developments and breakthroughs in various areas of biomedical research. It aims to help scientists and non-scientists alike appreciate what is being done, in technologies centered around human-specific cell, tissue and organ culture. Their major promise and evident advantage are that they provide more human relevance than, for example, animal models and simpler methods of cell and tissue culture (such as 2D monolayer cultures of immortalized cells). An unprecedented number of scientific publications now highlight the poor human relevance of data from these approaches. For example, greater than 90% of new drugs fail in human trials, mostly due to problems with efficacy and safety. This is despite encouraging data, largely from animal studies, that suggest good efficacy and safety profiles. Put simply: in drug development, preclinical approaches to establishing pharmacokinetics (PK) and pharmacodynamics (PD) that are used currently (and have been for many years) are not acceptably predictive of humans.^{1, 2} Further, the use of animal models cannot be significantly improved due to intractable species differences in genetics and biochemistry.³

A number of important classifications have been (and are still) used interchangeably, and there exists some overlap and a few gray areas in terminology. In this paper, the methods discussed are categorized as follows:

- “Classical” 2D tissue cultures: typically, flat monolayers of cells growing in a flat-bottomed flask to which the cells adhere.

- Spheroids: self-aggregating multi-cellular 3D structures, often containing cells of more than one type, which assemble into spherical forms and can replicate many functions of a tissue or organ in vivo.
- Organoids: typically derived from stem cells and considered to be functional “mini organs”, with greater complexity than spheroids. Organoids exhibit a high degree of organization, function and micro-anatomical fidelity, and which are more stable and long-lasting in culture than spheroids.
- Organ-on-a-chip (OOAC) and human body-on-a-chip (HuBoC) applications: microfluidic cell culture devices, with channels lined by living cells. Other physiological factors are brought into a cell culture system, such as circulatory systems, physiologically realistic flow, pressure and stress, and others (see below) (e.g.^{4, 5}).

2D Cultures



Many improvements of cell culture methods involve increasing physiological relevance via the development of 3D cultures. However, it is important to note that 2D cultures continue to demonstrate good human physiological relevance for some applications (see Table). Also, they are still being improved and optimized, sometimes on the path to the development of 3D cultures. For example, one major and ongoing issue with animal tests is

that they cannot reliably predict drug-induced liver injury (DILI), which results in many drugs (up to 50%) being terminated during development. This is the leading cause of drug withdrawal from market.⁶ Increasing the predictive power of animal tests for human DILI can only ever be slight, however, due to intractable species differences in pharmacodynamics and pharmacokinetics. Alternatives must, therefore, be sought, accepted and used. The use of primary and engineered human hepatocytes in this regard is established, though the predictive nature of tests using them, while good, stands to be improved. Some improvements are ongoing, but others have been achieved. For example, one group of researchers optimized a testing protocol based on 2D culture of hepatocytes on a number of levels, and used a “support vector machine” algorithm with the data to classify substances as toxic or non-toxic to the liver. This increased the sensitivity, specificity and accuracy of the test to 88-100%.⁷ Nevertheless, primary cells can suffer from a short culture life, which can limit disease modelling, and be disadvantageous where longevity is needed, for example in drug development.

3D Microtissues/Spheroids

3D microtissues have already been shown to be a significant improvement on 2D cultures in many areas. One example is in breast cancer research. The HER2 gene is over-expressed in around one-third of breast cancers and associated with poor prognosis of the disease, and

has consequently been a therapeutic target. 3D breast cancer cell lines treated by HER2-directed interventions and radiation showed more physiological relevance than 2D cultures. This led investigators to conclude that modelling human breast tumors more faithfully in this way would lead to more effective screening of breast cancer drugs, and thus more effective new drugs coming through development.⁸

Some Advantages and Disadvantages of 2D and 3D Cell/Tissue Cultures

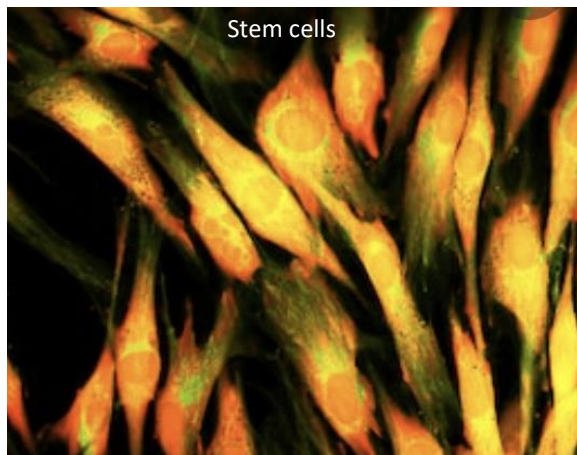
Characteristics	2D Cultures	3D Cultures
Physiological relevance	Good for some applications, but often poorer than 3D	Often superior to 2D: spatial arrangement, complexity, cell-cell contact, interactions, medium flow, barrier tissues (epithelia), etc.
Cost	Inexpensive	Can be more expensive
Ease of use	Established, well known & understood, easier techniques	Culture can be more specialist & time-consuming
Throughput	Can be higher than 3D	Can be significantly lower than 2D
Results	Easier to analyze	Can be more difficult to analyze
Predictive of in vivo	Often lower than 3D	Often higher than 2D
Shape	↓	↑
Medium/nutrient exposure	Similar	Different according to cellular location - more like in vivo
Differentiation	↓	↑
Metabolism	↓	↑
Proliferation	Can be too rapid	More realistic
Gene expression	Can be dissimilar to in vivo	More realistic
Replicability	↑	↓
Stability/longevity	↑	↓

“↓” and “↑” indicate relative attributes and e.g. physiological relevance of these aspects of 2D and 3D cultures. Source: [Caleb & Yong, Mol Biosci 2020](#) and [Mimetas](#)

Human liver spheroids are used for the detection of DILI.⁹ They are used in early drug development, in which 384-well plates containing only primary hepatocytes are used for high-throughput screening of many new candidate drugs. This provides data on which candidates are probably toxic to the liver, and which should progress to more detailed analysis. This initial screening also suggests which drug candidates should be used in more sensitive screens using co-cultured hepatocytes and Kupffer cells, or in highly detailed investigations of toxicity mechanisms and biomarkers. These spheroids have been shown to be stable in culture for several weeks, via various cell-function parameters, gene expression and enzyme function tests. They have also identified marketed drugs with the potential to cause DILI with 90% specificity and 64% sensitivity—twice the [sensitivity](#) of 2D cell models.¹⁰

Human heart spheroids, made from cardiomyocytes, endothelial cells and fibroblasts derived from induced pluripotent stem cells (iPSCs), can mimic heart morphology, biochemistry and

pharmacology.¹¹ Human brain spheroids are being used for basic neuroscience research and in research into bio-electronic interfaces. They are also proving their worth in Alzheimer's disease investigations and in brain tumor research, where the efficacy of new therapies can be tested.¹² 3D neural cultures of various types have generated exciting results, consistent with data from human patients, in neurodegenerative diseases. In Alzheimer's, Parkinson's and Huntington's disease, and in amyotrophic lateral sclerosis (ALS) research, 3D neural cultures have displayed pathologies characteristic of the diseases in patients, and have been used to test the therapeutic potential of interventions.¹³ These are being improved by, for instance, the development of [superior media](#) that better reflect the central nervous system extracellular environment, and which support synaptically active neurons.



“Hanging drop” microfluidics and microtissue formation involves the culture of cells in a drop of medium, for instance hanging from a cover slip on a glass slide. This approach has provided new approaches to conduct research into type 2 diabetes, specifically into the dynamics of insulin secretion by the islets of Langerhans of the pancreas. This is an area crucial to the understanding of type 2 diabetes pathology and evaluations of anti-diabetic therapies, and one in which shortcomings of animal models and other forms of human tissue culture are

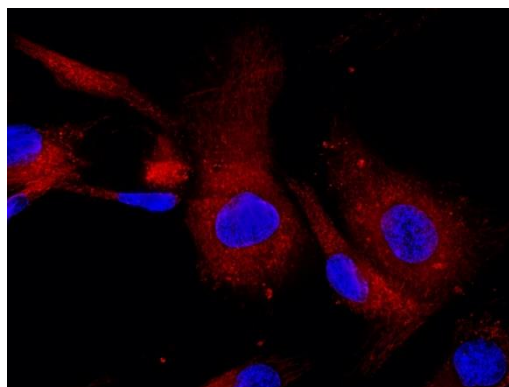
being overcome. Together, these new methods robustly and reproducibly mirror physiologically relevant insulin-secretion responses to glucose, in cultures stable for more than four weeks. Compared to other approaches, they preclude the need to pool islets to achieve quantifiable insulin secretion, which can cause dynamic issues due to the lack of coordination of individual islets. Small fluid volumes involving single-cell or homogenous small cell-aggregates enable precise control of the system, high reproducibility and viability, good-quality data of the temporal dynamics of single islets, and high physiological relevance. They have undergone proof-of-concept studies for pharmacological manipulation of islet dynamics, and so have great potential for the study of the efficacy and safety of new drugs.¹⁴

This hanging drop approach is also being used to advance embryotoxicity testing, i.e. the detection of adverse effects of new drugs and chemicals to unborn children. This area of testing remains focused on animal use, which has been known for years to be poorly predictive of human risk, and in spite of validated in vitro methods like the embryonic stem-cell test.¹⁵ Recently, metabolism has been introduced into the system by co-culturing primary human liver microtissues with embryoid bodies (which can be derived from iPSCs, instead of embryonic stem cells). By enabling human-specific metabolites of test substances to be present with the embryoid bodies in culture, this will increase the predictive accuracy of the embryonic stem-cell test from its already impressive 78%, and help to avoid false negative results.¹⁶

Spheroids are also being utilized in drug discovery generally, by virtue of their improved morphology, complexity, lifespan, and physiological relevance compared to 2D cultures. They are very simple to handle, and mimic diffusion gradients of nutrients and oxygen, etc. very

well. They also effectively model intercellular communication and interactions with the extracellular matrix (without the need for exogenous materials/scaffolds). Spheroids are increasingly used in high-content screening/imaging studies, to monitor how potential new drugs alter cellular function and phenotypes, and/or cause possible toxicities. For example, proof-of-concept studies have shown the utility and high degree of physiological relevance of multicellular 3D spheroids for drug discovery/development in human liver non-alcoholic steatohepatitis (NASH—associated with diabetes and obesity), and for lung and stomach cancers—to a degree that will make them routine in drug discovery in Pharma.¹⁷

Regarding cancer more generally, it has been demonstrated that 3D heterotypic cultures such as spheroids are much more physiologically relevant than 2D cultured human cells, by several measures. They more faithfully reproduce actual tumor microenvironments, including interactions between tumor and host/immune cells. They better model immune system activity, have more faithful expression of genes of various classes, simulate changes seen in the extracellular matrix of tumors, and more. Therefore, while 2D cultures remain valuable in screening candidate anti-cancer drugs, these 3D approaches greatly facilitate cancer research and testing of cancer immunotherapies. 3D methods provide more predictive information about a new drug's activity and potential efficacy and safety, specifically regarding how a new drug targets tumor cells, how immune cells are involved, and the associated kinetics of how they combine to destroy tumor cells.¹⁸ In lung cancer research, for example, 3D microtissues of non-small cell lung cancer cells, cultured using a hanging-drop system, provided greater accuracy for the testing of anti-cancer drug efficacy than 2D cultures. Similar findings exist for other tumors such as osteosarcoma, colorectal, breast, and head and neck squamous carcinomas. Researchers have noted that, due to the fact that some cancer therapies would be inactive in traditional monolayer-based drug screening, and therefore “missed” by researchers using only these conventional methods, a new approach to this process is essential.¹⁹



Organoids, and Organ on a Chip/Human Body on a Chip

Organoids and OOAC are included together here because they are frequently used together, complement one another, and are being developed to work synergistically.²⁰ The distinction between 3D microtissues/spheroids and organoids may often be vague, but centers on the degree of organization that any small 3D cell/tissue culture may have. Organoids may have more developed functional aspects than simpler 3D cultures, for example. Both are developing quickly and impressively, and will positively impact biomedical research and testing. One illustrative example of this involves a comparison of 2D and 3D cultures from patient-derived clear-cell renal-cell carcinomas. These cultures reliably mirror the heterogeneity of actual tumors, which underpins phenotypes and outcomes. Heterogeneity exists both between individual patients and within the tumors themselves, to a degree not seen or attainable in standard cell cultures typically used in research into this disease.²¹

Researchers concluded that in vitro modelling of clear-cell renal-cell carcinoma is crucial to its understanding and treatment, and will improve further with the development of next-generation cell culture. Similar findings exist for patient-derived bladder cancer organoids, which enabled the identification of specific genetic mutations at the root of the tumors, as well as screening each organoid with an array of drugs for susceptibility (both approved and investigational) and confirmation of tumor type.²² Advanced cultures offer a degree of throughput that would never be achievable with animal models, all the while facilitating the linkage of genetic mutations with different stages of tumor progression, response to treatments and ultimate outcomes.

OOAC technology involves the dynamic culture of cells on a “chip,” which can be patterned (e.g. by 3D bioprinting) to impart geometry and aid physiological relevance. Microfluidic channels contain the cultured cells, and aid the delivery of a blood substitute/nutrients as well as the disposal of waste products. Flow velocity, channel geometry and temporal and spatial biological gradients can be set up to mimic natural in vivo environments, including shear stress, physical pressure, and organ polarity.²³ Essentially, OOACs act as “three-dimensional cross-sections of major functional units of whole living organs.”²⁴ They have developed significantly in recent years, with varied, multiple refinements providing ever-more faithful models of human physiology and biology. Examples include the incorporation of automated fluid/media systems and transfers, co-culture of different cell types, involvement of endothelial barriers, and more. One salient illustration of this type of advancement is the liver. While primary human hepatocytes have provided an extremely productive and reliable means of studying, for instance, drug metabolism and toxicity, they have a short half-life, losing functionality and differentiating quickly. This is due, at least in part, to poor in vitro replication of the in vivo microenvironment, such as spatial arrangement of cells, signaling from other cells, interaction with the extracellular matrix, immune effects, and so on.²⁵

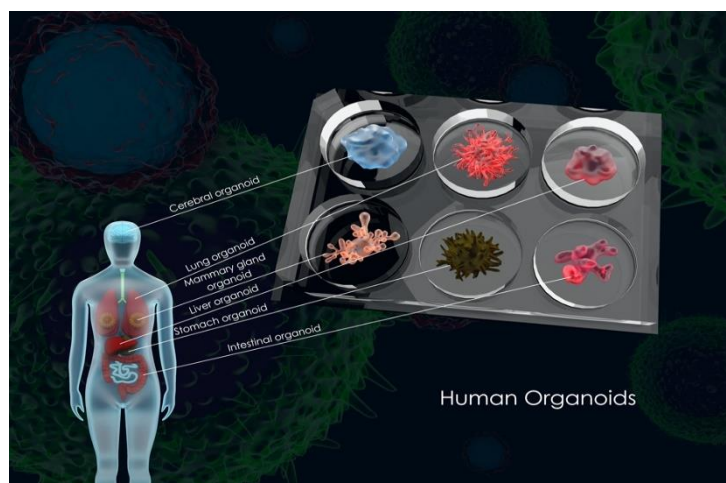
Progress in cell culture conditions and in OOAC techniques have resulted in, for example, a first-pass model of human absorption, metabolism and excretion of xenobiotics. This was achieved via the coupling of two-channel microfluidic gut, liver and kidney chips, which incorporate vascular endothelium in the lining of the channels to enable perfusion of the entire system with a blood substitute. These improvements also impart important physiological functions such as metabolic function, and a reservoir can be included to facilitate drug mixing and the measurement of blood and plasma concentrations of the drug being tested.¹ All of these refinements constitute a system that is highly reflective and predictive of human PK and PD, similar to observations from clinical trials. Indeed, it has been stated by international experts in the field that microphysiological systems (MPS) such as these are now “an enabling technology for the development of approaches to reliably predict the safety and efficacy of novel drug candidates prior to their use in humans.”²⁶

The use of OOAC in everyday laboratory settings at higher education, research and industrial establishments across the world has been facilitated by the efforts of various providers. Examples include [InSphero's Akura Flow](#) platform utilizing scaffold-free 3D-microtissue chips, [CN Bio Innovations' PhysioMimix](#) benchtop modular platform, the [Wyss Institute's Interrogator](#) system, and others. All incorporate physiologically relevant fluid flow and multi-organ capability, whereby different human OOACs can be connected together, with various

automated processes. These may include culture itself, and analysis and imaging of results—including quantitative prediction of human PK and PD data.

[Emulate's 'Zoë'](#) system, currently used by greater than 150 labs, enables the use of 'plug-in' organs chips for the human liver, kidney, intestine, lung, and soon to be brain. The user-programmable bench-top system can support 12 chips together at once, and provides conditions that assure maximum human relevance of experimental data. These conditions include simulation of blood flow, breathing, and digestive-tract movement. The 'Zoë' system has been particularly embraced in drug discovery and development, used to help predict effects of new drugs on the liver and kidney, and how those drugs are absorbed and metabolized. The duodenum intestine chip combined with intestinal organoids appears to model actual human intestinal function even more accurately than intestinal organoids alone, though the latter are still superior to using animals to model human responses to drugs. The poorer performance of animals is due to significant differences in the proteins and enzymes that transport and metabolize drugs in the gut,²⁷ and the poorer accuracy of the widely used 'gold standard' 2D-cultures of Caco-2 cells is due to the lack various features of more advanced 3D systems that provide more physiological fidelity. Moreover, the combined OOACs are derived from cells isolated from individual patients, and so they can be personalized to reflect person-to-person differences in drug responses, as well as factors such as age, sex, disease, diet and others that affect these differences.

The same company's (Emulate) [liver chip](#) is also demonstrating human accuracy. Tests showed it predicted human toxicities for eight known drugs that were not predicted by animal testing, as well as confirming mechanisms of toxicity, clinical biomarkers, and species differences between humans, dogs and rats.²⁸ Notably, the chips also revealed a lack of human toxicity for substances that had shown toxicity in animals. These results were achieved thanks to increasingly faithful 3D microenvironments in the organ cultures. These comprised multiple types of liver cells (primary hepatocytes, and sinusoidal endothelial, Kupffer, and stellate cells) with realistic architecture, tissue interfaces, perfusion, fluid flow, etc. Together, these aspects give OOACs a significant advantage in terms of physiological relevance and prediction of human response, even compared to other in vitro approaches such as 2D co-culture and 3D spheroids. These chips were maintained for two weeks or longer, and facilitated maintenance of more clinically relevant concentrations of test substances than



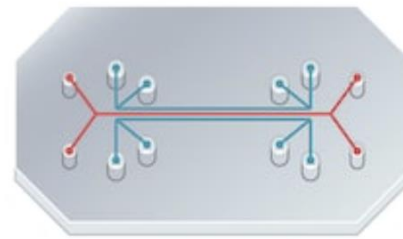
other methods. They also permitted the detection of diverse types of DILI via different and varied endpoints, including hepatocellular injury, steatosis, cholestasis, Kupffer-cell depletion, and stellate cell activation (a marker for fibrosis). Finally, in common with the intestinal (and other) chips, they are also amenable to the study of inter-personal variability and susceptibility.

Wyss's Interrogator "HuBoC" system involves automated culture, perfusion, liquid handling, sample collection and microscopic analysis of up to ten organ chips. These chips can be maintained as viable and functional for up to three weeks.²⁹ Such systems have recapitulated, for example, not only healthy human states, but also chronic obstructive pulmonary disease (COPD), exposure to tobacco smoke, asthma, lung infections, bacterial infections, and radiation exposure. [TissUse's HUMIMIC](#) system can perform automated culture and experiments with an unlimited number of modular OOACs. Most recently, this system has been involved in developing a bone marrow OOAC model. This model is helping to assess the safety of genome-edited hematopoietic stem cells, proposed as a therapy for blood diseases such as sickle-cell anemia, hemophilia and thalassemia.^{30, 31} A major issue with genome editing is that unexpected modifications can occur,³² causing the cells to be oncogenic. Normally, animal experiments are done for this type of safety assessment, but they suffer from reliability and reproducibility problems, and are often not predictive of human safety or risk.

This linking of OOACs has, among other things, been of importance in introducing hepatic metabolism into the system, much the same as in the examples above of the hanging drop/EST for developmental toxicity testing, and PK/PD modelling and testing for drug development. Often, it is not the test substance itself, but its metabolites/products of its biotransformation that may have therapeutic and/or adverse effects. For example, OOAC coculture of human testis and liver organoids has shown great promise for reproductive toxicity testing by the introduction of metabolic activity.³³ Other similar coculture systems, such as pancreas, skin, neurons, GI tract and airway, have shown stable development, physiological function and promise. It is now believed that MPS will undoubtedly greatly expand the ability to study human liver function, diseases, drug responses and toxicities. While many approaches have shown good results in the hepatic field, including complex 2D systems and static 3D cultures, flow-based mono- and co-culture systems provide increased physiological relevance.²⁵ The increased longevity of liver chips compared to culture of primary human hepatocytes is helpful in this regard. They have been used to investigate mitochondrial dysfunction, for example, and also hepatitis B infection and pathology (see e.g.^{34–36}).

Kidney and gut chips have presented problems due to the complexity of those organs. However, kidney chips have successfully modelled the functional unit of the glomerulus, and shown efficient cell differentiation and proximal tubule formation. Gut chips have been able to replicate important functional properties of the gut, as well as to model diseases and be used in drug toxicity tests.²³ Modelling of the female human reproductive system and menstrual cycle is possible, with interacting models of the ovary, fallopian tube, uterus, cervix and liver, which supports the generation of hormone profiles. A model of type 2 diabetes with linked human pancreatic islets and human liver spheroids is showing great promise, as is a model of colon tumor metastasis to the liver (see³⁷).

Some companies, instead of selling their platforms and technologies to users, offer their advanced OOAC/HuBoC technologies as a service. [TARA Biosystems](#) offers a service-based cardiac platform, that can assess drug safety and efficacy, and analyze cardiac function in healthy and diseased states. [Hesperos](#), for example, accept that, for some applications, the required technology may be too complex to transfer to research labs with untrained users. That said, it also seems clear that—while complexity is sometimes necessary to answer some researchers’ questions—sometimes it is not necessary. Simpler culture methods and models can, and do, suffice. Don Ingber, of the Wyss Institute, highlighted exactly this in a news article on the subject by using the lung, and lung diseases, as examples. Different conditions like flu, pulmonary edema or fibrosis need different, simpler solutions, such as airway or alveolus chips, with or without fibroblasts, for instance, instead of one complex “one size fits all” lung culture.³⁸



Organ on a chip

OOAC approaches have impacted biomedical research in many areas. In lung research, while simpler approaches can be apposite, OOACs are considered essential for many applications due to documented limitations of animal models of human lung diseases. Interspecies differences exist in human and murine airway anatomy on both a macro and micro level, and in biochemical pathways, development, pathology, physiological and metabolic rates. All of these differences lead to poor translation of data (see³⁹). Human primary cells, tissue, immortalized cells and stem cells are all used in lung research, and are valued, having informed many areas of investigation. Organoids, for example, have informed research in lung morphogenesis and tumorigenesis, lung regeneration, and various infectious agents of different kinds and their associated diseases. Organoids have also been used to screen therapeutics, including personalized ones. Lung-on-a-chip approaches improve on organoids further, by better mimicking physiological flow, stretch, stimuli, the air-liquid interface, and other factors. They have been used in many areas of research, for instance to model lung inflammatory diseases and discover new therapies for them, and to recreate asthmatic conditions. They have also been used to investigate the role of small-airway pathogens on COPD and asthma, and smoke pathophysiology, pulmonary edema, cystic fibrosis, and lung cancer.³⁹ Lung airway chips have recently identified two compounds that inhibit lung airway infection by SARS-CoV-2 pseudoparticles, at concentrations similar to those in clinical studies.⁴⁰

Like lung research, heart research also has issues regarding the translation of animal data to humans, and increasingly involves informative 2D and 3D cultures of human cells. 2D cardiomyocyte cultures have been productive for our knowledge of ischemic hypoxia, and in drug discovery and testing, which is important due to the major role cardiotoxicity plays in the failure of new drugs (see¹¹). While there appears to be a role for 2D cultures for the detection of secondary effects in humans, it seems clear that 3D cultures, due to their greater physiological relevance, perform better as predictors of long-term and chronic adverse effects. OOACs go one step further, recreating shear stresses and mechanical stimulation (for

example, from the heart beating) that are essential for a faithful phenotype of cardiac endothelial cells and myocytes.¹¹

Brain and spinal cord OOACs are also gaining a huge amount of attention. Like the other OOACs, they are proving capable of modelling many human neurological disorders, including some that are not achievable by other means, including with animals. The significant shortcomings of animal models in the field are widely appreciated. One expert opined that animal models in neuroscience are expensive, low throughput, and most importantly “offer limited insight into human physiology” due to a species gap that is a major underlying cause of translational failure in the field.¹³ This opinion reflects a growing appreciation that a shift toward the much greater and widespread use of human-specific methods may be not just desirable, but also essential.

Neural MPSs—like other OOACs—can be patient-derived, which brings intrahuman variability into consideration as well as overcoming interspecies translatability issues. Neural MPSs sidestep limiting factors such as biological sampling from animals and humans (due to their invasiveness, need for sacrificial samples, and so on), thus enabling greater knowledge of the dynamics of high-order neurological processes. They also provide a means of overcoming translational barriers in neurodegenerative disease research such as drug pharmacokinetics and the blood-brain barrier.

Microfluidic models, compartmentalized neuronal models, and hydrogel-based models (effectively, artificial extracellular matrices) are informing many areas of investigation. These include basic neuroscience, blood-brain barrier function, brain injury, and neurodegenerative diseases like Alzheimer’s and Parkinson’s. Additionally, these models enable the screening and testing of new drugs against brain tumors, especially in association with liver cultures for metabolic activity. Neural OOACs are also facilitating analysis and modulation of neuronal electrical activity, even down to single-cell resolution—something often (and incorrectly) used as a justification for invasive experiments on nonhuman primates.¹² Experts believe that the integration of microfluidic technology with 3D bioprinting and iPSCs will lead to unprecedented, detailed and faithful modelling of the central nervous system (CNS) microenvironment for use in neurodegenerative disease research.¹³ iPSCs, organoids and MPSs, together, are reported as generating breakthroughs in knowledge in many other neurological diseases and disorders, including autism, Down’s syndrome, schizophrenia, bipolar disease, and neural injury.

In Silico/Computer-Based Methods

The above advanced culture methods are often used with, and augmented by, a variety of in silico methods. One topical example of their power has been in the response to the SARS-CoV-2/COVID-19 pandemic, as the world tries to find effective preventive and therapeutic measures for it in advance of any successful vaccine development. [The Wyss Institute](#) (MA, USA), supported by The U.S. Defense Advanced Research Projects Agency (DARPA), has been charged with building on its existing computational and OOAC drug discovery/development programs to help repurpose existing drugs, and investigate human responses to the virus.⁴¹ A machine-learning algorithm known as DRUID (DRUG Indication Discoverer) is being used to

search gene expression data associated with tens of thousands of drug compounds, and detect drugs that may be able to revert a particular gene expression pattern back to normal based on gene expression data from SARS-CoV-2-infected human lung cells.⁴¹ Computers also simulate the spike protein that the virus uses to infect target cells, and virtually develop new molecules that may interfere with its function, potentially blocking infection. The result of this “molecular dynamics repurposing” is synthesized molecules that are tested in cultures of infected cells, some of which have already appeared to inhibit the infectious process. Existing drugs with similar structures are also being identified and tested, in the hope that they can be repurposed for COVID-19 treatment. Finally, computer-based comparative analysis of gene networks in healthy individuals and COVID-19 patients ([NemoCAD](#) – “Network model for causality-aware discovery”) is attempting to link differences in gene networks to drugs that may have the capacity to revert those in COVID-19 patients to those in healthy people.

Outlook and Future Developments

The wholesale uptake and implementation of various new and developing human-specific technologies such as MPS is undoubtedly a major challenge. This is in spite of the many advantages these methods offer compared to standard tissue culture methods and the use of animals, and the fact that they have been extensively developed for two decades and have, on many levels, met the needs of industry. It is now very widely accepted that MPSs, for instance, offer numerous advantages. These include more predictive and reproducible toxicity and efficacy testing, early exclusion of drug candidates in the drug development pipeline, the ability to perform testing on relevant human disease models, and a significant reduction of animal studies. All of this is with the concomitant reduction of development costs and time, and with the ability to obtain directly relevant human-specific results.⁴² Despite the advantages offered by human-specific techniques, there remain significant barriers to their adoption, which are discussed below.

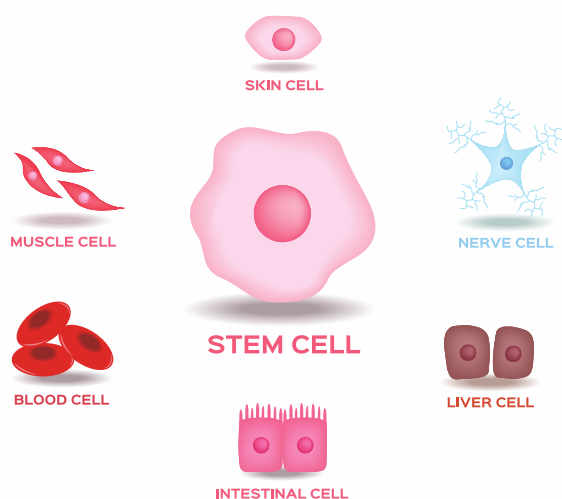
There is also considerable appreciation of the potential of the technologies discussed in this article to create a paradigm shift in biomedical research, as well as the urgency for it. It has been suggested, for example, that MPS technology could allow the generation of human clinical trial phase 2 data, and that establishing an MPS-based “universal physiological template” could replace the use of animals in laboratories in basic research and facilitate the development of personalized therapies.⁴³ The number of scientific publications on organoid cultures has increased exponentially in the past two decades (since 2000), and currently stands at greater than 25,000, as of September 2020 (Google Scholar). In the U.S., DARPA has been supportive of MPSs for several years, both ideologically, and financially. This support has been augmented by that of the National Institutes of Health (NIH), the National Center for Advancing Translational Sciences (NCATS) and the Food and Drug Administration (FDA). More than ten new human OOACs and 500 scientific publications resulted from this support and these collaborations. The MPS affiliate of the [IQ Consortium](#) (The International Consortium for Innovation and Quality in Pharmaceutical Development—a not-for-profit group of greater than 20 pharmaceutical and biotech companies) was established specifically to facilitate the development and implementation of MPS technology. The NIH and NCATS have shown commitment to developing MPS as disease models and in drug development and testing. The Environmental Protection Agency (EPA) is seeking, partly through MPS

technologies, to [eliminate](#) all of its requests for, and funding of, mammal studies by 2035. The FDA has established an agreement with several MPS suppliers to use their technologies in-house, and its Center for Toxicology Research is working with the German MPS company TissUse.⁴⁴

The t⁴ transatlantic think tank for toxicology published, earlier in 2020, the proceedings of a workshop on MPSs, and discussed many advances in the development and implementation of the technology.⁴⁴ These included 3D blood vessels; models for thrombosis, angiogenesis, metastasis, and inflammatory response; and various new co-culture models being used in PK studies and toxicity testing. The think tank also reported areas in which MPS data are being used by the pharmaceutical industry for internal decision making. These areas include MPS models of vasculature, bone marrow, gut, lung, liver, eye, kidney, pancreas, thyroid and skin. These models are being utilized in one or more of target identification and validation, discovery, compound selection, PK, pharmacology, safety testing, efficacy, and toxicology. Participants of the t⁴ workshop also highlighted a major and crucial benefit of MPS platforms over many other methods, including animal models, which is individual variation. One of the most attractive opportunities with the use of stem cells, 3D cultures and OOACs is their capacity to reflect this individual variation. Cultures and chips can be generated from cells from individual people, allowing intrahuman variation to be incorporated into general research programs in many fields.

MPS platforms facilitate personalized medicine, whereby specific molecular characteristics of an individual's biology or disease can be investigated, and treatments prioritized and tailored to them. The need to model human variability has been an issue for some time: scientists who use animal models are modelling "humans"—but which humans? Human genetic, biochemical and physiological variability is considerable: often, what is discovered in one

individual cannot be reliably translated to another. Intrahuman differences may be illustrated, for example, by the variability in efficacy and adverse effects of drugs in people. The scientific literature increasingly reflects the view that this is a crucial consideration, and it is one that only human-focused research can address (see, for example,^{45–47} and [Terasaki Institute](#)).



3D bioprinting is showing promise to adding structural complexity to OOAC/HuBoC approaches, including increases in scale and functionality. There is great excitement about the use of patient-derived stem cells, which provide a means to examine the

molecular basis of diseases in great detail, incorporating the inherent variations that are present among humans. Initial obstacles such as cost and difficulties in producing stem cells in significant numbers have been, and are being, overcome. It is now quite straightforward to take a biopsy of skin or a blood sample, and reprogram cells (via various methods, for example using a drug or chemical) to revert from a specialized, terminally differentiated cell to a

pluripotent stem cell. These iPSCs can then be directly differentiated into different types of cells, representative of the individual (healthy, or a patient with a specific disorder) from whom they came. iPSCs are used to research biological processes, diseases, and potential therapies in so-called “in vitro clinical trials”. Repositories have been set up to facilitate their acquisition by researchers.

Summary and Conclusion

There is a great deal of optimism and satisfaction in what has been achieved in this field of science. However, there is also significant and growing frustration among many and varied stakeholders, concerning the slow pace of adoption of MPS and associated human culture and modelling systems. The t⁴ think tank cited some potential reasons for this slow pace of change, and potential hurdles that need to be overcome to expedite it.⁴⁴ These obstacles include a reluctance by drug developers to invest in methods of generating data that are not absolutely required by regulators. Where there may be some incentive to develop and use human-specific methods, their use, and the submission of related data to regulators should be encouraged, as they could provide confidence in those approaches to enable more widespread use. Suggested scenarios include when MPS data may be able to rescue a drug whose development is at risk, when known models are irrelevant for a new drug, or for exploring new targets. The think tank also identified an urgent need for greater communication between stakeholders, with earlier engagement, clearer criteria of success, and greater harmonization, including globally.

Each new drug going through development typically takes almost 15 years and costs around \$2.5 billion dollars,⁴⁸ and so the current failure rate has a very high human, animal, societal and financial cost. It would serve all stakeholders for the drug development process to improve. Widespread use of, and greater reliance on, truly predictive research methods would make biomedical research—and therefore our understanding of human biology, diseases and responses to drugs and potentially toxic chemicals—cheaper and quicker. In turn, this would greatly facilitate and expedite the development of more and safer new drugs to treat human diseases.

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Notes

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