# Multi-Organ Microphysiological Systems are Poised for Expansive Integration

TISSUSE
Emulating Human Biology

**Drug development, disease modeling, and toxicological analysis are formidable ventures, because the human body with its multiple organ systems is a complicated, interconnected, interdependent, and idiosyncratic environment that is difficult to replicate with animal or *in vitro* models. Newer technologies culminating in the development of microfluidic microphysiological systems (MPSs) comprising connected organs-on-a-chip represent a great opportunity for streamlining these processes and more effectively recapitulating human physiology. Obstacles exist, but the technology is ready for increased and more widespread use.**

## Background

Pharmaceutical companies, academic labs, and researchers from various backgrounds want to understand how medicines, dietary exposures, cosmetics, and other chemicals behave and interact in a true physiological environment to influence human health. Traditionally, researchers have been able to grow and study authentic human cells in culture dishes, first in flat monolayers and later with more sophisticated 3D architectures, but the ability to replicate higher-order system physiology remains inaccessible. For instance, these static arrangements allow no blood flow with its associated nutrient exchange, have limited metabolic processing compared to that performed in the liver, do not excrete waste through kidneys, and have no immune system with which to interact. Alternatively, researchers can perform experiments in the true physiological environment of an animal model, but results from these studies often translate poorly to humans due to various species-specific differences. As testament to this disconnect, only 1 in 10 drug candidates that enter human clinical trials, having passed the hurdles of *in vitro* and animal experiments, proceeds to achieve FDA approval.[1]

In an effort to more closely model human physiology in a miniaturized *in vitro* environment, scientists have worked tirelessly to develop specific Organ-on-a-Chip systems using combined methods and knowledge gleaned from tissue engineering, microfabrication, and microfluidics. These Organ-on-a-Chip systems are typically the size of a microscope slide but contain sufficient architecture and appropriate cell types to reproduce the crucial activities of a full-sized organ with a simple “circulatory system” mimicking nutrient exchange. Since the first publication of a successful lung model in 2010[2], improved organ models with more representative physiology have been expanded to include blood vessels and vasculature, bone marrow, intestine, liver, kidney, heart, pancreas, skin, thyroid, the blood-brain barrier, and others. Most of these models consist of a single Organ-on-a-Chip, but physiological relevance increases as additional organ systems are successfully integrated. The ultimate goal is to model a complete human using the minimal necessary set of interconnected organ systems, forming what is now referred to as a universal physiological template (UPT).[3]

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## Challenges

Although relevant organ-specific physiology can be attained with the first relatively simple single-organ chips, a lack of connection to and crosstalk with other organ systems severely limits the scope of research and the applicability of findings. To recapitulate true biology, the organ systems must be linked, and while the concepts of UPT and even specific patient-on-a-chip models have existed for decades, their implementation has been incredibly challenging. For each organ type, the appropriate cell types must be identified, and a substantial and reproducible supply of these cells must exist. Additionally, organs do not consist of single cell types; the human lung, for instance, is composed of approximately 40 different cell types when considering vasculature and immune cells.[4] Researchers must weigh the added value of mixing these cell types using appropriate timing and growth factors or growing organoids or tissue-engineered constructs against the added associated difficulties. Models also increasingly use induced pluripotent stem cells (iPSCs), which can be directed to differentiate into specific organ types with various cell types while enabling the collection of patient-specific experimental information. MPS designs typically include micropumps or gravity-assisted flow to distribute a rich growth media representing blood, and thus a proper medium must be identified that will support all tissue types on the chip, complicated of course by each new addition. Mechanical stresses such as those induced from shear stress associated with flow help to reconstitute a more realistic physiological environment, but the control of fluid flow dynamics and tissue:fluid and tissue:tissue ratios must be carefully considered and controlled.

## Resistance to greater use

In addition to technical challenges, there is some hesitation to adopt these newer technologies. Researchers generally assume that as physiological relevance increases, handling, processing, and data interpretation will become more difficult. They think that individual scientists will be unable to effectively perform experiments, that teams of experts will be required, and that throughput will suffer. Perhaps the greatest impediment to MPS adoption is the need for end users to know that results obtained with MPSs will be translatable to results in actual humans. In the same vein, there are concerns regarding regulatory approval and industry standards. Newer technologies and the assay results obtained with them are not immediately acceptable by FDA, EMA, and other regulatory bodies. Finally, cost is a consideration. Will labs need to make large investments into new equipment?

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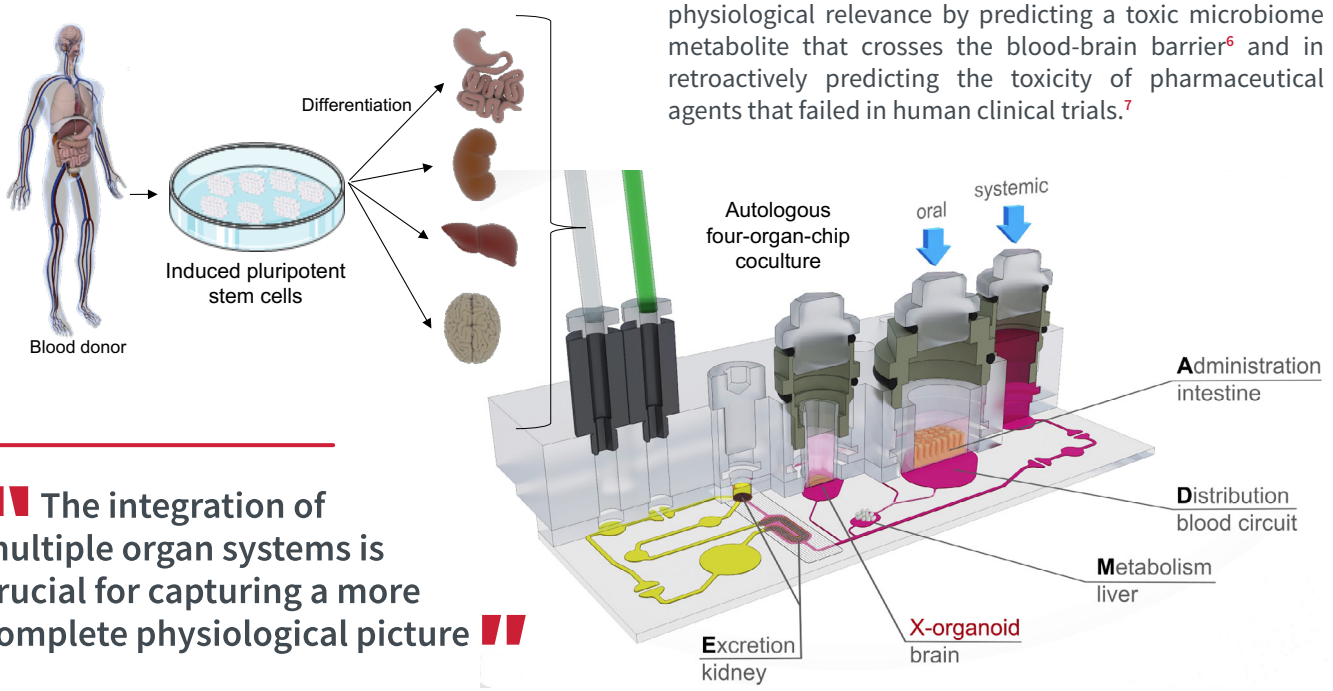
## Modern MPSs replicate human physiology to a high degree

The integration of multiple organ systems is crucial for capturing a more complete physiological picture, and several literature examples exist in which Multi-Organ-Chip (MOC) systems show realistic biology and responses to chemical agents. MOCs containing liver compartments in addition to other relevant target organ compartments can be especially useful in identifying both primary and secondary compound toxicity. Microfluidic systems enable nutrient exchange, the formation of biochemical gradients, and the establishment of pressure and shear stress. Pharmaceutical companies are increasingly adopting these technologies at several stages of their pipelines, including target identification, discovery, PK/PD, preclinical safety, and drug efficacy.[5] Additionally, MPSs, both in individual organ formats and multi-organ setups, have demonstrated physiological relevance by predicting a toxic microbiome metabolite that crosses the blood-brain barrier[6] and in retroactively predicting the toxicity of pharmaceutical agents that failed in human clinical trials.[7]

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## The regulatory players get on board

While regulatory concerns are well founded, thankfully agencies are eager to accept trustworthy data that accelerate the pace of discovery and reliably demonstrate safety and efficacy.[8] In 2017, the FDA published a 6-step predictive toxicology roadmap in which they indicated their desire to "foster the development and evaluation of emerging toxicological methods and new technologies", specifically including MPSs, and they seek to incorporate these methods and technologies into regulatory review. The FDA is also engaged with DARPA and NCATS programs and is collaborating with MPS suppliers to evaluate assays and methods. Other regulatory agencies are engaging in similar collaborations. In every case, the promise of adhering to the "3R" guidelines of animal research—replacement, reduction, and refinement—is a strong incentive for increased MPS use. Accordingly, the EPA has established a directive to reduce testing on mammals by 30% by 2025 and to eliminate it completely by 2035.[9]

## Unique strengths of TissUse

TissUse has developed successful proprietary MOCs using a platform that integrates up to four interchangeable organ systems, each scaled down by a factor of 1:100,000, with an on-chip micro-pump mimicking the heart. Chips with up to ten organs have already been designed, and a unique UPT will soon enable whole human and even specifically derived male and female Patient-on-Chip designs. A recent report using TissUse chips shows the promise of using cells from an individual donor to establish iPSCs that supply a four-organ intestine-liver-neuronal-kidney chip, which enables realistic ADME profiling and toxicity testing of substances.[10] The TissUse platform enables great flexibility in choice of organ types and input materials, including healthy or diseased tissues, primary or stem cells, commercial cell lines, and biopsies, depending only on the user's intended purpose. TissUse takes a customer-oriented flexible approach regarding how chips are used; assays can be performed in-house using established methods and chip arrangements, partnerships can be forged to establish new models, or chips can be taken to a user's laboratory and integrated into the same protocols they are accustomed to. Thus, a large investment in equipment is unnecessary, and MOC-based assays do not require teams of experts. Automation greatly reduces concerns regarding increased handling complexity; the HUMIMIC Autolab can process 24 chips per robot, and connected in groups of four, the throughput rivals that of pre-clinical animal experiments. TissUse is also well poised with regard to regulatory approval, and they are working directly with the FDA on bringing MPSs into regulatory acceptance. The National Institutes for Food and Drug Control of China has also recently evaluated TissUse chips in a liver-kidney proximal tubule model with repeated dose multi-drug toxicity testing.[11] The MPS design allows the interrogation of several parameters. During an assay, microscopy can be used to assess cell morphology or track tagged molecules, and media can be sampled for biomarker discovery or metabolite measurement. Typically at the end of an experiment, tissue samples can be subjected to histology, gene and protein expression can be assayed, or FACS can be performed to identify specific cell populations. These and many additional experimental assay end-points are accessible.

## TissUse chips in action

The physiological relevance of TissUse chips is demonstrated by a recent collaborative effort with AstraZeneca, in which researchers connected pancreatic islet microtissues to liver spheroids to demonstrate organ cross-talk that would not be possible in standard *in vitro* experiments.[12] When a glucose tolerance test was applied, islet cells secreted insulin, which increased glucose uptake by liver spheroids and thus reduced the glucose concentration in media. Then, as the glucose concentration decreased, the islet cells ceased insulin secretion, demonstrating a functional feedback loop mirroring human physiology. The experiment was reproducible in both independent laboratories, and cultures were maintained for 15 days with multiple glucose loading times. This experimental design has enabled further ongoing collaborative work toward type 2 diabetes models, including evolution to a heart-liver-pancreas model.

**"As a senior principal scientist at AstraZeneca, Gothenburg, I led a collaboration with TissUse from 2016-2020 to develop a physiological liver-pancreas microphysiological system (MPS) for metabolic research. The TissUse platform was chosen after a thorough evaluation as the most physiologically relevant MPS available for this purpose. The possibility to combine organs in a physiological system reflecting blood circulation while using organoids at relative proportions congruent with those found *in vivo* was especially crucial."**

– Tommy B. Andersson, PhD

## Conclusion

MPSs have made great strides in the last decade, and their relevance is evident from their increased adoption by pharmaceutical companies and interest from regulatory agencies. They hold great promise for expediting the drug discovery and validation process, for evaluating the safety and systemic effects of various chemicals and nutritional components, and for leading to improved human health while minimizing animal use and suffering. It is envisioned that their use will ultimately reduce the cost and time required for pharmaceutical development because of increased precision in target selection and earlier identification of toxicity.[13] TissUse uses a flexible, versatile, and customer-oriented approach to model design, and is the first dedicated Organ-on-a-Chip company to be certified under ISO EN 9001-2015, reflecting a dedication to quality, customer satisfaction, and ongoing improvement. TissUse is well equipped to incorporate several organ types to increase the relevance of experiments that require higher-order physiology and to ultimately establish UPTs.

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