

## PERSPECTIVE

# Multiorgan microphysiological systems as tools to interrogate interorgan crosstalk and complex diseases

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**Metabolic and inflammatory disorders such as autoimmune and neurodegenerative diseases are increasing at alarming rates. Many of these are not tissue-specific occurrences but complex, systemic pathologies of unknown origin for which no cure exists. Such complexity obscures causal relationships among factors regulating disease progression. Emerging technologies mimicking human physiology, such as microphysiological systems (MPSs), offer new possibilities to provide clarity in systemic metabolic and inflammatory diseases. Controlled interaction of multiple MPSs and the scalability of biological complexity in MPSs, supported by continuous multiomic monitoring, might hold the key to identifying novel relationships between interorgan crosstalk, metabolism, and immunity. In this perspective, I aim to discuss the current state of modeling multiorgan physiology and evaluate current opportunities and challenges.**

**Keywords:** complex diseases; human physiology; *in vitro* model; interorgan communication; microphysiological systems; multiorgan crosstalk; organ-on-a-chip; systems biology; tissue chip; tissue engineering

Some of the most significant biomedical urgencies of our time come in the form of metabolic, autoimmune, and neurodegenerative disorders. While these broad categories of complex diseases encompass a wide range of pathologies, many of them are influenced by a combination of genetic traits, environmental factors, and lifestyle choices. Even though their clinical manifestation can often be associated predominantly with one particular region of the body, they involve the interaction of an intricate network of different cell types, tissues, organ systems, and a disruption thereof [1]. These interactions are mediated in a multimodal fashion *via* cell-mediated and cell-extrinsic signaling to maintain systemic homeostasis and to address harmful processes. It is thus not surprising that organ systems

that are deeply connected in maintaining organismal homeostasis share the burden of systemic illness.

An illustrious example can be found in the gut–liver–brain axis, where the three-organ systems and the environment, in the form of the microbiome, are intimately interconnected [2]. This connection is established *via* the enteric nervous system, lymphoid system, systemic circulation of nutrients, metabolites, and humoral factors, and migration of a wide variety of cells [3]. Increasing evidence suggests a connection between the three-organ systems and several diseases even though, in the clinic, these are often considered unrelated and organ-specific. For example, patients who suffer from inflammatory bowel disease (IBD) are more likely to develop certain inflammatory diseases

## Abbreviations

IBD, inflammatory bowel disease; iPSC, induced pluripotent stem cell; MOMPS, multiorgan microphysiological system; MPS, microphysiological system.

of the liver and *vice versa* [4,5]. Moreover, animal models of metabolic-associated fatty liver disease show an increased risk of neuroinflammatory degeneration [6], while patients with dementia, Alzheimer's, and Parkinson's disease often first report gastrointestinal issues before any neurological symptoms have been identified [7-9]. While much progress has been made in the phenotypic description of such complex and overlapping diseases, their fundamental origin and cure remain a mystery.

Several reasons can be listed as to why a cure to these diseases remains elusive, such as incomplete understanding of the basic biology of interorgan cross-talk or lack of models relevant to human physiology. However, a particular contributing factor often remains unacknowledged—the basic challenge to derive causality in complex systems. Both the overbearing complexity and the many compensatory physiological mechanisms represent a significant hurdle in our understanding of clear causal relationships. As much as we have learned from genetically manipulated animals, we often find that the lack of a missing cell type or gene is compensated for by other cells or mechanisms, thus masking their true function and leading to misinterpretation.

The most widely used models in preclinical medical research can broadly be categorized as top-down or bottom-up approaches. A knockout mouse model can be considered a top-down approach, where an element from an otherwise complex system is removed, such as a particular cell population, in order to derive its biological role or contribution to a particular disease. On the other hand, cell cultures and cell-based models can be viewed as a bottom-up approach where from a position of relative simplicity, casual relationships can be inferred from the addition of new components.

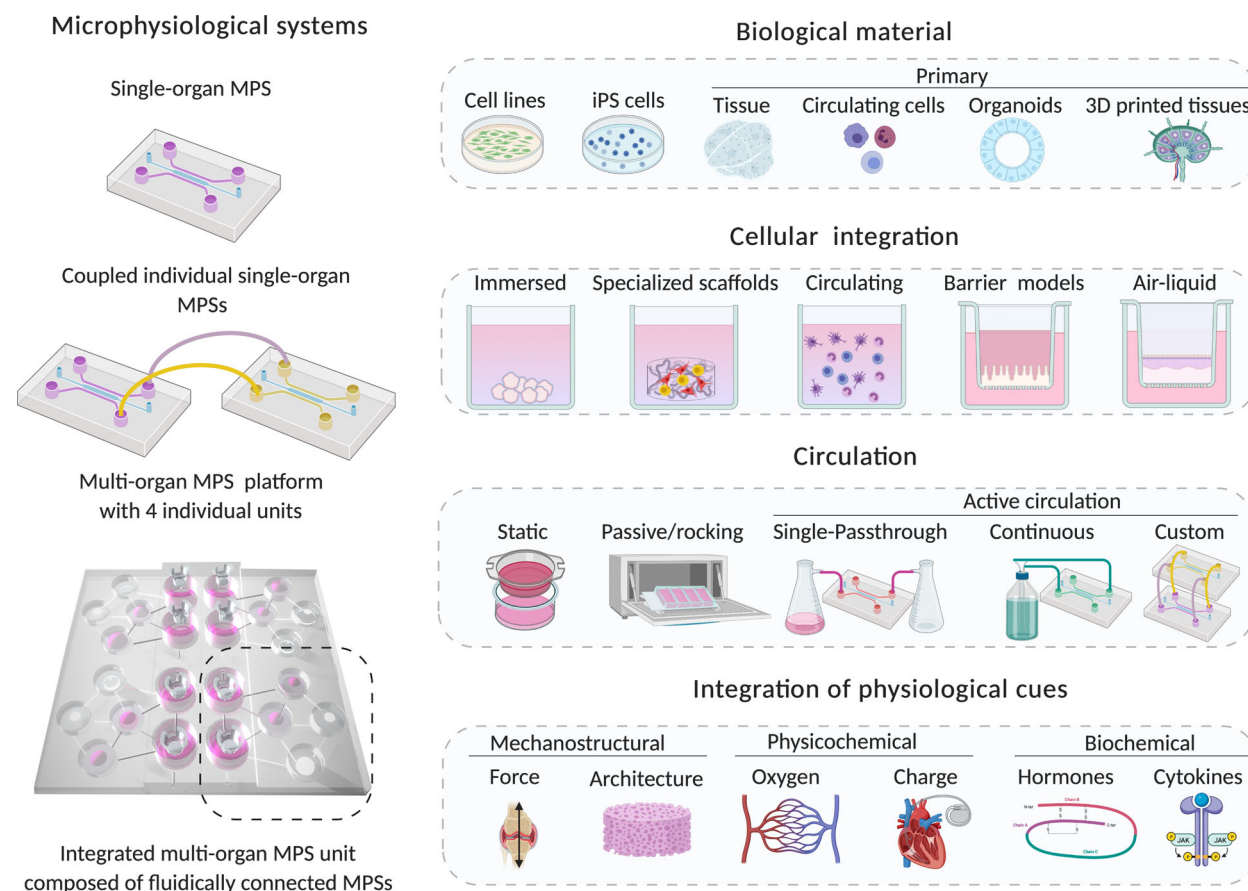
In both cases, the argument can be made that altering the natural state of the object in question, either in the form of a mouse that lacks a certain cell type or a cell culture that lacks all the otherwise present cues it receives from its neighboring tissues, leads to an unrepresentative system to begin with.

There is a limit to how many parameters can be excluded in a top-down approach (e.g., a mouse cannot function without a brain) and how many elements can currently be added to a bottom-up approach (e.g., we cannot upgrade a cell culture into a fully functional human). Hence, a need exists for models that support a greater degree of scalability regarding biological complexity. A scalable model system could bridge the knowledge gained from existing approaches and allow us to probe mechanistic relationships among its components.

One such technology is multiorgan microphysiological systems (MOMPS). Microphysiological systems are *in vitro* models that aim to replicate specific biological features of a tissue or organ of interest in a reductionist manner. These are often constructed on specialized substrates, in optimized media and under perfusion, that provide a more native environment for each tissue type [10]. Several detailed reviews have been published highlighting the technical and engineering advancements of MPSs, as well as the current state of the field and the various applications of MPS technology [11-19]. Thus, this current perspective aims to contextualize MOMPS through the narrow prism of investigating interorgan crosstalk within complex pathologies such as liver, cardiac, renal diseases, and metabolic disorders such as diabetes, metabolic syndrome, and others [20-22], while providing examples of interesting use cases that highlight their utility.

## Multiorgan microphysiological systems

In the past decade, a tremendous leap forward has been achieved in creating tools that better mimic human biology. This is possible due to a shared interest in these technologies from the academic, industrial, and regulatory side, where close collaboration between these stakeholders is fueling continued technological improvements as well as commercial growth [13]. While initially, a significant focus has been in developing MPSs for safety, efficacy, and toxicity studies, over the years, they also became important tools for fundamental biomedical research and personalized medicine [12]. Individual MPSs can be fluidically coupled to form a connected system of multiple linked devices. These can also be part of a standalone multiorgan MPS platform designed specifically for multiorgan interaction studies. Even though this technology can be discussed in broad terms, most devices are developed within a particular context use case in mind and specific for a particular task in a fit-for-purpose fashion (Fig. 1). Thus, the scientific question and the biology to be mimicked will guide the complexity and number of MPSs in a multiorgan setup and the overall design of the approach. For example, MOMPS can be constructed to support cell migration between compartments, support the integration of specialized scaffolds to provide an extracellular space reminiscent of the native tissue environment, or include physical cues such as electrical stimulation and increased fluid shear. MOMPS come in different sizes, materials, and shapes, where often one attribute comes at the expense of another one. For example, larger devices with greater



**Fig. 1.** Microphysiological systems and their integral components. MPSs come in a variety of setups such as individual, single tissue devices, or multiple connected MPSs and integrated multiorgan platforms. Depending on the device design, a number of cell and tissue types can be integrated. Individual compartments can be adjusted to increase resemblance to the *in vivo* environment with the use of biomaterials and membranes. Cell culture media in these devices can either be static, moved passively or actively. Active circulation can be directed as a continuous and even flow or customized to different distributions across individual compartments and adjusted media flow rates. Moreover, physiological relevance can be increased with the integration of physiological cues such as shear stress, pH, and signaling factors.

volumes and cell numbers tend to give less access to traditional imaging technologies. In comparison, smaller microfluidic devices often do not support an open-well design with easy access to embedded tissue. Open-well devices facilitate easy tissue and media retrieval, whereas sealed devices enable more complex flow patterns with higher fluidic and environmental control [23].

In theory, any cell or tissue that can be cultured *ex vivo* can also be integrated on a MOMPS. However, this requires to consider their physiological ratios and proportional scaling in terms of cell mass, cell-to-media volumes, and physiological media distribution [10,19,24].

Common or shared media, which is circulated between the different MPSs, can be distributed in

multiple arrangements as a single pass-through, continuous circulation with scheduled media changes at different ratios and intervals, or remain static altogether. A significant effort in the field has been directed toward the development of a universal medium, which could be suitable to support a majority of the different cell types included in a MOMPS. Progress has been made, but the specific needs of individual cell types make it difficult to devise a universal media formulation. Breakthroughs in developmental and cell biology allow us to replace cancer-derived cell lines, as models of healthy human biology, with more representative tissues such as primary isolated cells which can be maintained for longer periods than previously possible. This is exemplified by organoids and cells grown in specialized environments [25] or induced pluripotent

stem (iPS) cells that can be utilized as originators of many different cell types derived from the same donor [26].

However, while commonly used cell lines were easily cultured in relatively simple cell culture media, newer approaches require more specialized, tissue-specific factors to be included. This can become problematic in cases where factors needed for one cell type antagonize the function of a different cell population. A great example are factors commonly used to maintain a healthy stem cell pool of intestinal epithelial cells, namely Wnt, R-spondin, and Noggin [27]. In their basic function, they alter TGF-beta and NOTCH signaling, which can antagonize the behavior of immune effector as well as tolerance-inducing cells, such as cytotoxic CD8<sup>+</sup> T, CD4<sup>+</sup> T helper, and regulatory cells [28]. If constructing a MOMPS whose purpose is to model immune responses at mucosal barriers, the presence or absence of these factors in the universal media would have to be considered. As per the example above, Wnt and R-spondin signaling is concentrated at the very base of intestinal crypts, where the interaction between the epithelial stem cell niche interacts with other cells in the vicinity, regulating intestinal renewal [29]. It is thus of great importance to develop MOMPS, whose individual compartments can cater to the specific needs of an individual cell population yet in a controlled and localized fashion such as with the use of scaffolds that allow for the controlled release of required factors in terms of both timing and concentration. In a full human body, not all possible humoral factors are being always circulated in all locations but rather distributed across small areas and niches in a time-dependent manner. Ultimately, such a timed, localized distribution under various spatio-temporal gradients is critical for the development of higher organisms. As advancements are made in the field of modeling multiorgan crosstalk, we should also consider natural circadian rhythms of endocrine signaling, such as the automated and timed release of signaling molecules of interest [30].

### The cellular secretome in interorgan crosstalk

One of the first accounts of interorgan communication is that of Galen of Pergamon (129–216) who investigated the role of the pharyngeal nerves [31]. Ever since, interorgan communication in homeostasis and the role of its disturbance have been of great interest to humankind, from the gut–brain axis [32] to the communication between adipose tissue, the pancreas, and liver, to name a few [33]. A disruption in the crosstalk and homeostasis

between individual organ systems has been clearly associated with the development of metabolic, neurodegenerative, cardiometabolic, and immunometabolic diseases [20,34,35,36,37].

Since the early discovery of hormones and their role in the maintenance of our energy balance, a wide variety of extracellular messengers have been identified that can serve as intra- and interorgan communicators. In addition to hormones, hundreds of other molecules have been identified that serve as modulators in cross-organ communication, including metabolites, nutrients, cytokines, chemokines, and, most recently, extracellular vesicles [38,39]. The release of apoptotic bodies during apoptosis has been long known but the fact that healthy cells shed vesicles with important signaling molecules like small noncoding RNA, peptides, and metabolites, has only become recently appreciated [39,40]. While our understanding of extracellular vesicles has improved, we still do not know whether they are causal players or bystanders [41].

Incomplete understanding of the biology of interorgan crosstalk and difficulty in deriving causation among factors responsible for homeostasis and progression of complex diseases forms the basic need for tools that could help us better understand their intricate relationships [42] (Fig. 2).

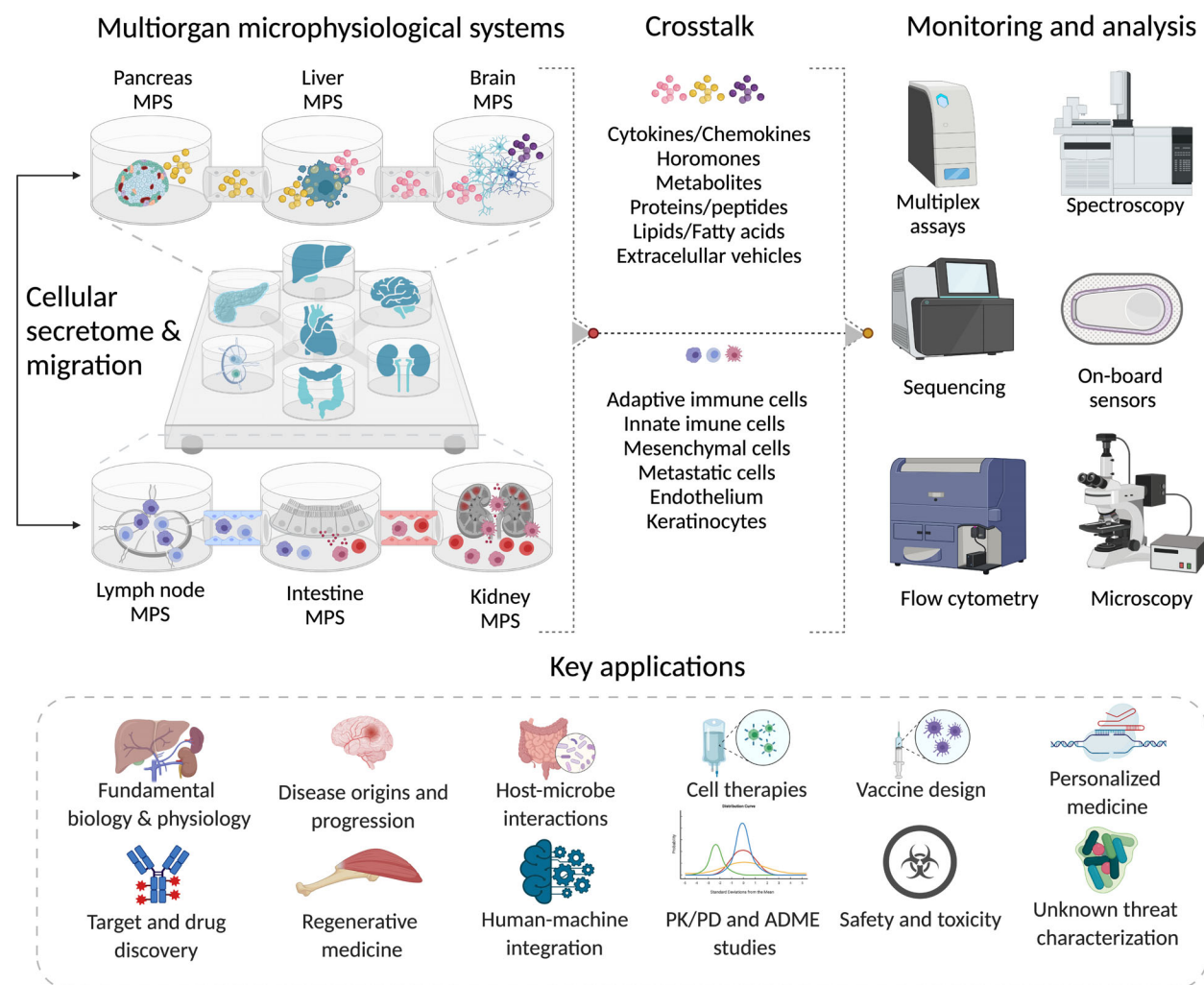
### Two-way tissue interactions

Multiorgan microphysiological systems allow us to connect a multitude of individual MPSs and thus to derive unique means of interaction and their effect on tissue function. As this is a new field, many insights into the unique behaviors arising of interorgan crosstalk are yet to be established for tissues taken out of the complex human environment. In this regard, two-way interaction studies, as opposed to those involving three, and more organ systems, are leading the way.

Interorgan communication has historically been centered around metabolic health, with a focus on responses to fasting, circadian rhythms, temperature control, and response to physical activity [33].

The liver serves as the central organ system maintaining metabolic and immune homeostasis while processing endogenous as well as exogenous material. With great interest in its role in drug toxicity and metabolism as well as a great number of diseases, it is a commonly incorporated MPS in the context of multiorgan interaction studies. A significant number of devices and approaches have been described to date, investigating its interaction with one or more tissues, in particular the gut–liver axis. In numerous studies, the interaction between a gut and liver MPS has been





**Fig. 2.** Multiorgan microphysiological systems for the studies of interorgan crosstalk. MOMPS can be used to investigate cellular and noncellular communication between integrated MPSs. Media and cell can be interrogated via a wider range of technologies and key applications.

described to significantly alter the function of both MPSs as compared to their behavior in isolation.

Gene expression data collected during a study incorporating human liver (hepatocytes and Kupffer cells) and intestinal (enterocytes, goblet cells, and dendritic cells) MPSs in a 2-week interaction experiment, showed significantly altered transcription related to hepatic bile acid release and cytochrome p upregulation and intestinal FGF19 secretion during the interaction. Moreover, during this interaction, significant non-linear modulation of cytokine responses was observed under inflammatory gut–liver interaction with enhanced CXCR3 ligand crosstalk [10]. A comparable approach further revealed the interaction between a liver and gut MPS to increase pathways related to intestinal nutrient uptake, increased hepatic

cytochrome P450 activity, and concurrent reduction in inflammation-associated pathways [43]. Increased hepatic metabolism has also been identified in gut–liver interaction studies lacking the inclusion of innate immune cell populations and absence of active perfusion [44,45] as well as in two-way studies between a liver and kidney MPS [46]. The presence of an intestinal barrier in such systems makes it possible to evaluate the first-pass metabolism of gut-derived products such as nutrients, metabolites, and oral medications. One example is the absorption of fatty acids and their influence on hepatic metabolism during inflammatory conditions [43,47]. The human microbiome, composed of trillions of individual microbial cells, is by many considered as an organ in its own right with a significant impact on human health and disease. From once

static co-culture models [48,49], recent advances in recreating host–microbe interactions under anaerobic conditions allow us to study complex microbial communities and their impact on the host's health [50,51]. However, integration of mucosal MPSs containing complex, patient-specific, microbial communities into multiorgan interactions remains a challenging task.

Mediators of the changes observed during gut–liver interaction studies remain to be fully elucidated. These phenomena warrant further investigation into how 2-way interactions of physiologically linked tissues give rise to altered functionality and whether this insight might inform new therapeutic strategies. Interestingly, a human microfluidic two-organ-chip model that incorporates human pancreatic islet microtissues and liver spheroids was able to maintain functional responses for up to 15 days in an insulin-free medium [52]. Moreover, such functional coupling was able to induce the release of insulin from the islet microtissues in response to a glucose load and to promote glucose uptake by the liver spheroids. The interacting system has maintained glucose concentrations in the circulation whereas glucose levels remained elevated in the MPSs during isolation [52]. A similar homeostasis was reported in a co-culture system of human liver tissues and neurospheres where after 6 days, the system achieved a stable equilibrium between glucose consumption and lactate production [53].

### Three and more interacting MPSs

Many multiorgan crosstalk studies focus on the reciprocal interactions between pairs of organs and the diseases affecting them; however, the crosstalk between two organs may involve mechanisms that go beyond those two sites. For example, the communication between the heart and kidneys in cardiorenal syndrome may involve the brain, adrenal glands, and bone marrow [35].

Given the current technical and computational abilities, two and three-way MPS studies remain the most insightful and carry the most utility. Nonetheless, technologies and tools have been developed that allow the simultaneous integration of up to fourteen different tissues [24,54,55,56,57]. As we expand our understanding of lower-level interactions between individual cells, tissues, and organ systems, the more utility such MOMPS devices will bear.

The possibility to integrate more than two MPSs allows for an increasing combination of various tissues and thus applications, depending on the scientific question of interest. The most common application thus far of MOMPS is the study of both integral systemic

metabolism and metabolism of xenobiotics as well as mechanisms governing tissue homeostasis and renewal.

In a model connecting MPSs of the gut, liver, kidney, and skin, metabolic establishment of homeostasis was achieved within four days and remained sustainable over at least 28 days independent of the individual cell line or tissue donor [58]. Furthermore, in a comparable approach where all tissues were pre-differentiated from iPS cells from the same healthy donor and integrated in one common medium deprived of tissue-specific growth factors, the system remained viable for over 14 days. Although there were no added growth factors present in the co-culture medium, the cells maintained defined marker expression [59].

The investigation of drug efficacy, safety, and metabolism has been a significant driver for the development of MOMPS. Their utility for studies of drug safety and drug metabolism [60] was demonstrated in a study that coupled MPS models representing the major absorption, metabolism, and clearance organs of the jejunum, liver, and kidney, along with skeletal muscle and neurovascular models [61]. In this reported model, terfenadine, trimethylamine, and vitamin D were evaluated and shown to be consistent with clinical data. The integration of a liver MPS in such studies is paramount as shown in a recent study that integrated organoid-based MPSs of the liver, heart, lung, endothelium, brain, and testes to study drug responses against capecitabine and ifosfamide [62]. When exposed to these drugs with a liver MPS present, each was metabolized into a product with downstream toxicity for MPSs of the heart, lung, and brain while removal of the liver MPS from the system did not lead to significant toxicity response in other tissues.

Interaction between various tissues affects their individual development *in vitro*. In a study where a brain MPS was integrated with a gut and liver MPS, the interaction led to the development of significantly more mature neurons and astrocytes and increased dopaminergic signaling [63]. Moreover, in a four-week interaction between a brain MPS and nine other MPSs, namely of the gut, endometrium, liver, lung, heart, pancreas, skin, kidney, and muscle, the brain MPS exhibited improved performance metrics during interaction on the platform compared with isolation as assessed by production of the metabolite N-acetyl aspartate which is indicative of neuronal function [24]. Even though these studies lack a blood–brain barrier, which tightly regulates the molecular exchange between the brain and the rest of the body, advances in modeling the blood–brain barrier have been made

[64–67]. For example, a multi-MPS integrating an endothelium, pericytes, neurons, and astrocytes has been used to study the effect of intravascular administration of the psychoactive drug methamphetamine and has identified a previously unknown metabolic coupling between the blood–brain barrier and neurons [68].

A further advance in modeling of endocrine signaling in MOMPS was achieved with a model of the female reproductive tract, which was able to mimic the 28-day follicular and luteal phase hormone synthesis [69]. The integrated MPSs of the ovaries, fallopian tube, uterus, cervix, and the liver under dynamic flow promoted ovarian steroid hormone production by follicles and their maturation. Interestingly, a similar conclusion of interorgan crosstalk was reached in a study that recreated the metabolic communication between the male testis and the liver [70]. Here, steroids released by the testis were metabolized by the liver.

### Migratory cells as messengers in interorgan communication

Most often, interorgan crosstalk is considered as non-cellular communication, but circulating and migratory cells also occupy an important role in orchestrating signaling between various tissues. The importance of the local and systemic immune environment, mediated *via* tissue-resident and circulating immune cell populations, drives the urgent need to consider these when modeling both individual tissues and multiorgan interactions. Not only are these important for the adequate representation of immune responses and immune tolerance, but also for the very fundamental homeostasis and renewal of tissues [71,72]. While some of the first MPS devices enabling cellular movement were geared toward modeling migration of metastatic cancer, technical advances in vascularization and peristaltic pump technologies are ushering in the era of MOMPSs which can accommodate primary and secondary immune tissues as well as systemic cellular migration (Fig. 2).

Innate and adaptive immunological processes that are of particular interest in a MOMPS setup are (a) localized, tissue-specific responses and how these inform the broader MOMPS system (e.g., tolerance-inducing mechanisms in one MPS influencing systemic inflammation and other MPSs); (b) migration of naïve and effector immune cell populations into tissue and their differentiation (e.g., monocytes replenishing the local macrophage population); (c) antigen presentation and effector T-cell function (e.g., CD8<sup>+</sup> T-cell expansion and clearance of pathogens *in vitro*); (d) humoral

antibody responses (e.g., mucosal vaccine development); and lastly (e) applications in immune oncology and metastatic disease (e.g., migration of metastatic cells and immune invasion of the tumor environment).

Microphysiological system devices that incorporate primary and secondary immune structures are in development additionally to MOMPS integrating immune cell populations only [18,73]. While cell migration can be facilitated *via* artificial means, such as pumping systems and channels that mimic the vasculature, the integration of natural vascularization and the lymphatic system is the ultimate goal. Both represent not only means for cells to move from one end to another but also actively participate in signaling and responses to pathology [74]. Single MPS models integrating components of both blood and lymphatic vessels have been developed. For example, a microfluidic device with two artificial circular channels that were lined with cells capable of angiogenic sprouting of blood and lymphatic vessels was reported [75]. Moreover, the antiangiogenic factor VEGF-R3 showed inhibition of sprouting in this model [75]. Similarly, a two-channel device lined with blood and lymphatic endothelial cells was used to model histamine-mediated modification of vascular permeability [76]. Investigating endothelial permeability in connection to immune signaling remains an important area of interest in the studies of tumor cell migration. A microfluidic-based assay has been created to replicate the tumor-vascular interface in three dimensions, allowing for high-resolution, real-time imaging, and precise quantification of endothelial barrier function and tumor cell migration [77]. A common objective among devices integrating endothelial linings is to model cellular migration within and through the vasculature in a gradient of circulating signaling molecules (e.g., cytokines and chemokines) and under shear stress. Such examples are monocyte adhesion and transmigration [78,79], endothelial interactions with activated and inactivated T cells [80], neutrophil migration [81], and chemotactic behavior of dendritic cells [82].

To this end, MPS of the bone marrow and lymph nodes, as a source of immune cells and site of antigen presentation, are of great interest. Multicompartment MPSs lined with endothelium were used to recreate a bone marrow harboring CD34<sup>+</sup> progenitor cells capable of differentiation into various blood cell lineages [83,84]. Moreover, MPSs for the studies of T-cell interactions with antigen-presenting cells were established [85–87]. While considerable advances have been made both in terms of technological developments as well as insight gained from vascularized models [18], their integration into MOMPS is at very early stages.

Despite the absence of primary and secondary lymphoid organs, individual immune and other migrating cell populations of interest can be integrated into existing MOMPS platforms. For example, a three-organ device with a liver module, cardiac cantilevers and stimulation electrodes, skeletal muscle cantilevers, and recirculating THP-1 monocytes was reported. In this study, nonselective damage in the three different organs was achieved through IFN- $\gamma$ -mediated activation of macrophages, as well as selective macrophage activation *via* tissue injury [88]. This MOMPS can emulate a targeted immune response to tissue-specific damage, and holistic proinflammatory immune response to proinflammatory compound exposure. Further, in a model of ulcerative colitis and concurrent inflammation of the liver, circulating CD4<sup>+</sup>T regulatory and Th17 cells were integrated as messengers between the gut and liver MPSs. Interestingly, in their absence the interaction between innate immune cell populations with their tissue counterparts led to a reduction in colitis-associated inflammation; however, the inclusion of adaptive CD4<sup>+</sup> T cells led to strong T cell-mediated responses and disruption of the gut barrier [43]. Contrary to those findings, circulating CD4<sup>+</sup> T regulatory and Th17 cells did not affect innate inflammatory processes associated with inflammation in a gut–liver–brain MOMPS of Parkinson's diseases. However, there the presence of CD4<sup>+</sup> T cells led to a significantly higher maturation of iPS-derived microglia [63]. These studies exemplify the utility that comes from the scalable complexity of MOMPS and further showcase how questions relevant to developmental biology can be asked and harnessed to gain better knowledge of basic human biology as well as processes relevant to tissue regeneration.

### Deriving causality one network at a time

The omics revolution and advancement of transcriptomic, proteomic, and metabolomic technologies stands behind the unprecedented advancement of our understanding of human biology and diseases. Integration of information from different omics technologies (multiomics studies) from multiple layers of biological data represents a new era of systems biology. Systems biology describes the studies of biological systems by controlled perturbation *via* different means, monitoring the gene, protein, metabolic, and informational pathway responses, and integrating these data into mathematical models and multilayered networks [89]. However, because of the network wirings of biology, a specific perturbation might not lead to one isolated

outcome but, given how signaling networks are interconnected, the perturbation can give rise to secondary effects and adaptation [90]. Thus, in order to understand a process of fundamental importance, it is critical to consider these biological layers as separate elements and to dissect how they interact with each other [91]. In the past, reductionism proposed that complex systems are composed of parts, and if we understand these parts, we can understand the entire system. We now accept that new methods are required to better understand complexity, and how the parts come together to give rise to something greater than their sum [92]. Studies of individual genes and proteins still dominate biomedical research today. The information from single-gene experiments is continually accumulating and providing information on how individual molecules and their interactions fit into a global picture of cell and tissue regulation. While such data collection suffers from research focus biases [93] and reproducibility concerns, systems biology and network-level approaches are gradually becoming the new standard and adopted into a number of specific use cases such as systems immunology and systems medicine. [92].

While attempts are made in systems biology to transition from the micro-, subcellular, and cellular interactions to whole-human epidemiological observations, there is a wide gap at the level of horizontal network interactions across organ systems [42]. An imperative challenge is thus to gain an understanding in how behavior at the molecular level determines physiological function at the level of not only cells but tissues and organs. Moreover, complex diseases are multifactorial involving a great number of influencing factors, cell, and tissue types, co-occurring pathologies, and biological processes at levels ranging from translation to systemic metabolism. Because of the inherent complexity of real biological systems, the development and analysis of multiscale experimental data are required to achieve this understanding [94]. Complexity arises from the number of involved agents and the number of connections between them [92]. The ability to scale the number of agents and their connections in the context of human biology brings forth an unmatched opportunity to gain granular insight into previously unknown casual connections between homeostasis and the fundamental origins of disease. As tools advance for studying single cells at the network level, new, improved computational models of cell–cell communication, cell heterogeneity, and multicellular properties are being developed [90]. However, the different mathematical formalisms and hybrid approaches under development on the computational side highlight the

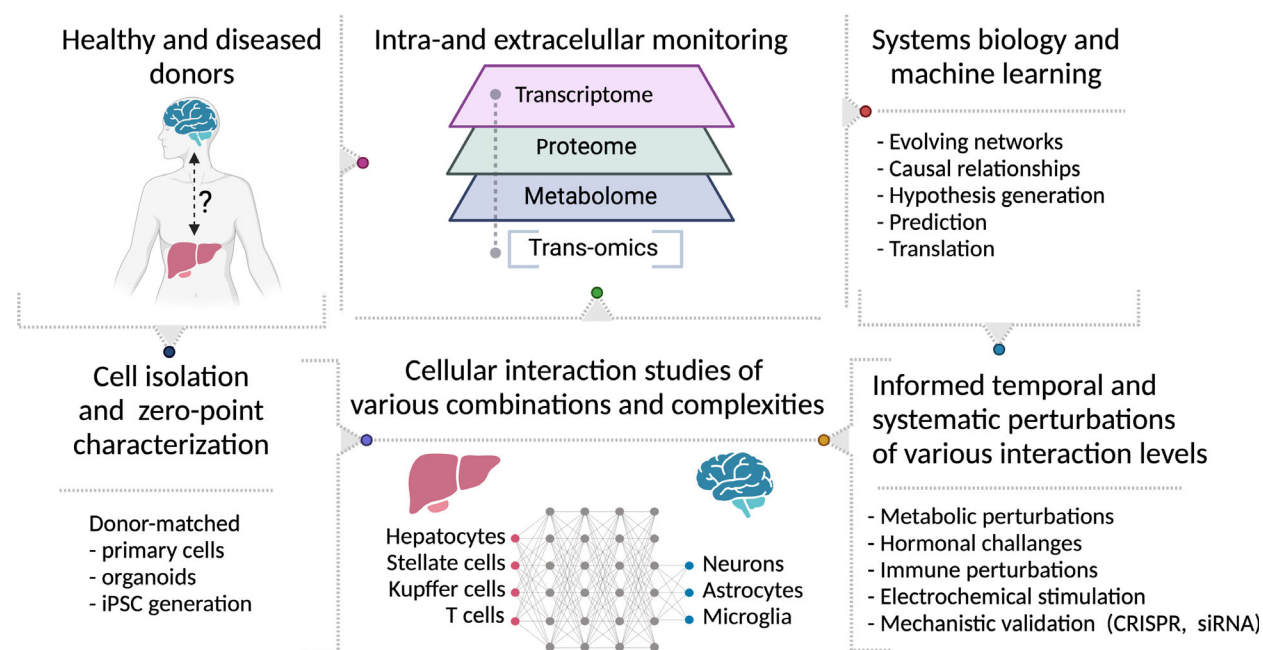


need for close cooperation of cell biologists, bioengineers, and medical researchers. Well-defined perturbation and interaction experiments under temporal multiomic observation, at levels from individual cells to MOMPS, could bring much-needed clarity. The modular and scalable nature of MOMPS allows the user to probe highly controlled interactions between as few or as many tissues and cell types as needed. This enables the investigator to exclude secondary and compensatory action by cells that are not of interest and thus construct a computational model of causation (Fig. 3). For example, a study investigating the physiological role of the microbial metabolites short-chain fatty acids performed in a MOMPS of the gut–liver and gut–liver–brain axis [43,63] has utilized a multiomic approach to parse the transcriptomic response of individual MPSs and changes in the systemic immunometabolic environment. This approach revealed the dual nature of short-chain fatty acids which under conditions of low inflammation can increase expression of tolerance-inducing pathways and fatty acid oxidation, while during acute T cell-driven inflammation, they promote increased glycolysis

and immune effector function. In a separate study, utilizing a six organ MOMPS and untargeted metabolomic profiling revealed a new connection between the metabolism of the drug tolcapone and brain metabolomics. Eighteen key biomarkers were significantly changed in human brain MPS after a simulated oral administration of tolcapone, which was due to the perturbation of tryptophan and phenylalanine metabolism and energy homeostasis [95]. MOMPS and systems biology can derive unique additive signaling phenomena arising from multiorgan interactions and help us understand how such responses give rise to new unique complexities that are not just linear sums of individual subunits [10]. Thus, a bioengineering approach to systems biology that comes with engineering design and predictive MOMPS could help us solve contemporary problems in an age of biomedical Big Data [96,97].

### The road to broad adoption

As with any technological leap, shortcomings are not only inherent companions of progress but its



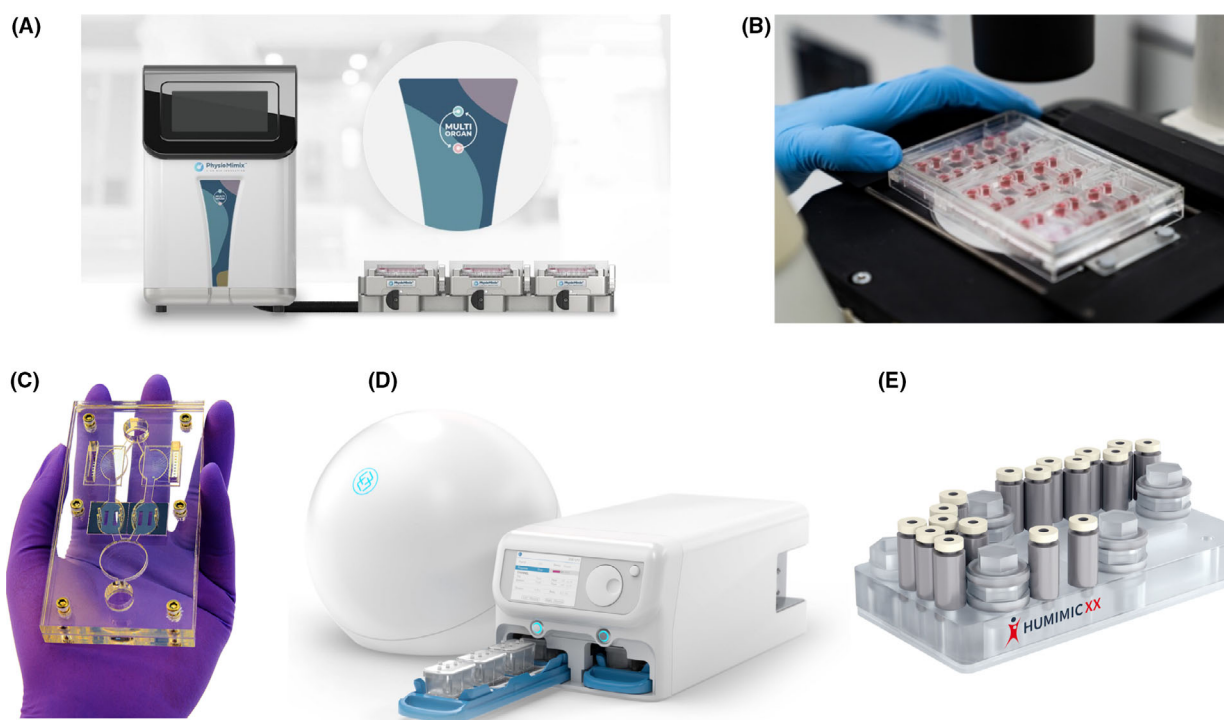
**Fig. 3.** Exemplary study approach to study liver-brain crosstalk. MOMPS allow for the systematic search for causal relationships between cells, tissues, and external factors due to their scalable biological complexity and integration of systems biology approaches. Collection of multiomic observations from the donor and donor-derived tissue allows to establish a 'point zero' description of the system for validation and temporal evolution. MOMPS can be used to reconstruct donor tissue at various levels of complexity which allows each level of complexity to be challenged via predetermined perturbations. Continuous observation of changes based on interaction and perturbation at each level of complexity allows for the construction of interaction networks that can reveal causal relationships among its agents. This process is iterative in that every next interaction is informed by the previous cycle until fundamental processes are identified that become the building blocks for computational prediction.

fundamental drivers. In the context of MOMPS, four broad but connected categories of challenges can be identified: (a) technical (e.g., increasing physiological relevancy, ease of use, reliability), (b) biological (e.g., devising single-donor MOMPS models by using the correct cell type, formulating a universal medium), (c) logistical (e.g., increasing access to these technologies to the wider community), and (d) standardization and validation (e.g., improving repeatability, transferability, and validation).

Today's MOMPS represent the most difficult to develop and to use systems in the microphysiological systems world. The successful use of such devices in most cases requires an interdisciplinary and well-coordinated team that has command over many different cell types and can navigate technical difficulties that might arise. Most studies utilizing MOMPS are performed in the laboratories of their originators, which speaks to the relative novelty of this field and the need for more commercially supported, validated, and standardized devices. Many aspects of MOMPS development and operation are not standardized nor sufficiently validated, at least to the degree where results obtained from different groups could easily be compared and reproduced.

While many of such issues hold true for the general microphysiological systems field, these become amplified in the context of MOMPS. For example, a decision must be made between integrating donor-mismatched cells in a MOMPS setup or deriving cells from stem cells of one single donor. In the latter scenario, the use of iPS cells creates the challenge of operating with tissue that might not be fully matured as their *in vivo* counterparts [13]. If patient-to-patient variability is clouding results from clinical studies, then five plus tissue donors per MOMPS introduce a significantly higher variation. Regardless of the approach, it is important to describe the system and findings in the context of humans when reporting and interpreting data obtained from such studies. Cells and tissues that are being integrated into MOMPS should be well characterized at the stage of integration. Moreover, findings from the interaction, such as transcriptomic profiles, can be compared to publicly available human data. Similarly, concentrations and dynamics of tracked signaling molecules, such as cytokines and chemokines, can be correlated with serum values reported in humans [43,63].

With individual MPSs, the decision regarding specialized substrates, cellular environments, and cell



**Fig. 4.** Examples of commercialized microphysiological systems. (A) CN-Bio PhysioMimix Multi-Organ System, Source: [www.cn-bio.com](http://www.cn-bio.com), (B) AIM Biotech AIM Chip, Source: [www.aimbiotech.com](http://www.aimbiotech.com) (C) Hesperos Human-on-a-Chip, Source: [www.hesperosinc.com](http://www.hesperosinc.com) (D) Emulate Human Emulation System, Source: [www.emulatebio.com](http://www.emulatebio.com) (E) TissUse HUMMIC Chip XX, Source: [www.tissuse.com](http://www.tissuse.com).

media is guided by the leading scientific question and biology to be replicated. However, in the context of multiple integrated MPSs, these questions are less straightforward and more convoluted. What would be the best option for one MPS, it might not be so for another. It also becomes a question of how to prioritize scaling. Should it be based on cell numbers of individual tissues, tissue mass against the mass of another MPS, cell-to-media volume, the concentration of a particular molecule of interest? Moreover, one must also decide on the role and distribution of the media shared between compartments. Should it represent the systemic or regional vasculature and lymphatic system and what will be the guiding principle in how and at what speeds the media is distributed and changed? This highlights how MOMPS depends on the progress of a wide variety of disciplines. A better understanding of stem cell biology, greater resolution of tissue 3D printing, new materials, and media, and increased interest in these devices from the biomedical community will help address many of the current challenges.

## Summary

From tools to reduce animal experimentation and improve preclinical toxicity assessments, today MOMPS represent an intriguing steppingstone in what are efforts to replicate our own biology. The future premise of MOMPS is clear—multicellular tissue models true to our own physiology would allow us to perform costly clinical trials at a fraction of the cost, reduce experimentation on animals, be used as a surrogate to explore the dangers of new environments such as deep space, offer insight into laws governing tissue homeostasis, repair, and emergence of complex diseases to name a few. While this sounds like a utopian dream, it is a reality to which the field is slowly working toward (Fig. 4). Examples of multiorgan interaction systems were reported that support the integration of a remarkable number of tissues [24,55], integrate MPSs constructed from one single donor [98], achieve multiorgan homeostasis even in the absence of tissue-specific humoral factors [52], and which showcase how the interaction of multiple MPSs leads to the rise of unique behaviors and maturity [63].

With improved computational tools and resolution into molecular underpinnings of cellular and tissue homeostasis, MOMPS represents a unique opportunity to systematically dissect how interactions at a lower order inform new behavior at the macroscale within- and between-organ systems. Such scalable complexity might yield new insight into causal relationships

between our genetic markup, the environment, our life choices, and the fundamental emergence of disease.

From the examples in this perspective, it is evident that most of these studies represent proof-of-principle experiments; however, we are now finally at a stage where these devices and approaches can be used to ask in-depth questions of human physiology and pathology. Increased use from the biomedical community and closer multidisciplinary collaboration between engineers, biologists, and computational scientists will be key to establishing MOMPS as a widely adopted technology in preclinical research.

While pursuing the development of MOMPS for particular use cases, the process of attempting to reverse engineer a system can lead to remarkable insight into how a system operates. Furthermore, the current interim phase of reverse engineering of human biology is a necessary step toward the forward engineering of new human-based organisms and the future human 2.0.

## References

- 1 Yang X. Multitissue multiomics systems biology to dissect complex diseases. *Trends Mol Med*. 2020;**26**:718–28.
- 2 Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol*. 2019;**16**:461–78.
- 3 Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. *Science*. 2005;**307**:1920–5.
- 4 Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. *Nat Rev Immunol*. 2006;**6**:244–51.
- 5 Loftus EV Jr, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR et al. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut*. 2005;**54**:91–6.
- 6 Mondal A, Bose D, Saha P, Sarkar S, Seth R, Kimono D et al. Lipocalin 2 induces neuroinflammation and blood-brain barrier dysfunction through liver-brain axis in murine model of nonalcoholic steatohepatitis. *J Neuroinflammation*. 2020;**17**:201.
- 7 Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm (Vienna)*. 2003;**110**:517–36.
- 8 Zhang B, Wang HHE, Bai YM, Tsai SJ, Su TP, Chen TJ et al. Inflammatory bowel disease is associated with higher dementia risk: a nationwide longitudinal study. *Gut*. 2021;**70**:85–91.

- 9 Kowalski K, Mulak A. Brain-gut-microbiota axis in Alzheimer's disease. *J Neurogastroenterol Motil.* 2019;**25**:48–60.
- 10 Chen WLK, Edington C, Suter E, Yu J, Velazquez JJ, Velazquez JG et al. Integrated gut/liver microphysiological systems elucidates inflammatory inter-tissue crosstalk. *Biotechnol Bioeng.* 2017;**114**:2648–59.
- 11 Picollet-D'ahan N, Zuchowska A, Lemeunier I, Le Gac S. Multiorgan-on-a-chip: a systemic approach to model and decipher inter-organ communication. *Trends Biotechnol.* 2021;**39**:788–810.
- 12 Marx U, Akabane T, Andersson TB, Baker E, Beilmann M, Beken S et al. Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. *Altex.* 2020;**37**:365–94.
- 13 Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA. Organs-on-chips: into the next decade. *Nat Rev Drug Discov.* 2021;**20**:345–61.
- 14 Jalili-Firoozinezhad S, Miranda CC, Cabral JMS. Modeling the human body on microfluidic chips. *Trends Biotechnol.* 2021;**39**:838–52.
- 15 Ronaldson-Bouchard K, Vunjak-Novakovic G. Organs-on-a-chip: a fast track for engineered human tissues in drug development. *Cell Stem Cell.* 2018;**22**:310–24.
- 16 Wang YI, Carmona C, Hickman JJ, Shuler ML. Multiorgan microphysiological systems for drug development: strategies, advances, and challenges. *Adv Healthc Mater.* 2018;**7**:1701000.
- 17 Tavakol DN, Fleischer S, Vunjak-Novakovic G. Harnessing organs-on-a-chip to model tissue regeneration. *Cell Stem Cell.* 2021;**28**:993–1015.
- 18 Hammel JH, Cook SR, Belanger MC, Munson JM, Pompano RR. Modeling immunity in vitro: slices, chips, and engineered tissues. *Annu Rev Biomed Eng.* 2021;**23**:461–91.
- 19 Malik M, Yang Y, Fathi P, Mahler GJ, Esch MB. Critical considerations for the design of multi-organ Microphysiological Systems (MPS). *Front Cell Dev Biol.* 2021;**9**:721338.
- 20 Priest C, Tontonoz P. Inter-organ cross-talk in metabolic syndrome. *Nat Metab.* 2019;**1**:1177–88.
- 21 Gancheva S, Jelenik T, Alvarez-Hernandez E, Roden M. Interorgan metabolic crosstalk in human insulin resistance. *Physiol Rev.* 2018;**98**:1371–415.
- 22 Romacho T, Elsen M, Rohrborn D, Eckel J. Adipose tissue and its role in organ crosstalk. *Acta Physiol.* 2014;**210**:733–53.
- 23 Vunjak-Novakovic G, Ronaldson-Bouchard K, Radisic M. Organs-on-a-chip models for biological research. *Cell.* 2021;**184**:4597–611.
- 24 Edington CD, Chen WLK, Geishecker E, Kassis T, Soenksen LR, Bhushan BM et al. Interconnected microphysiological systems for quantitative biology and pharmacology studies. *Sci Rep.* 2018;**8**:4530.
- 25 Clevers H. Modeling development and disease with organoids. *Cell.* 2016;**165**:1586–97.
- 26 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;**131**:861–72.
- 27 Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* 2009;**459**:262–5.
- 28 Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-beta: from conception to autoimmunity and infection. *Cold Spring Harb Perspect Biol.* 2017;**9**:a022236.
- 29 de Lau W, Peng WC, Gros P, Clevers H. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. *Genes Dev.* 2014;**28**:305–16.
- 30 Cyr KJ, Avaldi OM, Wikswo JP. Circadian hormone control in a human-on-a-chip: in vitro biology's ignored component? *Exp Biol Med (Maywood).* 2017;**242**:1714–31.
- 31 Bodine SC, Brooks HL, Bunnett NW, Collier HA, Frey MR, Joe B et al. An American Physiological Society cross-journal call for papers on "Inter-Organ Communication in Homeostasis and Disease". *Am J Physiol Lung Cell Mol Physiol.* 2021;**321**:L42–9.
- 32 Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell.* 2016;**167**:915–32.
- 33 Castillo-Armengol J, Fajas L, Lopez-Mejia IC. Inter-organ communication: a gatekeeper for metabolic health. *EMBO Rep.* 2019;**20**:e47903.
- 34 Wang A, Luan HH, Medzhitov R. An evolutionary perspective on immunometabolism. *Science.* 2019;**363**:eaar3932.
- 35 Oishi Y, Manabe I. Organ system crosstalk in cardiometabolic disease in the age of multimorbidity. *Front Cardiovasc Med.* 2020;**7**:64.
- 36 Cani PD, Knauf C. How gut microbes talk to organs: the role of endocrine and nervous routes. *Mol Metab.* 2016;**5**:743–52.
- 37 Man K, Kutyavin VI, Chawla A. Tissue immunometabolism: development, physiology, and pathobiology. *Cell Metab.* 2017;**25**:11–26.
- 38 Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol.* 2002;**2**:569–79.
- 39 Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol.* 2013;**200**:373–83.
- 40 Verweij FJ, Revenu C, Arras G, Dingli F, Loew D, Pegtel DM et al. Live tracking of inter-organ communication by endogenous exosomes in vivo. *Dev Cell.* 2019;**48**:573–589 e4.
- 41 Eckel J. The cellular secretome and organ crosstalk, 1st ed. San Diego, CA: Elsevier; 2018.



- 42 Ivanov PC. The new field of network physiology: building the human physiome. *Front Netw Physiol.* 2021;1:711778.
- 43 Trapecar M, Communal C, Velazquez J, Maass CA, Huang YJ, Schneider K et al. Gut-liver physiomics reveal paradoxical modulation of IBD-related inflammation by short-chain fatty acids. *Cell Syst.* 2020;10:223–239.e9.
- 44 Tsamandouras N, Chen WLK, Edington CD, Stokes CL, Griffith LG, Cirit M. Integrated gut and liver microphysiological systems for quantitative in vitro pharmacokinetic studies. *AAPS J.* 2017;19:1499–512.
- 45 Esch MB, Ueno H, Applegate DR, Shuler ML. Modular, pumpless body-on-a-chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. *Lab Chip.* 2016;16:2719–29.
- 46 Theobald J, Maaty MAA, Kusterer N, Wetterauer B, Wink M, Cheng XL et al. In vitro metabolic activation of vitamin D3 by using a multi-compartment microfluidic liver-kidney organ on chip platform. *Sci Rep.* 2019;9:4616.
- 47 Lee SY, Sung JH. Gut-liver on a chip toward an in vitro model of hepatic steatosis. *Biotechnol Bioeng.* 2018;115:2817–27.
- 48 Trapecar M, Leouffre T, Faure M, Jensen HE, Granum PE, Cencic A et al. The use of a porcine intestinal cell model system for evaluating the food safety risk of *Bacillus cereus* probiotics and the implications for assessing enterotoxigenicity. *APMIS.* 2011;119:877–84.
- 49 Trapecar M, Goropevsek A, Gorenjak M, Gradisnik L, Slak Rupnik M. A co-culture model of the developing small intestine offers new insight in the early immunomodulation of enterocytes and macrophages by *Lactobacillus* spp. through STAT1 and NF- $\kappa$ B p65 translocation. *PLoS One.* 2014;9:e86297.
- 50 Zhang J, Hernandez-Gordillo V, Trapecar M, Wright C, Taketani M, Schneider K et al. Coculture of primary human colon monolayer with human gut bacteria. *Nat Protoc.* 2021;16:3874–900.
- 51 Jalili-Firoozinezhad S, Gazzaniga FS, Calamari EL, Camacho DM, Fadel CW, Bein A et al. A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat Biomed Eng.* 2019;3:520–31.
- 52 Bauer S, Wennberg Hultdt C, Kanebratt KP, Durieux I, Gunne D, Andersson S et al. Functional coupling of human pancreatic islets and liver spheroids on-a-chip: towards a novel human ex vivo type 2 diabetes model. *Sci Rep.* 2017;7:14620.
- 53 Materne EM, Ramme AP, Terrasso AP, Serra M, Alves PM, Brito C et al. A multi-organ chip co-culture of neurospheres and liver equivalents for long-term substance testing. *J Biotechnol.* 2015;205:36–46.
- 54 Novak R, Ingram M, Marquez S, Das D, Delahanty A, Herland A et al. Robotic fluidic coupling and interrogation of multiple vascularized organ chips. *Nat Biomed Eng.* 2020;4:407–20.
- 55 Miller PG, Shuler ML. Design and demonstration of a pumpless 14 compartment microphysiological system. *Biotechnol Bioeng.* 2016;113:2213–27.
- 56 Shinha K, Nihei W, Nakamura H, Goto T, Kawanishi T, Ishida N et al. A kinetic pump integrated microfluidic plate (KIM-Plate) with high usability for cell culture-based multiorgan microphysiological systems. *Micromachines (Basel).* 2021;12:1007.
- 57 Rauti R, Ess A, Roi BL, Kreinin Y, Epshtein M, Korin N et al. Transforming a well into a chip: a modular 3D-printed microfluidic chip. *Apl Bioeng.* 2021;5:26103.
- 58 Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, Ramme AP, Hubner J et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip.* 2015;15:2688–99.
- 59 Ramme AP, Koenig L, Hasenberg T, Schwenk C, Magauer C, Faust D et al. Autologous induced pluripotent stem cell-derived four-organ-chip. *Future Sci.* 2019;5:FSO413.
- 60 Fowler S, Chen WLK, Duignan DB, Gupta A, Hariparsad N, Kenny JR et al. Microphysiological systems for ADME-related applications: current status and recommendations for system development and characterization. *Lab Chip.* 2020;20:446–67.
- 61 Verneti L, Gough A, Baetz N, Blutt S, Broughman JR, Brown JA et al. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci Rep.* 2017;7:42296.
- 62 Rajan SAP, Aleman J, Wan M, Pourhabibi Zarandi N, Nzou G, Murphy S et al. Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform. *Acta Biomater.* 2020;106:124–35.
- 63 Trapecar M, Wogram E, Svoboda D, Communal C, Omer A, Lungjangwa T et al. Human physiomic model integrating microphysiological systems of the gut, liver, and brain for studies of neurodegenerative diseases. *Sci Adv.* 2021;7:eabd1707.
- 64 Griep LM, Wolbers F, de Wagenaar B, ter Braak PM, Weksler BB, Romero IA et al. BBB ON CHIP: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function. *Biomed Microdevices.* 2013;15:145–50.
- 65 Park TE, Mustafaoglu N, Herland A, Hasselkus R, Mannix R, FitzGerald EA et al. Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. *Nat Commun.* 2019;10:2621.
- 66 Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, Rahnama M et al. Human iPSC-derived blood-brain barrier chips enable disease modeling and

- personalized medicine applications. *Cell Stem Cell*. 2019;**24**:995–1005.e6.
- 67 Brown JA, Pensabene V, Markov DA, Allwardt V, Neely MD, Shi MJ et al. Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor. *Biomicrofluidics*. 2015;**9**:54124.
  - 68 Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR et al. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat Biotechnol*. 2018;**36**:865–74.
  - 69 Xiao S, Coppeta JR, Rogers HB, Isenberg BC, Zhu J, Olalekan SA et al. A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. *Nat Commun*. 2017;**8**:14584.
  - 70 Baert Y, Ruetschle I, Cools W, Oehme A, Lorenz A, Marx U et al. A multi-organ-chip co-culture of liver and testis equivalents: a first step toward a systemic male reprotoxicity model. *Hum Reprod*. 2020;**35**:1029–44.
  - 71 Masopust D, Soerens AG. Tissue-resident T cells and other resident leukocytes. *Annu Rev Immunol*. 2019;**37**:521–46.
  - 72 Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol*. 2014;**14**:667–85.
  - 73 Morsink MAJ, Willemsen NGA, Leijten J, Bansal R, Shin SR. Immune Organs and immune cells on a chip: an overview of biomedical applications. *Micromachines (Basel)*. 2020;**11**:849.
  - 74 Henderson AR, Choi H, Lee E. Blood and lymphatic vasculatures on-chip platforms and their applications for organ-specific in vitro modeling. *Micromachines (Basel)*. 2020;**11**:147.
  - 75 Osaki T, Serrano JC, Kamm RD. Cooperative effects of vascular angiogenesis and lymphangiogenesis. *Regen Eng Transl Med*. 2018;**4**:120–32.
  - 76 Sato M, Sasaki N, Ato M, Hirakawa S, Sato K, Sato K. Microcirculation-on-a-chip: a microfluidic platform for assaying blood- and lymphatic-vessel permeability. *PLoS One*. 2015;**10**:e0137301.
  - 77 Zervantonakis IK, Hughes-Alford SK, Charest JL, Condeelis JS, Gertler FB, Kamm RD. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proc Natl Acad Sci USA*. 2012;**109**:13515–20.
  - 78 Srigunapalan S, Lam C, Wheeler AR, Simmons CA. A microfluidic membrane device to mimic critical components of the vascular microenvironment. *Biomicrofluidics*. 2011;**5**:13409.
  - 79 Zhang B, Peticone C, Murthy SK, Radisic M. A standalone perfusion platform for drug testing and target validation in micro-vessel networks. *Biomicrofluidics*. 2013;**7**:44125.
  - 80 Kim SK, Moon WK, Park JY, Jung H. Inflammatory mimetic microfluidic chip by immobilization of cell adhesion molecules for T cell adhesion. *Analyst*. 2012;**137**:4062–8.
  - 81 Wu XJ, Newbold MA, Gao Z, Haynes CL. A versatile microfluidic platform for the study of cellular interactions between endothelial cells and neutrophils. *Biochim Biophys Acta Gen Subj*. 2017;**1861**:1122–30.
  - 82 Haessler U, Pisano M, Wu M, Swartz MA. Dendritic cell chemotaxis in 3D under defined chemokine gradients reveals differential response to ligands CCL21 and CCL19. *Proc Natl Acad Sci USA*. 2011;**108**:5614–9.
  - 83 Chou DB, Frimantas V, Milton Y, David R, Pop-Damkov P, Ferguson D et al. On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology. *Nat Biomed Eng*. 2020;**4**:394–406.
  - 84 Sieber S, Wirth L, Cavak N, Koenigsmark M, Marx U, Lauster R et al. Bone marrow-on-a-chip: long-term culture of human haematopoietic stem cells in a three-dimensional microfluidic environment. *J Tissue Eng Regen Med*. 2018;**12**:479–89.
  - 85 Gopalakrishnan N, Hannam R, Casoni GP, Barriat D, Ribe JM, Haug M et al. Infection and immunity on a chip: a compartmentalised microfluidic platform to monitor immune cell behaviour in real time. *Lab Chip*. 2015;**15**:1481–7.
  - 86 Moura Rosa P, Gopalakrishnan N, Ibrahim H, Haug M, Halaas O. The intercell dynamics of T cells and dendritic cells in a lymph node-on-a-chip flow device. *Lab Chip*. 2016;**16**:3728–40.
  - 87 Mitra B, Jindal R, Lee S, Xu Dong D, Li L, Sharma N et al. Microdevice integrating innate and adaptive immune responses associated with antigen presentation by dendritic cells. *RSC Adv*. 2013;**3**:16002–10.
  - 88 Sasserath T, Rumsey JW, McAleer CW, Bridges LR, Long CJ, Elbrecht D et al. Differential monocyte actuation in a three-organ functional innate immune system-on-a-chip. *Adv Sci (Weinh)*. 2020;**7**:2000323.
  - 89 Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet*. 2001;**2**:343–72.
  - 90 Janes KA, Lauffenburger DA. Models of signalling networks - what cell biologists can gain from them and give to them. *J Cell Sci*. 2013;**126**:1913–21.
  - 91 Haas R, Zelezniak A, Iacovacci J, Kamrad S, Townsend S, Ralser M. Designing and interpreting 'multi-omic' experiments that may change our understanding of biology. *Curr Opin Syst Biol*. 2017;**6**:37–45.
  - 92 Ma'ayan A. Complex systems biology. *J R Soc Interface*. 2017;**14**.
  - 93 Spector-Bagdady K, Tang S, Jabbour S, Price WN, Bracic A, Creary MS et al. Respecting autonomy and enabling diversity: the effect of eligibility and enrollment on research data demographics. *Health*

- Affairs*. 2021;**40**:1892–99. <https://doi.org/10.1377/hlthaff.2021.01197>
- 94 Ideker T, Winslow LR, Lauffenburger DA. Bioengineering and systems biology. *Ann Biomed Eng*. 2006;**34**:1226–33.
  - 95 Wang X, Cirit M, Wishnok JS, Griffith LG, Tannenbaum SR. Analysis of an integrated human multiorgan microphysiological system for combined tolcapone metabolism and brain metabolomics. *Anal Chem*. 2019;**91**:8667–75.
  - 96 Janes KA, Chandran PL, Ford RM, Lazzara MJ, Papin JA, Peirce SM et al. An engineering design approach to systems biology. *Integr Biol (Camb)*. 2017;**9**:574–83.
  - 97 Brubaker DK, Proctor EA, Haigis KM, Lauffenburger DA. Computational translation of genomic responses from experimental model systems to humans. *PLoS Comput Biol*. 2019;**15**:e1006286.
  - 98 Vunjak-Novakovic G, Bhatia S, Chen C, Hirschi K. HeLiVa platform: integrated heart-liver-vascular systems for drug testing in human health and disease. *Stem Cell Res Ther*. 2013;**4**(Suppl 1):S8.