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Perspective

Complex or not too complex? One size does not fit all in next generation microphysiological systems

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SUMMARY

In the attempt to overcome the increasingly recognized shortcomings of existing *in vitro* and *in vivo* models, researchers have started to implement alternative models, including microphysiological systems. First examples were represented by 2.5D systems, such as microfluidic channels covered by cell monolayers as blood vessel replicates. In recent years, increasingly complex microphysiological systems have been developed, up to multi-organ on chip systems, connecting different 3D tissues in the same device. However, such an increase in model complexity raises several questions about their exploitation and implementation into industrial and clinical applications, ranging from how to improve their reproducibility, robustness, and reliability to how to meaningfully and efficiently analyze the huge amount of heterogeneous datasets emerging from these devices. Considering the multitude of envisaged applications for microphysiological systems, it appears now necessary to tailor their complexity on the intended purpose, being academic or industrial, and possibly combine results deriving from differently complex stages to increase their predictive power.

INTRODUCTION

Historically, the investigation of biological questions relied on two dichotomic approaches, i.e., a reductionist approach bringing forward the idea that only investigation of biological systems at the lowest possible level can generate significant knowledge, and a non-reductionist one highlighting systematic biases from overlooking relevant biological features inherent to simplified systems.[1,2]

This dichotomic approach also persists in current pre-clinical research whereby reductionist *in vitro* models and non-reductionist *in vivo* models deal with the study of pathologies and the discovery of potential drugs against them. One key challenge for reduction originates from the fact that the effects of a biological mechanism generally depend on the context in which it occurs,[3] making it difficult to find a suitable balance between model simplicity and relevance. On the other side, *in vivo* models fully consider the biological complexity of a living organism, although pinpointing which specific parameter combination affects cell/tissue behavior might be extremely challenging and potentially biased by the biological background of a given animal species.

In this context, microphysiological systems (MPS) can be conceived as an innovative bridge between these two opposite approaches, holding the promise to fully mimic the functional tissue units of an entire human organ at a micro- to meso-scale[4] and thus representing a half-way entity between the defined but simplistic *in vitro* models and the comprehensive but not fully controllable *in vivo* systems. Evolving from the traditional paradigm of tissue engineering defined by Langer and Vacanti, i.e., "the development of biological substitutes to restore, maintain or improve function",[5] MPS can be employed to identify biological mechanisms underlying patho-physiological processes,[6] perform drug development/screening studies,[7] and build patient-specific models for personalized clinical treatments.[8] Several leading experts in the field of MPS have recently suggested that it is now time for a higher consideration of the contribution that MPS have brought to the biomedical research field.[9,10]

To reach the goal of bridging the gap between traditional 2D culture assays and animal models, and then effectively complement/substitute current pre-clinical research standards, MPS have experienced a steady increase in complexity.[11] From pioneering models represented by extremely simplified systems based on 2.5D cell cultures embedded within microfluidic devices (e.g., hydrogel matrices surrounded by monolayers of endothelial cells[12–14]), the scientific community has been developing increasingly complex 3D, multi-culture models reproducing organ-specific microenvironments.[15,16] More recently, systems embedding organoids,[17] cancer spheroids,[18] vascularized matrices (e.g., blood vessels and lymphatics[19,20]) and even multi-organ devices[21–23] have been described, attempting at improving physiological fidelity. These complex microenvironments can integrate biochemical and biophysical stimulations[24,25] and finely recapitulate the architecture and function of several tissue units under patho-physiological conditions, including the alveolar-capillary interface,[26] the intestinal barrier,[27]

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skeletal myofibers,[28] and cartilage.[29] A further advantage of these models resides in the use of human cells, allowing to recapitulate tissue-specific functions that cannot be properly analyzed in animal models, mainly due to species-specific differences. Indeed, many differences exist between humans and mice,[30] such as, but not limited to, renal drug clearance,[31] blood homeostasis,[32] immune system, and inflammatory responses.[33] Furthermore, specific features of human diseases can be difficult to replicate in animals as it happens for osteoarthritis, Ewing sarcoma[30,34] and more generally in studies about tumor development/response to therapy.[35]

A few recent examples of MPS showed a high potential when replicating highly specific biological processes, screening compounds in high-throughput and even providing highly translational results. For instance, the Ingber group developed a simplified lymphoid follicle (LF)-on-a-chip within a two-channel device which allowed to stimulate with flow a cell-laden hydrogel. This system was able to reproduce the organization of B and T lymphocytes into 3D functional aggregates that matured into plasma cells producing antibodies in response to influenza vaccination. Cells from multiple human donors were then introduced into the chip and were able to produce antibodies. Strikingly, the level of antibody production in a group of donors was comparable to the traditional tonsil preparation.[36] Using a commercially available high-throughput microfluidic platform, Soragni and colleagues generated an array of human blood vessels that were then exposed to a library of 1,537 kinase inhibitors to monitor the angiogenic ability of endothelial cells and the integrity of larger microvessels. Importantly, the screening revealed a subset of drugs that showed high anti-angiogenic activity with low toxicity. Furthermore, a group of these drugs was not previously associated with angiogenic pathways, hence showing the potential of these pre-clinical screenings in the identification of potential novel therapies and drug repurposing.[37] Moving toward more translational applications, Barrile and co-authors employed a simple vessel-on-a-chip that was used to analyze the effect of a monoclonal antibody targeting CD40L. This antibody had previously showed serious side effects (i.e., thrombotic events) that were not detected in classical pre-clinical testing and its clinical development was then terminated. The vessel-on-a-chip based on a monolayer of endothelial cells allowed to quantify key parameters involved in thrombosis, including endothelial cell activation and platelet aggregation, and demonstrated a potential risk associated with the use of such antibody.[32] Overall, this study demonstrated that MPS could be employed to collect relevant information to speed-up and better direct the drug development process. Going beyond this significant contribution, the Hickman group developed MPS embedding human induced pluripotent stem cell (iPSC)-derived motor neurons and human Schwann cells. This system was characterized by microscale tunnels directing axon outgrowth over a microelectrode array. The MPS was employed to analyze two neuromuscular diseases, namely chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy. The model was challenged with serum obtained from human donors affected by these diseases, which is rich in auto-antibodies targeting the cells and making the motor neuron signals to move more slowly. The MPS demonstrated that treatment with TNT005, a drug that blocks the immune system reaction, was able to rescue the functional deficit.[38] It is important to highlight that this study represents one of the first examples of an MPS that provided data supporting a Food and Drug Administration (FDA) Investigational New Drug application.

Although these promising results point to a growing importance of MPS in translational research, could their increasing complexity help in overcoming the biases of the reductionist approach? Or rather it risks to hamper its advantages in terms of control and reproducibility of the obtained results? From a different point of view, could complexity be the key to finally fully replace animal models or it is just a useful feature to better complement available *in vitro* and *in vivo* approaches?

Rather than providing a comprehensive analysis of MPS to recapitulate specific features of human physiology and disease (the reader is invited to consider recent review articles for a biological description[39,40] and for a translational point of view[41]), here, we will try to provide an answer to the aforementioned questions by considering different parameters contributing to the complexity of recently developed *in vitro* 3D systems in terms of:

(1) System architecture
(2) System composition
(3) System functionality
(4) System readouts

In the following sections we will provide a critical overview of these layers of complexity (Figure 1) when applied to examples dealing with systemic (i.e., blood vessels), organ-specific (i.e., musculo-skeletal tissue) or pathological (i.e., cancer) models.

Systemic models

Blood vessels represent a compelling example to highlight the relevance of complexity when designing an effective *in vitro* model. Physiological vascular beds are generally organized into hierarchical structures. Hence, the architecture of the model should take into account this organization and mimic it at different hierarchical levels. In this context, it is possible to reproduce the architecture of the microcirculatory system in different ways: (1) to replicate the single capillary vessel, a monolayer of endothelial cells flanking an acellular 3D hydrogel represents the easiest way, nonetheless allowing the basic study of circulating cell flow, adhesion to the endothelium, and transmigration into the adjacent compartment;[42] (2) to mimic a whole district of the microvascular circulation, capillary networks can be created through the self-assembly of endothelial cells and supporting mural cells (e.g., fibroblasts, mesenchymal stem cells, pericytes) embedded within a natural or synthetic extracellular matrix and injected in a microfluidic channel[43–45]; (3) to also add large vessels to microcirculation, hierarchical microvascular networks can be designed through printing techniques (e.g., omnidirectional printing,[46] multiphoton ablation[47]) or through the functional interconnection between millimetric vessel-like scaffolds and self-assembled microvascular networks.[48,49] The increased level of architectural complexity allows to observe phenomena that are not reproducible with the simplest models, such as analyzing the influence of

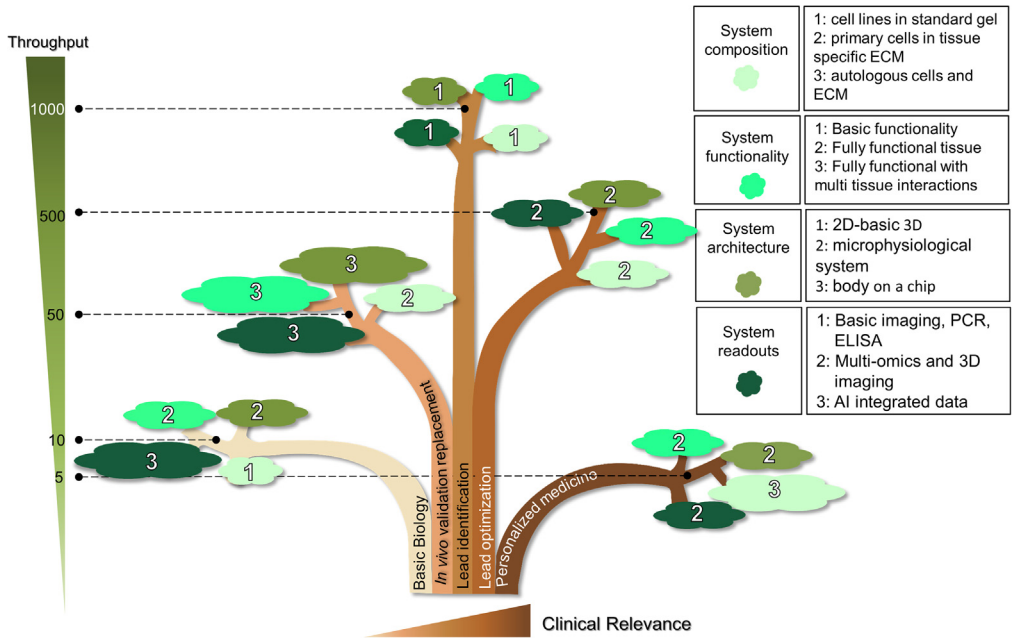

Figure 1. The MPS Tree

Each ramification represents a specific application in which MPS can be envisioned (i.e., basic biology, animal replacement, lead identification, lead optimization, personalized medicine). Each of these applications is characterized by a different level of clinical relevance (represented by the gradient bar under the tree) and throughput (represented by the gradient bar on the left). Each parameter defining system complexity can range from 1 (the lowest complex level, corresponding to a 2D system or a basic 3D architecture composed by cell lines embedded into or flanking a standard hydrogel, such as an endothelial monolayer, characterized by basic functionality and with simple readouts like imaging, standard qPCR and ELISA assays) to 2 (intermediate complexity, corresponding to a single tissue MPS based on primary cells embedded in a tissue-specific hydrogel, achieving the replication of tissue functionality such as muscle contraction and producing high content readouts such as transcriptomic analyses). The ultimate level of complexity is 3, characterized by a body-on-a-chip system, autologous cells and extracellular matrix, reproducing the interactions between different functional tissues, such as the contraction of the muscle stimulated by action potentials from motor neurons, and exploiting AI techniques for the analysis and integration of heterogeneous datasets.

different levels of shear stress and complex flow patterns on circulating cells. On the other hand, the increased complexity of the system can make it difficult to analyze what is going on (e.g., to track the behavior of single circulating/adhering and extravasating cells into a complex vascular network).

Complexity goes far beyond the architecture of an engineered microenvironment. Indeed, cellular composition and cell/biomaterial source can dramatically influence the behavior of a biological system allowing to observe effects that might be neglected within simplified models. In this context, besides the proper mixing of multiple cell populations, it is also critical to select the most adequate source for each of the populations considered. Recent studies in the vascular field have demonstrated that microvascular endothelial cells have organ-specific features[50,51] and significantly differ from widely used umbilical vein endothelial cells in terms of barrier function and angiogenic potential. As an example, brain microvascular endothelial cells showed lower permeability and formed microvascular networks characterized by reduced total length and number of branch points compared to umbilical vein endothelial cells.[52] Similarly, human induced pluripotent stem cell-derived endothelial cells showed different behaviors compared to primary cells during barrier disruption assays or when challenged with inflammatory stimuli. Indeed, endothelial cells derived from pluripotent stem cells did not respond to histamine stimulation, while both umbilical vein endothelial cells and dermal microvascular endothelial cells showed a marked increase in barrier permeability.[53] Direct reprogramming of fibroblasts into endothelial cells demonstrated to be another powerful technique to easily obtain patient-specific vascular cells, although transcriptome analyses still highlighted differences with primary cells.[54]

Moving from cellular to biomaterial sources, the importance of selecting the optimal extracellular matrix is generally underestimated, although it represents a critical feature for the generation of complex microenvironments. Indeed, this selection is a key for generating MPS which accurately model a specific disease and for effectively testing drugs. For instance, the vast majority of vascular-related studies employ the pro-angiogenic fibrin while neglecting the complex, organotypic matrix characterizing specific tissues. This selection is motivated by the existence of well-established protocols that allow to easily generate microvascular networks and customize their features (e.g., network length, surface area, number of branches).[55] However, vascular cells are generally exposed to different tissue-specific proteins *in vivo*, including collagens, hyaluronic acid, and laminin, and microvascular networks showed different morphologies when cultured in different hydrogels.[13] Similarly, the sprouting potential of microvascular endothelial cells was completely different in fibrin-based compared to collagen-based matrices. In particular, endothelial cells formed multicellular sprouts when embedded in fibrin hydrogels supplemented with vascular growth factors, while collagen hydrogels induced single cell migration events without the formation of complex structures.[56]

Tissue functionality represents another key aspect when optimizing the level of complexity of the model. The presence of perfusable vessels within large scale tissues is fundamental to increase the size of the model and properly nourish the cells within. In this context, it was reported that multimaterial bioprinting allowed to design a network of perfusable channels for the vascularization of centimeter-thick bone-like tissues.[57] However, bioartificial, endothelialized microchannels only allow to achieve a limited level of functionality because they do not consider the hierarchical organization of the microcirculation and its consequences in terms of wall shear stress gradients and complex flow patterns at bifurcations or curvatures. These features have consequences for several biological processes occurring through blood vessels, such as immune and cancer cell flow.[58,59] An additional level of functional complexity is represented by the possibility to setup continuous flows that counteract the spontaneous regression of biofabricated blood vessels.[60] These flows can be generated by connecting external pumping systems or by establishing pressure gradients through reservoirs.

The possibility to tune the complexity of these models from an architectural, compositional, and functional point of view leads to a predicted increase in the amount of data that can be theoretically extracted from MPS (i.e., readout complexity). In this scenario, the increasingly higher number of variables involved poses serious challenges for the analysis, interpretation and validation of the results. This aspect is even more challenging considering that novel MPS are embedded within high-throughput platforms able to host tens or hundreds of samples. In this scenario, it will be of paramount importance to develop novel data analysis pipelines that can help scientists to pinpoint which data have the highest chance to contain meaningful information and extract relevant conclusions from a specific experiment. As an example, in a recent study a high-throughput platform containing 96 units consisting of two overlaid microchannels separated by a membrane seeded with up to two cell monolayers on each side was described. One of these monolayers can be represented by endothelial cells, which are also exposed to programmable flow. This platform was reported to be compatible with RNA sequencing techniques, being able to discriminate two cell populations based on the expression of cell identity genes.[61] Further, electrical sensors were embedded to quantify the barrier properties of the monolayer, while an automated imaging pipeline was setup through a high-content imaging system to provide a wide set of information (e.g., cell body and nuclear morphology, cell activation, cell orientation). Other simpler systems characterized by similar throughput mainly focused on imaging-based analyses, rather than on their complex integration with omic techniques and other on-chip assays.[62,63]

Organ-specific models

The musculo-skeletal system represents another tissue whose complexity can be replicated *in vitro* at multiple levels.[64] At the architectural level, both skeletal muscle and bone are characterized by a hierarchical organization and mechanical properties that are rarely captured *in vitro*. For instance, the development of trabecular bone-mimicking tissues should consider the microfabrication of trabecular structures that recapitulate both the architectural complexity and the mechanical properties of spongy bone, rather than the simplistic optimization of hydrogel composition. Similarly, skeletal muscle is composed of fascicles, which are formed by fibers each surrounded by endomysium and vascular structures. In this scenario, most available systems only recreate bundles of suspended muscle fibers,[65,66] while just a few consider the presence of vascular structures and endo/perimysium.[28,67]

Considering the system composition, it is important to choose the proper cell type and source to guarantee the optimal performance of the model. For instance, the differentiation of osteoblasts and osteoclasts was significantly increased when they were co-cultured within 3D matrices embedding endothelial cells compared to monoculture conditions.[68] Similarly, in the skeletal muscle the co-culture of muscle cells and endothelial cells improved both myogenesis and angiogenesis.[69]

A system behavior is not only determined by its components, but rather it is strongly influenced by their dynamic interactions. Indeed, even though specific components (e.g., the presence of contractile myoblasts and motor neurons) are needed for a proper behavior of the system, it is the nature of their interactions that generates specific system functions (e.g., electrical coupling between muscle cells and motor neurons that generates muscle contraction). In this context, it should be carefully considered the minimal level of tissue functionality that must be achieved within MPS for a specific application. For instance, several *in vitro* models of skeletal muscle reported no contraction of the generated muscle fibers or limited functionality of isolated myotubes, allowing to investigate phenomena not related to contraction, such as toxicity of injected drugs or differentiation and growth of neural and vascular networks.[28,70,71] On the other side, a few models fully reproduced the contraction of an entire muscle fiber/bundle, allowing more complex investigations such as the muscle contractile response to tetanic stimulation or drugs.[72,73] Overall, the functional complexity of a model should be balanced according to the results that need to be achieved: while partially mature muscle fibers expressing typical markers of muscle differentiation could be sufficient to drive the organ-specific differentiation of endothelial cells or to model fibrosis,[28] non-contracting fibers or isolated, contracting myotubes might not represent a suitable system to properly test drugs targeting muscle activity or to study basic mechanisms of muscle contraction.

As discussed for systemic models, increasing the complexity of the system leads to an exponential growth of data. Indeed, traditional image-based analyses can be integrated with omic techniques including single cell RNAseq, secretome analyses, and functional tests of muscle contraction to identify molecular pathways involved in muscle maturation and performance.[66] This huge amount of data requires the development of analytical methods to maximize the amount of information that can be extracted in order to go beyond simplistic correlations and generate mechanistic models of muscle patho-physiology.

Pathological models

In the following section, the discussion on complexity will mainly focus on cancer-related models as a general example of *in vitro* modeling dealing with the identification of potential therapies.

It is now well established that 2D cultures of cancer cells do not represent a reliable model to test anti-cancer therapies,[74] but research is starting to appreciate that also a different 3D organization can affect cancer cell behavior. Indeed, cancer cells organized into 3D spheroids have a transcriptional profile that is completely different compared to the same cancer cells embedded within 3D hydrogel microcapsules.[75] Nevertheless, the presence of a hydrogel surrounding tumor cells can be a factor to carefully consider since it can interfere with cell recovery for further transcriptional analyses. Together with the architecture, it is important to highlight how the composition of the tumor microenvironment can heavily affect the outcome of the model. For instance, the proliferation rate and response to FDA approved anti-cancer drugs were dramatically different when triple negative breast cancer cells were cultured within 3D bone-like cellular microenvironments compared to 3D monocultures.[76] In addition, the immune compartment should also be considered due to the tight interactions that it can establish with the surrounding cells in the tumor microenvironment. For instance, the presence of breast cancer cells was reported to skew the polarization of macrophages[76] or impair the cytotoxic behavior of natural killer cells.[77] Platelets and neutrophils can affect the extravasation of cancer cells and the formation of micrometastases.[78] These interactions can have huge consequences on the response to therapies and on the progression of tumors *in vivo*.[79] Moreover, since the properties of the biomaterial can skew the behavior of specific cell populations (e.g., endothelial cells grown in collagen or fibrin matrices), it can be argued that *in vitro* tumor angiogenesis studies testing the effects of drugs affecting both sprouting formation and cancer cell migration should carefully consider the composition of the matrix to extract meaningful information. Finally, while the combination of autologous matrix and autologous cells (i.e., primary or derived from pluripotent stem cells) is a pre-requisite for the development of personalized medicine models, the use of cell lines combined with standard matrix proteins (e.g., fibrin) represents the only feasible approach for those studies requiring higher-throughput (e.g., lead optimization).

Overall, extensive comparisons are still required to properly identify the advantages and drawbacks of different 3D configurations and to evaluate their degree of similarity compared to human tissues. However, it is now clear how the architecture and composition of the system can potentially affect the experimental outcome in terms of marker expression, response to therapy and drug resistance. Finally, it is important to tune the complexity of the architecture and model composition based on the specific application, whereby a large-scale campaign on the identification of a new lead could require simpler systems, while a basic cancer biology study might need the highest possible level of complexity (Figure 1).

Increased complexity and higher throughput both lead to a significant increase in the number of variables/samples that need to be analyzed, possibly through one or more omic techniques (e.g., transcriptome, epigenome, proteome, secretome). For instance, by combining the biofabrication of tumor vasculature in an injection-molded plastic array (i.e., IMPACT platform) with a high-content profiling of cellular features (e.g., vascular network area, network connectivity, tumor area) it was possible to analyze the effect of different cancer cell lines on vasculature formation and to screen anti-tumor drugs (i.e., 5-FU, Axitinib, Cetuximab) at different concentrations on both tumor and blood vessels.[63] Importantly, the resulting datasets are highly heterogeneous because they combine molecular, imaging and clinical data, hence posing novel challenges for their effective combination and interpretation. Furthermore, the use of human cells/tissues as well as the validation with clinical data often result in high inter-donor variability, which requires proper data classification to extract meaningful information. In order to fully analyze the huge amount of data that can be theoretically collected and develop predictive models, it is then necessary to introduce the use of artificial intelligence (AI) methods. Although not yet applied to MPS, several examples of AI for the interpretation of complex biological datasets are available. For instance, starting from bulk and single-cell RNAseq data of hundreds of cancer cell lines, a deep neural network-based approach was recently developed to predict the outcome of specific anti-cancer treatments. More into details, using transcriptomic data available for three melanoma patients pre- and post-treatment with RAF and MEK inhibitors, an AI model was able to predict the resistance to dabrafenib and trametinib.[80]

Overall, it is expected that basic biology studies generating huge heterogeneous datasets will mostly benefit from the effective integration with AI methods for the extraction of meaningful information. On the other side, those applications closer to the clinic (e.g., lead optimization, personalized medicine) will probably rely on simpler correlations between omic data and high-content imaging (Figure 1).

Regulatory compliance

As a final consideration, innovative pre-clinical models such as MPS must undergo validation to be implemented in the drug discovery pipeline. Hence, proper requirements should be defined from a regulatory point of view. Indeed, despite all the promising results, even the simplest MPS have not yet received any regulatory approval in order to be routinely used for drug discovery/screening or inform clinical decisions, although some early adopters in pharma industry are starting to exploit MPS for internal decision-making in various phases of drug development.[81] Considering this complex scenario, regulatory agencies have started to work together with researchers developing MPS to set acceptable quality standards for these novel approaches to finally exploit their full potential in drug discovery.[82] Importantly, before MPS can satisfy regulatory acceptance, they must fulfill the requirements for industrial acceptance, that is, they must be reliable, robust, and reproducible in their response.[83] In this context, some pioneering projects on liver and renal MPS have been carried out aiming at setting standards on quality controls, comparing MPS with traditional cultures and performing reproducibility studies in different laboratories. Challenges were encountered to assure the translatability of procedures, materials (especially for biological elements such as cell sources) and equipment, highlighting what needs to be done before widespread adoption can be envisioned in drug discovery applications.[84,85] In this context, the increasing complexity of MPS presents a further significant hurdle for standardization, since every system has its own peculiarities that are difficult to reproduce in different laboratories for independent qualification. A step forward can be the implementation of systems compatible with already existing laboratory standards, such as multiwell plates, fully compatible with biological and pharmaceutical workflows.

Future outlook

Open questions still linger for the MPS community: how far should we go in developing less reductionist *in vitro* models? In other terms, is complexity a mandatory requirement, intrinsically increasing the value of a model? Or rather complexity (i.e., architectural, compositional, functional, analytical) should be carefully balanced according to the specific application (e.g., basic biology, personalized medicine, lead identification/optimization, replacement of animal studies) and the desired throughput (Figure 1)? In order to find a proper answer, it would be critical to understand how well results obtained through high-throughput, simpler systems are in accordance with data collected from single-unit models, which are often already validated in animal studies and are generally characterized by higher complexity in terms of architecture, composition, and functionality. Furthermore, although with available technologies it is possible to biofabricate very complex replicates of native tissues, it is still necessary to validate whether the answers obtained by those models are predictive of what happens in human diseases. Indeed, one potential scenario is that even an extremely complex biofabricated tissue cannot fully replicate the effect of a specific drug observed in patients due to the fact that it is neglecting some features of the systemic complexity of the human body. These features include among the others a functional immune compartment, blood and lymphatic microcirculation, peripheral nervous system, signals regulating the endocrine and metabolic systems, as well as circadian rhythms. Thus, modeling the reciprocal interactions among these components could be the missing piece to properly mimic tissue function in physiology and disease. If this is the case, an effective *in vitro* model mimicking the response to drugs would necessarily connect multiple tissue units in a body-on-a-chip system.[86,87] Introducing such level of organ interconnection and complexity would imply a significant advancement both at the biofabrication and analytical level. For instance, the biofabrication of multiple tissue units would require different maturation windows for each compartment (e.g., the maturation of contractile heart tissue would be significantly different compared to the mineralization of bone), hence posing severe challenges regarding their proper integration into a single system. The generation of such a complex model would also require a significant step forward in the analyses that should be performed. Indeed, it would be fundamental to employ AI to develop advanced analytical models to analyze the relation between all the variables involved, from the molecular to the functional level (e.g., changes in heart rate associated with increased skeletal muscle exercise and glucose metabolism). This interaction network could be constructed exploiting probabilistic graphical modeling techniques (e.g., Bayesian networks). Such models can integrate different data types (e.g., heterogeneous omic data, high-resolution 3D imaging, electrical activity) and infer direct associations between them, for instance by testing for conditional pairwise independence relations.[88] Clarifying these aspects would be fundamental to properly design the next generation of pre-clinical models, with the final goal of fostering a more effective and efficient translation of R&D products into the clinical setting.[89]

Conclusions

The FDA has recently approved the Modernization Act 2.0, which sets a landmark for biomedical research. With this document the FDA does not ban the use of animals, rather it highlights the relevance of alternative, innovative approaches (e.g., MPS, organoids, digital twins) to speed up the development of novel drugs and reduce their currently disappointing failure rate during clinical trials. This Modernization Act is in line with the 3R principle of refining, reducing, and replacing animal studies and emphasizes that human-based alternative systems should be seen as complementary to animal models, rather than a threat for the more traditional biological community.[90] Having established the key contribution that MPS can have in drug development, it is now envisioned that the rational design of experiments using MPS goes beyond the simplistic selection of cells, reagents and basic culture conditions. Rather, a broader analysis that embraces complexity at multiple levels (e.g., architectural, compositional, functional, analytical) should be carefully performed and know-how acquired to tailor the model according to the desired throughput and clinical relevance of the specific application. In this view, the next generation of MPS should improve the reproducibility of the assays and implement a smoother integration of experimental results with clinical data toward a more effective translation of laboratory outputs.

LIMITATIONS OF THE STUDY

This perspective raised critical points related to the integration of complexity, throughput and clinical-biological impact of next-generation MPS. However, this discussion was streamlined through a few specific examples related to systemic, organ-specific, and pathological models. In this context, the authors mainly focused on microcirculation, musculo-skeletal tissues and cancer models, respectively. Additional discussions including other tissue/organ models are envisioned to provide an even more accurate scenario.

DATA AND CODE AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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AUTHOR CONTRIBUTIONS

S.B., C.A., and M.M. conceptualized the article and the figure; S.B. and C.A. wrote the draft; G.T., C.A., and S.B. prepared the figure; C.C. and M.M. revised the final draft.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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