



t⁴ Workshop Report *

Biology-Inspired Dynamic Microphysiological System Approaches to Revolutionize Basic Research, Healthcare and Animal Welfare

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Abstract

The regular t⁴ workshops on biology-inspired microphysiological systems (MPS) have become a reliable benchmark for assessing fundamental scientific, industrial, and regulatory trends in the MPS field. The 2023 workshop participants concluded that MPS technology as used in academia has matured significantly, as evidenced by the steadily increasing number of high-quality research publications, but that broad industrial adoption of MPS has been slow. Academic research using MPS is primarily aimed at accurately recapitulating human biology in MPS-based organ models to enable breakthrough discoveries. Examples of these developments are summarized in the report. In addition, we focus on key challenges identified during the previous workshop. Bridging gaps between academia, regulators, and industry is addressed. We also comment on overcoming barriers to trust and acceptance of MPS-derived data – the latter being particularly important in a regulatory environment. The status of implementation of the recommendations detailed in the 2020 report was reviewed. It is concluded that communication between stakeholders has improved significantly, while the recommendations related to regulatory acceptance still need to be implemented. Participants noted that the remaining challenges for increased translation of these technologies into industrial use and regulatory decision-making will require further efforts on well-defined context of use qualifications, together with increased standardization. This will make MPS data more reliable and ultimately make these novel tools more economically sustainable. The long-term roadmap from the 2015 workshop was critically reviewed and updated. Recommendations for the next period and an outlook conclude the report.

Plain language summary

The regular t⁴ workshops on biology-inspired microphysiological systems (MPS) have become a reliable benchmark for assessing trends in the field. Participants at the 2023 workshop concluded

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that the technology as used in academia has matured significantly, but that broad industry adoption of MPS has been slow. The primary goal of academic research is to accurately recapitulate human biology in MPS-based organ models to enable breakthrough discoveries. Participants commented on overcoming barriers to trust and acceptance of MPS-derived data, the latter being particularly important in a regulatory environment. They reviewed the status of implementation of the recommendations detailed in the 2020 report and conclude that communication between stakeholders has improved significantly, while recommendations related to regulatory acceptance still need to be implemented. Participants highlighted the need for further qualification and standardization. The long-term roadmap from the 2015 workshop was updated. Recommendations for the next period conclude the report.

1 Introduction

Workshops organized by the Center for Alternatives to Animal Testing in Europe (CAAT-Europe) on biologically-inspired microphysiological systems (MPS) have been held regularly every four years since 2015 (2015, 2019, 2023). They have become an effective overarching communication platform for all stakeholders. The results have been published as peer-reviewed, open access¹ workshop reports and provide guidance for the respective next phase of MPS development. The first report, published in 2016, introduced the novel field, provided definitions, reviewed the *status quo* against industry needs, and outlined an MPS roadmap for the next 25 years (Marx et al., 2016). Figure 1 illustrates the roadmap sketched in 2015.

The 2015 workshop participants expected the first single- and multi-organ chip MPS (Fig. 1 red and blue arrow, respectively) to be adopted for in-house toxicity testing and mode of action studies by end users in industry by around 2020. Subsequently, the advancement of the first single- and multi-organ chip-based tests into validated safety assays informing regulatory decision-making in drug development was envisioned to become a reality from 2020 onwards. The first human body-on-a-chip models (white arrow) were expected to be developed by academic laboratories by 2020, subsequently entering pharmaceutical laboratories for systemic toxicity testing, disease modeling, and on-chip clinical trials. Finally, academic research was expected to develop novel personalized “you-on-a-chip” solutions by ensuring an autologous single-donor nature of organs in body-on-a-chip solutions within the period from 2020 until 2030. Such personalized tools were expected to inform patient-specific precision medicine approaches. This roadmap reflected the US National Institutes of Health (NIH)/National Center for Advancing Translational Sciences (NCATS) vision of the use of MPS for disease modeling, efficacy studies, and on-chip clinical trials funded by the NIH Tissue Chip program¹ since 2012.

Following further development and expansion of the MPS field, the second report, published in 2020, discussed the achievements and challenges along the roadmap (Marx et al., 2020; Roth and MPS-WS Berlin, 2021). A decrease in research activities to establish body-on-a-chip systems was observed in academia, likely due to their extreme biological complexity and the much higher value and probability of the commercialization of single- and multi-organ chip solutions. Indeed, workshop participants observed an exponential increase in research and development of single- and multi-organ chip tools, with the first robust commercial platforms entering academic and industrial laboratories worldwide (Nguyen et al., 2023). The 2019 workshop identified a global stakeholder communication gap in the field of translational science and MPS adoption by the industry and significant hurdles in the qualification and validation of regulatory accepted MPS-based decision-making. The workshop provided detailed recommendations on how to approach these challenges.

Traditionally, the term MPS, also referred to as organ-on-a-chip, multi-organ chip or body-on-a-chip systems, has been used over the two previous reporting periods only for the most complex *in vitro* systems that involve at least one fluid flow through one or more three-dimensional (3D) tissue culture compartments. The latter can be mechanically or electrically coupled. With recent significant improvements in mimicking physiology in conventional static 3D tissue cultures, static bio-printed tissue cultures, static organoid or assembloid cultures, and static membrane-based, multicellular, barrier-organ model cultures, all these culture systems have been classified as MPS. The distinction is now made between static and dynamic MPS, and the focus of this CAAT workshop remains on dynamic MPS. Definitions of new approach methods, microphysiological systems, dynamic MPS, context of use (CoU), Good Laboratory Practice (GLP), and standardization are provided in the supplementary file².

The establishment of dynamic single- and multi-organ chip MPS has advanced significantly over the last four years. On the

¹ <https://ncats.nih.gov/research/research-activities/tissue-chip> (accessed 29.08.2024)

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Abbreviations: ADME, absorption, distribution, metabolism and excretion; AI, artificial intelligence; CNKI, China National Knowledge Infrastructure; CoU, context of use; GLP, Good Laboratory Practice; ICH, International Conference on Harmonization; IND, investigational new drug; iPSC, induced pluripotent stem cells; MPS, microphysiological systems; NAMs, new approach methodologies; QIVIVE, quantitative *in-vitro*-to-*in-vivo* extrapolation

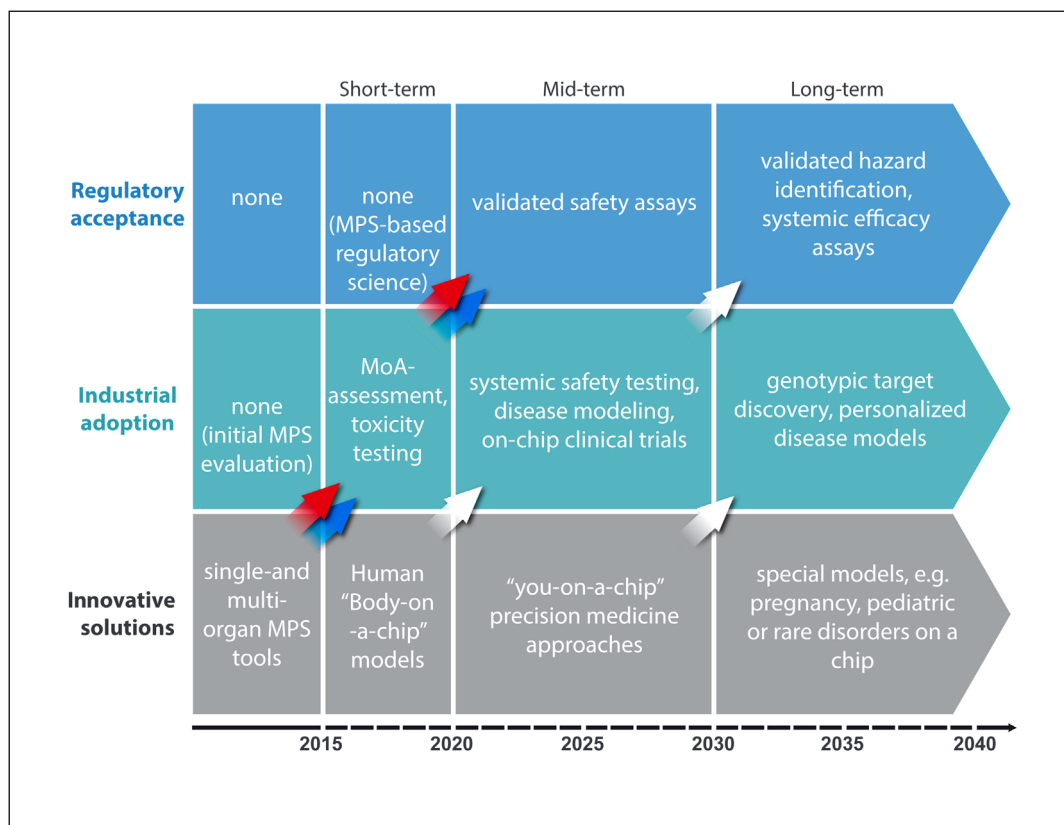


Fig. 1: Roadmap for the reduction and replacement of animals sketched in 2015

Red, blue and white arrows – translational impact of single-organ, multi-organ and human body-on-a-chip MPS-based approaches, respectively. The dotted black line marks the time the current workshop was held (adapted from Marx et al., 2016).

one hand, a wide range of new dynamic MPS developments have focused on not yet established single- or multi-organ models, with an ever-increasing degree of mimicry of human organ biology. On the other hand, the number of commercially available dynamic MPS platforms operating established MPS methods and their widespread distribution to academic research labs has increased exponentially, enabling an unprecedented surge in basic academic research and discovery activities using these dynamic MPS (Nguyen et al., 2023).

Consequently, the three working groups of the 2023 CAAT workshop focused their discussions on the following three questions:

- 1) How does one match human biology in MPS-based organ modeling? The latest trends and challenges in the field of novel single- and multi-organ chip model development were analyzed and summarized.
- 2) Do MPS-based models enable disruptive discoveries in human life science? Participants discussed whether the use of existing dynamic MPS tools in academic and industrial research has already led to new insights into disease mechanisms or new therapeutic discoveries.
- 3) How can MPS-based decision-making in healthcare, including regulatory acceptance, be achieved? Participants analyzed the progress in the use of dynamic MPS in drug development and regulatory aspects thereof. They reviewed the status of implementation of the recommendations from the previous workshop during the reporting period.

The workshop brought together 27 representatives from academia, the supplier industry, the end user industry, and regulators from nine countries for review and discussion and the subsequent writing of this report.

2 How does one match human biology in dynamic MPS-based organ modeling?

MPS have undergone a rapid evolution as *in vitro* tools to recapitulate human physiology by virtue of microsystems engineering and cell biology, yielding cell culture models that can display 3-dimensional organ-like architecture, enable multicellular interactions, establish tissue-tissue interfaces, provide fluid flow, and recapitulate aspects of organ-level mechanical cues.

The approximation of human biology by MPS along their entire development track has evolved in two directions. On the one hand, modeling of human organ and sub-organ structures has increasingly focused on the integration of fluid flow into their complex architecture and composition. Vascularization of organoid-on-chip models has been recently reviewed (Li et al., 2024). On the other hand, some laboratories aspired to increase the number of organ compartments in chips to ten and even more, with the goal to host the minimal number of essential “organ equivalents” interconnected by a physiologically arranged fluidic network for a potential self-contained homeostatic system resembling that of a human body. Several groups, including validation centers, are

focusing on the robustness and reliability of such highly complex networks in models and assays. However, due to significant complexity and lack of resources to establish all organ equivalents from a single donor, these body-on-a-chip development attempts have not progressed as envisaged during the previous reporting period. Nevertheless, some success towards physiology-based multi-organ chips comprising autologous single-donor organ equivalents has been reached with a four-organ chip MPS based on pluripotent stem cell (iPSC)-derived cells and organoids from a single donor (Koenig et al., 2022; Ramme et al., 2019). The status of body-on-a-chip developments necessary to achieve the vision was summarized in the previous report and elsewhere (Dehne and Marx, 2020; Marx et al., 2020). Workshop participants confirmed that progress in body-on-a-chip modeling was limited over the last four years. In addition to the enormous complexity of such systems, intellectual property issues associated with the use of iPSCs are hampering development. Instead, a recent trend emerged to use computational simulation and modeling to inform on existing gaps. However, the theoretical background and principles to establish biological body-on-a-chip models, now also called “organismoids,” have been published, providing guidance for the next development steps (Marx et al., 2021). Experts at the second MPS World Summit in Berlin 2023 estimated that the first proof-of-concept studies of organismoid MPS tools would take place within the next decade.

Modeling of a growing number of organs or organ substructures using dynamic single- or multi-organ MPS has recently been summarized in a meta-analysis of organ-on-a-chip research. Shoji et al. (2023) found that the range of organs and sub-organs recreated as dynamic MPS already includes 107 organs and organ substructures, yielding 1515 articles. The same meta-analysis identified 147 organs and organ substructures using static organoids published in 5714 research articles. The integration of organoids into dynamic MPS is an important current trend to improve human-like physiology of organ models and has recently been reviewed (Wang et al., 2024). The potential of organoid-on-chip for oncology research has recently been highlighted in another review (Zhu et al., 2023). Based on these trends, the workshop participants estimated that dynamic MPS could enable scientists to integrate and eventually study models of almost all relevant human organs and sub-organ structures within the next five to ten years.

Other equally important trends to improve human-like physiology of dynamic single- and multi-organ MPS are the integration of vascularization (Zhao et al., 2021), immunocompetence (Wang et al., 2023), mechanical coupling to mimic, e.g., lung breathing motion, gut peristalsis, skin stretching, mechanical loading of the skeleton by weight and movement, and electrical coupling to induce heartbeat or mimic neural signaling and innervation, as schematically illustrated and abbreviated as systemic biology in Figure 2. Many reports have addressed almost all aspects of the progress made in recent years in single- and multi-organ modeling. Comprehensive guides to this organ-on-a-chip landscape have recently been published (Ingber, 2022; Leung et al., 2022).

Workshop participants believe that progress in this field could be multiplied if developers leverage other disruptive technologies such as 3D bioprinting, materials science, automation and

robotics, nanotechnologies, artificial intelligence, and micro-actuator technologies to improve organ and system modeling. The integration of advanced imaging, digital pathology, omics technologies, and real-time and online-biosensor readout tools is expected to significantly increase the readout options and data quality and quantity of dynamic MPS (Fig. 2). The encouraging progress made in recent years in coupling and integrating sensors into MPS devices and platforms (Fuchs et al., 2021; Nahon et al., 2024) is a cornerstone for significantly improving the volume and quality of input data for computational simulation and feedback between computational and biological organ models.

To resemble human biology of dynamic single-organ MPS more closely, the workshop participants propose to further integrate healthy as well as diseased organoids, and to include adult stem cell niches and connective tissue of the respective organ. Furthermore, it was suggested to increase the complexity of physiological models beyond organoid complexity itself, and to establish pathophysiological models by integrating disease with support from *in silico* simulation. Prime examples of such a combination of experimental MPS data with computational modeling have been published for a gut-liver-on-a-chip device to model the quantitative *in vitro* pharmacokinetics of mycophenolate mofetil (Milani et al., 2022), and a liver-pancreatic islet chip device to model the dynamics of glucose-insulin regulation observed in the system, which provides mechanistic insight into disease progression features such as insulin resistance and β -cell dynamics. Furthermore, these *in silico* simulation and modeling approaches supported quantitative *in-vitro-to-in-vivo* extrapolation (QIVIVE), and thus, provided a tool for translating experimental insights to human outcome (Casas et al., 2022). Multi-organ chip systems, for example, have been used to quantitatively predict human drug pharmacokinetic parameters (Herland et al., 2020). Multi-organ MPS approaches should be improved through the integration of organoid and human-like single-organ MPS models, an expanded portfolio of stem cell organs and tissues, the establishment of systemic disease models, and increasing the *in vitro* longevity of MPS models to cover relevant clinical exposure regimens. The combination of organoids and tissues from autologous adult stem cells and iPSCs is another way to resemble human biology more closely. For example, the hDMT INFRA StemCells project, supported by the Dutch Research Council, integrates adult stem cell-derived organoids with iPSC-derived vasculature, immune cells, and/or neural cells from the same patient. Such models have the potential to achieve a paradigm shift by contributing to (personalized) disease cures based on correction of the underlying cause of a disease instead of treating its symptoms.

The integration of vasculature, immunocompetence (Goyal et al., 2022; Morrison et al., 2024; Wang et al., 2023), innervation, and mechanical and electrical signaling for dynamic single-organ and multi-organ MPS has been an important goal since the last review period (Marx et al., 2020), but a dynamic MPS platform covering all these aspects does not exist so far. Each step in this direction approximates the respective chip-based biological twin to its human patient counterpart regarding their treatment response. To this end, a deeper understanding of the dynamics driving the single- and multi-organ-level function in MPS mod-

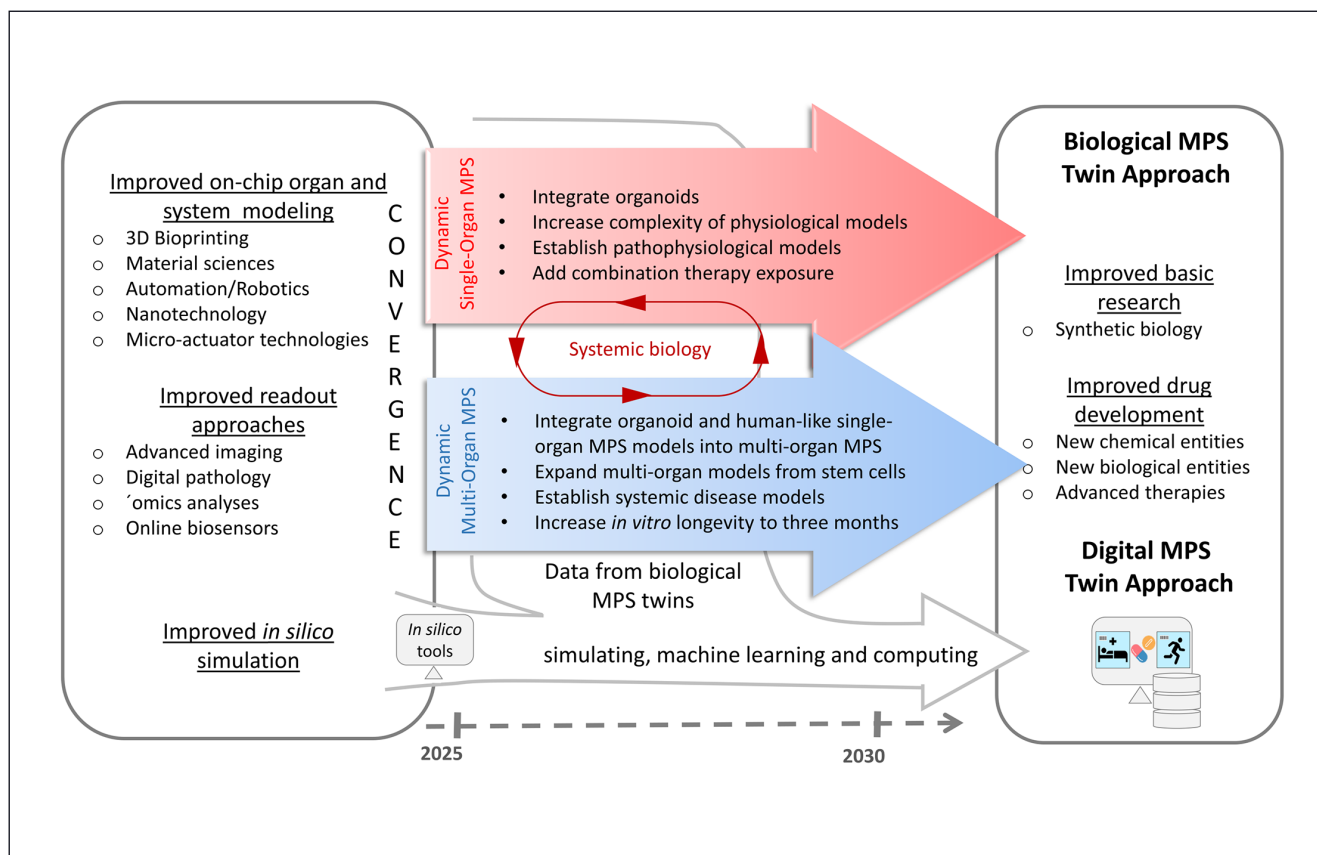


Fig. 2: Proposed technology convergence to match human biology and enable breakthrough discoveries

Human dynamic single-organ (red arrow) and multi-organ (blue arrow) MPS development along the proposed pathways (red and blue arrow, respectively) should include the establishment of systemic biology (circle with red arrows). Convergence with disruptive technologies supporting organ modeling and monitoring (left box), and basic research and drug development (right box) is essential for exponential progress towards biological MPS twins mimicking the patient's treatment response. The following integrated approach is the establishment of a corresponding digital MPS twin by simulating, machine learning and computing of relevant data within a certain context of use (white arrow).

els, in both stable and perturbed conditions, will be generated. More complex and time-resolved readouts will be established to provide the necessary database. The supplementary information from the meta-analysis mentioned above (Shoji et al., 2023) provides a search tool for readers who wish to learn more about the progress in some of these aspects.

Furthermore, a big chance and challenge for the coming development periods is the creation of digital MPS twins along each CoU. Digital twin technology is the process of using datasets to create a digital representation of a real physical object or process. Such virtual twins are created using software tools that combine simulation, data, machine learning and computation in a feedback data loop.

In brief, each MPS technology consists of

- 1) a physical object: the chip including culture compartments, pumps, and sensors;
- 2) a biological object: for example, healthy and diseased organ models, vasculature, and surrogate blood flow; and
- 3) a process for maintaining the biological object and its time-de-

pendent exposure to treatment (e.g., new chemical and biologic entities, advanced therapies).

The most relevant goal for such digital MPS twins is to create a digital representation of a biological MPS twin's response to a given therapeutic patient treatment to improve information access and prediction of therapy outcome. The well-known problems of interpretation and explainability of the results, which are currently inherent in most highly complex and opaque learning models, should be overcome with very large training data sets from the respective biological MPS twins. Here, the described implementation of sensors and complexification of biological MPS models may become a key success factor for the creation of such digital MPS twins. The increased platform costs of such an implementation are arguably fully rewarded by the added value of the established digital twin tools. Long-term data collection periods are envisaged to accumulate sufficient database sizes.

Finally, this approach of simulation, machine learning, and computation applied to disease modeling driven by biological MPS twins and patient data is envisaged to provide optimized

patient-centric predictions for a treatment outcome in a specific disease.

3 How will MPS-based models enable breakthrough discoveries in human life sciences?

In order to enable breakthrough discoveries in life sciences, a technology should have disruptive potential, transcend human (patho-)physiology, and combine value-added technology. The groundbreaking potential of dynamic MPS technologies for life sciences is undisputed: A recent meta-analysis of organ-on-a-chip research has shown that dynamic MPS already go beyond normal human physiology and have started to incorporate aspects of pathophysiology (Shoji et al., 2023). Sixty-six different disease types have been explored using dynamic MPS technologies, and results have been published in 2,181 peer-reviewed journal articles. In addition, dynamic MPS tools have been used in a significant number of articles on drug discovery in cardiovascular, hepatic, pancreatic, biliary, musculoskeletal, neural, respiratory, renal, and dermal areas. Thus, the expectation that dynamic MPS bear the potential for groundbreaking discoveries to a wide range of research applications, including the desired enablement of more human-relevant efficacy and safety studies of drug candidates and providing greater insight into the mechanisms of human disease, has been realized.

A recent review by the IQ MPS Affiliate introduced the present status of MPS disease models and described notable examples in six disease areas: cancer, liver/kidney diseases, respiratory diseases/COVID-19, neurodegenerative diseases, gastrointestinal diseases, and selected rare diseases (Baker et al., 2024; Irrechukwu et al., 2023). Research highlights of the use of dynamic MPS have been reviewed, *inter alia* in vascular disease modeling (Ingber, 2022; Shakeri et al., 2023) and basic genetic research (Palasantzas et al., 2023). Individual laboratories and research programs have applied various synergistic disruptive technologies over the reporting period to help improve the overall outcome of dynamic MPS experiments for drug discovery and development.

Moreover, workshop participants see significant benefit regarding speed and breadth of the drug discovery process if synthetic biology approaches – a combination of engineering principles with biotechnology techniques, such as genome editing to modify organisms or create new ones – converge with dynamic MPS to enhance basic research platforms (Fig. 2, right-hand box). In addition, the latest new medical developments could be explored using dynamic MPS to accelerate their adoption: Antibody formats, oligonucleotide therapeutics, extracellular vesicle therapies, and cell and gene therapies are prime candidates. The use of human lung chips to assess the delivery of adenoviral vector-mediated gene therapies (Li et al., 2019) and discover new broad-spectrum antiviral RNA therapeutics (Si et al., 2022) as well as a human kidney and liver organoid-based multi-organ-on-a-chip model to study the therapeutic effects of mesenchymal stromal cell-derived extracellular vesicles (Nguyen et al., 2022) are prime examples of the latter. A comprehensive review by the IQ MPS Affiliate describes how dynamic MPS can accelerate the development of

oligonucleotide therapeutics (Ramsden et al., 2022) and cell therapies (Candarlioglu et al., 2024).

Content-wise, the opportunities for new dynamic MPS approaches lie in the fields of:

- 1) Modeling complex human physiology and pathophysiology (e.g., developmental, metabolic, immune, behavioral neuroscience) and characterizing long-term, systemic and developmental health effects of environmental and drug exposures.
- 2) Translating across species in critical areas (e.g., cancer, psychiatry, ophthalmology, aging, reproductive health).
- 3) Human-specific models for assessment of biological therapeutics that are not adequately modeled in animal studies because the targets, therapeutic mechanisms, and toxicities are mostly human-specific.

Dynamic MPS approaches in the long term could also leverage their full potential in the area of precision medicine. The interplay between the *in vitro* simulation of, for example, treatment episodes, by biological MPS twins and *in silico* simulation of the same treatment episode for the respective CoU has the potential to generate the corresponding digital twin, which supports the optimization of biological twin-based treatment trials through a feedback loop. The combined use of the biological and digital twin approaches will lead to an ultimate level of prediction for the respective human counterpart twin – the patient.

Consequently, workshop participants sketched a hypothetical “best-cure” prediction based on individualized biological MPS twin therapy trial data. In addition, they proposed a strategy for stepwise *in silico* simulations, machine learning, and computing to generate a digital MPS twin for each patient’s treatment episode along such an MPS life cycle (Fig. 3).

A hypothetical “best-cure” prediction case was made exemplarily for two individuals of different ethnicities. It was hypothesized that the two patients donated tissue samples, allowing the creation of iPSC banks and the generation of adult stem cell-containing healthy and diseased organoids to better visualize the entire potential future life cycle of such a case. The iPSC banks are already widely used in basic research. They provide repeated, unlimited access to donor cells of well-characterized origin and quality. In addition, information on ethnicity, gender, genetics and much more is often available. However, reprogrammed iPSCs lose most of the epigenetic signature that is critical for development of a clinically relevant disease phenotype and therefore may not reflect changes in physiology relevant for diseases acquired during lifetime that do not have a genetic cause.

In a first step, a vial of iPSCs from each donor is thawed and all organoids and cell types of interest are generated to the required scale to assemble the respective healthy multi-organ set of identical MPS chips from each donor. The resulting organoids and cells are then loaded into the multi-organ chips, and long-term cultivation of a physiologically “healthy” MPS set is initiated (Fig. 3, upper left panel). In a second step, the disease in question is implemented in the required number of chips of this MPS set, for example, by infection, integration of a primary tumor or diseased primary organoids, or by the use of cells from donors with genetically inherited diseases. This results in a pathophysiological “diseased” MPS set that mimics the key features of the disease on

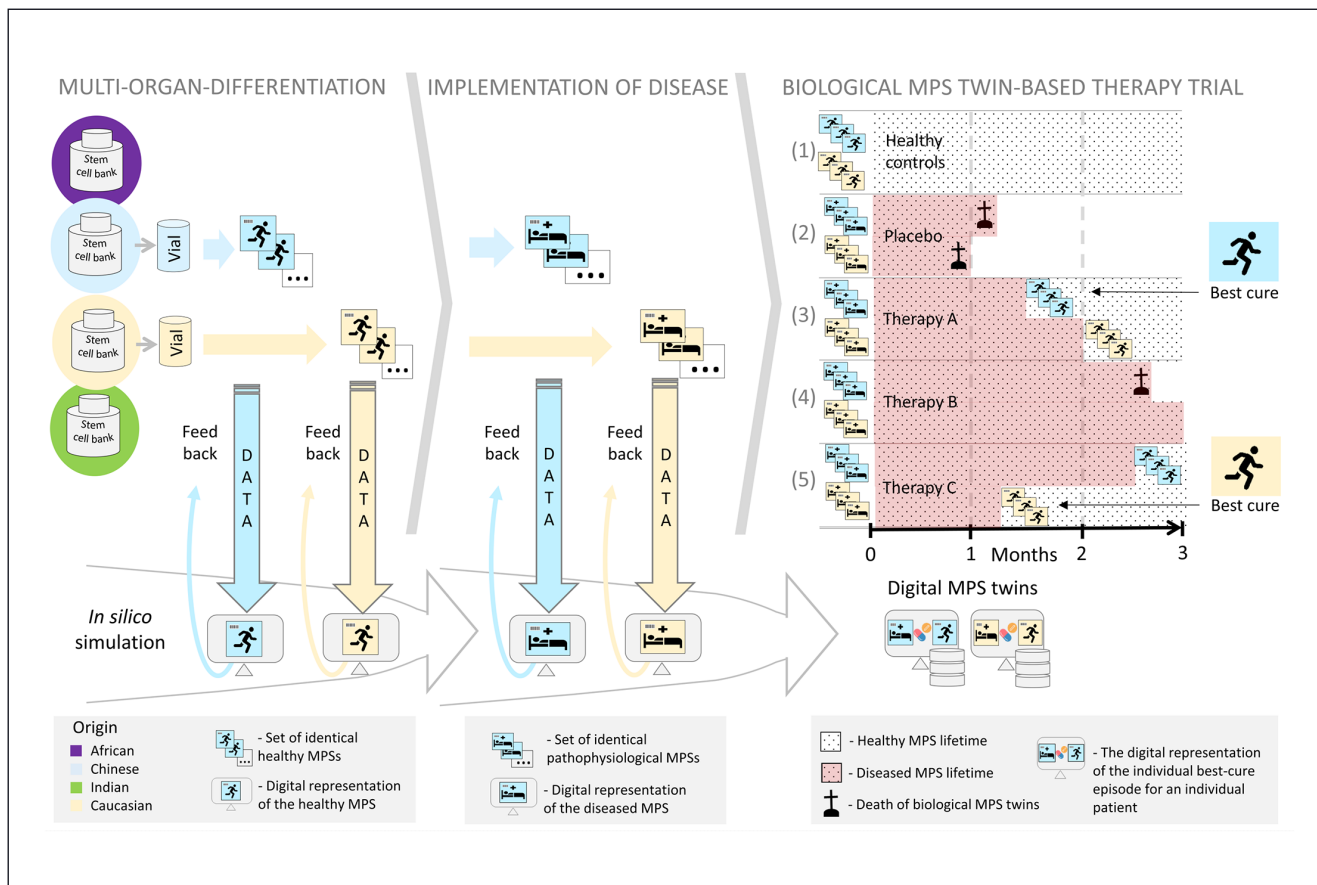


Fig. 3: The combined biological and digital MPS twin approach to revolutionize precision in healthcare and human life science

The upper section of the scheme illustrates the entire life cycle of a hypothetical biological MPS twin-based therapy trial to select the best cure for two patients of different origin (blue – Chinese; cream – Caucasian). It covers the multi-organ differentiation from stem cells donated by the patients (left panel), through the establishment of the patients' diseases (middle panel), finally exposing the biological MPS twins of the cell donors to different relevant therapies, resulting in time-dependent survival or death for each MPS set (right panel). The lower section illustrates the potential of the biological MPS twin approach to support the establishment of a digital twin approach. The latter includes *in silico* simulation of aspects of each patient's behavior based on data derived from the healthy and diseased biological MPS sets (left and middle panel). Finally, a digital representation of the treatment outcome for both patients (the digital MPS twins) can be generated.

the MPS-based multi-organ background of each of the two donors (Fig. 3, upper middle panel).

In a final step, the diseased biological MPS twin-set of each patient is divided into cohorts for each arm of the therapeutic MPS-based trial. Therapies may range from chemical and biological agents to cell and gene therapies or combinations of all of these. A healthy control arm consists of a cohort of the healthy MPS set. The long-term culture of the cohorts is continued until the primary endpoint, such as survival/death, is reached, and the data generated provide evidence for the selection of the best therapy, for example, the best approximation to the physiology of the healthy control cohort (Fig. 3, upper left panel).

The digital MPS twin approach illustrated in the lower panel of Figure 3 implies the creation of digital MPS twins for the patient-specific treatment episodes of each donor based on i) the *in silico* simulations of the first two steps, ii) the biological MPS treatment trial data of the third step, and iii) modeling of any other relevant

real-world data available for each patient and the respective type of disease. The digital MPS twins are generated through the application of *in silico* simulation, machine learning, computing, and artificial intelligence, whilst the *in silico* simulation itself can also provide feedback to optimize the respective biological MPS twin trial design or advance toward purely *in silico* clinical trial models.

Workshop participants are confident that if elements of such a hybrid biological and digital MPS twin strategy become a reality for the existing advanced biological MPS models at research level in academia and industry, the cross-fertilization of data in the field will grow exponentially. An ever-growing data stream from such healthy, diseased and treated MPS twins will enable training and feedback loops between the dynamic MPS and *in silico* models, subsequently accelerating breakthrough discovery research. Database needs and infrastructure should be considered early on to succeed with this strategy in discovery and research. Relevant

Tab. 1: Key performance indicators of the first three MPS World Summits

Numbers of:	New Orleans 2022	Berlin 2023	Seattle 2024
Countries represented	26	39	31
Attendees	665	1300	991
Posters	189	549	428
Exhibitors	33	78	78
Sponsors including exhibitors	52	97	99

qualification measures for both the biological and digital twin approaches should be considered in the longer term to translate the strategy to an ever-improving predictivity in drug development and precision medicine.

The workshop participants provided an overview of further recommendations for improving dynamic MPS modeling during the next period (see Box 1 in Section 7).

4 Has the global stakeholder communication gap been solved?

Participants of the previous workshop concluded that there was no effective global communication among the four key stakeholder groups: academia, suppliers, user industries, and regulators (Marx et al., 2020). As a consequence, they made several recommendations to improve the situation, and the current status is summarized below.

Recommendation

– *To establish an International MPS Society (IMPSS) representing stakeholder activities in North America, Europe, Asia, and other parts of the world, building on existing local society structures in the field.*

Achievement

The NCATS took the lead in a targeted solicitation of applications (NOT-TR-20-005) to identify a suitable candidate to establish and operate a world congress on MPS and an international society for MPS. NCATS also provided funding of US \$450,000 to expedite the process (Johns Hopkins University CAAT was awarded after the NIH peer review process).

The first MPS World Summit was subsequently held in New Orleans, USA, in 2022, the second in Berlin, Germany, in 2023, and the third in Seattle, USA, in 2024³. In 2023, the European Organ-on-Chip Society (EUROoCS) skipped its annual meeting in favor of the MPS World Summit in Berlin and integrated its topics into the MPS World Summit program. Table 1 illustrates the successful start of the series.

During the first two MPS World Summits, the International MPS Society (IMPSS⁴) was established; it was legally installed and fully operational by 2023. The IMPSS includes continental chapters, such as the Asia-Pacific Chapter or the European Chapter, where local societies, such as the Japan MPS Initiative⁵, coordinate activities and collaboration at their geographical level. The IMPSS maintains an overview of major activities and new developments in the field worldwide and shares and promotes knowledge to support the integration of end user requirements into early development to maximize the outcome and use of a given MPS-based model, method or assay. The IMPSS is responsible for organizing not only MPS-focused meetings, but also special sessions at other international conferences, such as the Society of Toxicology Annual Meeting and the World Congress on Alternatives and Animal Use in the Life Sciences.

Recommendations and respective achievements on the major goals of the society

– *Organization of a bi-annual world congress on biology-inspired MPS providing a communication platform for all stakeholders under the leadership of EUROoCS (with rotating locations).*

The first three MPS World Summits started on an annual basis, with locations in the US and Europe. The one in Berlin was co-organized with EUROoCS. In years in which the summit is organized outside Europe, EUROoCS will organize its own annual meeting at a European location. During the past few years, the number of participants at the annual EUROoCS meeting increased from 464 (EUROoCS 2022, Grenoble, France) to 664 (EUROoCS 2024, Milan, Italy), and the number of abstracts from 218 (56 oral, 162 posters) to 370 (64 oral, 274 posters).

– *Involvement of new stakeholders, such as patient groups, as soon as MPS platforms have matured to serving their needs.*

Patient groups will be involved in the IMPSS with the goal of communication and outreach and to increase the involvement of end users.

– *Active development and supervision of global ethical standards for the use of MPS-based technologies emulating human biology.* Ethical issues were occasionally raised in individual presentations and the roundtable discussions, but a sustainable platform for ongo-

³ <https://mpsworldsummit.com/> (accessed 14.08.2024)

⁴ <https://impss.org/> (accessed 14.08.2024)

⁵ https://www.nih.gov/phar/lab/MPS-kyogikai_HP/index1_en.html (accessed 24.09.2024)



ing discussion of ethical issues and agreement on standards remains a recommendation for the next period (see Box 1 in Section 7).

– *Coordinated information of policymakers and the guidance of governmental, nongovernmental organization-based and philanthropic funding programs (e.g., the Defense Advanced Research Projects Agency, Innovative Medicines Initiative 2, NC3Rs, Bill & Melinda Gates and Mark Zuckerberg Foundations) regarding funding bottlenecks in the field.*

NCATS has continued to pursue open and transparent information on tissue chip research in the US involving all interested groups. Comprehensive information on tissue chip projects and initiatives is openly accessible on the NCATS website⁶. As a result, US funding agencies/entities are informed about developments in the field at an early stage. NCATS continues to partner with US federal agencies, such as the Food and Drug Administration, National Aeronautics and Space Administration, Biomedical Advanced Research and Development Authority, Defense Threat Reduction Agency, National Institute of Standards and Technology, Environmental Protection Agency, and the Department of Veterans Affairs. The Bill & Melinda Gates Foundation has also been involved for some time in MPS-based project funding. In the current reporting period, for example, the University of Maryland, Baltimore, USA⁷ and TissUse GmbH, Berlin, Germany⁸ have been among the recipients of new funding for MPS-based development projects. At the same time, funds in Europe have also become more active in this area. On the one hand, large collaborative projects of the European Union, such as RISK-HUNT3R⁹, geneTIGA¹⁰, and TOP-GUT¹¹, have included MPS components in their programs, and on the other hand, projects fully based on MPS have been approved in the European Union, such as FLAMIN-GO¹², GUTVIBRATION¹³, EMAPS-Cardio¹⁴, TumorLN-oC¹⁵, and UNLOOC¹⁶, funded by the Chips Joint Undertaking and its members. In the Netherlands, two grants (€13.5 and €3 million) were awarded by the Netherlands Organization for Scientific Research to establish the national infrastructure hDMT INFRA with service centers of expertise (SCEs): hDMT INFRA StemCells¹⁷ and hDMT INFRA OoCDev¹⁸. In addition, the National Dutch Growth Fund awarded €76 million for the NXTGEN Hightech Biomedical Production Technologies pro-

gram¹⁹ and €125 million for a new Center for Animal-Free Biomedical Translation. The Wyss Institute at Harvard has also received funding from the US Biomedical Advanced Research and Development Authority²⁰ as well as the Gates Foundation²¹. The NC3Rs has awarded projects in different calls on MPS technology²². A comprehensive review of organ chip research in Europe was published recently (Leonel da Silva and Blasimme, 2023). A project in Japan focusing on the development of a key evaluation technology aiming at the industrialization of regenerative medicines and gene therapies has been funded by the Japanese Agency for Medical Research and Development (Ishida, 2021).

Thus, improved communication effectively bringing together these target groups, particularly in the US, Europe and Japan, has been established. The experiences gained using these communication channels can now be disseminated worldwide through the regional chapters/sections of IMPSS.

– *Establishment of sustainable workshops and training programs for young scientists and technicians in the field, across the globe.*

This recommendation refers primarily to the activities of the regional chapters and societies active in a certain geographical region or country. In Europe, EUROoCS has set up the first training activities for new professionals entering the field of dynamic MPS in the form of the entry-level EUROoCS Academy as well as the theoretical/hands-on EUROoCS Summer School series. In addition, the monthly EUROoCS webinar series has started, including research updates from senior and junior experts in the MPS/organ-on-a-chip field.

In Korea, the Korea Advanced Alternative Test Conference was held in 2023 to contribute to the development of the national biohealth industry by becoming a venue for meaningful international academic exchange and the networking of domestic and foreign industries, academia, research centers, and hospitals related to advanced alternative tests under the theme of “Meet the Future: The Advanced Alternative Test.”

Further recommendations on closing the communication gap

– *Academia: to support open access journals solely focusing on basic and applied science discoveries with microfluidic MPS.*

⁶ <https://ncats.nih.gov/research/research-activities/tissue-chip/projects> (accessed 14.08.2024)

⁷ <https://www.gatesfoundation.org/about/committed-grants/2020/06/inv016638> (accessed 14.08.2024)

⁸ <https://www.gatesfoundation.org/about/committed-grants/2022/10/inv-046428> (accessed 14.08.2024)

⁹ <https://cordis.europa.eu/project/id/964537> (accessed 14.08.2024)

¹⁰ <https://cordis.europa.eu/project/id/101057438> (accessed 14.08.2024)

¹¹ <https://cordis.europa.eu/project/id/101119911> (accessed 14.08.2024)

¹² <https://cordis.europa.eu/project/id/953121> (accessed 14.08.2024)

¹³ <https://cordis.europa.eu/project/id/953201> (accessed 14.08.2024)

¹⁴ <https://cordis.europa.eu/project/id/953138> (accessed 14.08.2024)

¹⁵ <https://cordis.europa.eu/project/id/953234> (accessed 14.08.2024)

¹⁶ <https://unlooc.eu> (accessed 14.08.2024)

¹⁷ <https://www.hdmt.technology/2023/02/20/13-5-million-euros-for-hdmt-infra-stemcells/> (accessed 14.08.2024)

¹⁸ <https://www.hdmt.technology/2023/05/10/over-e3-million-for-organ-on-chip-development/> (accessed 14.08.2024)

¹⁹ <https://nxtgenhightech.nl/en/biomed/> (accessed 14.08.2024)

²⁰ <https://wyss.harvard.edu/news/wyss-institute-at-harvard-university-wins-barda-contract-to-leverage-human-organ-chips-to-advance-knowledge-and-drug-discovery-for-broad-range-of-health-security-threats/> (accessed 14.08.2024)

²¹ <https://wyss.harvard.edu/news/human-cervix-modeled-in-microfluidic-organ-chip-fills-key-womens-health-gap/> (accessed 14.08.2024)

²² <https://nc3rs.org.uk/crackit/vitro-tdar>; <https://www.nc3rs.org.uk/crackit/sensoochip> (accessed 14.08.2024)



– *CAAT Europe: to repeat the CAAT stakeholder workshop on biology-inspired MPS on a four-year basis.*

The CAAT workshops on biology-inspired MPS have been held on a regular four-year basis so far, and respective t⁴ workshop reports are published with open access accordingly. The next CAAT workshop on biology-inspired MPS is envisioned for 2027.

In summary, the workshop participants concluded that the stakeholder communication gap, referred to in the 2020 report, has been effectively closed by the implementation of an annual MPS World Summit since 2022 and the establishment of the IMPSS including its regional chapters. There has been less progress regarding the recommendations on regulatory acceptance and related qualification activities. However, the FDA Modernization Act 2.0²³ in December 2022 opened the possibility that qualified organ-on-a-chip (MPS) solutions could serve as valid preclinical evaluation tools. Ultimately, such use of alternative test methods would help reduce the number of animals used in drug testing. The European Medicines Agency (EMA) clearly outlines in its *Regulatory Science Strategy to 2025*²⁴ recommendations to leverage the use and qualification of novel *in vitro* and *in silico* methods, such as complex *in vitro* systems, including MPS, in adherence with the 3Rs. These efforts are coordinated by the EMA 3Rs Working Party and encompass applications for human and veterinary medicinal products.

The workshop participants provided a breakdown of their further recommendations for improving community communication through IMPSS in the next period (see Box 2 in Section 7).

5 How does one achieve regulatory accepted MPS-based decision-making?

The 2019 workshop (Marx et al., 2020) identified a “regulatory acceptance dilemma” as, from a regulatory perspective, none of the MPS-based assays used in industry for internal decision-making had been either qualified or validated such that data could be used for the drug approval process. The low level of standardization of MPS instruments and chips and the still limited in-house level of characterization of MPS-based assays were identified as major challenges. The participants of the 2019 workshop recommended actions along two lines to accelerate regulatory acceptance of MPS-based decision-making in the future: i) supporting MPS-based assay qualification in industry and ii) addressing the regulatory acceptance dilemma at the level of regulatory authorities. Here, we summarize the progress made on each of the recommendations to achieve regulatory accepted MPS-based decision-making.

Recommendations supporting MPS-based assay qualification activities, followed by the respective progress achieved within the reporting period

– *All stakeholders: to foster the further growth and establishment of centers for the characterization and qualification of MPS-*

based methods in America, Europe and Asia under the guidance of regulators. Involve all stakeholders in co-funding of such centers and ensure the coordination of the characterization and qualification programs of such centers to avoid redundancy across the globe.

– *End-users (pharma, biotech): to jointly fund or support existing funding for MPS-based assay qualification in various settings, including centers of excellence (virtual contract research organizations are also to be considered), to qualify MPS-based assays centrally for a given CoU. This should lower the hurdle for late adopters of MPS-based assays in large pharmaceutical firms and for smaller pharmaceutical companies and biotechs.*

The COVID-19 pandemic severely limited the ability to establish new testing centers during this reporting period. However, progress has been made with the existing US Tissue Chip Testing Centers: the Texas A&M Tissue Chip Validation Centre and the Translational Center of Tissue Chip Technologies for Quantitative Characterization of Microphysiological Systems at Massachusetts Institute of Technology received start-up funding from the NIH in 2016²⁶. Tissue Chip Testing Center teams from both centers became financially independent and self-sustaining by 2021. The Massachusetts Institute of Technology group founded Javelin Biotech, a venture-backed company focusing on tissue modeling for predictive drug discovery. The Texas A&M University team formed the Texas A&M Tissue Chip Validation Consortium to promote the use of tissue chips by industry and regulatory agencies. At the same time, a Tissue Chip Data Center was funded at the University of Pittsburgh to establish databases for each organ platform. It recently reported significant success in using such databases to create *in silico* models of complex diseases (Negi et al., 2023) and for the mechanistic understanding of the mode of action of macromolecular drug candidates (Lefever et al., 2022). These success stories were shared with the community during the MPS World Summits. As a result, activities to establish such centers in other regions of the world have been intensified since 2023. A first step was taken in Korea to establish a dynamic MPS qualification center: Osong-Korea MPS Validation Center, which is collaborating to support Korea’s leap as a global biohealth powerhouse by becoming the main driving force in the commercialization of 3D MPS²⁶. A national infrastructure of SCEs, called hDMT INFRA, is now being established in the Netherlands that will make MPS/organ-on-a-chip-related technologies available to all users from academia and industry. The first SCEs are currently being set up within hDMT INFRA and will be focusing on human adult stem cells and iPSCs. The hDMT INFRA StemCells enable a population of organ-on-a-chip models with adult stem cells and iPSC derivatives to generate optimal human organ and disease models. This will be realized by providing a broad range of services to the research community via three complementary SCEs located at the medical centers in Leiden, Utrecht and Rotterdam. The services will include independent testing and qualification of the models.

²³ <https://www.congress.gov/bill/117th-congress/senate-bill/5002>

²⁴ https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection_en.pdf

²⁵ <https://ncats.nih.gov/research/research-activities/tissue-chip/projects/centers> (accessed 14.08.2024)

²⁶ <https://www.ok-mps.kr/> (accessed 14.08.2024)



- *End-users (pharma, contract research organizations): to become members of the IQ Consortium and actively pursue MPS-based assay qualification strategies there.*

The IQ MPS Affiliate has grown to 26 members as of the end of 2023, with Boehringer Ingelheim, UCB, Incyte, Roche, Servier and Daiichi Sankyo joining during this reporting period. The IQ MPS²⁷ is a collaboration of pharmaceutical and biotechnology companies created as an affiliate within the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ-Consortium). It provides a global venue for appropriate cross-pharma collaboration and data sharing to facilitate the industry implementation and qualification of MPS models. The five working groups are active in the most pressing translational areas for MPS: strategic industrial partnership, regulatory outreach, pilot projects, organotypic manuscripts, and landscape survey. The most recent work of the IQ MPS Affiliate relevant to the topics of this report is referenced in the appropriate chapters.

Recommendations addressing the regulatory acceptance dilemma and respective progress achieved within the reporting period

- *End users and suppliers: to make internal feasibility studies on MPS-based tests with a given fit-for-purpose publicly available, wherever possible.*
- *End users, suppliers and regulators: to generate use cases for MPS-based assays under the supervision of an end user.*

The first dynamic MPS data being submitted in 2019 as supporting evidence in an investigational new drug (IND) application was based on a dynamic MPS-based model applied for a rare disease with Sanofi (Rumsey et al., 2022). The only new pharmacology data in this particular IND was from the Hesperos conduction velocity model for chronic inflammatory demyelinating polyneuropathy. This supported a Phase II clinical trial²⁸ that has proceeded to a Phase III trial²⁹. Since then, a growing number of feasibility studies and use cases for the application of dynamic MPS-based assays have been initiated by end user industries.

Data from a study using a human lung alveolus chip that experiences cyclic breathing-like deformations to investigate whether physical forces influence innate immune responses to viral infection were included in an IND application to the FDA requesting entry of azeliragon into human clinical trials for the treatment of viral pneumonia in hospitalized COVID-19 patients (Bai et al., 2022).

Another prime example is the effort of qualifying a dynamic liver organ chip in the prediction of small molecule drug-induced liver injury CoU published by scientists from Emulate Inc., an organ-chip developer (Ewart et al., 2022). The liver-chip demonstrated 87% sensitivity and 100% specificity in this CoU, following exposure to 27 small molecules, across three independent hepatocyte donors.

Furthermore, a proof-of-concept study combining 3D models, microwells, and a standardized microfluidic platform into a liver

chip has been published by scientists from the University of California, Berkeley (Cox et al., 2022).

A recent example using a dynamic human MPS-based bone marrow model for toxicity assessment has been published by AstraZeneca scientists (Cairns et al., 2023).

A dynamic MPS modeling a perfused blood vessel has been applied to model the effects of snake venoms to establish an efficacy assay for the treatment of snake bites (Bittenbinder et al., 2024)

A team at Beiersdorf evaluated the route-specific metabolism and toxicodynamic effects of genistein in a human skin-liver chip. The rate of metabolism was in accordance with the short half-life observed *in vivo* in humans. The route-specific information on the metabolic fate and toxicodynamics derived from this MPS may be relevant to safety assessment (Tao et al., 2024).

A team at Abbvie investigated an MPS approach to determine pharmacokinetics of pro-drugs, a strategy used to address poor permeation following oral administration. The MPS approach outperformed conventional models, such as isolated enzymes and transwells (Sharma et al., 2024).

The preparation of another use case exploring a dynamic MPS platform was announced recently. After a successful EMA Innovation Task Force briefing held on April 24, 2024, an interdisciplinary consortium of scientists from six pharmaceutical companies and an MPS platform supplier started the preparation of a human ring trial to evaluate the reproducibility and accuracy of a liver MPS in predicting drug-induced liver injury and intrinsic clearance³⁰.

While these individual drug program examples or proof of concept studies are encouraging, the majority of such studies remain unpublished or – in the case of an active drug molecule under development – are published only after a defined lead time due to their confidential nature. When included as supporting evidence data in an IND or clinical trial application, or later in a marketing authorization application, they are, at least, becoming known to the respective regulatory authorities at a certain point in time.

Workshop participants expect the community to witness exponential growth of the number of publicly accessible industrial case studies exploring dynamic single- and multi-organ MPS in a defined CoU in the coming years. A panel of 27 authors representing the combined expertise of 16 pharmaceutical companies in absorption, distribution, metabolism and excretion (ADME) science, preclinical safety assessment, investigational toxicology, clinical pharmacology, pharmacokinetics, dynamics and metabolism, early development, and discovery biology elaborated on the *status quo* and perspectives of *in vitro* new approach methodologies (NAMs), including dynamic MPS, as alternatives to animal use (Stresser et al., 2024). They predicted the path to CoU qualification, routine regulatory acceptance, and widespread deployment of *in vitro* NAMs in the safety assessment of drug candidates to be gradual and span decades. These considerations of timelines in the qualification of *in vitro* NAMs, including dynamic MPS-based assays, in the context of their use for safety and toxicity assessment

²⁸ <https://clinicaltrials.gov/study/NCT04658472> (accessed 14.08.2024)

²⁹ <https://clinicaltrials.gov/study/NCT06290128> (accessed 14.08.2024)

³⁰ https://www.pressebox.com/pressrelease/tissue-gmbh/Novel-Liver-Ring-Trial-Set-to-Revolutionize-Drug-Safety-Assessment/boxid/1202701?utm_source=Belegmail&utm_medium=Email&utm_campaign=Aktiv (accessed 29.08.2024)

to support regulatory decisions have been supported by an FDA/Center for Biologics Evaluation and Research perspective (Avila et al., 2023). In addition to detailed explanations of technical validation requirements, the publication highlights that biological and toxicological models and assays can be qualified. The qualification process for NAMs at the Center for Drug Evaluation and Research is intended to establish that a method can be used repeatedly for a particular regulatory CoU without requiring submission of validation data for each use. The EMA has set up a specific free-of-charge forum for early dialogue under the Innovation Task Force. This is open to all stakeholders, including pharmaceutical companies, small and medium sized enterprises, academics and researchers, and public/private funded consortia, such as the Innovative Medicines Initiative³¹. This dialogue can guide stakeholders towards EMA qualification. Ultimately, the level or rigor of qualification that a dynamic MPS-based method must achieve depends on how the resulting data will be used within the regulatory decision-making process (i.e., the CoU). If a NAM is submitted for review as supporting evidence, qualification may not be necessary, and if it is used to explore the potential pharmacology of a drug or to screen out candidates with a potential safety liability, it most likely will not require qualification by a regulatory agency such as the EMA or FDA. The EMA already accepts MPS data use to demonstrate the pharmacology of a drug. The FDA encourages the submission of such data, but these uses, unlike a NAM used to inform a regulatory decision, will be less critical to regulatory safety decisions.

A brief look at the validation history of the only static MPS-based human complex *in vitro* model to be included in an Organisation for Economic Co-operation and Development (OECD) test guideline for chemical/cosmetics testing is instructive in terms of the approach and timeline for achieving regulatory acceptance in this particular area. The MPS-based model is a static, insert-based, reconstructed model of the human epidermis at the air-liquid interface. Validation efforts spanned 13 years until an initial assay was validated and translated into OECD TG 431 to test chemicals for skin corrosion activity in 2019 (OECD, 2019). Subsequently, OECD TG 439 was released in 2021 to test skin irritation using the same model (OECD, 2021). Cosmetics Europe, the European trade association for the cosmetics and personal care industry, engaged in a program in 2017, proposed by the leadership of Beiersdorf AG, aiming at evaluating the added value of a dynamic MPS co-culture of such reconstructed human epidermis models integrated into the commercially available microfluidic HUMIMIC platform of the supplier TissUse GmbH. The Chip2 platform supports circular interconnection of the validated human skin model with a qualified human spheroid 3D liver model. The validation program led by the end user Beiersdorf and supervised by Cosmetics Europe's board has resulted in distinct qualification expertise and generated a set of unique data investigating the pharmacokinetics, first path metabolism, toxicokinetics, and toxicodynamics of se-

lected substances (Brandmair et al., 2024; Kühnl et al., 2021; Tao et al., 2021, 2024). This qualification process, driven by various use cases, continuously improved the performance parameters of the model and the assays for the respective CoU. It has since led to investigations replacing the epidermis model in the dynamic two-organ chip with a reconstructed human full thickness skin model. The program generated reproducible data on selected CoUs in a human dynamic two-organ model test setting, and thus provides a good basis for further validation efforts in the field of chemicals testing, for example, toxicokinetics. The expertise gained can now be applied equally effectively to validation programs in drug development. A validation process (including continuous quality control measures) that deviates from the classical OECD procedure, but promises to be much faster, has been proposed (Pamies et al., 2024)

– *Regulators: to produce a position paper on aspects of the regulatory acceptance of biology-inspired MPS-based assays for drug testing under the supervision of the US FDA involving American, European and Asian regulatory agencies. This should guide sponsors to learn how the agencies deal with the data based on case examples.*

Again, the COVID-19 pandemic severely limited international face-to-face exchanges for at least two and a half years. Nevertheless, the well-established communication structures in the US between the IQ MPS Affiliate and the FDA, as a result of the NIH Tissue Chip Program, enabled a workshop to be held on the perspectives of the evaluation and adoption of complex *in vitro* models in drug development (Baran et al., 2022). Interaction with regulators from other countries and regions was resumed immediately after the end of the COVID-19 pandemic at the three MPS World Summits. The recommendation remains in place for the next period (see Box 1 in Section 7).

An EMA guideline on the principles of regulatory acceptance for 3Rs testing approaches was drafted in 2011. Regulatory acceptance should be seen not only as the incorporation of a new 3R testing approach into a regulatory testing guideline, which can be an EMA guideline, but also as an international guideline issued by the Human (ICH) or Veterinary International Conference on Harmonization. Regulatory acceptance also encompasses the case-by-case acceptance of results obtained by using a new 3Rs testing approach not (yet) included in a specific guideline for regulatory decision-making. High-level criteria for regulatory acceptance are provided in the guideline, including the availability of defined test methodology, the demonstration of both relevance and limitations within a specific CoU, and reliability and robustness. The guideline also allows for the voluntary submission of data generated with the 3R testing approach in parallel with the data generated using existing methods, for the sole purpose of the evaluation of regulatory acceptability. The EMA held a workshop in 2017 to foster early dialogue between the regulatory environment in Europe, end users, and method developers to drive collaboration between all

³¹ [https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines#ema's-innovation-task-force-\(itf\)-section](https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines#ema's-innovation-task-force-(itf)-section) (accessed 14.08.2024)



the stakeholders involved on the qualification needs for MPS technologies. The EMA 3Rs Working Party is currently revising its guideline on the principles of regulatory acceptance for 3Rs testing approaches to include annexes comprising specific CoU qualification criteria for MPS, including organ-on-a-chip models³². In addition, an International Medicines Regulators Working Group on 3Rs has been set up to strive towards harmonization regarding regulatory acceptance criteria for NAMs.

– *Regulators: to establish a standardized annual meeting format to convene a group of regulators from America, Europe, Asia and other geographies in the drug, food and biologics space to coordinate regulatory science, track and analyze MPS-based data arrival at a regulatory level (e.g., Investigational Medicinal Product Dossier, IND), and organize the development of ICH guidelines for those MPS-based assays which make it to replace existing animal-based ICH guidelines.*

This recommendation could not be implemented in the previous reporting period due to the pandemic and will, therefore, be carried forward to the next period (see Box 3 in Section 7).

The workshop participants concluded that significant progress was made in qualifying MPS-based assays for the nonclinical testing of drug candidates and internal decision-making in the pharmaceutical and biotechnology industries. In addition, *in vitro* NAMs in general and dynamic MPS-based assays in particular have gained political support and global visibility, at least in the US and Europe, in the context of reducing the use of animals in testing. This was evidenced by new high-level policy statements during the reporting period: the EMA Actions to Reduce Animal Testing in 2021³³ and the US FDA Modernization Act 2.0²³. The latter did not change any existing regulations, but specifically mentioned cell-based assays, MPS (such as organ chips), and bio-printed or computational models as part of the nonclinical (previously preclinical) testing portfolio (Adashi et al., 2023). These activities have attracted a level of public attention only matched by the full European Union (EU) ban on animal testing for cosmetics, which entered into force in 2013, a deadline set by the EU Council and Parliament in 2003, and by the commitment of the US Environmental Protection Agency in 2019 to phase out all mammalian testing by 2035 (The US Environmental Protection Agency has since abandoned the latter deadline (Grimm, 2024)). Nonclinical testing methods have also already encompassed the models in the EU mentioned above. The current proposal for the reform of the EU pharmaceutical legislation³⁴ explicitly defines “nonclinical” as a study or a test conducted *in vitro*, *in silico* or *in chemico*, or a nonhuman test related to the investigation of the safety and efficacy of a medicinal product. Such a test may include simple and complex human cell-based assays, MPS, including organ-on-a-chip, computer modeling, other nonhuman or human biology-based test methods, and animal-based tests.

Despite these statements and some corresponding programs, there are only a few examples of NAMs, none of which are from

the MPS universe, that have already been submitted to CDER and contributed to product safety evaluation, for example, *in vitro* assessments of skin sensitization and phototoxicity potential. However, these and other readily available and validated NAMs only address a limited number of nonclinical issues. Nonclinical data generated from NAMs can currently be submitted to existing applications along with the information necessary to demonstrate their validity (Avila et al., 2023). A hurdle for dynamic MPS is the limited commercial availability of only a few well-characterized MPS models for industrial decision-making. By comparison, about 50 tissues are typically evaluated in nonclinical animal studies. Great progress has been achieved here with the implementation of a wide organ portfolio into dynamic MPS in the academic setting over the last few years (Shoji et al., 2023). However, their CoU qualification remains a mammoth long-term task.

In practice, none of the dynamic MPS-based methods used in the pharmaceutical industry have yet been qualified for pivotal decision-making in an informed regulatory environment, such as an approval process. Figure 4 illustrates the quality management landscape envisioned for the use of dynamic MPS in healthcare.

The ICH guidelines provide guidance on the requirements for the approval of new drugs, such as new chemical entities, biological entities, or cell and gene therapies, while precision medicine approaches for patients are governed by *in vitro* diagnostics regulations supported by quality requirements, such as ISO 13485 / 21CFR820. GLP standards are mandatory for pivotal safety studies in the pharmaceutical industry and for performing *in vitro* diagnostic tests for patients in clinical test laboratories. In addition to these two end user groups of dynamic MPS, the contract research organization industry could establish contract safety testing for pharmaceutical sponsors who require this high-end quality background (Fig. 4, upper dashed squares with yellow filling).

GLP laboratories are subject to regular external audits by certifying bodies. To the best of the workshop participants’ knowledge, dynamic MPS are not currently operated in these laboratories. Most of the research and development activities with dynamic MPS are carried out in conventional research laboratories, with or without established in-house standardization rules. The expertise of the scientists involved defines the level of quality in these laboratories. Laboratories from all stakeholder groups – academia, suppliers, end users and regulators – fall into this category (Fig. 4, dashed squares with white filling). Quality standards in laboratories in the pharmaceutical industry are driven primarily by legal and intellectual property rights considerations.

Guidance documents to standardize *in vitro* technologies used in the field have been developed in the past. A Good Cell and Tissue Culture Practice (GCCP) 2.0 guideline was developed for the operation of *in vitro* NAMs, including dynamic MPS (Pamies et al., 2022), to which many of the research laboratories using dynamic MPS voluntarily adhere. This has been further specified particularly for MPS (Pamies et al., 2024). The OECD GD 289 on

³² https://www.ema.europa.eu/en/documents/scientific-guideline/concept-paper-revision-guideline-principles-regulatory-acceptance-3rs-replacement-reduction-refinement-testing-approaches_en.pdf (accessed 14.08.2024)

³³ <https://www.ema.europa.eu/en/news/ema-implements-new-measures-minimise-animal-testing-during-medicines-development> (accessed 14.08.2024)

³⁴ https://health.ec.europa.eu/medicinal-products/pharmaceutical-strategy-europe/reform-eu-pharmaceutical-legislation_en (accessed 14.08.2024)

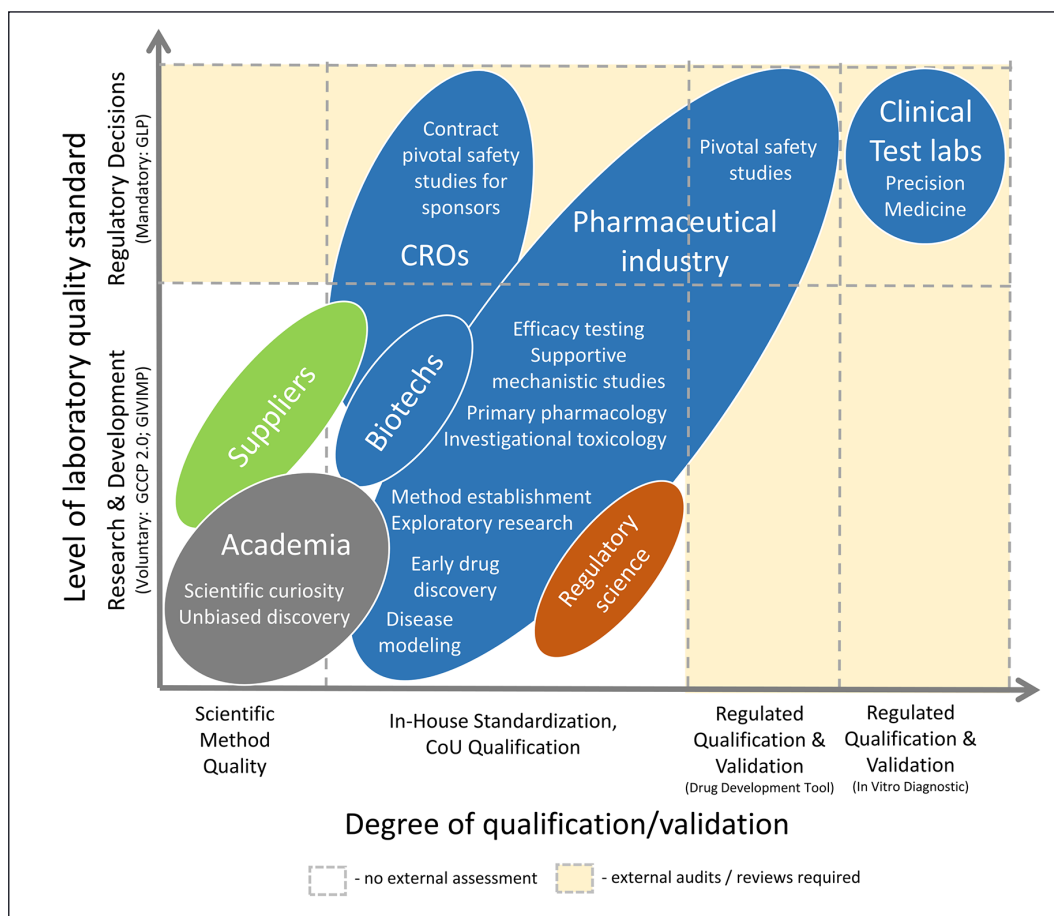


Fig. 4: The quality management landscape envisioned for the use of dynamic MPS-based methods in healthcare

The level of the quality standard of academic (grey), supplier (green), regulatory (brown) and end user (blue) laboratories is plotted against the proposed degree of standardization of methods, depending on their intended use. Dashed squares filled with yellow indicate the quality levels that require external audits.

Good In Vitro Method Practices for the development and implementation of *in vitro* methods for regulatory use in human safety assessment (GIVIMP) was published in April 2018³⁵. The guidance aims to reduce the uncertainties in predictions derived using cell and tissue-based *in vitro* methods by applying all the necessary scientific, technical and quality practices from *in vitro* method development to *in vitro* method implementation for regulatory use. Finally, the IQ MPS Affiliate has recommended guidelines for the development, qualification and implementation of complex *in vitro* models, including dynamic MPS, to promote qualification efforts (Ekert et al., 2020). Workshop participants emphasized that the time to qualified regulatory accepted methods for use in decision-making for pharmaceuticals can be very different, depending on the industry segment (modality-dependent). This results in different qualification requirements that can be met by a flexible quality management and fit-for-purpose qualification approach (Pamies et al., 2024).

Groundbreaking basic research, early discoveries, and most scientific working hypotheses on disease mechanisms supported by

dynamic MPS originate from academic laboratories. Such pharmaceutical and biotechnology laboratories can use dynamic MPS for disease modeling, early drug discovery, exploratory research, primary pharmacology, prospective and retrospective investigational toxicology, and efficacy testing. Regulatory agencies conduct regulatory science based on dynamic MPS in these types of laboratories. The MPS supply industry operates such laboratories to develop and standardize biological models and CoU assays.

The workshop participants provided a breakdown of their further recommendations for accelerating regulatory decision-making in the next period (see Box 3 in Section 7).

6 How can important MPS-related research published in China be made accessible?

China has caught up with the USA in recent years as the world's leading country in terms of both scientific research output and high-impact studies (Wagner et al., 2022)³⁶. The country pub-

³⁵ <https://www.oecd-ilibrary.org/docserver/9789264304796-en.pdf> (accessed 14.08.2024)

³⁶ <https://sj.jst.go.jp/news/202309/n0928-03k.html> (accessed 14.08.2024)



lishes a significant proportion of its scientific literature in Chinese. A recent global meta-analysis of organ-on-a-chip research publications deposited in the literature databases EMBASE via Ovid³⁷, PubMed³⁸, Scopus³⁹ and Web of Science⁴⁰ after 2010 identified 2,181 relevant research articles, of which the USA accounts for 812 articles (37%), followed by the European Research Area with 746 articles (34%), and China with 202 articles (9%) (Shoji et al., 2023). The meta-analysis used only English language databases.

Workshop participants were interested in finding out whether there is a valuable Chinese language literature stream on biology-inspired MPS that those community members who are not fluent in Chinese would miss due to language barriers. To get a rough understanding of the organ-on-a-chip landscape that is only publicly available in Chinese, the workshop participants compared the number of hits for scientific publications in the China National Knowledge Infrastructure (CNKI) database with the hits in PubMed using the same organ-on-a-chip-related search term catalogue. The CNKI covers various disciplines, including science, engineering, agriculture, medicine, social sciences, law, education, humanities, economics, management, engineering, and technology. The database includes academic journals, master's and doctoral theses, conference proceedings, scientific reports, and technical standards, primarily published in the Chinese language. In particular, CNKI has collected more than 400,000 doctoral dissertations and 5 million master's theses, most of which contain unpublished research not covered by EMBASE, PubMed, Scopus or Web of Science. In comparison, the PubMed database contains more than 36 million citations and abstracts of biomedical literature. It does not include full-text journal articles; citations in PubMed are primarily from biomedical and health sciences and related disciplines, such as life sciences, behavioral sciences, chemical sciences, and bioengineering. These two databases are, therefore, very different in scope and based on source material of varying detail. However, they provide a first source of information for MPS researchers in China and internationally.

The survey was conducted by applying relevant terms to the PubMed and the CNKI databases on June 24, 2023 according to the methodology described in the supplementary material². A total of 43 survey terms related to nine of the ten human systems yielded 967 hits in the PubMed database, of which 17 terms related to eight human systems yielded 3,606 hits in the Chinese database. This brief, cursory snapshot provided the workshop participants with sufficient evidence that the organ-on-a-chip literature deposited in the Chinese CNKI database may well contain valuable scientific results that are currently inaccessible to members of the global MPS community who are not fluent in Chinese. The same may hold true for other regions, for example, Japan, where MPS-based research is ongoing and native language articles are a common tool of scientific interaction. Therefore, the participants recommended mechanisms and actions to make relevant high-

quality datasets from the Chinese academic MPS publication portfolio available to the English-speaking MPS community, and, vice versa, to inform interested Chinese scientists about developments in the global MPS community from time to time in their native language (see Box 1 in Section 7). The recommendations also apply to other regions where relevant scientific literature on MPS may be published in the local language, such as Japan or South America.

7 Take home messages and recommendations for the next period

Expectations stated in the 2015 roadmap that organismoids would be industrially applicable from 2020 onwards have not materialized. Instead, static and dynamic single- and multi-organ MPS are now widely used in laboratories worldwide for multiple applications. The first hybrid approaches combining MPS-based models and assays with computational modeling and *in silico* simulation have been reported. Strong alliances such as the IQ MPS Affiliate advocate CoU qualification for in-house end user decision-making and, in the long term, for informed regulatory authorization. However, despite this unprecedented increase in research activities and reasonable progress in method development and standardization for in-house decision-making in end user laboratories, none of the methods have yet reached a level of qualification that supports critical, informed regulatory decision-making. Thus, another expectation of the 2015 roadmap has not yet materialized. Accordingly, the participants of the 2023 workshop updated the MPS roadmap for the next 15 years to the best of their knowledge and belief (Fig. 5).

The short-term focus within the rapidly growing academic landscape is on increasing the biological complexity of MPS, e.g., vascularization, innervation, immunity, mechanical and electrical cues, and microbiome research, and implementing new functional readouts such as noninvasive optical analyses. The development of human organismoid body-on-a-chip solutions has become a long-term goal, while advanced disease modeling integrated with computational pharmacokinetic, pharmacodynamic, ADME and QIVIVE modeling, and *in silico* simulation is the medium-term focus in academia. This will provide the basis for the creation of digital MPS twins. Digital MPS twins are the main challenge for progress within the next ten years. The standardization of organ-on-a-chip systems is envisioned at all levels and along well-defined standardization roadmaps.

The MPS supplier industry is currently focusing on optimized chip designs and a growing portfolio of standardized organ models. Mid-term goals are to increase the level of automation and implement novel readouts in their MPS platforms.

A continuous flow of optimized MPS solutions from academia and the supplier industry, combined with in-house standardiza-

³⁷ <https://ovidsp.ovid.com/> (accessed 14.08.2024)

³⁸ <https://pubmed.ncbi.nlm.nih.gov/> (accessed 14.08.2024)

³⁹ <https://www.scopus.com> (accessed 14.08.2024)

⁴⁰ <https://www.webofscience.com> (accessed 14.08.2024)

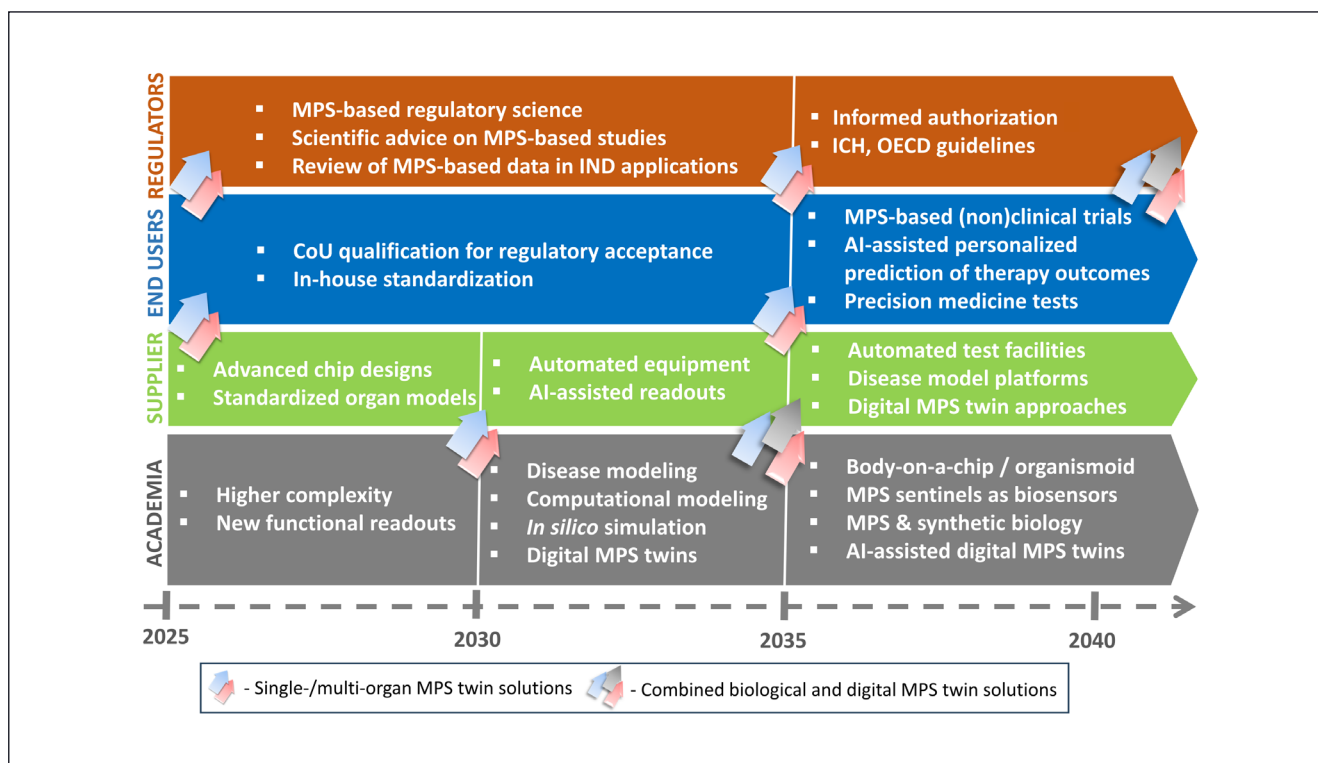


Fig. 5: A roadmap toward dynamic MPS-based patient benefit and animal welfare

Expected dynamic MPS-based progress highlights in academia (grey), the supplier industry (green), the end user industry (dark blue), and regulators (brown) over the next 15 years. The translation of new dynamic single-organ (light red arrow) or multi-organ (light blue arrow) biological MPS approaches into end user and regulatory acceptance is illustrated. Furthermore, the transfer of combined biological and digital MPS twin approaches between stakeholders (combination of grey, light blue and red arrows) is shown.

tion processes, will enable end users to generate valuable data in mechanistic studies, primary pharmacology, efficacy testing, computational pharmacokinetic, pharmacodynamic, and ADME and QIVIVE modeling for in-house decision-making. There will be an increasing number of instances over the next decade where MPS-based assays need to be qualified for a particular CoU. As a result, interaction with regulatory agencies to obtain scientific advice is expected to increase concurrently with a focus on pivotal safety studies. The strong interest and commitment of industry to invest in MPS as alternative models for drug testing is evident and underlined by numerous targeted investments by different companies, a prime example being the recent opening of Roche's Institute of Human Biology (IHB) that is dedicated to research in organoids, human model systems, and translational bioengineering.

Attractive long-term goals for the pharmaceutical industry and the healthcare system in general are also biological MPS twin-based (non-)clinical treatment trials and the implementation of personalized prediction of therapeutic outcomes. Exchange between developers and end users on needs and gaps to achieve this will be required.

Regulatory authorities will continue to increase their involvement in MPS through regulatory science. Increased efforts in providing scientific advice for MPS-based studies and increasing engagement with MPS-based datasets to be reviewed in IND appli-

cations are expected over the next decade. However, the informed approval of new medicines based on validated MPS-based assays has a very long-term regulatory horizon. The generation of ICH or OECD guidelines for MPS-based assays is the longest-term regulatory goal.

Recommendations were developed for the coming years to address bottlenecks in access to scientific tools and sources (Box 1), to further improve community communication through IMPSS and EUROoCS (Box 2), and to accelerate actions towards dynamic MPS-informed regulatory decisions (Box 3).

Box 1: Improving access to scientific dynamic MPS tools and sources

- Advocate for open resource platforms, such as biomaterial and media databases, and 3D print files.
- Support accessibility and democratization, transparency and openness (sharing anonymized data in publications and workshops) to move forward. Get the IMPSS to gather publications/documents on qualification standards. Evaluate ways for easier access to patient cells for use in biological MPS models for personalized precision medicine.
- Call for a Human Exposome Project.



- Develop new international standards based on the recently published Roadmap for Organ-on-a-Chip Standardization⁴¹.

English presentation of native language scientific literature databases in China and Japan (and possible translation software) by representatives of the Asia-Pacific Chapter of the IMPSS at an MPS World Summit to showcase relevant MPS literature only available in native languages.

Box 2: Improving MPS community communication through the IMPSS

- Integrate new stakeholders at the IMPSS level, such as physicians/clinics, health insurance bodies, patients, patient advocates, policymakers, philanthropic and charitable organizations, and other societal supporters based on expanding MPS applications, for example, precision medicine.
- Implement the active development and supervision of global ethical standards for the use of MPS-based technologies, develop communication channels tailored to the audience, and establish MPS education platforms.
- Identify supporting measures to fund the Human Exposome Project / Exposome Atlas.

Increase the visibility of MPS / IMPSS / EUROoCS in other scientific communities by, for example, hosting satellite sessions at conferences, such as AACR to showcase achievements, use cases, and limitations.

Box 3: Accelerating regulatory accepted decision-making

- *All stakeholders*: Foster the further implementation of centers for independent standardized characterization and qualification of microfluidic MPS-based methods in America, Europe and Asia. Ideally, expand the scope of the centers to NAMs in general, and implement global data sharing mechanisms among the centers and with the regulatory authorities. Use the MPS World Summit platform for progress reporting of the local centers across the globe.
- *Regulators*: Produce guidance on aspects of the regulatory acceptance of biology-inspired MPS-based assays for drug testing. This should guide sponsors to learn how agencies deal with the data based on case examples.
- *End users and regulators*: Develop a strategy to evaluate the potential place of MPS data in weight of evidence approaches and their implementation, for example, for sys-

temic toxicology. Actively involve end users and regulators from the start of the development of an MPS/organ-on-a-chip model.

- *Pharmaceutical industry*: Integrate data from qualified MPS models in weight of evidence building for specific target organ toxicity, for example, carcinogenicity (ICH S1B⁴²).
- *Pharmaceutical industry*: Include MPS data, for example on cardiotoxicity, in the current safety pharmacology package to support waiving further clinical studies in some cases (ICH E14/S7B Q&A⁴³).
- *End users and regulators*: Decide on how MPS should become a standard tool to avoid human “experimentation” in extreme circumstances lacking animal tests.
- *All stakeholders*: Establish a clear presence for MPS on an ISO level to develop standards to facilitate the standardization of building blocks and use and evaluation of the models, based on the published Roadmap for Organ-on-a-Chip Standardization⁴¹.

8 Outlook

MPS have the potential to revolutionize basic research, healthcare, and animal welfare. The focus for the next five years is increasing the biological complexity, improving existing and adding novel functional readouts, and advancing the corresponding chip designs. Attractive short-term development opportunities lie in the following areas:

- 1) Development of systems to address areas that are not yet well-defined (e.g., neuroscience and behavioral research).
- 2) Modeling complex human (patho-)physiology (e.g., developmental, metabolic, immune) and characterizing long-term, systemic and developmental health effects of environmental and drug exposures.
- 3) Cross-species translation (e.g., oncology, psychiatry, ophthalmology, ageing, reproductive health).
- 4) Establishing human-specific (patho-)physiological models for the evaluation of biological therapeutics that are not adequately modeled in animal studies because targets, therapeutic mechanisms, and toxicities are human-specific.

The mid-term (5-10 years) focus for dynamic MPS is on complex human disease modeling, artificial intelligence (AI)-assisted readouts, CoU qualification of assays, and the creation of digital MPS twins based on *in silico* simulation, machine learning, and computational modeling. As MPS technologies increasingly encounter AI platforms at all stages of their life cycle, training and feedback loops between the resulting *in vitro* and *in silico* human models are envisaged to advance the field.

⁴¹ <https://www.cencenelec.eu/media/publication-july-2024-fg-ooC-roadmap.pdf> (accessed 14.08.2024)

⁴² https://database.ich.org/sites/default/files/S1B-R1_FinalGuideline_2022_0719.pdf (accessed 14.08.2024)

⁴³ https://database.ich.org/sites/default/files/E14-S7B_QAs_Step4_2022_0221.pdf (accessed 14.08.2024)



Improvement of the infrastructure is required for these mid-term goals relating to the following needs to increase the speed and diversity of innovation in the field:

- 1) Cell bank of standardized primary and stem cells from diverse populations (e.g., ethnicity, gender, developmental age).
- 2) Cell and tissue bank from animal models.
- 3) Centralized and interoperable database and analysis tools.
- 4) Platform automatization with built-in sensors to capture phenotype dynamics.
- 5) Preclinical and clinical training and validation datasets for *in silico* simulation and modeling.
- 6) Data-sharing mechanisms between end users and regulatory authorities.

The long-term (10+ years) focus for biological MPS twin approaches is their combination with synthetic biology, the creation of personalized MPS sentinels as integrated sensors, and the development of organismoid body-on-a-chip solutions and their combination with AI-assisted digital MPS twin approaches. Prospects to use MPS for early product development, e.g., frontloading toxicity testing as investigative toxicology (Beilmann et al., 2019) or green toxicology (Maertens et al., 2024), or discussions toward a Human Exposome Project (Hartung, 2023; Sillé et al., 2024) represent further opportunities to broaden MPS application.

The workshop participants are convinced that hybrid biological and digital MPS twin approaches, which model the real level of human (patho-)physiology, are the ultimate tool to revolutionize human basic research, drug development, and personalized precision medicine. This would bring dynamic hyperphysiological MPS systems within reach that are unbound by phylo- and embryogenesis, as well as open to both new architectures, such as the artificial combination of donor functional units, and novel inputs, such as optogenetics or brain-machine interfaces. Such new designer MPS aim to create novel artificial functions, such as insulin secretion by the liver, muscle cells that sense tension, or novel biological neural circuits for monitoring. These approaches could open unprecedented new opportunities for discovery in the future within and far beyond the human life sciences to satisfy academic curiosity.

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Conflict of interest

Thomas Hartung is the named inventor on Johns Hopkins' patent application for a BrainSphere model, licensed to AxoSim Inc., New Orleans, LA, where he serves as Consulting Vice President of Scientific Affairs, holding shares in the company. Lena Smirnova consults AxoSim. Thomas Hartung is also a consultant of American Type Culture Collection (ATCC); InSphero, Zurich, Switzerland; Crown Biosciences, San Diego, CA; and was until recently consultant for AstraZeneca on advanced cell culture methods. Donald E. Ingber holds equity in Emulate Inc. and chairs its Scientific Advisory Board; he also consults to Roche. Uwe Marx is a shareholder and CSO of TissUse GmbH, which commercializes MPS platforms.

Data availability

No datasets were generated for this work.

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