Harnessing Human Microphysiology Systems as Key Experimental Models for Quantitative Systems Pharmacology

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Contents

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Abstract

Two technologies that have emerged in the last decade offer a new paradigm for modern pharmacology, as well as drug discovery and development. Quantitative systems pharmacology (QSP) is a complementary approach to traditional, target-centric pharmacology and drug discovery and is based on an iterative application of computational and systems biology methods with multiscale experimental methods, both of which include models of ADME-Tox and disease. QSP has emerged as a new approach due to the low efficiency of success in developing therapeutics based on the existing target-centric paradigm. Likewise, human microphysiology systems (MPS) are experimental models complementary to existing animal models and are based on the use of human primary cells, adult stem cells, and/or induced pluripotent stem cells (iPSCs) to mimic human tissues and organ functions/structures involved in disease and ADME-Tox. Human MPS experimental models have been developed to address the relatively low concordance of human disease and ADME-Tox with engineered, experimental animal models of disease. The integration of the QSP paradigm with the use of human MPS has the potential to enhance the process of drug discovery and development.

Keywords

Computational models of ADME-Tox · Computational models of disease · DILI · Drug development · Drug discovery · Drug repurposing · Induced pluripotent stem cells · Microphysiology systems · Omics analyses · PBPK · Personalized medicine · Quantitative systems pharmacology · Toxicology

1 Introduction

Over the last 30 years, the primary drug discovery and development paradigm has been based on target-centric discovery methods and the use of simple 2D cellular models along with animal models of disease and ADME-Tox (Sorger et al. 2011; Stern et al. 2016). Although some very valuable therapeutics have been discovered and delivered to patients based on this paradigm, the efficiency has been very low. In fact, after the investment of significant time and money, the failure rate is still ca. 80% for those new drug candidates that enter phase 2 clinical trials (Arrowsmith and Miller 2013), although in recent years there has been some improvement concurrent with an increase in the percentage of biologics and a more critical triage of candidates (Smietana et al. 2016). The primary causes of failure have been identified as a lack of efficacy with some unpredicted toxicity (Alex et al. 2015; Arrowsmith and Miller 2013). This knowledge has led to a widely held view that there is need for a new paradigm, together with the use of more sophisticated human multicellular, 3D experimental tissue/organ models (Sorger et al. 2011; Stern et al. 2016). This chapter explores the application of QSP as an alternative approach to

drug discovery and development and the role of human MPS (e.g., organs-on-a-chip) to complement animal models of disease and ADME-Tox in the practice of QSP.

1.1 Quantitative Systems Pharmacology

The identification of small molecules that modulate disease-relevant animal phenotypic models, which can then serve both as probes of pathophysiology and starting points for drug discovery and development, has its roots in classical pharmacology (Sorger et al. 2011). Historically, the structure-activity profiles of these small molecules were used to classify receptors, infer their biological functions, and then guide their molecular characterization (i.e., "target identification") (Ahlquist 1948; Black et al. 1972; Lands et al. 1967; Sorger et al. 2011). Recent technological advancements including genomics and high-content screening (phenotypic screening) have resulted in the development of more sophisticated experimental models (human cells, human 3D, MPS models, and small organisms) exhibiting quantifiable, clinically relevant features (phenotypes) (Haasen et al. 2017; Horvath et al. 2016; Taylor 2012). Phenotypic discovery has been enabled with more extensive structurally and mechanistically diverse chemical libraries (https://drugdiscovery.msu.edu/facilities/addrc/compound-libraries/) and orthogonal methods that facilitate target identification of phenotypic screening "hits" (Mateus et al. 2016; Schenone et al. 2013). Juxtaposed to classical pharmacology, recent pharmacology has evolved an alternative reductionist approach, exploiting phenotypic screening, extensive datasets, chemical libraries, and antibody collections, to identify small molecules and biologics that directly target well-annotated receptors to effect specific biological responses (Sorger et al. 2011).

Phenotypic and target-centric pharmacological approaches are complementary, can be used in tandem, and have resulted in the approval of nearly 4,000 drugs having a profound benefit on human health worldwide. The reduction in mortality among a large segment of our population, through the pharmacological management of cardiovascular risk factors, and the modification of the lethal HIV infection into a clinically manageable chronic disease through combination therapy targeting the virus life cycle are two remarkable examples among many. Despite this success, diseases range widely in their complexity and prevalence, from cancers, opioid addiction, Alzheimer's disease, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) to the more than 7,000 rare diseases for which there are no effective treatments. To address this extensive unmet need, the field of pharmacology continues to evolve, incorporating an explosion of knowledge and unprecedented advances in technology in this post-genomic era, into a modular, highly integrated platform termed quantitative systems pharmacology (QSP) (Gadkar et al. 2016a; Hansen and Iyengar 2013; Iyengar et al. 2012; Sorger et al. 2011; Stern et al. 2016; Zhao and Iyengar 2012).

QSP focuses on determining disease mechanisms and drug modes of action and their intrinsic relationships, to facilitate the repurposing of existing drugs, as well as

to develop novel therapeutics and therapeutic strategies. Discovering and developing therapeutics is a multiscale challenge. QSP addresses this challenge through the iterative use of experimental and computational models, starting with analysis of human clinical data and continuing with the analysis of molecular results from in vitro experimental and animal models relative to the human data.

Historically, advancements in the development of preclinical human models of barrier epithelia, coupled with the development of mathematical models of pharmacokinetics, improved our ability to predict human pharmacokinetic (PK) profiles, optimizing compound and dose selection for early stages of clinical development (Ferreira and Andricopulo 2019; Kola and Landis 2004; Sorger et al. 2011; Torras et al. 2018). The combined use of human, cell-based experimental models and computational models of physiologically based pharmacokinetics (PBPK) paved the way for overcoming a major hurdle in traditional drug development (Lave et al. 2016; Rowland et al. 2011). Addressing this particular challenge has nevertheless unmasked others. Today, attrition in the drug discovery pipeline results mainly from lack of efficacy in phase 2, as well as toxicity that can be observed at any stage of development including post-market surveillance (phase 4). A lack of efficacy can be observed despite evidence for drug-target engagement and that toxicity has often been determined to be on-mechanism, suggesting that medicinal chemistry per se is not limiting drug development, but that our knowledge of underlying human biological mechanisms and pathophysiology is insufficient.

Paradoxically, it appears that for complex diseases a phenotypic approach may be particularly useful in combination with elements of the target-based approach (Haasen et al. 2017). This observation suggests, at least for certain diseases and targets, that cellular context at the level of network regulation may be an important determinant for achieving efficacy and that a more comprehensive systems-based approach, in contrast to a focused yet restrictive target-based approach, may be indicated to identify emergent biology (Hopkins 2008). Likewise, mechanism-based toxicity can also be context-dependent, resulting from the expression of the target in different tissues/organs or in the presence of particular comorbidities (Ferdinandy et al. 2018; Marnett 2009). In addition to these intricacies, successful drug development requires the study of a drug candidate's mode of action in systems that span a wide range of biological complexity and diversity (i.e., from purified subcellular components to patient populations), involving timescales from milliseconds to life spans (Sorger et al. 2011). Together these considerations emphasize the need to make comprehensive systems-based measurements in experimental models that are also iteratively coupled to computational models, resulting in predictions that lead to new experiments. This iterative process leads to the refinement of the computational models with the goal to define mathematically the alterations leading to disease and toxicity (Woodhead et al. 2017). In QSP, hypotheses are tested across experimental models of increasing clinical relevance, including increasingly sophisticated human cell-based MPS, to help verify a mechanistic link between drug mode of action and the underlying pathophysiology in patients (see Sect. 1.2). The identification of pharmacodynamic (PD) markers that take into account context-dependent emergent properties to quantitate drug-target interactions and reliably predict efficacy is an

important deliverable for QSP. It is important to note that the pharmaceutical industry has been implementing QSP (Visser et al. 2014).

Figure 1 describes the implementation of QSP based on a platform for repurposing drugs and developing novel therapeutics. QSP begins with a focus on *patient sample analytics* (Fig. 1a) where patient biospecimens (adjacent "normal" and disease biopsies and longitudinal samples where possible) are obtained and analyzed by methods including DNA sequencing, transcriptomics, epigenetics, proteomics, metabolomics, and computational pathology (Spagnolo et al. 2016, 2017). Despite the challenge of obtaining longitudinal tissue biopsies, single time point measurements can often provide valuable insights into disease progression. For example, in the case of rapidly evolving diseases such as metastatic cancer, mutational analysis of the primary tumor and patient-matched metastases could indicate those clones from the primary tumor with the highest metastatic potential, routes of dissemination from one site to the other, and the selection of therapy-resistant clones (Macintyre et al. 2017). More generally, noninvasive blood sampling and single cell "omics" (Keating et al. 2018) can be used to compute real- and pseudo-time trajectories (Trapnell et al. 2014), and an in situ proteomics-based computational pathology platform (Keating et al. 2018) can exploit spatial heterogeneity to infer the evolution of disease-associated phenotypes.

These analyses are used to *infer pathways of disease progression* (Fig. 1b). In the example of RNASeq from "normal" and disease tissue samples, the outputs from comprehensive data analyses are differentially expressed gene sets that can be used to infer disease-associated pathways through the implementation of validated systems-based computational tools (Ge et al. 2018; Lee et al. 2008). Large numbers of inferred pathways can be reduced to a smaller most significant number by applying thresholding statistics and allow making inferences on causal molecular networks (Hill et al. 2016). The selected pathways allow identification of known molecular targets that serve as candidate targets for pharmacological and/or genomic perturbations to investigate disease mechanism. Although superficially this stage of QSP implementation may appear to take on the character of the target-centric approach, there are significant differences that may ultimately bear on the high rate of attrition due to lack of efficacy. For example, many candidate targets, in contrast to one or a limited few, are being considered in parallel, and each is inferred from a comprehensive unbiased dataset derived directly from patient samples.

Machine learning (ML) tools can then *predict drug-target interactions* (*DTIs*) or chemical-target interactions (Fig. 1c) from databases such as DrugBank (Wishart et al. 2018) and STITCH (Kuhn et al. 2010), in order to identify a focused library of disease mechanism "probes" that includes known and predicted drugs (Chen et al. 2016; Cobanoglu et al. 2013, 2015; Keiser et al. 2009; Liu et al. 2016) and is complemented by RNAi- and cDNA-based probes (Martz et al. 2014).

The predicted drug/chemical "probes" are then investigated as *test drugs/chemicals in human MPS* (Fig. 1d) that recapitulate critical functions of normal organs and clinically relevant disease states. The disease state MPS can be constructed using patient-derived cells and/or by exposure to established disease-potentiating environmental factors (see Sect. 1.2 below). MPS experimental models

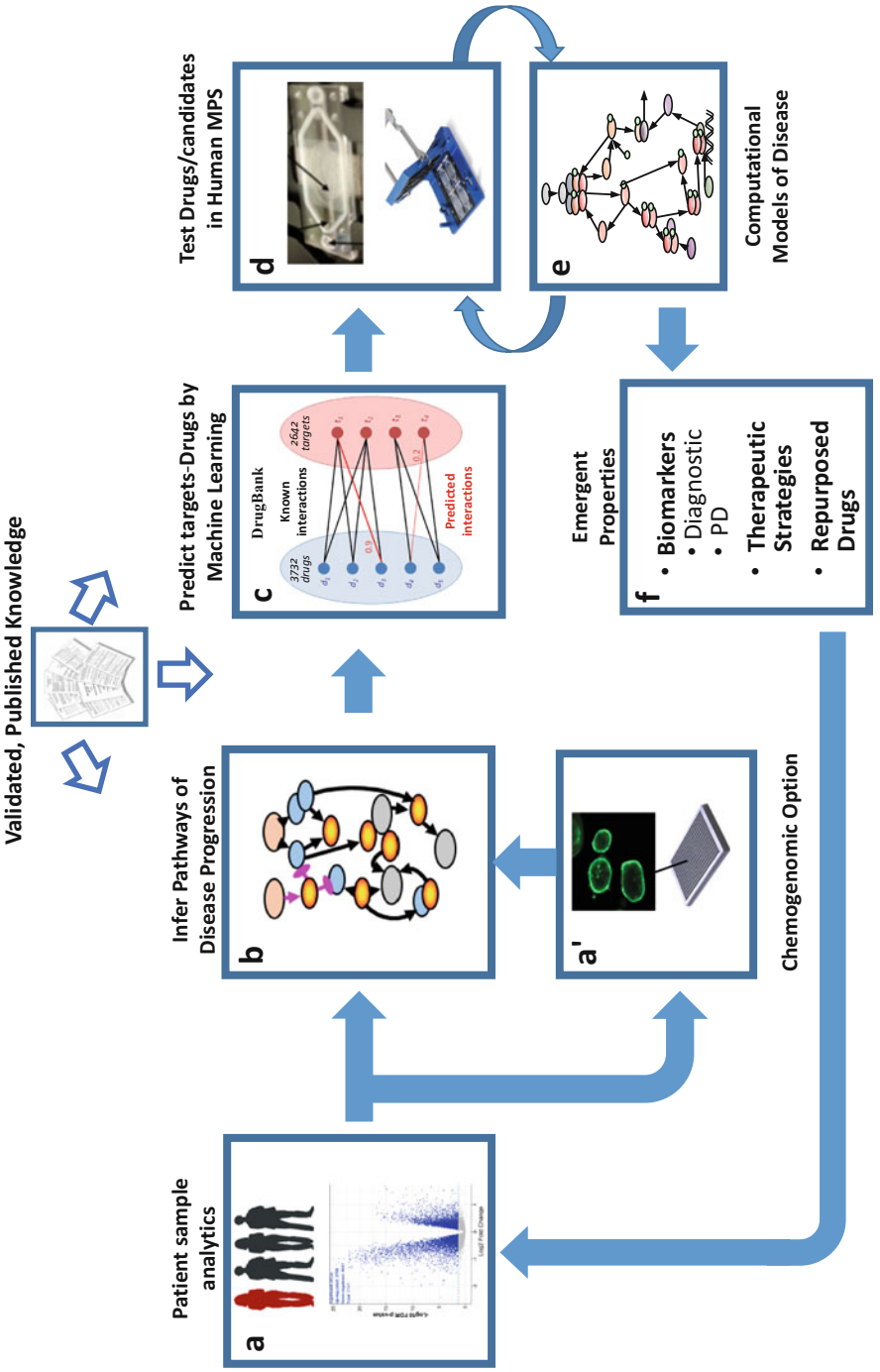


Fig. 1 Quantitative systems pharmacology platform for repurposing drugs and developing novel therapeutics

are amenable to phenotypic screening with the set of "probes" using high-content screening (HCS) platforms to quantify disease-specific phenotypes. The goal of these "probe" studies is to identify probes or probe combinations that reverse the phenotype or genotype of the disease experimental models back to "normal." The selection of "probes" can be expanded through the use of computational medicinal chemistry tools such as homology modeling, druggability assessment (Bakan et al. 2012; Volkamer et al. 2012), pharmacophore modeling (Sanders et al. 2012), and molecular simulations (De Vivo et al. 2016). The predicted "probes" can also be modified through medicinal chemistry to identify drug candidates that selectively modulate specific molecular targets rather than the canonical targets. The quantification of pharmacodynamic and disease-modifying effects of each probe enables drug mode of action to be studied in relation to disease mechanism. Successful probes from the in vitro studies can also be tested in animal models of disease. Probes or probe combinations that are approved drugs can be the starting point for drug repurposing.

The datasets resulting from use of the "probes," coupled with publication-validated knowledge, are used to construct *computational models of disease* (Fig. 1e), which are refined and optimized through iterative experimental and computational analyses (Sorger et al. 2011). The computational models can make predictions based on selected perturbations, and these can be tested in the experimental models (e.g., using well-annotated drug sets and gene/protein knockdown studies).

The computational models ultimately predict *emergent properties* (Fig. 1f), including diagnostic and pharmacodynamic biomarkers associated with the disease, and therapeutic strategies (including drug combinations) that utilize novel and/or repurposed drugs. These strategies can be tested in personalized MPS experimental disease models using patient-derived cells (primary, adult stem cell-derived, and induced pluripotent stem cells (iPSCs)) in a "preclinical trial" on a range of patient genetic and disease backgrounds (see Sect. 1.2 below). The results from the "preclinical trial" studies and clinical trial data are used to refine hypotheses of the mechanisms of disease progression. Since some of the measurements made in the experimental models are the same as those made in the patient samples, biomarkers identified in the model can be retrospectively analyzed in the patient samples to cross-validate the preclinical studies and establish a strong rationale for clinical trial design. In the case of rare diseases where biospecimens may be scarce, the implementation of QSP could be initiated with MPS models. Furthermore, as discussed below, MPS models could be used to predict both on-mechanism and off-target toxicities (Vernetti et al. 2016). It is important to note that validated, published knowledge about the disease, targets, pathways, biomarkers, and drugs can be used as input information at any point in the QSP platform (Stern et al. 2016). Selected key examples of applying QSP in developing therapeutic strategies are presented in Table 1.

Chemogenomic option (Fig. 1a'). A specific chemogenomic version of the platform can be applied at the beginning of the pipeline, preferably using higher-throughput human, 3D models of disease. In silico chemogenomic approach

Table 1 Selected key examples of applying QSP in developing therapeutic strategies

Authors	Title
Schoeberl et al. (2009)	Therapeutically targeting ErbB3: a key node in ligand-induced activation of the ErbB receptor-PI3K axis
Gadkar et al. (2016b)	Evaluation of HDL-modulating interventions for cardiovascular risk reduction using a systems pharmacology approach
Dziuba et al. (2014)	Modeling effects of SGLT-2 inhibitor dapagliflozin treatment versus standard diabetes therapy on cardiovascular and microvascular outcomes
Howell et al. (2014)	A mechanistic model of drug-induced liver injury aids the interpretation of elevated liver transaminase levels in a phase 1 clinical trial
Pei et al. (2017)	Connecting neuronal cell protective pathways and drug combinations in a Huntington's disease model through the application of quantitative systems pharmacology
Vaidya et al. (2019)	Combining multiscale experimental and computational systems pharmacological approaches to overcome resistance to HER2-targeted therapy in breast cancer
Yin et al. (2018)	Quantitative systems pharmacology analysis of drug combination and scaling to humans: the interaction between noradrenaline and vasopressin in vasoconstriction
Pei et al. (2019)	Quantitative systems pharmacological analysis of drugs of abuse reveals the pleiotropy of their targets and the effector role of mTORC1

(Fig. 1a'–f), inferring the molecular mechanisms of a phenotype of interest based on a collection of chemicals identified through phenotypic screening, offers an alternative framework to identify novel therapeutics (Bredel and Jacoby 2004; Brennan et al. 2009; Digles et al. 2016; Pei et al. 2017; Prathipati and Mizuguchi 2016). The collection of chemicals is used therein to mine the DTI or chemical-target interaction databases (Gaulton et al. 2017; Kooistra et al. 2016; Szklarczyk et al. 2016; Wishart et al. 2018) and extract ML-based information on associated targets (Cobanoglu et al. 2015; Gfeller et al. 2014; Nickel et al. 2014; Yamanishi et al. 2014), which may be further linked to enriched pathways and gene ontology (GO) annotations (Huntley et al. 2015; Kanehisa et al. 2017; Slenter et al. 2018). Thus, the cellular pathways and environment and the biological functions and processes affected by the chemicals are systematically explored. Such system-level analyses (Bian et al. 2019; Pei et al. 2017, 2019; Wei et al. 2018; Wu et al. 2019; Xu et al. 2016) assist in deciphering polypharmacological effects and disease mechanisms (Fig. 2).

1.1.1 Challenges and Opportunities in Applying QSP

The unprecedented molecular and cellular characterization of patient samples, in conjunction with well-documented electronic health records, enables the comprehensive and unbiased QSP platform to determine complex disease mechanisms and inform optimal therapeutic strategies, including the identification of emergent properties (Fig. 1). The paradigm of iterative experimental and computational modeling provides testable mechanistic hypotheses serving to connect the actual pathogenesis to the ensemble of modules comprising QSP, despite the large spatio-temporal scales they encompass. For example, the presence in the MPS models of the disease-specific pathways inferred from the patient data can be determined,

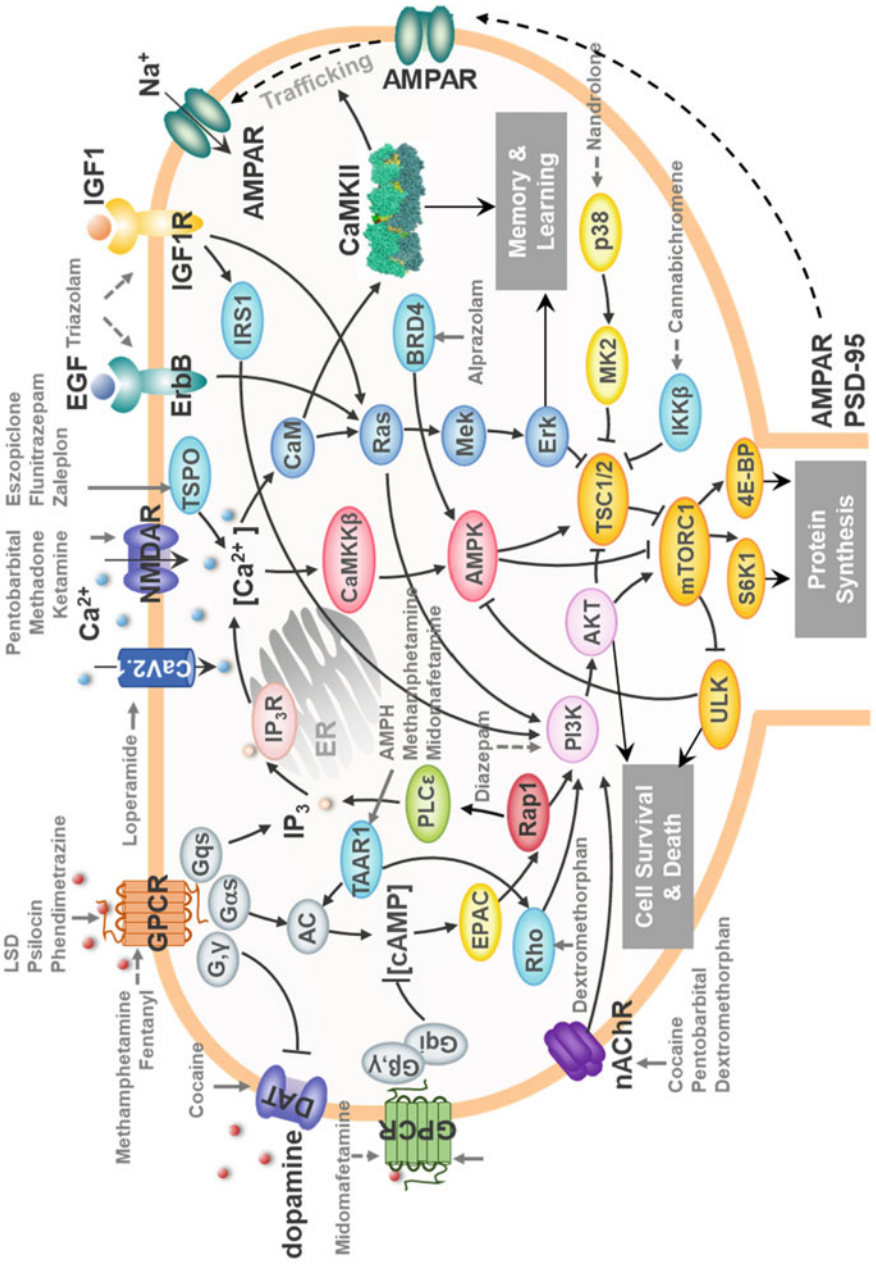


Fig. 2 A unified signaling network generated through the chemogenomic approach (see Fig. 1a' and Pei et al. 2017, 2019) in investigation of drugs of abuse. *Black arrows* represent the activation, inhibition, and translocation events during signal transduction. *Solid gray arrows* represent the known drug-target

Fig. 2 (continued) interactions. *Dashed gray arrows* represent predicted drug-target interactions. The diagram illustrates the targets of several drugs of abuse belonging to different categories: loperamide, fentanyl, heroin, morphine, and methadone from opioids; midomafetamine, ketamine, dextromethorphan, LSD, and psilocin from hallucinogens; triazolam, diazepam, alprazolam, pentobarbital, eszopiclone, flunitrazepam, and zaleplon from CNS depressants; cannabichromene, 2-AG, cannabinol, and dronabinol from cannabinoids; methamphetamine, cocaine, AMPH, and phendimetrazine from CNS stimulants; and nandrolone from anabolic steroids. mTORC1 emerges as a hub where the effects on several targets of addictive drugs appear to be consolidated to lead to cell death and/or protein synthesis in the CNS and in particular AMPAR/PSD95 synthesis that induces morphological changes in the dendrites. Figure originally published in Pei et al. (2019)

thereby providing one level of cross-validation. Chemical and genetic probes predicted to modulate these pathways can then be tested in the MPS models to determine their effect on disease phenotypes recapitulated in the model, providing a second level of clinical relevance. In parallel, systems modeling of these inferred pathways could be used to predict disease-specific biomarker profiles that can form the rationale for an observational study, establishing yet another critical connection between the preclinical model and the patient. Finally, epidemiological analysis of clinical outcome in those patients being treated for a comorbidity could provide complementary evidence for a particular disease mechanism and could establish a strong basis for drug repurposing. The QSP platform provides the critical nexus between pharmacodynamic markers and disease mechanism that promises to reduce attrition and facilitate the regulatory process. This focus on connecting disease mechanism with drug mode of action enables QSP to be effective for identifying drugs that can be repurposed and for optimizing combination therapies, particularly for the treatment of complex diseases. This approach also functions as a starting point for harnessing medicinal chemistry to evolve novel therapeutics that have higher specificity and efficacy than the DTI taking advantage of the polypharmacology of drugs. A key target other than the canonical target may be critical, and the "other" target engagements can be optimized.

The strength of QSP lies in its transdisciplinary approach, and this presents its greatest challenge, in the form of organizational barriers. For the full potential of QSP to be realized, multidisciplinary teams need to be assembled, likely across two or more institutions, under leaders with expertise not only in one particular field but also possessing the sophisticated set of skills to manage critical interfaces across several disciplines. The requirement for this paradigm shift in basic research and translational medicine is increasingly being recognized by industry, academia, and government. Consequently, we anticipate that the organizational barriers to full implementation of QSP will be significantly reduced.

1.2 Human Microphysiology Systems (MPS)

The development of human MPS has grown out of the recognition that animal models and simple 2D monocultures of cells do not reflect the complexity and specificity of human physiology, toxicology, and disease mechanisms (Hartung 2009; Seok et al. 2013; Sorger et al. 2011; Stern et al. 2016). The challenge has been to develop in vitro human experimental models using patient-derived cells, either primary, tissue-resident adult stem cells (AdSCs), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), that recapitulate enough tissue/organ functions to serve as useful models in the drug discovery and development pipeline. There is the added potential to create a personalized platform for preclinical trials using these patient-derived cells. Another opportunity is to evolve these personalized MPS models into tissue replacement therapeutics (Xie and Tang 2016). The use of HCS methods to acquire temporal-spatial information and quantitative phenotypes from the 3D, multicellular MPS systems has been critical (Stern et al. 2016; Taylor 2012).

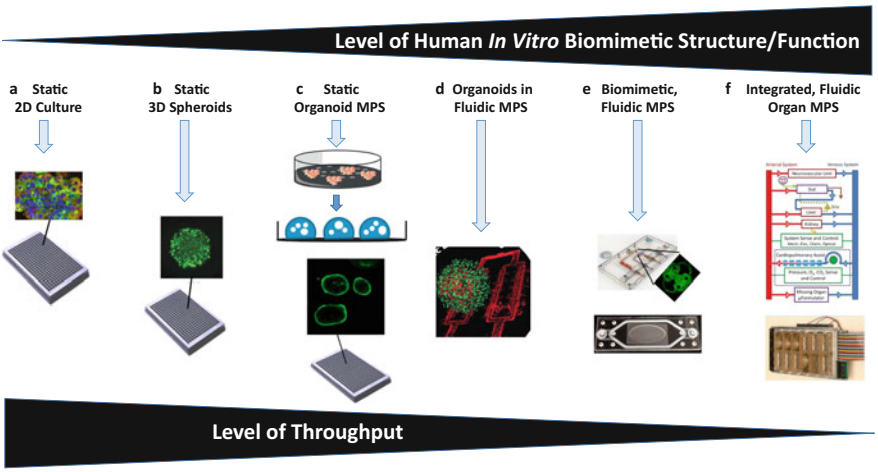


Fig. 3 Human in vitro experimental models span a broad range of experimental throughput and biomimetic structure and function

Figure 3 illustrates a range of in vitro human experimental models that increase in tissue/organ biomimetic structure and function from left to right. A common goal in the discovery and development pipeline is to select the optimal model for the stage ("fit-for-purpose"). The continuum in Fig. 3a–f involves sacrificing throughput vs biomimetic complexity.

Static 2D cultures (Fig. 3a), typically consisting of cell lines analyzed in microplates, have dominated biomedical research for over 30 years. These static 2D cultures have been used extensively in high-throughput screening (HTS) and high-content screening (HCS) applications for target ID, target validation, screening, hit to lead, and early toxicology testing (Fang and Eglen 2017). However, there has been a shift away from static 2D cultures since they do not adequately reflect human physiology/pathology. The more recent use of 3D experimental models dates back to the early 1900s, and the historical timeline of the evolution of 3D approaches has recently been summarized (Simian and Bissell 2017).

Static 3D spheroids (Fig. 3b) were originally developed by Sutherland and collaborators to better recapitulate the functional phenotypes of human cancer cells in response to radiation therapy and as a general model for tumors (Fang and Eglen 2017; Sutherland et al. 1970). Spheroids are produced by a variety of methods that form spheres of cells (ca. 100–400 um diameter) that have more physiological cell-cell and cell-matrix interactions and can generate gradients of nutrients, oxygen, signaling molecules, and metabolites from the outer layers of cells to the center, mimicking a solid tumor better than 2D models (Fang and Eglen 2017). Most spheroids use a single cell type such as cancer cells or hepatocytes, but it is possible to construct spheroids with more than one cell type. Static 3D spheroids have been used for both HTS and HCS in microplates, but confocal imaging is required for single cell resolution.

Static organoid MPS (Fig. 3c) have been defined in multiple ways (Simian and Bissell 2017); however, we prefer the following broad definition: an organoid is an in vitro 3D cellular cluster derived exclusively from primary tissue, ESCs, AdSCs, or iPSCs, optimally capable of self-renewal and self-organization, and exhibiting similar organ functionality as the tissue of origin (slightly modified from Fatehullah et al. 2016). Static organoids developed from either AdSCs, ESCs, iPSCs, or primary patient cells (sometimes mixed with some human cell lines for selected cell types) recreate a partial biomimetic for many types of organs, which can be used for drug discovery, development, and the exploration of disease mechanisms (Dutta et al. 2017; Low and Tagle 2017; McCauley and Wells 2017; Prestigiacomo et al. 2017; Schwartz et al. 2015; Shamir and Ewald 2014; Skardal et al. 2016; van den Berg et al. 2019). Like static spheroids, static organoids can be investigated by HTS and HCS in microplates using confocal imaging.

The same principles used in developing static organoids can be applied to *organoids in fluidic MPS* (Fig. 3d). In fact, it is possible to combine the organoid technology with *biomimetic, fluidic MPS* (Fig. 3e) engineering principles to better address the limitations of each approach (Edington et al. 2018; Takebe et al. 2017; Wevers et al. 2016). Organoids in fluidic MPS enable the physiologically relevant shear stress required by many tissues, coupled with at least partial spatial cell-cell and cell-matrix interactions. They can also be linked to other organ MPS for integrated functions using fluidic connections.

Biomimetic, fluidic MPS (Fig. 3e) are devices designed to maximize physiologically relevant structural relationships between cells, natural gradients of physiological parameters (e.g., oxygen tension, hormones), matrix materials, mechanical cues including shear stress of vascular flow, mechanical movements, innervation, and immune system communication (Bhatia and Ingber 2014; Low and Tagle 2017; Watson et al. 2017). The focus is on constructing an organ model that is as close to a functional unit (e.g., liver acinus, cardiac muscle fibers, lung) as possible (Huh et al. 2010; Li et al. 2018; Lind et al. 2017). Early advances were stimulated in particular by research of Don Ingber and his colleagues at the Harvard Wyss Institute, including a lung biomimetic, fluidic MPS (Bhatia and Ingber 2014; Huh et al. 2010).

Presently, static organoids, organoids in fluidic MPS, and biomimetic, fluidic MPS are being created for most normal and diseased organs (Esch et al. 2015; Fang and Eglen 2017; Low and Tagle 2017; van den Berg et al. 2019). The complexity of current biomimetic, fluidic MPS models is not amenable to high-throughput studies, yet the high content of the structure and functionality are optimal to validate findings from higher-throughput models, as well as to investigate the mechanisms of disease progression using HCS over extended time periods (ca. 1 month or longer).

The most ambitious platform is the *integrated, fluidic organ MPS* (Fig. 3f) where multiple organ MPS are linked together either functionally (Vernetti et al. 2017) or physically (Edington et al. 2018; Low and Tagle 2017; Oleaga et al. 2019; Satoh et al. 2017; Skardal et al. 2016). Michael Shuler and his colleagues have been pioneers, demonstrating in the 1990s that linking multiple organ systems allowed organ-organ communications that could be used to identify toxicity and to perform physiologically based pharmacokinetics (PBPK) (Sin et al. 2004; Sweeney et al.

Table 2 Selected examples of human experimental MPS disease models

Authors	Title
Jain et al. (2018)	Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutic clinical pharmacology and therapeutics
Blutt et al. (2017)	Gastrointestinal microphysiological systems
Workman et al. (2017)	Engineered human pluripotent stem cell-derived intestinal tissues with a functional enteric nervous system
Hachey and Hughes (2018)	Applications of tumor chip technology
Clark et al. (2018)	A model of dormant-emergent metastatic breast cancer progression enabling exploration of biomarker signatures
Vernetti et al. (2016)	A human liver microphysiology platform for investigating physiology, drug safety, and disease models
Atchison et al. (2017)	A tissue-engineered blood vessel model of Hutchinson-Gilford progeria syndrome using human iPSC-derived smooth muscle cells

1995). This concept has been extended and applied with an integrated, fluidic organ MPS to explore ADME and PK/PD using QSP approaches (Yu et al. 2015). There are many challenges and opportunities in developing and applying these "body-on-a-chip" systems, but the progress over the last 5 years has been impressive (Low and Tagle 2017; Shuler 2017; Skardal et al. 2016; Wikswo et al. 2013b).

A consortium of pharmaceutical company representatives (IQ Consortium), participating in the National Center for Advancing Translational Sciences (NCATS) microphysiology systems program, recently wrote an article discussing the translation of MPS models from the laboratory to commercial use by the pharmaceutical industry (Ewart et al. 2017). It is clear that the industry understands the great potential of these systems and is giving important guidance to the field. In addition, the FDA and the EPA have collaborated with NCATS to learn of the potential of these systems and to provide their insights into the needed functionalities and reproducibility. Furthermore, the dramatic advances in the development of the biology, materials science, and microfluidics have led to the formation of numerous companies offering platforms that will accelerate the biomedical sciences, drug industry, and clinical applications based on some emerging standards (Zhang and Radisic 2017). Recently, May et al. (2017) explored the advantages and disadvantages of organoids, biomimetic, fluidic MPS, and integrated, fluidic organ MPS. Table 2 lists selected examples of human experimental MPS disease models.

1.2.1 Challenges and Opportunities in Developing and Applying MPS

The MPS field has exploded during the last 5 years, and dramatic advances have been made in microfluidic devices, in-line as well as cellular biosensors, the development of renewable cells from iPSCs (where there is still the need to mature the iPSCs to the adult genotype and phenotype), optimizing matrix biochemical content and stiffness, the optimization of media for normal and disease states in different organs, the exploration of a "universal" medium for connected organs, the

involvement of the innate and adaptive immune systems, as well as the role of chemical, electrical, and mechanical cues on functions. NCATS has involved the pharmaceutical industry, the FDA, and the EPA in the MPS programs, and there has been great feedback to guide developments. Further technical developments, as well as the demonstration of reproducibility of the models from day to day and between distinct sites, will position MPS to have a major impact on the drug discovery and development process, as well as to help to define the progression of diseases in human, in vitro models. MPS models are projected by many to refine, reduce, and ultimately replace animal models of disease and ADME-Tox sometime in the future.

2 QSP Involves Iterative Application of Experimental and Computational Models

The iterative use of experimental and computational models of disease and ADME-Tox is the hallmark of the practice of QSP (Fig. 1). This section discusses in more detail the key role of computational methods in the QSP platform, while Sect. 3 discusses in more detail the application of MPS in the QSP platform.

2.1 Identifying Differential Omics from Patient Samples

2.1.1 Early Omics and Implications for Human Disease: The GWAS Era

Omics generally refers to technologies that profile the entirety of the biological domain of interest (Hasin et al. 2017), which allows the investigator to take an unbiased data-driven, instead of a focused hypothesis-driven, approach to research. The first omics field to emerge was genomics, driven by the "SNP chip" (reviewed by LaFramboise 2009), which allowed high-throughput genotyping of individuals across common variants, termed genome-wide association studies (GWAS) (Visscher et al. 2012a). Some early-disease GWAS results were translational successes. The best example is age-related macular degeneration, where over half the disease heritability was explained by the GWAS results that guided drug discovery (Black and Clark 2016). However, this was not true for other complex diseases as the results could only explain a tiny portion of heritability. For schizophrenia (Visscher et al. 2012b) and obesity (Weedon et al. 2006), only 1–2% of heritability could be attributed to the GWAS-identified SNPs (Visscher et al. 2012a). This limitation applies to complex traits as well. For example, a study examining height across 253,288 individuals found 697 SNPs, which together explained ~20% of heritability (Wood et al. 2014). Further, the effect sizes of identified SNPs from most GWAS are typically vanishingly small, which necessitates huge sample sizes (Visscher et al. 2012a). Taken together, the leading paradigm is that complex diseases are polygenic and are therefore caused by complex interactions of genes as opposed to single genes (Wray et al. 2018). While the knowledge obtained using

GWAS has provided priceless insights into the biology of complex disease and drug discovery (Floris et al. 2018), it is only one piece of the puzzle.

2.1.2 Post-GWAS Era Omics Technologies and Strategies for Their Use in Human Disease

GWAS results often implicate numerous variants which have some degree of association with the disease; however, a mechanistic understanding of how these variants contribute to the disease phenotype remains largely incomplete (Wray et al. 2018). Other omics technologies (summarized in Table 3) offer the chance to close the gap left by GWAS (Karczewski and Snyder 2018). For example, the independent role of the epigenome in type 1 diabetes was shown by identifying differentially methylated regions using monozygotic twins as case controls (Paul et al. 2016). In another example, transcriptome data from patients with inflammatory bowel disease was used to identify potentially repurposable drugs (Dudley et al. 2011). Metabolomics has emerged relatively recently and shows great potential in further characterizing human disease (Wishart 2016). Lipidomics is another discipline which gained importance in the last decade with advances in mass spectrometry, driven by the tight association of lipids with many diseases including cardiovascular diseases, diabetes, stroke, NAFLD, neurological disorders, and cancer (Yang and Han 2016).

Since the cost of omics technologies continues to fall, investigators are increasingly combining multiple types of omics to obtain a more complete picture of the underlying biology (Hasin et al. 2017; Karczewski and Snyder 2018). One approach is to combine gene expression profiling with GWAS to identify quantitative trait loci, that is, variants which are associated with gene expression (Karczewski and Snyder 2018). A number of studies, reviewed in Sharma et al. (2015), have tied several genes – most notably PNPLA3 – to NAFLD progression. Interestingly, metabolic profiles associated with risk variants do not directly correlate with risk of disease (Sliz et al. 2018), underscoring the complex nature of the disease and the value of complementary multi-omics approaches for studying NAFLD.

Table 3 List of key omics analyses and reviews

Domain	Applications	Reference
DNA	Genome: Variant calling, GWAS, SNPs	Laurie et al. (2016) and He et al. (2017)
	Epigenome: Chip-Seq, BS-seq	Bailey et al. (2013) and Kurdyukov and Bullock (2016)
RNA	Transcriptome: gene expression profiling	Conesa et al. (2016) and Koch et al. (2018)
Protein	Proteomics: protein abundance	Larance and Lamond (2015)
Metabolites	Metabolomics: metabolite abundance	Johnson et al. (2016) and Wishart (2016)
Lipids	Lipidomics: lipid classes and pathways	Yang and Han (2016)

Most of the early omics technologies have been based on tissue samples that do not preserve the spatial relationships between cells, matrix, and tissue structures (e.g., blood vessels, ducts) and "average" the analyses among many cells. For example, the omics sampling of tumor samples has until recently relied on cores of tissue that do not consider the spatial heterogeneity in the tumors. We now understand that heterogeneity within a tumor is critical to understanding the evolution of the tumor. Recently, a variety of single cell methods have emerged to address this challenge (Keating et al. 2018). One of these methods is hyperplexed fluorescence imaging (Gough et al. 2014; Spagnolo et al. 2016, 2017). This method is based on computational and systems pathology using iterative fluorescence labeling of specific targets within formalin-fixed paraffin-embedded (FFPE) tissue sections or tissue microarrays (TMAs), imaging, quenching of the fluorescence, and then repeating the cycle for dozens of biomarkers in the same sample (Gerdes et al. 2013). Spatial analytics are then applied to the samples (Spagnolo et al. 2016). This method preserves the spatial relationships within tissues while allowing omics analyses based on the spatial connections within microdomains. Recently, this platform has been applied to a colon cancer patient cohort and a risk recurrence prognostic analysis demonstrated (Uttam et al. 2019).

2.1.3 Remaining Challenges of Using Omics to Study Human Disease

While omics continue to further our understanding of human disease, there are remaining challenges. Omics datasets are high-dimensional, with many more observations (e.g., genes, metabolites, proteins, lipids, etc.) assayed than samples (e.g., patients) taken (Teschendorff 2018). However, there has been considerable effort in developing specialized statistical methods for omics including the development of mixed graphical models (Manatakis et al. 2018) for learning disease models. Batch effects or technical confounding factors introduced by experimental design have historically been (Lambert and Black 2012) and continue to be (Goh et al. 2017; Goh and Wong 2018) the bane of omics studies. In a study examining epigenome of obese men as compared to lean controls, the authors found ~5.5% to be differentially methylated; however, these differences were entirely attributable to batch effects (Buhule et al. 2014). In another dramatic example, failure to account for technical variability introduced by using a different platform for the cases and controls led to the retraction (Sebastiani et al. 2011) of a paper originally published in *Science* (Sebastiani et al. 2010). There is great potential power in the use of omics approaches, but significant controls and data optimization steps are required.

2.2 Inferring Pathways of Disease from Omics Data

There is increasing interest in using patient-derived omics data for drug discovery to help increase therapeutic efficiency (Floris et al. 2018; Hodos et al. 2016). GWAS data has been used to help guide drug discovery; however, these data alone do not usually provide sufficient information for rational drug design (Pushpakom et al. 2018). Gene expression data can be an excellent type of omics to use for drug

discovery, and transcriptomic data was found to predict drug sensitivity of breast cancer cells better than genomic, epigenomic, and proteomic data (Costello et al. 2014). Other omics data still have their value: proteomics data, for example, provide details on posttranslational modifications that are not visible at the transcript level yet may provide insights into the nature of signaling in disease (Erdem et al. 2016).

Inferring pathways of disease progression begins with defining the difference between "diseased" and "healthy" states in terms of specific omics measurements. For example, in transcriptomic analysis, one might identify differentially expressed genes (DEGs) as those genes with transcript levels that change significantly between disease samples and healthy controls. Exactly defining "diseased" and "healthy" states themselves however is often difficult due to the inherent noise of biological data and inter-sample variability. Once statistically significant differences between diseased and healthy states are identified, the biological mechanisms that give rise to these differences can be hypothesized. For example, pathways containing higher than expected numbers of DEGs are commonly implicated in disease progression and subject to further investigation. Similarly, pathways upstream of transcriptional regulators of DEGs may also be implicated in disease progression. Connectivity mapping can then be used to find drugs which "reverse" the gene expression pattern (Musa et al. 2017).

2.3 Identifying Drugs, Targets, and Pathways by Machine Learning for Drug Repurposing and as a Starting Point for Developing Novel Therapeutics

Drug repurposing (also known as drug repositioning) refers to the process of identifying new therapeutic indications for approved drugs or investigational drugs (Allarakhia 2013; Ashburn and Thor 2004; Keiser et al. 2009). Drug repurposing takes advantage of established pharmacology of existing drugs to drastically reduce risk and cost of development, making it an attractive track for drug discovery and development (Pushpakom et al. 2018). A well-known example of drug repurposing is the anticancer drug imatinib, which was originally developed in 2001 for the treatment of chronic myeloid leukemia and, later in 2008, approved by the US Food and Drug Administration (FDA) for treating gastrointestinal stromal tumors (Al-Hadiya et al. 2014). A key step of drug repurposing is to identify new DTIs. However, experimental identification of DTIs is time-consuming, costly, and limited. For example, the current version (v5.1.1) of DrugBank (Wishart et al. 2018) contains data on 16,959 interactions between 10,562 drugs and 4,493 targets, while the presence or absence of the remaining interactions (99.96% of the complete space of interactions) is yet to be determined. Therefore, developing machine learning (ML)-based computational methods (Fig. 1c) for efficient DTI prediction is of great need.

To date, both supervised and semi-supervised ML methods have been adopted in DTI predictions (Chen et al. 2016, 2018). Most supervised learning methods, including kernel regression (Yamanishi et al. 2008), random forest (Cao et al.

2014), bipartite local models (Bleakley and Yamanishi 2009), regularized least-square classifier (van Laarhoven et al. 2011), kernelized Bayesian matrix factorization, and similarity-based deep learning (Zong et al. 2017), use the known DTIs as positive samples and consider the rest as negative ones. The structural and physicochemical properties of drugs, such as 2D fingerprints, 3D conformations, topological descriptors, and the sequence, structure, and expression data of targets such as protein sequence and structural motifs and gene expression profiles, are utilized to generate feature vectors of drugs or targets or to calculate drug-drug similarities and target-target similarities. Other supervised learning methods such as probabilistic matrix factorization (Cobanoglu et al. 2013) and integrated neighborhood-based method (Chen et al. 2016) utilize the known DTI patterns to compute drug-drug similarities and target-target similarities and predict novel DTIs, independent of the structural or physicochemical properties of drugs and targets. Semi-supervised methods, on the other hand, use labeled data (known DTIs) to infer labels for unknown DTIs, and these inferred DTIs play a role in the training process. Examples include the manifold Laplacian regularized least-square method (Xia et al. 2010) based on integrated data from known DTIs, chemical structures and genomic sequences, and the deep learning-based framework (Wen et al. 2017).

Most current ML-based methods simply regard DTI as an on-off relationship. Development of selective and potent drugs may require further consideration of specific binding poses and affinities. ML-based DTI prediction serves as a first step for identifying new associations, while further computational biophysical and medicinal chemistry tools help characterize the mechanistic aspects and specificities of predicted DTIs. For example, if the drug-binding site on the target is unclear or new (e.g., allosteric) sites beyond those (orthosteric) traditionally targeted are of interest, a useful method of approach is to perform druggability simulations (Bakan et al. 2012; Ivetac and McCammon 2012; Lexa and Carlson 2011; Loving et al. 2014). These simulations are conducted in the presence of a series of probes representative of drug-like fragments, whose simulated binding properties disclose the high-affinity binding sites as well as favorable binding poses on the target. Statistical analysis of these binding events permits us to build pharmacophore models (see, e.g., Bakan et al. 2015; Mustata et al. 2009), which, in turn, are used for screening virtual libraries of small compounds and identifying best matching compounds, termed "hits." Top hits identified at this stage are experimentally tested (e.g., via binding affinity assays (Pollard 2010)), and the feedback from experiments is used to revise computational models. In addition, with a set of bioactive hits, a numerical description of molecular structure/properties to known biological activity can be generated via quantitative structure-activity relationship (QSAR) (Wang et al. 2015) analysis, which further guides the rational structural optimization of the hits into lead compounds. The combined computational and experimental methods are performed iteratively until the refinement of the compounds to achieve desirable biological activity in the MPS models.

2.4 Computational Models of Disease

A central element of QSP is the iterative computational/experimental feedback loop. In order to understand the biological mechanisms of disease onset and progression, it is helpful to formalize certain aspects of the experimental system into a mathematical model that can be manipulated in silico. When dealing with in vitro systems, computational disease models are usually limited to interactions within and between a small number of cells and most often take the form of either agent-based models (ABMs) or systems of ordinary differential equations (ODEs). In an ABM, each biological cell is represented as an autonomous entity that interacts with its environment and neighboring cells according to pre-defined rules. The behavior of the system as a whole is therefore an emergent property of this collection of agents. Although computationally more expensive than ODE models, ABMs are easily interpretable in terms of cellular features and are readily adaptable to novel geometries such as those found in MPS experiments. ABMs have been used to explore a range of diseases, including tumor growth (Szabo and Merks 2013) and liver fibrosis (Dutta-Moscato et al. 2014). ODE models are typically higher resolution than ABMs and represent the system at the level of molecules rather than cells. As they are computationally efficient and mathematically straightforward, these are a popular choice for modeling signaling pathways and regulatory networks. The standard ODE approach assumes that the molecular components of cellular chemistry are contained in a well-mixed system that obeys mass action kinetics, although more complex, spatially realistic models (represented by partial differential equations, PDEs) and/or stochastic models (described by stochastic differential equations) are gaining popularity, especially in the description of complex microphysiological processes (e.g., MCell for modeling synaptic transmission) (Bartol et al. 2015; Kaya et al. 2018).

In the context of the computational model, the difference between "diseased" and "healthy" states arises from changes in parameters, such as reaction rates or molecule numbers. For example, differences in computationally predicted transcript profiles between healthy and diseased cells might arise as the result of an altered binding affinity and/or posttranslational modification in the computational model (Fig. 4). Changes in the computational model that promote the disease phenotype indicate hypothetical mechanisms of disease progression. If rectifying these changes (e.g., via drugs) in the in vitro system reverses the disease state, then the computational model has successfully identified a disease mechanism; if not, then the computational model is refined, and another hypothesis is generated and experimentally tested. For example, by using separate compartments, an ODE was able to capture the effects of liver zonation on steatosis (Ashworth et al. 2016).

2.5 Computational Models of ADME-Tox

Since the days of Fortran programs such as MODFIT (Allen 1990), drug discovery researchers recognized the advantages in storing, managing, and analyzing large

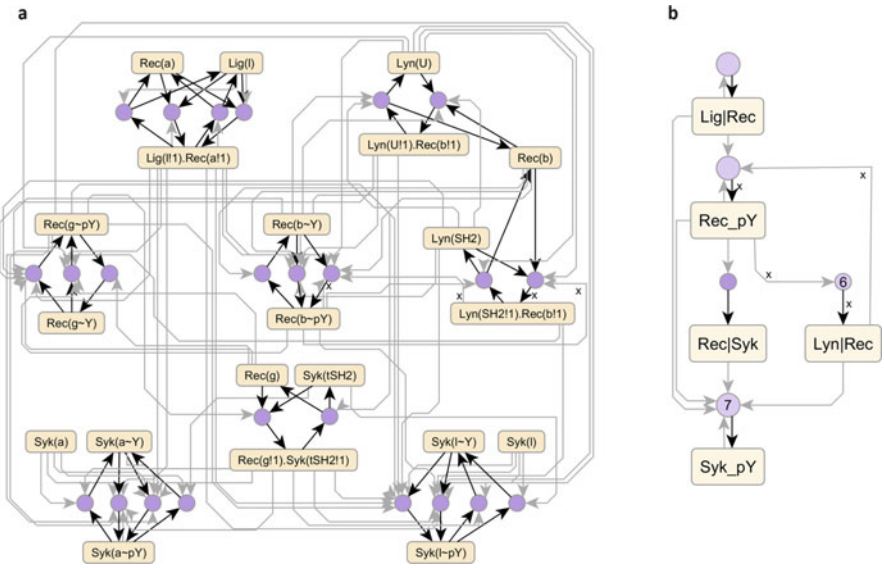


Fig. 4 Two views of a detailed computational model of immunoreceptor signaling mediated by the high-affinity receptor for IgE (Fc epsilon R1). Panel (**a**) shows the molecular components (yellow rectangles) and processes (purple circles) that govern the flow of activity in the network. Each process represents either a binding interaction between the components or posttranslational modification of a component (e.g., phosphorylation). Enormous complexity is generated just from the basic interactions that include binding and phosphorylation. Although this complexity does not limit our ability to simulate the dynamics of such systems, it does limit our ability to understand the dynamics. Through a process of static analysis, we can reduce the complexity and interpret the dynamics in terms of simple motifs and mechanisms, such as the positive feedback loop that is illustrated in panel (**b**) (edges marked with "x"). Modified from Sekar et al. (2017)

amounts of pharmacokinetic (PK) data. Drug developers require computational tools that have a good correlation between in silico, in vitro, and in vivo absorption, distribution, metabolism, and excretion (ADME) data to address the challenges of predicting PK behavior in drug development in order to determine dosing regimens, target organ exposure, and identify compounds or their reactive metabolites for off-target liabilities. The current computational modeling for drug development is evolving from simple classical PK compartmental models that describe the disposition of drugs in the body and the component ADME properties to physiologically based PK (PBPK) models that predict PK based on the physiochemical properties of the drugs and knowledge of the physiology of the organism. Although the concept of PBPK modeling has been around since 1937, it is the relatively recent advances in computing power and preclinical physiologic data that enable effective PBPK modeling. Computational approaches now include in silico predictors for drug metabolism, pharmacokinetics, and toxicology using ordinary differential equations, machine learning neural networks, Bayesian, recursive partitioning, and support vector machine algorithms (Byvatov et al. 2003; Hou et al. 2001; Li et al. 2007;

Muller et al. 2005; Sadowski and Kubinyi 1998; Wagener and van Geerestein 2000; Walters and Murcko 2002; Zernov et al. 2003). There are many commercial and academic computational tools available (R Project, GastroPlus, DILIsym, Simcyp, and MATLAB among others) for PBPK prediction (Lin et al. 2017; Tan et al. 2018; Zhuang and Lu 2016). Toxicology tools based on R-group structural alerts (DEREK), QSAR (MC4PC, MDL-QSAR, TopKat, and ADMET Predictor® among others) and molecular descriptors (PaDel) are also being developed for predicting human organ and systemic toxicity (Chen et al. 2014; Wu and Wang 2018).

All of these computational models depend on the availability of experimental data accurately representing the clinical physiology. The advanced physiological relevance of human MPS models is well suited to providing such data. In particular, liver MPS models are useful in predicting intrinsic hepatic clearance, which can then be applied to predict other PK parameters (Ewart et al. 2018; Tsamandouras et al. 2017). In addition to predicting PK, data from MPS models also allow for modeling of pharmacodynamic (PD) properties, enabling PK/PD modeling to guide drug development decisions. Finally, as MPS models can utilize patient-specific cells, PK/PD and toxicology modeling can be applied to individual genetic and physiologic backgrounds to guide the development of precision medicine models (Tsamandouras et al. 2017). The combination of MPS models and the advancing computational modeling will aid in reducing the time and cost of preclinical drug discovery.

3 Human Organ Microphysiology Systems (MPS) Complement Animal Models of Disease and ADME-Tox

As discussed above, the minimal concordance between animal models of disease and toxic liabilities, and human disease and toxicity, is one of the factors in the low success rate for drug candidates entering phase 2 clinical trials. However, animal models are still the gold standard in research and development; and regulatory agencies still require animal data before going into humans. Continued developments in the MPS field have the potential to initially complement animal models and then refine, reduce, and ultimately replace animal testing.

3.1 Designing Human Organ MPS

As illustrated in Fig. 2, MPS models include a continuum from simple 2D models to complex, integrated multi-organ systems. The design and implementation of an MPS is a "systems engineering" challenge that must take into account the complete platform consisting of microdevices, control systems, cells, extracellular matrices, media, readouts, and data analysis (Wikswo et al. 2013a). This becomes more important and challenging when integrating multiple organ MPS, requiring consideration of issues of organ scaling, sequencing, media composition, volume, and flow

(Wikswo et al. 2013b). The rapid growth in the development of MPS is partially driving, and partially driven by, the rapid development of component technologies, which provides a diversity of choices, but can also complicate the design and optimization of the model. The ultimate goal is to create a multi-organ human-on-a-chip that will recapitulate a wide range of human physiology for experimentally modeling complex systemic diseases and toxicities, but such a complex model is not needed for many studies. Because all experimental models have limitations, and the simplest model that provides the required information is usually the best choice, perhaps the most important considerations in designing an MPS are how the model will be used and what the key functional indications will be.

Models can be roughly divided into two types: (1) self-assembly models that range from cells spreading on a 2D substrate to multilayer organoids in fluidic chambers and (2) biomimetic models in which the design of the device and/or the assembly of the model promotes cellular organization that mimics the in vivo organization. Generally, self-assembly models are easier to apply in high-throughput applications, while biomimetic models provide deeper functional information. In either case, many choices go into the design of an MPS. Here, we will focus on the design of biomimetic models, though many of the same considerations apply to simpler models.

A major focus in the development of biomimetic models is the engineering of the device to recapitulate the organization of cells in vivo and also, in some cases, to engineer active elements that mimic functions such as breathing in the lung (Huh et al. 2010), contraction of muscle (Truskey et al. 2013), the beating of the heart (Benam et al. 2015; Lind et al. 2017), as well as others. To facilitate the prototyping of these systems, polydimethylsiloxane (PDMS) has been the material of choice due to low cost and ease of rapid casting in a laboratory setting. PDMS is also oxygen permeant, reducing the need to provide for additional oxygenation in the design of the model. However, PDMS is hydrophobic and readily absorbs hydrophobic molecules including some drugs and other test molecules, especially those with a higher logP and few or no hydrogen-bond donor groups (Auner et al. 2019). There are now many commercial devices that are glass and/or plastic, reducing the likelihood of compound binding (Lenguito et al. 2017; Ribas et al. 2018). Existing commercial devices have less flexibility for customizing model architecture and require more attention to oxygenation of the cells in the model, but many have already been used to implement specific organ models and therefore provide a good starting point for design or development. Driving flow in the MPS is also an important consideration and has been accomplished by using gravity, either through rocking or media transfers between outlet and inlet, pressurized systems, syringe pumps, and peristaltic pumps. In all cases, it is important that the pumping system can provide the required range of flow rates and that a physiological shear stress on the model tissues is attained.

Because a major goal in the development of MPS is to model human physiology, the focus has been on the use of human cells. While there is some interest in developing MPS models using animal cells, both for validation of the model with respect to the larger number of compounds that have been tested in animal models

and for the prediction of preclinical animal safety, relatively few MPS have been constructed with animal cells. For human cells, the choice is between primary cells, ESC, AdSC, iPSC, and cell lines. Primary cells are still the gold standard for adult-like human organ function, but specific functions may vary from donor to donor, and therefore a specific lot of cells may need to be selected and then used for the duration of a project to minimize variability from the cells. iPSCs hold promise to provide an unlimited supply of human cells, including isogenic cells for models with multiple cell types, but improved protocols to generate adult-like cells are still in development (Besser et al. 2018). The inclusion of one or more human cell lines in a multicell MPS is still an attractive option for higher-throughput applications or where the functional role of the cell type is adequately provided by a cell line. The media used in an MPS is typically selected to support the cells used, but this can become difficult in multicellular models where different cell types require different media compositions. This is further complicated in integrated organ systems. Mixing media has been one approach (Vernetti et al. 2017), but there is some evidence that creating vascularized organ systems with media that is optimized for the endothelial cells in the vascular channel, while parenchymal cells are perfused with a cell-specific media, may be a good solution, especially in coupled organ systems. In addition to the media consideration, selecting and optimizing an appropriate extracellular matrix (ECM) material is important in most models. Collagen 1 is widely used, but other hydrogels have also been used, and achieving physiologically relevant biochemistry and stiffness has been shown to be an important factor in some models (Barry et al. 2017; Kalli and Stylianopoulos 2018; Sun et al. 2018).

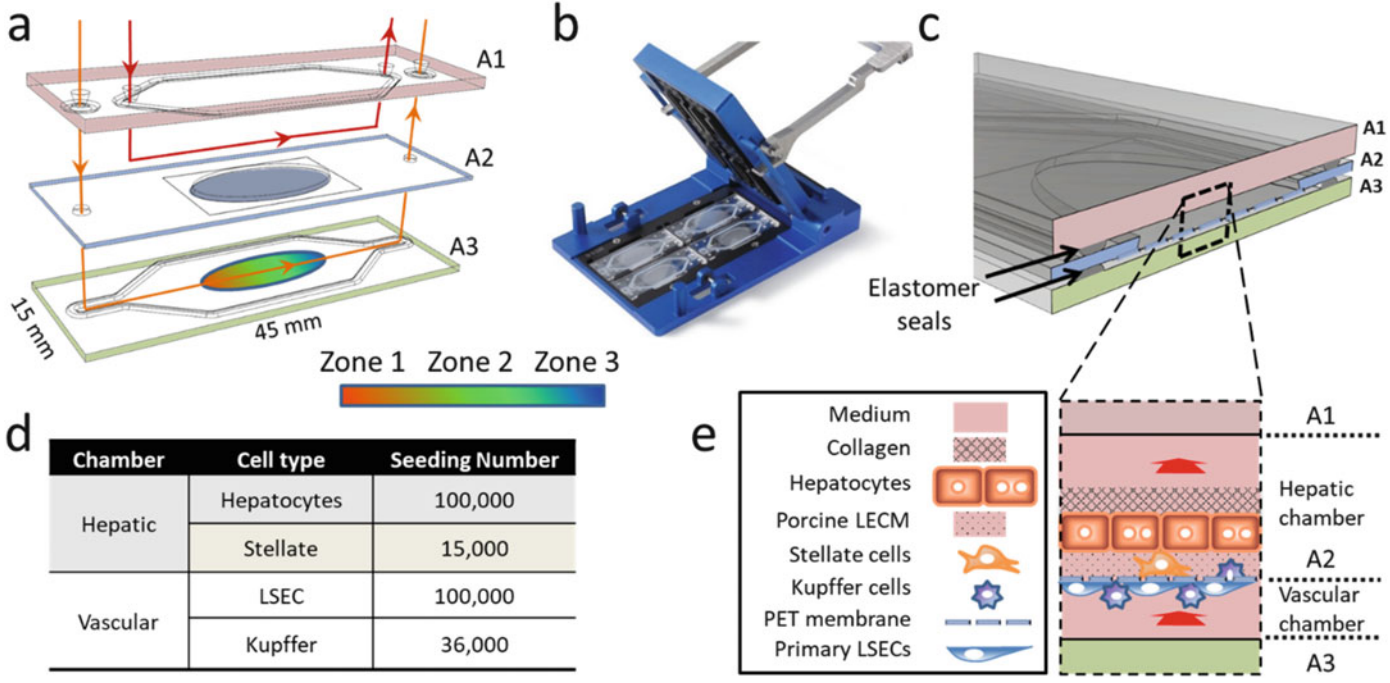
The most important aspect of an MPS is the functional performance in the particular application. A wide range of assay types have been developed and used in MPS to demonstrate basic organ functions as well as disease- and toxicity-associated responses. From a systems perspective, it is important to consider the planned readouts in the design of the model. Readouts in MPS often include secreted factors (proteins, cytokines, free fatty acids, etc.), imaging, biosensors, expression profiling, metabolism, and spatial characterization. Sampling the media efflux or from the media recirculation in microfluidic systems is typically sufficient to allow assays of secreted factors, metabolites, and cytokines, although sensitivity may be limiting in systems with high flow rates or large media volumes, key considerations in system design. Imaging, especially with the many commercial and custom biosensors (Newman and Zhang 2014; Senutovitch et al. 2015), can provide important real-time functional readouts including cell tracking, protein expression, ion concentration, enzyme activity, ROS, apoptosis, and other functions, provided the device design supports online imaging. Imaging of the 3D spatial relationships in the model can be important in establishing the organization of the cells, and interrogating subsets of the cells, such as the growth of cancer cells in an organ model of a metastatic niche (Miedel et al. 2019; Rao et al. 2019). For high-resolution confocal imaging, it is important that the device is constructed with an optical-quality, coverslip-thick "window" through which to image the cells and that the cells in the device are within the working distance of the objective, which may be >1 mm at $20\times$ and <0.2 mm at $40\times$ (Vernetti et al. 2016).

3.2 Example of a Liver MPS

The optimal MPS design will likely result from an evolution of models of increasing capability with respect to organ functions and its intended use (Beckwitt et al. 2018; Clark et al. 2016). As an example, the vascularized liver acinus MPS (vLAMPS) model (Fig. 5) currently in use at the University of Pittsburgh (Li et al. 2018) started as a micro-grooved prototype cast from PDMS and bonded to a glass coverslip for imaging (Bhushan et al. 2013). Although the prototype was functional by several metrics, the connections were unreliable, and the evaporation rate from the large surface area of PDMS was too high. To address these issues, we moved the model into the Nortis (Seattle, WA) chip, which is also cast from PDMS and attached to a coverslip but encased in plastic with metal ferrules for tubing connections. The robustness of this device provided a reproducible model for further optimization that included, along with the primary human hepatocytes and endothelial cells, the addition of human stellate and Kupffer-like cells. This model was shown to be stable out to 28 days and provides multiple functional readouts. It responded appropriately to toxic compounds (binding of test compounds to PDMS was tested); exhibited canalicular efflux, a fibrotic response (Vernetti et al. 2016); and supported the development and validation of multiple biosensors (Senutovitch et al. 2015). Further development of this model included the addition of a space of Disse using a porcine liver ECM, the incorporation of liver-specific endothelial cells, and alteration of flow rates, by which the oxygen tension in the device could be controlled to simulate oxygen zones in the liver, enabling the demonstration of zone-specific biology (Lee-Montiel et al. 2017; Soto-Gutierrez et al. 2017). However, the oxygen permeability of the PDMS made it difficult to create the continuous zonation of the in vivo liver and complicated the use of the device for screening compounds, due to the potential for absorption discussed above. Furthermore, although the model was successfully used to demonstrate organ-organ interactions (Vernetti et al. 2017), the lack of a vascular channel limited the prospects for direct coupling with other organ models, a key application for a metabolically competent liver model. To address these limitations, the model was transferred to the Micronit (Enschede, Netherlands) organ-on-a-chip platform which is glass, supports continuous oxygen zonation, and has a vascular channel for connection to other organs, as well as introduction of circulating immune cells (Li et al. 2018). Presently, multiple liver disease models, both stand-alone liver MPS and liver coupled to other organ MPS, containing isogenic primary cells or iPSC-derived cells from normal and diseased patients are in development. The liver biomimetic MPS will continue to evolve based on technological advances.

3.3 Human Liver MPS Experimental Model of Nonalcoholic Fatty Liver Disease

The liver performs ca. 500 critical functions making it vulnerable to many diseases including NAFLD, a disorder that is rapidly increasing in parallel with the



Chamber	Cell type	Seeding Number
Hepatic	Hepatocytes	100,000
	Stellate	15,000
Vascular	LSEC	100,000
	Kupffer	36,000

Fig. 5 The vascularized liver acinus microphysiology system (vLAMPS). (**a**) The vLAMPS model is assembled in a three-layer glass microfluidic device from Micronit. The center layer (A2) has an 8 × 16 mm elliptical hole with a porous PET membrane on which matrices and cells are layered. The media flow in the hepatic and vascular chambers, combined with the oxygen consumption by the hepatocytes, creates an oxygen gradient mimicking the in vivo liver acinus, creating Zones 1–3 microenvironments. (**b**) The three layers are held together in a clamp for robust connections and imaging. (**c**) The independent flow channels are sealed with elastomer. (**d**) The proportions of the four human cell types used to construct the model were chosen based on the proportions in the human liver. (**e**) The organization of the cells and matrices in the assembled model. Adapted from Li et al. (2018)

worldwide obesity and diabetes epidemics. NAFLD encompasses a spectrum of liver damage ranging from simple steatosis (or NAFL) to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC). Genetic and environmental factors, as well as disease drivers such as inflammatory cytokines, including adipokines, bacterial products, and metabolites originating from the intestine and adipose tissue, contribute to the development and progression of NAFLD (Satapathy and Sanyal 2015). The pathologic hallmarks of NAFLD include steatosis, inflammatory infiltrate, fibrosis, and hepatocyte ballooning, leading to decreased hepatocellular functions and eventually cirrhosis and HCC (Jain et al. 2015; Pacana and Sanyal 2015).

The application of QSP to NAFLD starts with studies of patient data (Fig. 1a) that have identified several NAFLD associated single-nucleotide polymorphisms (SNPs) (Speliotes et al. 2011) and gene signatures. The most relevant and reproducible SNP identified across GWAS studies is in the patatin-like phospholipase domain-containing 3 (PNPLA3) gene (rs738409 C>G p.I1e148Met) which is strongly associated with hepatic steatosis, fibrosis, cirrhosis, and HCC (Schulze et al. 2015). Despite its strong association with NAFLD, the functional significance of the PNPLA3 SNP is unknown. A major limitation in the elucidation of a mechanistic role of PNPLA3 in NAFLD has been the interspecies differences in its expression and tissue-specific distribution (Anstee and Day 2013). In particular, PNPLA3 is expressed predominantly in the human liver, whereas in mice it is mainly expressed in the adipose tissue (Smagris et al. 2015). Therefore, human patient-derived MPS are needed to study the pathogenesis of NAFLD and to test novel therapies. The use of molecular manipulation technologies is currently being used to engineer human IPSCs for specific gene knockouts and knock-ins to generate specific genetic disease models (Wu et al. 2018).

Based on network inference (Erdem et al. 2016; Grimes et al. 2019; Lezon et al. 2006; Subramanian et al. 2005), molecular interactions and signaling pathways are identified (Fig. 1b) that may be involved in the progression of early NAFLD. A consensus gene network is constructed using published interaction information (*see* Fig. 1), including regulation, protein-protein interactions, and functional relationships that are used to define on the network a disease neighborhood. Pathways containing members of the disease neighborhood are flagged as potentially disease-associated. Potential DTIs in these pathways are computationally predicted with a latent factor model such as BalestraWeb (Fig. 1c) (Cobanoglu et al. 2015), and then predicted drugs are screened in the vLAMPS models (Fig. 1d), along with compounds currently in development, to identify drugs that halt and/or reverse the disease phenotypes.

The experimental strategy is to recapitulate the early stages of human NAFLD progression (NAFL and NASH) in the vLAMPS experimental models using primary human hepatocytes and non-parenchymal cells (LSECs, stellate and Kupffer cells) from patients. The models are investigated over a 1-month period with and without the addition of known molecular and cellular drivers of NAFLD progression. Early NAFLD models are compared with normal liver models, along with clinical findings in the MPS-Db (see below) using a panel of phenotypic/functional measures.

vLAMPS models are also post-processed to produce H&E stained sections to compare the pathology with the original patient tissue. The resulting data are used to create computational models of disease progression (Fig. 1e) that are iteratively used to refine the selection of biomarkers and potential therapeutics (Fig. 1f).

3.4 Testing Drugs in Human MPS

Human MPS models are projected to have great potential to bridge the efficacy gap between animals and humans by offering drug testing in a complex, physiologically relevant human organ or multi-organ system. For many decades, animal models have served the pharmaceutical industry well for testing single target therapeutics for antibiotics, blood pressure control, or cholesterol reduction but were ineffective or even misleading when testing compounds for complex human diseases such as cancer, obesity, liver diseases, and neural degenerative diseases (van der Worp et al. 2010). Although the biomimetic fluidic MPS platforms are not high-throughput at this time, progress is being made in that direction (Satoh et al. 2017; Trietsch et al. 2013; Wevers et al. 2016). Importantly though, many biomimetic MPS models have been tested and shown to be sufficiently robust and repeatable for routine use in compound testing (Sakolish et al. 2018). Progress toward confirming correlation between the test systems and human safety and efficacy is expected to reduce the number of drugs that fail in clinical trials, despite promising findings in preclinical test species (Cirit and Stokes 2018). Preclinical animal models for toxicity assessment are still required, despite multiple examples of lead compounds that failed in clinical trials due to toxicity and despite demonstrated safety in animal models. Human MPS organ models and multi-organ models will increasingly be used along with animal models for toxicology assessment and disease efficacy models. Finally, biologic therapies such as peptides, proteins, antibodies, and cells are notoriously difficult to assess for safety liabilities in the standard preclinical toxicology models due to foreign antigen recognition and immune response. Here, again, human MPS models will offer a convenient and species-specific method to assess off-target liabilities.

3.5 Critical Role of the Microphysiology Systems Database (MPS-Db)

To accelerate the development and application of MPS in the biopharmaceutical and pharmaceutical industries, as well as in basic biomedical research, a centralized resource is required to manage the detailed design, application, and performance data that enables industry and research scientists to select, optimize, and/or develop new MPS solutions. We have built and implemented a microphysiology systems database (Gough et al. 2016) which is an open-source, simple icon-driven interface as a resource for MPS researchers (accessible at https://mps.csb.pitt.edu). The MPS-Db enables users to design and implement multifactor, multichip studies,

capture and standardize MPS experimental data and metadata (description of the experimental design and conditions), and provide tools to analyze, model, and interpret results in the context of human physiology and toxicology. The MPS-Db is designed to capture and aggregate data from multiple organ models using any type of platform from microplates to sophisticated, microfluidic devices and associate that data with reference data from chemical, biochemical, preclinical, clinical, and post-marketing sources, in order to support the design, development, validation, and interpretation of organ models. A key benefit of the MPS-Db is the standardization of metadata and data, which simplifies intra- and inter-study comparison of the results for testing and validating the performance of MPS models.

The vision for the MPS-Db is to support all MPS technologies, from organ model design to applications. Portals have been developed to aid in the design of organ and disease models by linking to databases to collate information on organs or disease biology, along with MPS data. This new information, together with the existing links to compound and clinical information, enables the user to more efficiently design and analyze proof-of-concept studies, in order to establish the model performance. Independent validation of the models is supported by tools to design studies, for example, by selecting compounds and concentrations to test and distributing those to the chips in the study, identify the best or most relevant clinical readouts, and apply statistical tools to assess the reproducibility of the model. Links to clinical data enable evaluation of clinical concordance and developing physiologically based pharmacokinetics (PBPK) models that will provide a basis for predicting exposure and clearance.

In summary, the MPS-Db supports data providers (e.g., academic and industry researchers) with tools to capture, manage, and disseminate data from experimental models, and data consumers (e.g., researchers and regulatory agencies) with a platform to analyze data and interpret results in the context of human physiology, and design computational and experimental models and studies. The variety and types of data collected and incorporated into the MPS-Db allow scientists to build predictive tools that will link the pathways or molecular events of drug toxicity and efficacy to higher-order pathways, cells, tissues, and organs. The MPS-Db is an innovative advancement for the MPS community and is the first and only publicly accessible, comprehensive resource for sharing and disseminating data and information on MPS.

4 Summary and Conclusions

The last decade has seen an explosion in the number of computational studies in the field of quantitative systems pharmacology, with the realization that current challenges in drug discovery and development require approaches well beyond traditional chemically driven efforts at the single-molecule level. In parallel, there has been progress in experimental models from classical animal models to human microphysiology systems (MPS) based on the use of human primary cells, adult stem cells, and/or iPSCs, as a powerful tool to mimic not only the structure and

morphology of human cells, tissue, and organs but also their biological or physiological functions. The combined use of these novel computational and experimental methods, complemented by classical PK and PD approaches, QSAR analyses, and ADME-Tox assessments, holds promise for overcoming the attrition effect that has long stalled progress in rational design of new therapies.

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