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Stem cells, organoids, and organ-on-a-chip models for personalized *in vitro* drug testing

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Abstract

Breakthroughs in stem cell biology and microfluidics technology have opened doors to *in vitro* screening platforms for personalized testing of safety (pharmaceuticals, nutrients, chemicals) and efficacy (pharmaceuticals, nutraceuticals). Major breakthrough technologies include development of induced pluripotent stem cells, the development of induced pluripotent stem cell-derived organoids and adult stem cell-derived organoids, and the generation of organ-on-a-chip and multi-organ-on-a-chip models to mimic human physiology *in vitro*. These technologies are highly complementary and offer tremendous potential for improved efficiency in drug development and chemical safety testing. In the current review, we will provide an overview of recent advances in *in vitro* modeling for personalized drug testing based on stem cell and organ-on-a-chip technologies and illustrate how these developments will eventually lead to the replacement of animal testing. Particular focus will be on multi-organ-on-chip human disease models, which have the potential to be the gold standard of the future for the investigation of safety, toxicity, and efficacy of newly developed medicines.

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Personalized medicine

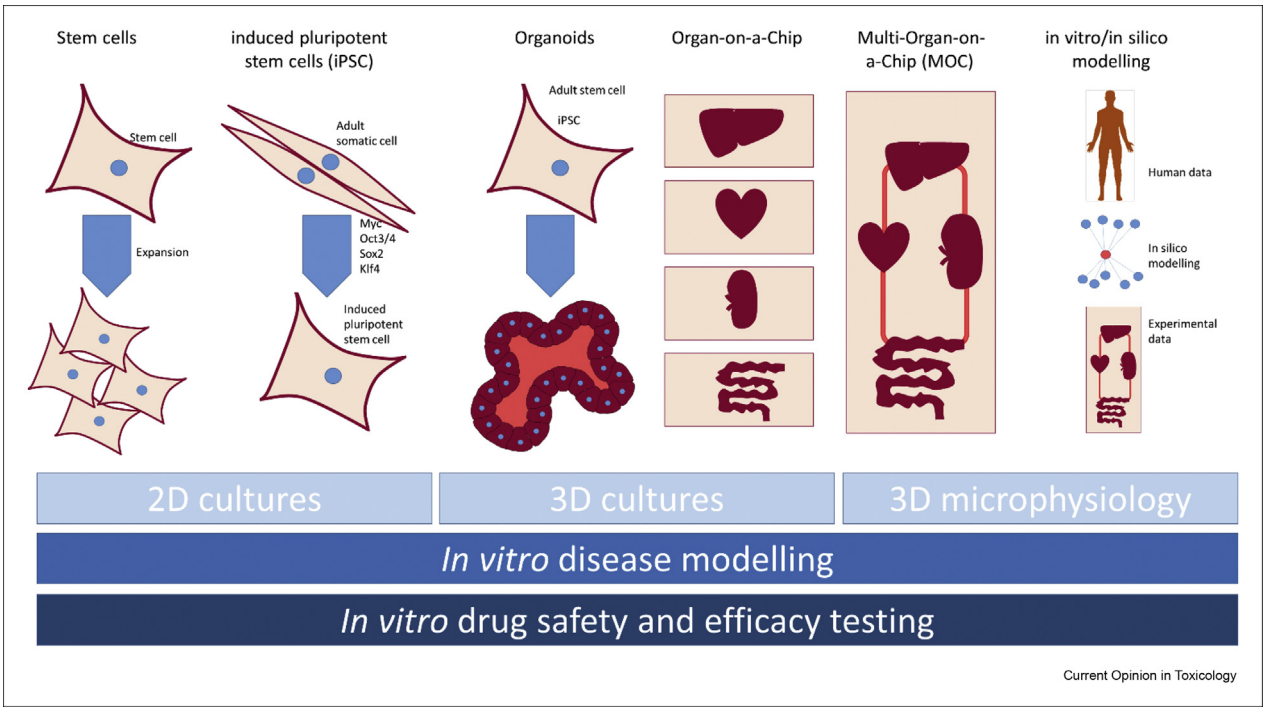
Although the term 'personalized medicine' was initially used to advocate improved attention to the patient among practitioners [1], the current definition rather describes the optimal matching of drug regimens to individuals and genetically unique patients [2]. Personalized medicine nowadays attracts much attention in drug development to optimize effects and reduce costs for new and existing drugs. Generally, drugs have thus far been approved based on their safety and efficacy in a wide range of patients. Personalized medicine is the opposite of this 'one drug fits all' approach, taking into account individual variability in response to medications, aiming to develop patient-tailored treatment strategies. For this purpose, patient- and disease-specific drug testing platforms based on recent developments in stem cell, organoid, and organ-on-a-chip (OoC) technologies would provide a very useful asset, especially regarding *ex vivo* prediction of treatment response and corresponding optimization of drug dosing (Figure 1).

Stem cells

Drug testing platforms using patient-derived cells and tissues would enable direct assessment of drug efficacy on a personalized level. Developments in stem cell technologies, such as induced pluripotent stem cells (iPSCs) and mature/adult stem cell (ASC) culture, provide an excellent basis to develop such platforms.

In 2006, Shinya Yamanaka [3,4]described how the introduction of four specific genes could reprogram adult somatic cells into iPSCs. After this breakthrough, in 2007, James Thomson et al. [5] used a different set of genes to reprogram human skin cells into pluripotent stem cells and subsequently developed several protocols using advanced three-dimensional (3D) culturing techniques for the differentiation of pluripotent stem

Figure 1



Innovative *in vitro* models for personalized medicine. With the availability of human (induced) stem cells, advanced 3D- and microfluidics-based culture systems, and *in silico* modeling of human physiology, personalized drug testing in the laboratory has become within reach. 3D, three-dimensional.

cells, for example, to aortic endothelial cells and hepatocytes [6,7]. The generation of highly complex functional multicellular renal organoids from iPSCs, consisting of nephrons (glomerulus, proximal, and distal tubule) with a collecting duct, surrounded by renal and endothelial interstitium is illustrative for the continuing strive to develop physiologically relevant organ models from patient-derived cells [8]. Apart from the initially envisioned application which aimed at regenerative medicine purposes, iPSCs have demonstrated great potential for personalized drug testing, illustrated by the establishment of iPSC-based models for Parkinson's disease, Duchenne muscle dystrophy, schizophrenia, nonalcoholic steatohepatitis, type 1 diabetes, and polycystic kidney disease [9–12].

Important progress has also been made in the understanding, culture, and application of ASCs, which are found in the human body and can replicate and differentiate to replace dead or injured cells, regenerating damaged tissue. They particularly reside in specific organs or tissues where a high cell turnover is required. At these sites, called niches, ASCs proliferate and differentiate into different cell types present in their organ of residence to maintain functional and structural integrity. Compared with iPSCs, they offer practical advantages because they do not require extensive genetic reprogramming, which is time-consuming and has a high failure rate.

Organoids

Although stem cell-derived cell monoculture offers excellent possibilities for personalized safety and efficacy testing, such cell cultures still lack structural organization and cellular variety, limiting their physiological relevance. Stem cell-based organoid technology provides an improved representation of tissue and organ physiology. Organoids are stem cell-derived, self-organized 3D tissue cultures serving as miniature representations of (human) organs [13,14]. Organoids are characterized by the presence of multiple organ-specific cell types which are organized similarly (to a certain degree) to their respective organ and recapitulate specific organ functions such as contraction, endocrine secretion, filtration, or excretion. In 2006, the first organoid-like structure was created by recruiting hepatocytes to endothelial vascular structures cultured on Matrigel, generating a structure showing high physiological similarity to the *in vivo* liver. These liver organoids could be cultured for more than two months with retained cytochrome P450 activity [15], a vast advantage over cultured primary hepatocytes, the gold standard for *in vitro* liver research, which quickly lose their metabolic capacity upon isolation and culture. Two years later, the discovery of mature stem cells in intestinal crypts allowed the generation of intestinal organoids, from single stem cells, in the absence of their nonepithelial niche [13,16]. These organoids resemble the crypt-villus intestinal structure much closer than

monocultures and are valuable tools to investigate drug-specific processes such as absorption, nutrient transport, and secretion of the hormone incretin [17].

Organoid technology is becoming more and more refined and diverse, providing a broad platform for *in vitro* drug testing. Organoid cultures can nowadays be derived from various sources, such as embryonic stem cells, iPSCs, and ASCs, and represent various organs, such as kidney, brain, heart, and other organs have been established and are explored for drug testing, like cidofovir treatment of BK virus-infected ASC-derived renal tubuloids [18]. A striking example that organoids can be extremely powerful to assess safety and efficacy of drugs in a personalized medicine setting is the use of patient-derived intestinal organoids for the personalized testing of cystic fibrosis drugs [19], which led to the initiation of a large-scale screening study of cystic fibrosis drugs in patient-derived organoids with rare mutations. Another example is provided by the development of lung cancer organoids to assess potentially different responses to drugs based on the patients' genetic alterations [19]. Interestingly, patient-derived organoids have already made their way into influencing decisions regarding patient treatments. After *in vitro* treatment using patient-derived cancer organoids showed effects of a specific drug retreatment, the patient received the retreatment, and, indeed, it proved to be effective as well *in vivo* [20]. However, a limitation that needs to be taken into consideration is that many drug responses correlated with patients' genetic mutations are generated by individual organoids and not by a representative large diverse group that can predict the response of all the organoids carrying the specific mutations [21]. Genetic variations of patients' cells can also render the standardization of *in vitro* models challenging because they rely on the genetic signature of each patient aiming to the production of patient-specific data.

Organ-on-a-chip/microphysiological systems

Although organoids exhibit a degree of structural organization, that is, similar to their tissue-of-origin, the rich cellular variety within many organs is not completely recapitulated. Vascularity is lacking or insufficient, and the immune component is usually absent. The absence of immune cells limits the organoids' application for studies of pathophysiological processes that are immune-mediated, like the idiosyncratic drug-induced liver injury, a disease that accounts for drug withdrawals and cases of acute liver failure [22]. Although organoids represent a model for a single organ or for a structure within that organ, drug testing platforms for safety and efficacy require multiple interconnected organ systems. Advanced stem cell culturing and organoid culturing technologies and developments in microfluidics now allow the establishment of microphysiological OoC models able to capture human physiology *in vitro*.

OoC models often combine a biological component consisting of a cell line, an organoid, or primary cells representing the organ of interest, with a microfluidic component simulating (pulsatile) blood flow [16].

Cell systems for OoC models are generally cultured in 3D, similar to the organoid/stem cell-based culture systems described previously, and feature improved, more physiologically similar cell population, structure, and environment than classic *in vitro* systems, combined with appropriate levels of shear stress via microfluidic flows that improve cell viability, differentiation, and polarity in various *in vitro* organ models. The systemic flow conditions mimic blood flow that 1) facilitates controlled delivery of chemicals or drugs to induce or to treat disease-mimicking conditions and 2) in combination with cellular barriers/compartmentalization allows for subsequent modeling of exposure of secondary target organs (i.e. organs distal from the site of initial exposure). The tumor environment could be adequately mimicked by the integration of multiple cell types, patient-derived cells, physiological matrices, hemodynamic mechanical shear stress, and perfusion in a 3D scaffold, allowing the analysis of biological transport and tumor-specific hemodynamics *in vitro* [23,24].

In addition, OoC models allow manipulation of the microenvironment of the cells, for example, by varying flow (and thus induced shear stress) or by introducing mechanical oscillation, mimicking breathing motion, or heartbeat [25]. During long-term culturing experiments, enabled by the continuous supply of oxygen and nutrients via the systemic flow, subacute, subchronic, and possibly even chronic exposure to a compound of interest can be evaluated.

OoC mimicking many different organs systems have been developed, for instance, for heart, lung, kidney, brain, bone, cartilage, and skin [26,27]. Aiming at personalized testing of drug safety and efficacy, a number of OoC disease models have been developed, such as for glioblastoma [28], iPSC-derived pancreatic islets for diabetes [29], intestinal organoids for cystic fibrosis [30], and primary liver cells for liver cancer [31]. Next to drug testing platforms for safety and efficacy, OoC models have been used to study drug absorption, distribution, metabolism and excretion, drug metabolism, and pharmacokinetics. For example, several blood–brain barrier OoC models have been developed [23,24] to study the delivery of drugs through the blood–brain barrier, a major physical hurdle for brain-targeting drugs. These models are based on a barrier formed by endothelial cells and may incorporate separate vascular and brain chambers to investigate the

interaction with other brain cells such as pericytes and astrocytes [32].

Organ-on-a-chip technology involves a wide range of expertise and requires the collaboration of experts from multiple research fields including (stem) cell biology, microfabrication, microelectronics, microfluidics, as well as computer modeling, and liquid physics. With so many different fields of expertise involved and many different system designs, standardization and validation clearly pose tremendous challenges.

Multi-organ-on-a-chip systems

Although OoC models can recapitulate various physiological processes of a single organ system, many diseases (e.g. diabetes, cystic fibrosis), (patho) physiological processes, and pharmacological effects involve multiple organs and cannot be investigated with a single organ-on-a-chip system.

To effectively investigate new treatments for such systemic diseases *in vitro*, systems connecting multiple organ models need to be developed. Multi-organ-on-a-chip (MOC) systems connect multiple *in vitro* organ systems (OoC models) via microfluidic channels; this enables modeling of organ—organ interaction in health and disease and provides a platform for *in vitro* testing of the effect of potential therapies on different organ systems.

The potential of MOCs to mimic physiological interactions between different organs was elegantly illustrated by Bauer et al. [33], who demonstrated that in an MOC combining pancreatic islets and liver spheroids connected via microfluidic circulation, sugar metabolism of the liver could be regulated by the insulin secreted by pancreatic islet microtissues. Recently, a microfluidic platform was presented in which gut (including the microbiome), liver, and brain OoC models were connected to investigate interactions between these organs in the context of Parkinson's disease [34]. The same group developed an MOC system to investigate organ—organ interactions in inflammatory bowel disease (e.g. ulcerative colitis), by including the gut, the liver, and an immune component represented by circulating regulatory T cells and T helper 17 (Th17) cells [33,35]. MOC systems can be particularly informative in the assessment of drug safety. Lin et al. [36] used a combination of the liver and renal proximal tubule, the major sites in drug metabolism and excretion, to investigate the toxicity profile of cyclosporine A/rifampicin administration, demonstrating reduced toxicity of the combination therapy. A combination of six organs, namely, heart, liver, brain, testes, endothelium, and lung, has been demonstrated to remain stable and viable for 21 days. This MOC system was used to investigate toxicity 5-fluorouracil, the liver metabolite of capecitabine, which is toxic for the heart and liver. Similarly, liver metabolites of ifosfamide showed neurotoxic effects in this complex MOC setup, highlighting that complex models can accurately assess drug metabolism and toxicity and provide a valuable alternative for the use of animals in preclinical drug testing [37].

One of the main challenges in the development of MOC systems is to extend the number of organs that are combined within a system which requires culture conditions (e.g. shear stress, medium composition, mechanical stretch, scaffold materials) suitable for all organ representatives. A'human-on-a-chip' or 'body-on-a-chip' system integrating all major organ systems and all of their functional units is an attractive prospect which may render animal tests obsolete. Such a system is very hard to develop and validate, and many applications do not require this level of fidelity and complexity. For instance, to investigate pharmaceutical safety, MOC systems including the major target organs for drug-induced toxicity, such as the heart, liver, kidney, intestine, and the central nervous system, could already provide a major asset for preclinical testing. For orally administered drugs, integration of stomach, gut and liver representatives in MOC models would provide useful information on drug metabolism along the digestive tract, whereas, for dermally or intravenously administered drugs, other MOC components will be required. Next, OoC modules representing (secondary) target organs would allow for assessment of efficacy and toxicity after systemic translocation, notoriously difficult to investigate *in vitro*.

Clearly, standardization and validation of a MOC system can be very difficult and time-consuming. Different rates of cell growth and differentiation time and the requirement of different flow rates, culture media, and biochemical stimuli for different cell types make MOC development and standardization very complex. Functionality and viability of all components need to be established and monitored, preferably during each experimental repeat. In addition, the physiological biomass of each biological compartment (organ) cannot be fully controlled and estimated, rendering the interpretation of obtained data challenging. Incorporation of biosensors to measure and control parameters such as medium flow, barrier integrity, viability, and the concentration of a compound of interest is very promising and can greatly facilitate MOC validation and implementation in the pharmaceutical industry [38]. Currently developed OoC and MOC models impose additional challenges regarding the suitability of the biomaterials used for their fabrication. The design of many microfluidic models is based on polydimethylsiloxane, a commonly used synthetic polymer for the fabrication of OoC models owing to its low-cost production, flexibility, and chemical inactivity [39]. However, this polymer has high affinity toward hydrophobic compounds that can lead to nonspecific binding of molecules and inaccurate

estimations hampering drug testing and rendering the standardization and validation of these models challenging [40]. Further efforts on developing fabrication materials and improving the integration of biosensors in MOC models are essential to move toward more efficient long-term drug screening and more precise predictions of drug efficacy and toxicity. All these technical and biological challenges are an integral part of the transformation process of prototype OoC and MOC models into robust drug efficacy and safety platforms in the pharmaceutical and biotechnology industry.

Multi-organ-on-a-chip models in pharmaceutical industries and regulatory agencies

Application of the 3D human (multi)cellular assays, including organoids and MOC models, in the pharmaceutical industry will allow the study of human biology, identification of therapeutic targets, and the safety and efficacy testing of specific drugs.

Advanced 3D *in vitro* assays have great potential to bridge the 'translational gap' between preclinical studies and patients and are intended to be used as human disease models, reflecting relevant human (patho)physiological mechanisms and targets, providing biomolecular and functional readouts. Currently, advanced *in vitro* models are used by pharmaceutical companies to aid internal portfolio decision-making (i.e. which drug candidates are most promising and should be developed further); an overview of OoC models that are used as such is presented in a recent t4 (Transatlantic ThinkTank for Toxicology) Workshop Report [41].

As the standard research and development workflow in the pharmaceutical industry is often based on high-throughput methodologies, hence, innovative models should be able to be implemented in such processes. Indeed, several OoC- and organoid-based models allowing high-throughput analysis have been developed. Such models are mostly based on multiwell plate formats which are modified to integrate a barrier between different compartments for cell/organoid culture and use passive (by gravity or surface tension) or active (minipump-driven) induction of flow and shear stress [42–45]. Microsensors can be incorporated to assess certain endpoints in real-time, such as trans-epithelial electrical resistance which is indicative of barrier integrity/toxicity [38,45]. Various pharmaceutical and biotechnology companies, often in collaboration with academic research groups, are currently developing organ-mimicking systems that can be implemented in the drug development workflow, based, for example, on Caco-2 cells as a gut model, primary human proximal tubule epithelial cells, or ASC-derived kidney organoids (called 'tubuloids') as a kidney model, primary human or iPSC-derived hepatocytes, and primary human intestinal cells [42,43,45–47].

Such innovative *in vitro* high-throughput methods will have applications beyond drug development; for instance, they can be used as screening methods for novel food ingredients and as a screening tool to facilitate implementation of novel chemicals that are produced as per the Safety-by-Design principle that is advocated in the European Green Deal/European chemical strategy for sustainability.

Although advanced *in vitro* models are increasingly used in (preclinical) research, regulatory agencies still request data from animal experiments before providing market approval for drugs. Stakeholders, including pharmaceutical companies, biotech companies, researchers, and policy makers are actively exploring the possibilities, and the potential, of accepting safety tests based on experiments using advanced *in vitro* models. Ironically, regulatory agencies are willing to evaluate this type of evidence but indicate that their experience and knowledge in this field are lagging behind because very few applications include evidence from advanced *in vitro* methods. Indeed, the industry, to increase the chance for market approval, does not rely on their application to contain only *in vitro* evidence [41]. A similar paradox can be identified with regard to funding proposals and research articles in the academic world, where especially (the reviewers for) high impact journals and funders of large research grants request animal experiments before publication or funding can be granted. In addition, here, the relative novelty of organoid models and especially OoC models, based on the absence of standardized cell culture protocols, acceptance criteria for tissues/cell quality, biomarkers, and endpoints for functional assessment, leads to the need for 'true' validation *in vivo* [48].

Future directions

Advances in stem cell culturing, microfluidics, and MOC models open the door to preclinical and/or *in vitro* personalized drug testing. The use of patient-derived cells, tissue, or organoids makes them a valuable tool with great potential to markedly reduce or abolish animal tests that can only provide a flawed prediction for the human population in general. In addition, they can be applied early on in pharmaceutical development, for instance, in drug discovery. MOC models allow for thorough preselection of drug candidates in an individual human, rather than general animal background. Although these *in vitro* models show great potential to serve as an additional tool in the toolbox for drug screening applications, their predictive value compared with *in vivo* models still needs to be demonstrated, and animal testing is still considered the gold standard for

preclinical safety and efficacy testing. Here, a critical trade-off is encountered: animal models often do not fully recapitulate human physiology in health and disease [49]. A solution may lie in the growing amount of human clinical and experimental data. This information allows advanced and specialized bioinformatic tools to model human physiology *in silico* and subsequently integrate such models with experimental *in vitro* data to translate these into relevant clinical situations [50].

Such bioinformatics tools use parameters from curated databases, including pharmacodynamics, gene function, and expression data, for quantitative systems pharmacology and physiologically based pharmacokinetic modeling of direct and indirect effects of several variables, including disease state, body composition, and gender [51,52]. As such, the influence of factors that cannot be modeled in MOC systems by themselves, such as physiologically relevant exposure levels to drugs, can be extrapolated. On the other hand, standardization in the collection and reporting of human clinical, biochemical, and molecular data is crucial to provide sufficient and high-quality data for computational modeling and establish a robust linkage between clinical- and *in vitro*-generated data. With these developments, the ultimate goal of a 'human-on-a-chip' may be realized as a hybrid system in which complex MOC systems, available human data, and advanced bioinformatics tools are combined. Such hybrid systems are envisioned to be self-learning and warrant generation of input for further refinement. Ultimately, they may be used for validation of less complex stem cell models, OoC models, or MOC models, thereby, playing a pivotal role in the reduction and refinement, and, in some cases, even replacement (3R) of animal experiments [41,53,54].

To successfully validate complex advanced *in vitro* models, it is necessary to build strong collaborations between academia, regulators, contract research organization, and the industry. Academia is highly innovative but lacks the facilities, budget, and experience required to move beyond a very early prototype and achieve a higher technology readiness level for ultimate replacement of animal testing. The regulatory experience from the EU Reference Laboratory for alternatives to animal testing (EURL-European Centre for the Validation of Alternative Methods (ECVAM)), the practical experience from the associated European Union Network of Laboratories for the Validation of Alternative Methods-accredited contract research organizations (CROs)/institutes that perform such *in vitro* validation studies and the needs and Research and development (R and D) experience (including a wealth of human-relevant data) from the chemical and pharmaceutical industry need to be integrated within the framework of consortia — or even a specific validation facility for innovative *in vitro* methods.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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