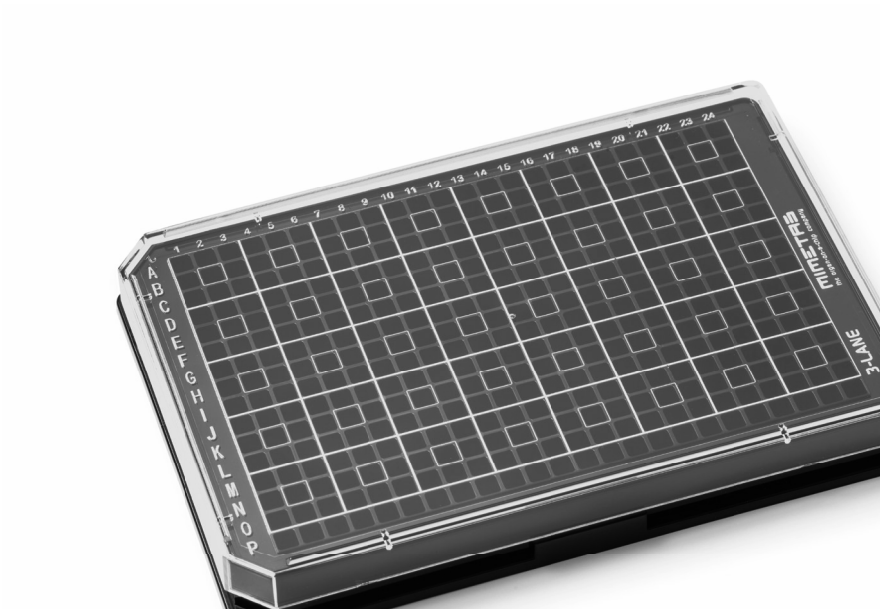
Vasculature and flow in microfluidic systems

Kramer, B.

Chapter 1

General introduction



The drug development pipeline

The drug development pipeline (Figure 1) is a complex and lengthy process that requires the use of various preclinical model systems to identify potential drug candidates, usually utilized by pharmaceutical companies. The process starts with thousands of potential drug candidates, which are subsequently narrowed down to a select few through multiple preclinical tests aimed at assessing efficacy and safety.[1] Subsequently, the selected drug candidates from the preclinical tests undergo clinical trials, that are divided in 3 phases. In phase 1, the safety of the drug candidate, including its pharmacokinetics (how the body absorbs, distributes metabolizes and excretes the drug) and pharmacodynamics (the drug's biological effects) are evaluated in a small group of healthy volunteers, with the goal of establishing an appropriate dosage range. Moving forward, phase 2 trials focuses on assessing the drug's efficacy in treating the targeted disease, identifying optimal dosing strategies and evaluating further safety. This phase often involves hundreds of patients with the specific condition to determine if the drug candidate has the intended therapeutic effect with manageable side effects. Finally, if the drug is effective and does not show toxicity in phase 2, it will proceed to phase 3 trials, where the drug is tested in an even larger group of patients to confirm its dosage, efficacy and toxicity. Then, the data from the whole process is submitted to the regulatory committees for approval to be released to the public. On average, it takes 10-15 years and costs €1-2 billion for each drug to obtain clinical approval.[2]

These high costs and lead times are mostly due to the high failure rate of drugs in the (preclinical) pipeline.[3] There are several possible explanations for the high rate of clinical failures in drug development, with the following factors being the most commonly cited: insufficient clinical efficacy (40-50%), excessive toxicity (30%), inadequate drug-like characteristics (10-15%), and insufficient market demand or ineffective strategic planning (10%).[2,3] The inefficiency of the drug development process is evident from the high failure rate of drugs in

the preclinical pipeline, requiring the exploration of more effective approaches to address these challenges, such as better translatable preclinical models. To further address these challenges, traditional preclinical models commonly used in drug development, including 2D cell culture models and animal models, will be discussed. Additionally, the emerging role of complex in vitro systems as translatable alternatives will be explored, highlighting their potential to improve predictive accuracy in the drug development pipeline.

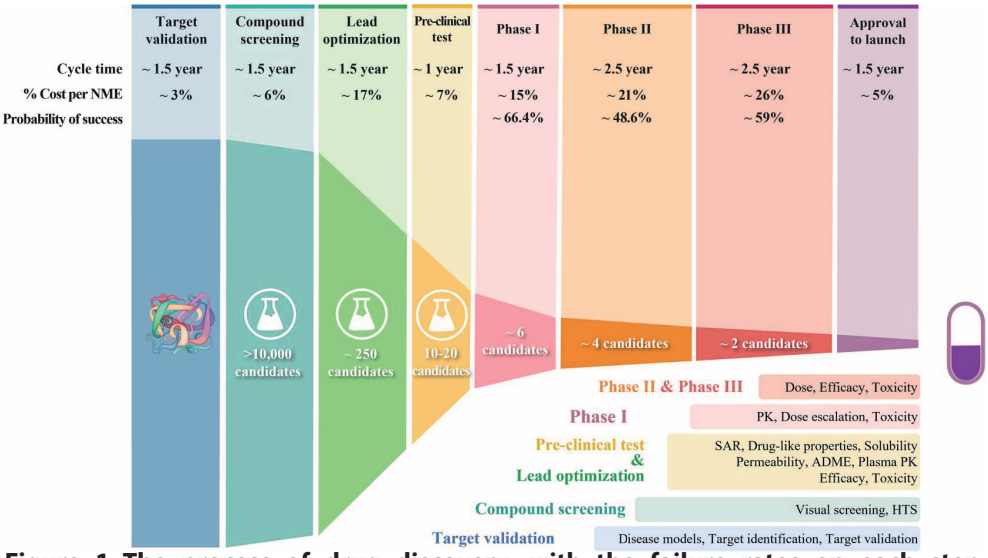


Figure 1 The process of drug discovery, with the failure rates on each step. Abbreviations: NME: New Molecular Entity, PK: Pharmacokinetics, SAR: Structure-Activity Relationships, ADME: absorption, distribution, metabolism and excretion, HTS: High-throughput screening.[2]

2D cell culture models

2D cell culture models have been used for decades in preclinical drug development,[4] and are still widely used in preclinical research to study disease mechanisms and drug development.[5] Due to the relative simplicity of the culture method and the widespread availability of primary cells and cell lines, they are an effective tool to assess thousands of compounds for their potential effectivity and safety as a drug.[6] However, these systems have many limitations. A few examples are the lack of 3D tissue architecture,[7] sufficient cell differentiation[7] and an unnaturally high proliferation rate.[4] This all impacts the accuracy of predicting which compounds should be selected for further evaluation during a compound screen.

Despite these limitations, 2D cell culture models remain a valuable tool in preclinical drug development and basic research, particularly for initial screening of large numbers of compounds or for studying cell behaviour under controlled conditions.[1] However, it is important to recognize that the aforementioned limitations of 2D culture could lead to an incorrect prediction of the efficacy of a potential new drug. Supplementing these models with more physiologically relevant models, such as animal models or 3D cell culture, to validate findings and ensure that they are translatable to human disease is needed, especially in the context of developing new drugs but also for ensuring the successful repurposing of existing drugs for new therapeutic applications.[5]

Animal models in preclinical studies

Animal models are currently an essential tool in preclinical studies, serving as a bridge between basic research of 2D cell cultures and clinical trials. By using animal models, researchers gain insight into the drug pharmacodynamics, pharmacokinetics and toxicity. Animal research can not only provide information about the potential drug's efficacy, but also its off-target effect on other organs after metabolism, as well as potential interactions with other drugs.[8]

However, there are also some limitations for the use of animal models in preclinical development. Firstly, there are ethical concerns for the use of animal models that are widely recognized by the public.[9] Secondly, animal experiments are costly and time consuming. Lastly, the translation of the results of a drug study from animal models to humans is an issue. The physiology and genetics are vastly different between humans and animals, and drugs proven safe in animals may still cause unexpected adverse effects in humans. These complexities can substantially impact the drug discovery pipeline, requiring a cautious and thorough approach to ensure both efficacy and safety in human applications.

An example is the clinical trial for the CD28 superagonist antibody TGN1412, which was hypothesized to treat multiple autoimmune diseases. After preclinical studies performed on multiple animal models, including rhesus monkeys, TGN1412 was tested in a phase I clinical study on 6 human volunteers. After administering a dose 500 times smaller than was found safe from animal studies, all humans suffered from severe allergic reactions within minutes, and faced life-threatening conditions when they were moved to the intensive care unit.[10] It was hypothesized that the difference in observed effect was caused by a 4% difference in the amino acid sequence of the C"D loop of the CD28 receptor in humans and monkeys.[11]

Bridging the gap between basic cell culture models and animal studies

Bridging the gap between basic cell culture models and animal studies requires the use of more complex in vitro models that incorporate physiological cues that better mimic in vivo conditions. These models include organoids, Organ-on-a-Chip systems, and Transwells.[5,6] Recent advances in stem cell biology, tissue engineering, and microfabrication techniques have enabled the development of these more sophisticated models, which have the potential to accelerate drug discovery and provide more reliable predictions of drug efficacy and toxicity. These advancements are particularly significant in the context of replacing or reducing the reliance on animal studies. Organ-on-a-Chip technology, for example, can replicate organ-level functions, offering a promising alternative to animal models.

The evolving landscape of advanced methodologies offers several advantages over conventional approaches, such as improved physiological relevance, reproducibility, and high-throughput capabilities. Moreover, they can provide a better understanding of the mechanisms of drug action, toxicity, and efficacy, thereby facilitating the identification of promising drug candidates early in the drug development process.

Advancements in in vitro modeling have increasingly focused on developing systems that better replicate the complexity of human physiology. The complexity of complex in vitro systems extends beyond their structural complexity and arises from their ability to incorporate additional features that mimic physiological conditions. For instance, these systems allow for the inclusion of multiple cell types to establish co-cultures, enabling the study of cell-cell interactions. Furthermore, external factors such as fluid flow, compartmentalization of cells and media and integrated measurement tools, such as electrodes for real time monitoring, can be integrated in some systems to enhance functionality.

Legislation for complex in vitro systems

The innovative momentum of complex in vitro systems was recognized with the recent progression in legislation. On 29 December 2022, the US government passed the FDA Modernization Act 2.0.[12] This bill follows up on the original 'Federal Food, Drug and Cosmetics Act' of 1938, which included mandatory animal testing for every new drug development study. Where this originally was included to improve the safety of new drug developments, the recent scientific advancements in alternatives for animal testing, such as Organ-on-a-Chip technology now offers a realistic alternative to animal testing.[13] The new act opens the door to allow clinical testing of drugs without performing preclinical animal test studies. Nevertheless, these models need to be validated if companies want to replace animal testing in preclinical research with alternatives such as Organ-on-a-Chip systems.

The current state of complex in vitro systems would not be able to immediately replace all animal studies. In the aforementioned example of systemic immune-mediated failures – the TGN1412 cytokine storm (which depended on human-specific CD28 receptor biology and tissue-resident T-cell populations) – these types of immune failures would likely remain undetected in current complex in vitro systems lacking functional adaptive immunity. However, the possibility of using human-derived cells in these systems could overcome species-specific genetic differences in protein structures between humans and animals, potentially preventing similar failures from occurring. In the following section, examples of complex in vitro systems are described.

Transwell system

One of the most widely adopted complex system in biomedical research is the Transwell system. Comprising a permeable membrane insert placed within a well, the Transwell system facilitates the creation of a bi-phasic environment, allowing researchers to investigate the interactions between different cell types, cellular layers, and their respective media (Figure 2).[14] One of the key applications of this system is the use for barrier function studies of epithelial and endothelial cells, as the easy access to the apical and basal side of these cells allow for the measurement of Transepithelial (or endothelial) Electrical Resistance (TEER).[15] This system is also widely used for cell migration assays and drug transport studies. Although the system is relatively simple (for a 'complex' system), reproducible and adaptable to various studies, it also has some limitations. The throughput is relatively low and to initiate and maintain the cultures a high amount of medium and cells are needed. The static nature of the system does not recapitulate the dynamic flow conditions that are present in vivo. Moreover, the system lacks the architectural complexity of three-dimensional tissues, which can impact cellular behavior and response to stimuli.[16]

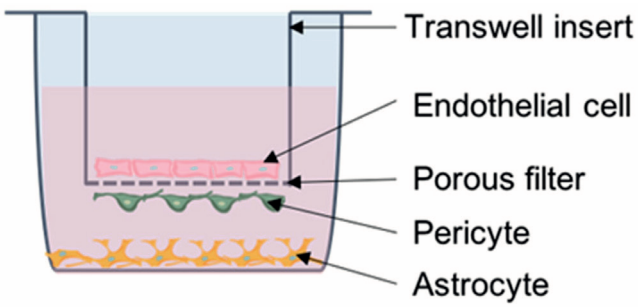


Figure 2 Schematic image of the Transwell system. A Transwell insert with a porous membrane is inserted in a well filled with medium. Cells (in this case endothelial cells, pericytes and astrocytes) are loaded on either side of the membrane, allowing for crosstalk between the cell types while keeping them separated. (adapted from[17])

Bioprinting

Another example of such complex systems are bioprinting based systems. These systems offer a technological platform to create more physiologically relevant 3D tissue models. Bioprinting involves the use of 3D printing technology to deposit cells, biomaterials, and supporting components in a layer-by-layer fashion to construct tissue-like structures (Figure 3).[18,19] This technology is used by researchers to fabricate 3D cellular constructs that mimic the architecture and functionality of native tissues, thereby providing a more accurate platform for studying tissue biology, disease modeling, and drug testing. The main advantage of bioprinting is their highly customizable nature, allowing researchers to tailor the model to their specific research questions. The created microenvironments can closely represent the architecture of the in vivo structures, and therefore adequately mimic the cellular complexity of tissues, enabling the study of cell-cell interactions.[20] However, although the complexity of this system is high, it is difficult to scale this technique to replace the current preclinical in vitro systems. The specialized equipment needed is another barrier that limits accessibility and widespread adaption.[21]

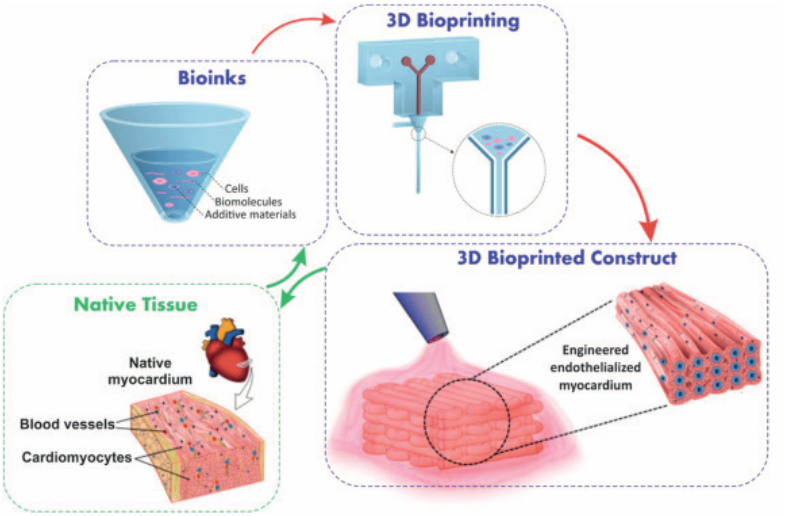


Figure 3 Schematic overview of the bioprinting process. Cells, biomolecules and additive materials are mixed into a bioink, which is 3D printed into a layered construct.[22]

Organoids

Organoids, often referred to as "mini-organs," have emerged as a revolutionary tool in the field of biomedical research, providing a 3D complex in vitro platform that mimics the structural and functional aspects of real organs. Derived from stem cells, organoids have the capability to self-organize and differentiate into various cell types, thereby recapitulating the architecture and functionality of their in vivo counterparts.[23]

A major advantage of organoids is their ability to model human disease, with cells directly derived from the patient, which makes it an interesting platform for investigating pathogenesis and developing new therapeutic interventions, known as personalized medicine. This can be used to predict the most favorable treatment option for the patient.[24] A key example of personalized medicine approaches for organoids is in the case of cystic fibrosis (CF), where organoids have demonstrated profound efficacy in tailoring personalized treatment approaches. Organoids can be used to functionally test the CF transmembrane conductance regulator (CFTR) gene, and predict treatments aimed at restoring

its function.[25] A limitation of the use of organoids is the lack of vascularization which limits the ability to mimic the in vivo environment fully.[24] Additionally, (iPSC-derived) organoids often remain in a fetal-like developmental state, lacking the full functional maturation and functionality of adult tissues. This immaturity can affect their ability to accurately model diseases and limits their applicability in certain therapeutic contexts.[26]

Organ-on-a-Chip systems

Advancing at the forefront of these technologies is Organ-on-a-Chip (OOC) technology. This approach enables the development of robust and reliable model systems that accurately recapitulate the complex physiological and biochemical features of the target organ. By incorporating relevant cell types, extracellular matrix components, and microfluidic channels that enable controlled flow of nutrients and metabolites, Organ-on-a-Chip technology facilitates the development of robust and reliable model systems and accurately recapitulates the physiological and biochemical features of the target organ.

OOC systems can roughly be divided into 2 classifications: a lower throughput system that offers high complexity in the use of cell types and configuration and a class of higher throughput devices, that standardized the configuration of the microfluidic channels, but offer higher throughput, which could be used to perform drug screening. In the following section, examples of both classifications will be discussed.

Low throughput with high complexity OOC systems

The first class of OOC systems, the lower throughput systems that offer high complexity, are often Polydimethylsiloxane (PDMS) based. PDMS is a material that is often used in OOC systems because of its ease of use, which makes it a favorable material to make quick adaptations to the chip design due to iterative prototyping, allowing researchers to easily create complex chip layouts.[27] PDMS is inexpensive, elastic and is optically clear, which makes it compatible with

(fluorescent) microscopy imaging.[28,29] A limitation of PDMS based systems is that the material absorbs small hydrophobic molecules, which makes it a less favorable material to use for drug testing.[28]

A major player in the field of PDMS based OOC devices is Emulate, a company that manufactures Organ-on-a-Chip devices that are suitable for a wide range of applications. They offer a wide range of organ models on the chip, ranging from Brain and Colon to Kidney and Liver (Figure 4).[30] The chips are connected to an ecosystem of hardware that provide medium to the chips, regulate flow, provide strain and control the humidity and temperature.[31] In addition to the commercialized platforms, there are many academic groups developing chips for their research needs.[32,33]

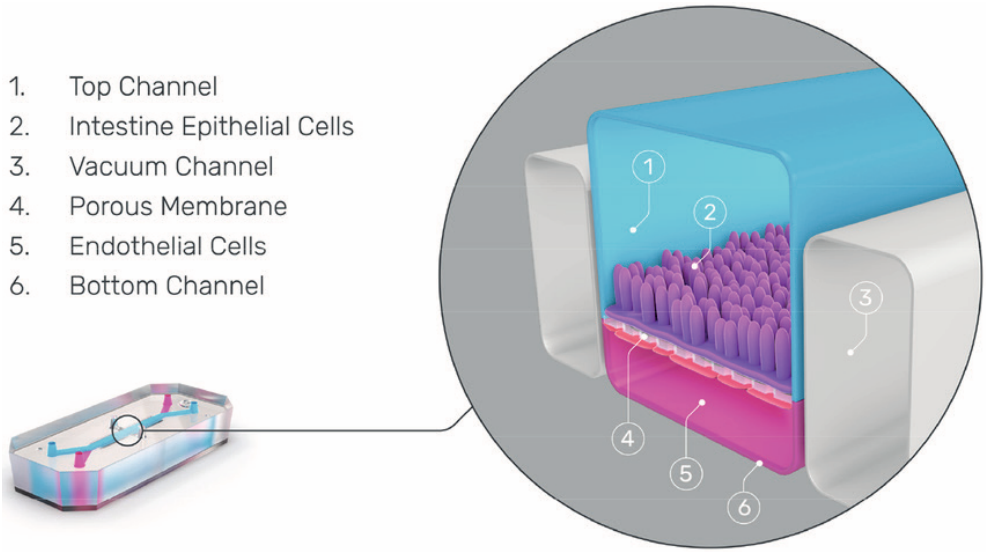


Figure 4 The Emulate OOC platform. Two parallel microfluidic channels are separated with a porous membrane. Different cell types can be loaded into the channels, allowing for cross talk between the cell types. Flow is applied through the channels.[35]

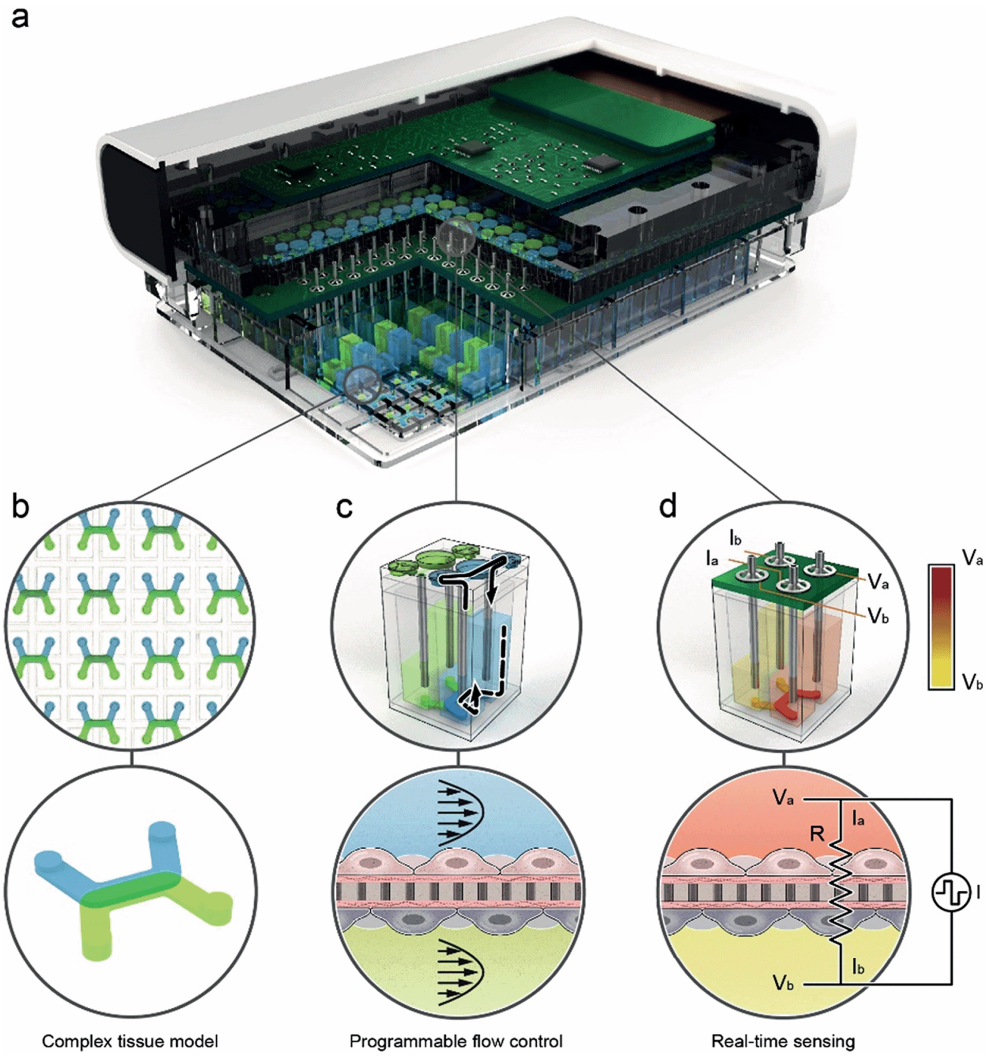


Figure 5 The Draper OOC platform. The system consists of multiple chips, which can be loaded with multiple cell types. The system is integrated with a pumping system for flow and sensing of electrical resistance (TEER).[37]

High throughput OOC systems

The second class of OOC devices focuses on high-throughput applications. This class is usually characterized by the accumulation of multiple similar chips in one larger entity, so that handling of large amounts of cultures is made possible by hand or can be automated by robotics. This opens the door for screening of large

amounts of compounds to evaluate the efficacy of new potential drugs, or screen for toxic effects of compounds.[35] The disadvantage to the first class of systems is that this limits the complexity of the design of chips more compared to first class. The high-throughput nature prioritizes volume and speed over detailed, individualized analysis. As a result, while these systems are excellent for broad screenings and preliminary evaluations, they might not capture the full depth of organ-specific responses or nuanced cellular behaviors that more complex, specialized chips can.

One example of a high-throughput Organ-on-a-Chip system is developed by Draper. Their platform, Predict96, is an advanced microfluidic platform designed to mimic the human physiological environment on a scalable platform (Figure 5). It is possible to establish 96 individual cultures on a titer plate, which is compatible with standard laboratory equipment and microscopy. Flow in the cultures is established with a pneumatic pump, that is added as a lid on top of the plate. The lid is connected with tubing for air and vacuum connections with a main control unit.[36,37]

Another illustration of a microfluidic platform is the OrganoPlate, developed by MIMETAS (Figure 6). This system also has the footprint of a titer plate, which houses 40 to 96 chips, depending on the plate design. The microfluidic channels are separated by small ridges, PhaseGuides, that act as a pressure barrier, allowing the channels to be patterned individually, without the use of artificial membranes.[38] This offers a versatile and scalable solution for applications in biology and drug discovery. MIMETAS has developed models for a wide range of organs, ranging from neuronal[39] and kidney[40], to vasculature[41] and liver.[42] Flow is added to the system in a gravity driven manner, by placing the plate on a rocking platform in the incubator. The incorporation of flow is important for mimicking the in vivo conditions, which is underscored by recent research highlighting the significance of flow dynamics in microfluidic devices.[43,44] The standard titerplate based platform and the pumpless system to generate flow demonstrate the system's scalability,

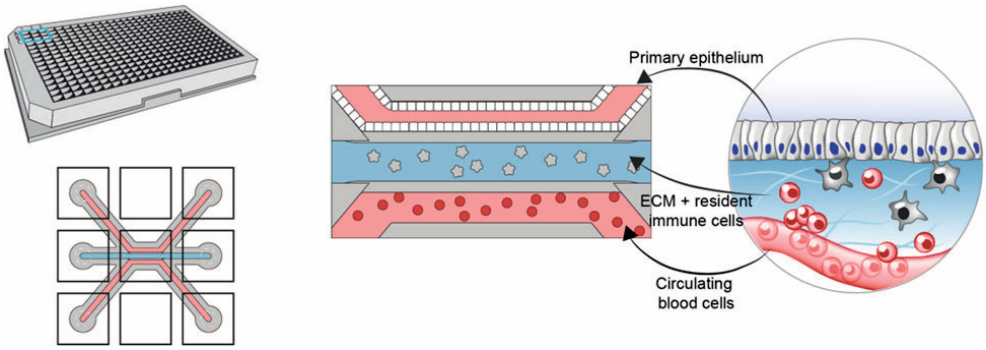


Figure 6. The microfluidic platform the OrganoPlate 3-lane 40 (MIMETAS). The platform consists of microfluidic channels separated by PhaseGuides, which allow direct interaction between the different channels without the use of membranes.[46]

as exemplified by its successful execution of a comprehensive screening of 1537 kinase inhibitors to assess their impact on angiogenesis.[45]

In order to gain insights into the potential of Organ-on-a-Chip technology for the future of drug development, it is crucial to understand the key requirements for developing physiologically relevant models and the advantages and limitations they have over the currently established models. To this end, extensive research and model optimization are needed to develop physiologically relevant in vitro models in Organ-on-a-Chip technology.[47] Another challenge lies in ensuring the translatability of these models to real-world human situations. The incorporation of flow in Organ-on-a-Chip technology could significantly boost its physiological relevance, addressing one of the limitations of static 2D cell culture models.[48] However, for Organ-on-a-Chip technology to effectively replace or supplement animal models in the drug development pipeline, the model must not only possess complexity but also demonstrate scalability. The ability to rapidly generate large scale data through high-throughput Organ-on-a-Chip platforms will be essential for integrating these models into drug screening processes, enabling more efficient and predictive testing of both new and repurposed drugs.

Scope of this thesis

The aim of the research described in this thesis is to develop physiological relevant models that utilize the capabilities of Organ-on-a-Chip technology, such as the incorporation of vasculature and fluid flow. Fluid flow is pivotal in various physiological processes, including the transport of molecules and cells, cell signaling, and tissue development. The hypothesis of this thesis is that incorporating flow in Organ-on-a-Chip systems is crucial for accurately mimicking the physiological conditions of in vivo organs. The dynamic environment created by fluid flow will contribute to model tissue specific functions by enabling the formation of gradients, to promote cellular (re)organization and to enhance the development physiologically relevant tissue structures. This incorporation enhances the predictive power of drug responses, thereby ultimately improving the efficiency and reliability of the drug development pipeline.

Almost all tissues in the human body are supplied with oxygen and nutrients by a network of blood vessels, known as the vascular network. Cells outside the diffusion range of this network will be subjected to hypoxia and apoptosis. To this end, the OrganoPlate (Figure 6) is used to create different microphysiological models that utilize vasculature and flow, aiming to more accurately replicate these in vivo conditions and validate our hypothesis.

In Chapter 2, the aim is to develop a three-dimensional Pancreatic Ductal Adenocarcinoma (PDAC) model that is suitable to assess drug resistance of existing and new therapies. Special focus is placed on the incorporation of interstitial flow, which is an important characteristic of PDAC. A difference in chemoresistance in two-dimensional versus three-dimensional cell culture is observed, and in addition the relevance of interstitial flow on drug resistance in the three-dimensional culture is demonstrated.

In Chapter 3, the focus shifts from fluid flow to vasculature. A vasculature-on-a-chip model for the toxicological assessment of substances on the early stage of

atherosclerosis is described. A vascular model with coronary artery endothelial cells is developed and a method for assessing the presence of adhesion molecules and oxidative stress is evaluated. In addition, a functional assay for the live assessment of adhesion of monocytes to the endothelium is established. Finally, the adverse effect of cigarette smoke conditioned medium on the developed readouts is studied as a proof-of-concept.

Another application of the use of vasculature in Organ-on-a-Chip systems is developed in Chapter 4. The aim of this chapter is the development of a microvessel-on-a-chip to study defective angiogenesis in Systemic Sclerosis (SSc). Angiogenesis is often dysregulated in Systemic Sclerosis, and most of the research regarding angiogenesis in SSc is performed in animal models. A microfluidic angiogenesis model with dermal endothelial cells is developed and the use of human serum is optimized. The effect of compounds and inhibitors is studied on the stability of the angiogenic sprouts. Finally, the effect of serum from SSc patients is assessed on the model as a proof of concept.

Incorporating Organ-on-a-Chip technology into the drug development pipeline requires not only thorough model development but also the acquisition of precise quantitative data. In chapter 5, a robust and high-throughput approach for the quantification of three-dimensional vascular beds is developed. Vascular beds comprising of Human Umbilical Cord Endothelial Cells (HUVECs) and pericytes are generated on the OrganoPlate Graft. The vascular beds are imaged with high-throughput confocal imaging in 3D, and the images are processed to assess the characteristics of the vascular bed. The effect of withdrawing (part of) the angiogenic sprouting cocktail and addition of the pericytes on the characteristics of the vascular bed is studied.

In chapter 6, a summary is provided of the findings of the previous chapters. The limitations of the thesis are discussed, as well as directions for future research are proposed.

References

1. Mayr, L. M. & Fuerst, P. The future of high-throughput screening. Journal of Biomolecular Screening vol. 13 443–448 Preprint at https://doi.org/10.1177/1087057108319644 (2008).
2. Sun, D., Gao, W., Hu, H. & Zhou, S. Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica B vol. 12 3049–3062 Preprint at https://doi.org/10.1016/j.apsb.2022.02.002 (2022).
3. Dowden, H. & Munro, J. Trends in clinical success rates and therapeutic focus. Nature reviews. Drug discovery vol. 18 495–496 Preprint at https://doi.org/10.1038/d41573-019-00074-z (2019).
4. Langhans, S. A. Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. Frontiers in Pharmacology vol. 9 Preprint at https://doi.org/10.3389/fphar.2018.00006 (2018).
5. Jensen, C. & Teng, Y. Is It Time to Start Transitioning From 2D to 3D Cell Culture? Frontiers in Molecular Biosciences vol. 7 Preprint at https://doi.org/10.3389/fmolb.2020.00033 (2020).
6. Costa, E. C. et al. 3D tumor spheroids: an overview on the tools and techniques used for their analysis. Biotechnology Advances vol. 34 1427–1441 Preprint at https://doi.org/10.1016/j.biotechadv.2016.11.002 (2016).
7. Imamura, Y. et al. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 33, 1837–1843 (2015).
8. Denayer, T., Stöhrn, T. & Van Roy, M. Animal models in translational medicine: Validation and prediction. New Horiz Transl Med 2, 5–11 (2014).
9. Ormandy, E. H. & Schuppli, C. A. Public attitudes toward animal research: A review. Animals vol. 4 391–408 Preprint at https://doi.org/10.3390/ani4030391 (2014).
10. Attarwala, H. TGN1412: From discovery to disaster. Journal of Young Pharmacists vol. 2 332–336 Preprint at https://doi.org/10.4103/0975-1483.66810 (2010).
11. Hansen, S. & Leslie, R. G. Q. TGN1412: scrutinizing preclinical trials of antibody-based medicines. Nature 441, 282 (2006).
12. https://www.congress.gov/bill/117th-congress/senate-bill/5002. (2022).
13. Han, J. J. FDA Modernization Act 2.0 allows for alternatives to animal testing. Artif Organs 47, 449–450 (2023).
14. Rohrschneider, M. et al. Evaluation of the Transwell System for Characterization of Dissolution Behavior of Inhalation Drugs: Effects of Membrane and Surfactant. Mol Pharm 12, 2618–2624 (2015).
15. Linz, G. et al. Cell barrier characterization in transwell inserts by electrical impedance spectroscopy. Biosens Bioelectron 165, (2022).
16. Youhanna, S. & Lauschke, V. M. The Past, Present and Future of Intestinal In Vitro Cell Systems for Drug Absorption Studies. J Pharm Sci 110, 50–65 (2021).
17. Ippolitov, D., Arreza, L., Munir, M. N. & Hombach-Klonisch, S. Brain Microvascular Pericytes—More Than Bystanders in Breast Cancer Brain Metastasis. Cells vol. 11 Preprint at https://doi.org/10.3390/cells11081263 (2022).
18. Daly, A. C., Prendergast, M. E., Hughes, A. J. & Burdick, J. A. Bioprinting for the Biologist. Cell 184, 18–32 (2021).
19. Gungor-Ozkerim, P. S., Inci, I., Zhang, Y. S., Khademhosseini, A. & Dokmeci, M. R. Bioinks for 3D bioprinting: an overview. Biomater Sci 6, 915–946 (2018).
20. Seol, Y. J., Kang, H. W., Lee, S. J., Atala, A. & Yoo, J. J. Bioprinting technology and its applications. European Journal of Cardio-Thoracic Surgery 46, 342–348 (2014).
21. Sun, W. et al. The bioprinting roadmap. Biofabrication 12, 022002 (2020).
22. Ashammakhi, N. et al. Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs. Materials Today Bio vol. 1 Preprint at https://doi.org/10.1016/j.mtbio.2019.100008 (2019).

23. Clevers, H. Review Modeling Development and Disease with Organoids. Cell 165, 1586–1597 (2016).
24. Li, Y., Tang, P., Cai, S., Peng, J. & Hua, G. Organoid based personalized medicine: from bench to bedside. Cell Regeneration 2020 9:1 9, 1–33 (2020).
25. Dekkers, J. F. et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nature Medicine 2013 19:7 19, 939–945 (2013).
26. Xu, Z. et al. Merits and challenges of iPSC-derived organoids for clinical applications. Front Cell Dev Biol 11, 1188905 (2023).
27. Cameron, T. C. et al. PDMS Organ-On-Chip Design and Fabrication: Strategies for Improving Fluidic Integration and Chip Robustness of Rapidly Prototyped Microfluidic In Vitro Models. Micromachines (Basel) 13, (2022).
28. Campbell, S. B. et al. Beyond Polydimethylsiloxane: Alternative Materials for Fabrication of Organ-on-a-Chip Devices and Microphysiological Systems. ACS Biomater Sci Eng 7, 2880–2899 (2021).
29. Cameron, T. C. et al. PDMS Organ-On-Chip Design and Fabrication: Strategies for Improving Fluidic Integration and Chip Robustness of Rapidly Prototyped Microfluidic In Vitro Models. Micromachines (Basel) 13, (2022).
30. Emulate. Emulate Product Page. https://emulatebio.com/products-services/ (2023).
31. Workman, M. J. et al. Enhanced Utilization of Induced Pluripotent Stem Cell-Derived Human Intestinal Organoids Using Microengineered Chips. Cell Mol Gastroenterol Hepatol 5, 669-677.e2 (2017).
32. Leung, C. M. et al. A guide to the Organ-on-a-Chip. Nature Reviews Methods Primers 2022 2:1 2, 1–29 (2022).
33. Driver, R. & Mishra, S. Organ-on-a-Chip Technology: An In-depth Review of Recent Advancements and Future of Whole Body-on-chip. BioChip Journal 2022 17:1 17, 1–23 (2022).
34. Wang, H. et al. 3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory consideration. Clinical and Translational Science vol. 14 1659–1680 Preprint at https://doi.org/10.1111/cts.13066 (2021).
35. Du, G., Fang, Q. & den Toonder, J. M. J. Microfluidics for cell-based high throughput screening platforms—A review. Anal Chim Acta 903, 36–50 (2016).
36. Tan, K. et al. A high-throughput microfluidic microphysiological system (PREDICT-96) to recapitulate hepatocyte function in dynamic, re-circulating flow conditions. Lab Chip 19, 1556–1566 (2019).
37. Azizgolshani, H. et al. High-throughput organ-on-chip platform with integrated programmable fluid flow and real-time sensing for complex tissue models in drug development workflows. Lab Chip 21, 1454–1474 (2021).
38. Trietsch, S. J., Israëls, G. D., Joore, J., Hankemeier, T. & Vulto, P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13, 3548 (2013).
39. Wevers, N. et al. 3D networks of iPSC-derived neurons and glia for high-throughput neurotoxicity screening. Toxicol Lett 258, S157 (2016).
40. Vormann, M. K., Gijzen, L., Hutter, S. & others. Nephrotoxicity and kidney transport assessment on 3d perfused proximal tubules. AAPS J 20, 90 (2018).
41. van Duinen, V. et al. Perfused 3D angiogenic sprouting in a high-throughput in vitro platform. Angiogenesis 22, 157–165 (2019).
42. Bonanini, F. et al. In vitro grafting of hepatic spheroids and organoids on a microfluidic vascular bed. Angiogenesis 25, 455–470 (2022).
43. Wilkinson, J. M. A review of complex in vitro cell culture stressing the importance of fluid flow and illustrated by organ on a chip liver models. Frontiers in toxicology 5, (2023).
44. Regmi, S., Poudel, C., Adhikari, R. & Luo, K. Q. Applications of Microfluidics and Organ-on-a-Chip in Cancer Research. Biosensors (Basel) 12, (2022).
45. Soragni, C. et al. Phenotypic screening in Organ-on-a-Chip systems: a 1537 kinase

inhibitor library screen on a 3D angiogenesis assay. Angiogenesis 1, 1–13 (2023).
46. Lewin, T. D. et al. An in silico Model of T Cell Infiltration Dynamics Based on an Advanced in vitro System to Enhance Preclinical Decision Making in Cancer Immunotherapy. Front Pharmacol 13, (2022).
47. Vulto, P. & Joore, J. Adoption of organ-on-chip platforms by the pharmaceutical industry. Nat Rev Drug Discov 0123456789, (2021).
48. Dellaquila, A., Le Bao, C., Letourneur, D. & Simon-Yarza, T. In Vitro Strategies to Vascularize 3D Physiologically Relevant Models. Advanced Science vol. 8 Preprint at https://doi.org/10.1002/advs.202100798 (2021).