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Microfluidic 3D cell culture for high throughput screening

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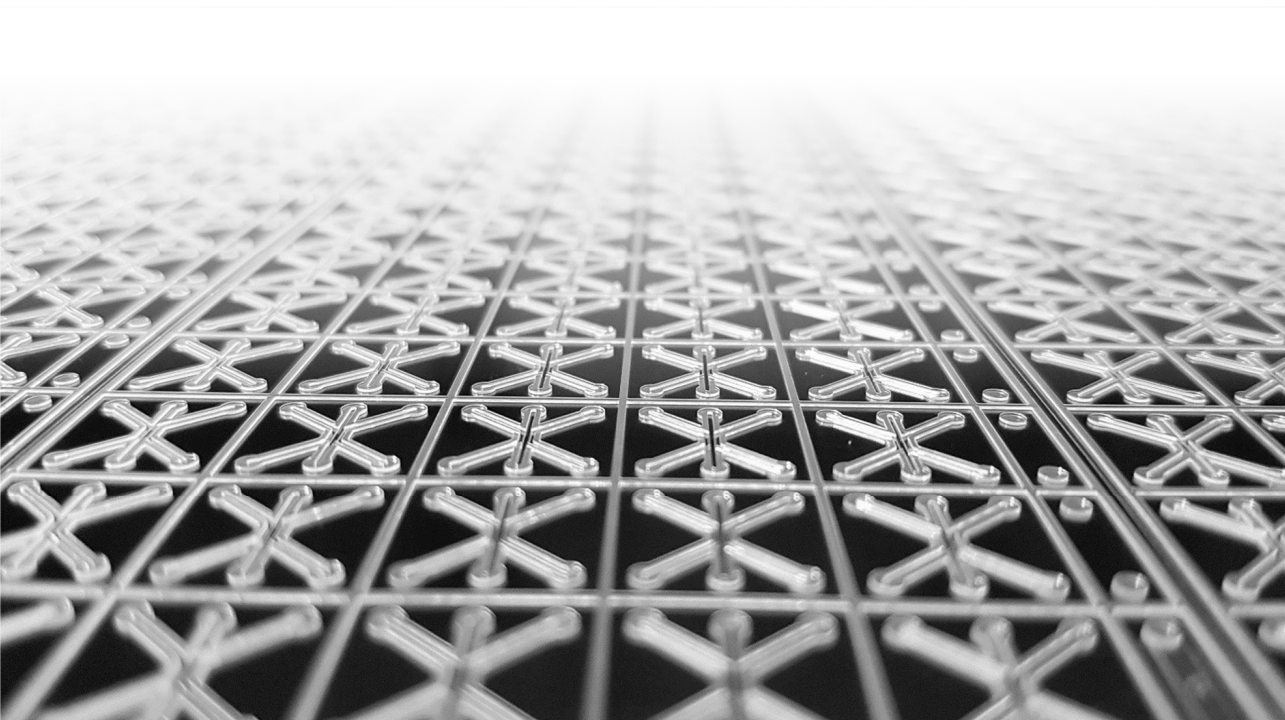
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Chapter 7

Conclusion and outlook



Conclusion and outlook

In this thesis, microfluidic technology was leveraged to develop a high throughput 3D cell culture platform. This 3D cell culture platform was designed to be used in the discovery and preclinical phases of the drug discovery pipeline. It should improve the predictivity of models that are used for efficacy and toxicity testing of compounds. These models are comprised of human cell cultures that are cultured in the relevant microenvironment. Critical factors to mimic human physiology comprise extracellular matrix embedment, 3D morphology, interaction with multiple cell types and flow. Such complex tissue models should enable selection of better drug candidates in a more efficient manner. Primary constraints to satisfy criteria of physiological relevance of these tissues were following:

- 3D tissue morphology
- Extracellular matrix embedment
- Co-culture of multiple cell types
- Integration of tubules and vessels
- Absence of artificial membranes
- Continuous flow

In addition to constraints of physiological relevance, an equally important aspect in the design of the platform was the fact that the platform could be adopted by end-users that are not necessarily an expert in microfluidics. Therefore, strong emphasis was put on the ease of use of the platform and compatible with standard laboratory equipment and routines. This led to a set of additional design constraints:

- Multiple data points in parallel
- Compatible with high throughput applications
- Stand-alone operation
- Compatibility with standard liquid handlers and imaging equipment
- Inert and biocompatible materials
- Usability by non-experts

To achieve the goal of designing a microfluidic 3D cell culture platform for use in preclinical drug development and screening, we had to select the most appropriate technology to suit these demands. A review of the state of the art in the field of microfluidics in chapter 2 showed a strong trend towards the development of complex operations in microfluidic systems. Categorizing the efforts in the field by fluid control mechanisms and detection methods gave clear insights into the trends in the field: a focus on complex, flexible systems with advanced readout. During this review of the literature the value of gaining throughput by parallel operations was clearly observed. It became, however, also clear that widespread implementation of these technologies in biomedical or pharmaceutical research had not yet occurred. We are convinced that this lack of implementation can be overcome by focusing more on ease of use and ease of adoption of microfluidic technologies. Based on these observations we concluded that for widespread adoption of any microfluidic platform, one should deviate from the search for ever more complex devices, as these are usually low throughput, require dedicated infrastructure and require specialized skills from end-users. Instead, a focus is needed on devices that can be used by non-experts using standard equipment, while maintaining and increasing the throughput in the experimentation needed for research.

Triggered by the conclusions of Chapter 2, in Chapter 3 a passive microfluidic device for screening purposes was developed. The mechanism and possibilities of phaseguide-based microfluidics were studied and extended. Both empirical studies and simulations were used to define the relation between phaseguide shape and stability. The acquired insights were used to guide the design of microfluidic networks with unprecedented passive liquid routing complexity. Selectively filling hundreds of chambers according to predetermined patterns provided a proof of principle for stand-alone, passive microfluidic devices of a complexity previously only achieved with active actuation. The detailed understanding of the functioning of phaseguides, as well as the demonstration of complex operations using passive elements only, further showed the possibilities of performing advanced microfluidic operations without increasing complexity of interfacing the device to the outside world or reducing ease of use. These complex networks can be useful for many applications in the life sciences. We expect that these networks can be used for handling of cells, chemical reactions, sample handling, sample preparation, single cell analysis, 3D cell culture, and probably many more.

The knowledge of passive liquid control gained in chapter 3 was further leveraged in the development of a microfluidic platform using a microtiter plate interface for stratified 3D cell culture in chapter 4. The so-called OrganoPlate was designed on a 384 wells plate footprint and contained up to 96 individual microfluidic networks. Phaseguides were used to pattern extracellular matrix and cells for 3D cell culture, leaving adjacent channels open for perfusion with medium. Gravity was utilized to drive flow by means of reciprocal passive leveling between two reservoirs. This eliminated the need for tubing and pumps. (Co-)culture of various cell types was demonstrated to create organized tissue architecture without usages of artificial membranes. Instead, phaseguides were used to control tissue architecture. A dose dependent response to a known toxic compound was observed and proofs of principle for transmitted light, epifluorescence and confocal microscopy based readouts were shown. The platform developed in this chapter demonstrated the range of applications that can in principle be achieved using a passive, stand-alone microfluidic device. The combination of perfused microfluidic 3D cell culture with high throughput readouts and compatibility with standard equipment formed the basis for the development for a plethora of tissue models.

In chapter 5 of this thesis, the scope of applications developed on the microfluidic platform as described in chapter 4 was expanded from (co-)culture of solid tissues to include barrier type tissues in the form of perfused tubules. The OrganoPlate was utilized to culture human Caco-2 cells as intestinal tubules, with membrane-free basal and apical access. Perfusion in these tubules was prolonged using an interval rocker platform.

In addition to immunohistochemical characterization of the tubes, an assay was developed to monitor their barrier function. The permeability of the tubule could be monitored by perfusing the lumen of the tube with a fluorescent marker. At increased permeability, diffusion of the probe across the cell barrier could be observed. Monitoring of the barrier function during exposure to toxic compounds yielded EC50 curves over time. Such dose response curves could be generated by real time imaging of the barrier integrity up to 8 hours, or be expanded into chronic exposure by intermittent monitoring for over a week. As part of this single study, over 20.000 data points based on more than 350 gut tubes were generated. This yielded the largest published organ-on-chip dataset to date according to our knowledge. The drug-induced loss of epithelial integrity by staurosporine and aspirin was demonstrated, and proved the good predictivity of this model to human physiology.

The platform and culture strategies described in chapter 4 and 5 have been expanded to a multitude of tissue models as described in chapter 6. These include models for solid tissues such as liver, neurons and cancer, but also epithelial and endothelial models including (micro-)vasculature, kidney and gut. In addition to this, complex cultures including organoid and double and triple co-cultures are being developed towards models for e.g. the blood brain barrier, renal clearance and vascularized tissues. These tissue models demonstrated that the platform is generic in nature and many more tissue models are expected to be developed in the future. End-users can decide to use any combination of cells, ECMs and levels of interaction between them to model their tissue of interest. The challenge of building the most physiologically relevant models has thus become one of biological insights and understanding, instead of microfluidic engineering.

As with any design, decisions made to increase ease of use, robustness and throughput of the platform also enforce some limitation such as a limit to the maximum size of the tissues, the maximum achievable flow, absence of mechanical cues and direct access to the tissues. The wide application of the OrganoPlate shows that this combination of strengths, including the limitation imposed by them, has a valuable place between the other platforms and research efforts in the organ-on-a-chip field.

For the various models that were developed on the OrganoPlate, a wide spectrum of assays has been, and is continuously being developed including complex morphological analysis, angiogenesis, neuronal activity, mitochondrial, metabolic function and mass spectrometric molecular analysis. The compatibility with high quality optical readouts is used in high throughput, high content assays, but other types of readout including off-plate (bio-)chemical analysis or e.g. electrical readout have also been developed.

The combination of the unprecedented level of robustness and throughput demonstrated by the platform, combined with the fact that it can be easily adopted by non-expert end-users has enabled wide spread implementation of the OrganoPlate platform developed in this thesis.

Current OrganoPlate models are comprised of human cells that are used to create healthy or diseased models. Cell sources may be a cell line, primary cells, induced pluripotent stem cells or organoids. All these techniques have been shown compatible. Primary cells, organoids and iPS derived cells offer the promise to generate disease models for some of today's most complex diseases, including

Alzheimer's and Parkinson's Disease. Techniques such as Crispr/CAS could be exploited as well to induce specific disease phenotypes. In a next stage, we predict that the OrganoPlate will become a useful tool in diagnostics: patient-derived material can be transferred to the OrganoPlate and used to establish organotypic 3D cell culture models to diagnose the presence of a disease, to predict the development of a disease, and probably most important, to predict the treatment outcome by exposing them to a panel of treatment options. First steps towards these applications have been shown in Chapter 6.

Probably the most valuable outcome of the research in this thesis is the wide spread adoption of the OrganoPlate in both academic labs, pharmaceutical industry, consumer-goods companies and chemical industry. The platform is well underway to become an industry standard and prove its value to increase efficiency in drug development and select better therapies for patients.

