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Executive Summary

Organ-on-chip (OoC) is a promising technology that might have a potential strong impact on drug discovery. This document discusses opportunities for OoC systems in drug discovery, today and in the future. These are based on opinions and discussions from experts at the ORCHID workshops, and literature examples[1,2]. Finally, we discuss how the European Organ-on-Chip Society (EUROoCS) can play a role in facilitating adoption of OoC in this scheme, and what the expected impact in the future can be.

Introduction and current status

Organ-on-chip (OoC) is a promising new technology with a potential large impact on how we will evaluate and develop drugs in the future. In the ORCHID project OoC technology related opportunities and challenges were examined from all possible perspectives, i.e. societal, economical, ethical, regulatory, etc. During several workshops we consulted experts and stakeholders in the field and together with them we defined key areas of use for OoC technology: prediction of drug efficacy & toxicity, understanding human disease and the route towards personalized medicine[2]. Figure 1 demonstrates the drug discovery process and the opportunities for OoC technology as discussed by a report of t[4] – the transatlantic think tank for toxicology (Beilmann et al. 2019).

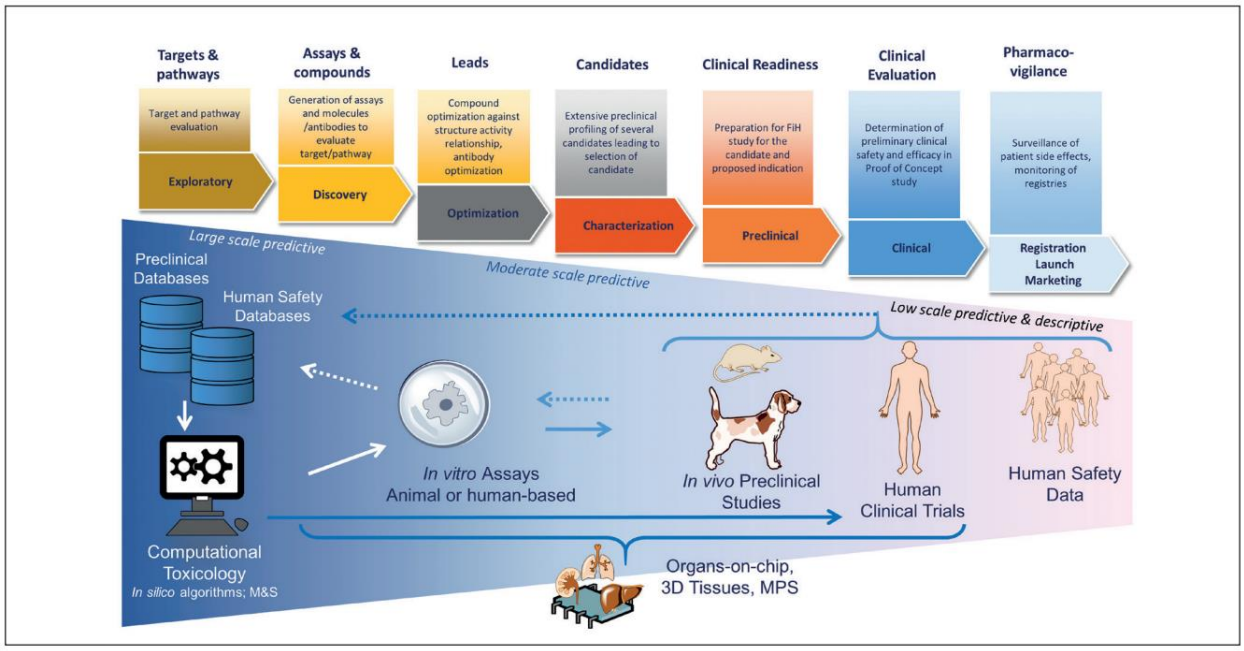

Fig. 1. The drug discovery process and opportunities for OoC systems. Reproduced from *Beilmann et al. 2019.*

[1] Organ-on-Chip in Development: Toward a roadmap for organs-on-chip
[2] Building blocks for a European Organ-on-Chip roadmap

In the short term it is likely that especially drug safety or toxicology might represent a *beach head market* for qualification of OoC devices and technology. Drug safety/ adverse effects are typically performed in the pre-clinical stage of drug development. In this stage, drug candidates already selected through the initial discovery process (lead optimization) are being validated before they move into the clinical phase (testing on human individuals). Novel methods can then be used in parallel with existing, animal- or non-animal-based methods (Valentin et al. 2010; Hardwick et al. 2017). This might also be the case for OoC devices. Drug safety always needs to be assessed in the right context, i.e. using the correct set of reference compounds. A good example of introduction of novel methods and subsequent international harmonization in the drug safety field is the CiPA initiative[3] (Servick 2016). This process could be used as a blueprint for the introduction of new technology adoption (such as OoC) in the drug discovery development, specifically for safety.

At this moment, for OoC devices there is a need for evidence-based data and qualified models. OoC methods can be introduced in two steps: (1) by producing missing, i.e. complementary data next to established methods before (2) replacing some of the established methods later on. This allows for a gradual transition that would put pharma & regulators more at ease and creates the necessary evidence where OoC methods could lead to reduction or even replacement of animal testing. There are several key academic efforts available today that take the necessary leap forward by assessing the OoC device/system in the relevant context of use and comparing it to ground root data sets, such as animal based and *in vivo* human data (Maass et al. 2017; Prantil-Baun et al. 2018; Maass et al. 2019; Foster et al. 2019; Ewart et al. 2018). Quick wins can be identified first – e.g. a complementary device or method to an existing approach. This allows for quicker adoption compared to long developments.

The OoC field is still relatively new so that most publications to date concern basic research, technology development, design and integration of appropriate sensors and the introduction of relevant biology into the devices such that long term tissue/cell survival and monitoring is feasible. There are already some significant examples of how OoC models are being used to gain insight into human disease, and in some cases identify drug target pathways. Barrile et al. (2018; Ingber's group) found evidence for low potential thrombotic risk for a monoclonal antibody (Hu5c8) in their vessel-on-chip model, which could not be detected otherwise. In addition, Song et al. (2018) show that OoC can be used to study how breast cancer cells leave solid tissue and enter the blood stream during metastases. The results show that HIF 1a via hypoxia was crucial for transmigration and that blocking this pathway could prevent metastases, validating this as a target for future therapy. Vormann et al. (2018) developed a functional 3D perfused proximal tubule model (kidney-on-chip) with advanced renal epithelial characteristics in which the principle features of kidney toxicity induced by the chemotherapeutic cis-platinum are expressed: this OoC model is now being used as a reference for other potential kidney toxins. Wevers et al. (2016) describe the use of a similar OoC platform by industry to culture three-dimensional networks of spontaneously active neurons and supporting glial cells in a microfluidic platform for parallel evaluation of compound effects in drug discovery. Zhang et al. (2018) describe how a lung-on-chip model can

[3] https://cipaproject.org/

accurately predict the toxicity of nanoparticles. Osaki et al. (2018) provide an example which showcases use of OoC in drug discovery for presently intractable diseases like ALS.

More recently, a gene therapy approach was tested for the cardiac arrhythmia condition "Catecholaminergic Polymorphic Ventricular tachycardia" (CPVT) (Pu, 2019). Inhibition of abnormal calcium waves that cause arrhythmias in patients was observed very rapidly following genetic inhibition of the Ca/calmodulin-dependent kinase II on "strips" of patient-derived human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes in a Heart-on-Chip model. Similar experiments took 3 months in mice and did not provide evidence that the approach would work in humans where cardiac physiology differs from rodents. The evidence provided by the human Heart-on-Chip data was sufficient for the researchers at Harvard to approach the regulators for permission to use the gene construct in CPVT patients. There is no other therapy for these individuals.

In another recent example, a common antibiotic was identified that could be "repurposed" to protect against the development of schizophrenia, in particular in vulnerable families (Maoz et al., 2018; Sellgren et al., 2019). Here, in a Blood-Brain Barrier (BBB)-on-Chip model under conditions of microfluidic flow which mimicked the neurovascular unit of the brain, it was shown that when microglia in the model were treated with minocycline, a treatment of acne in adolescence, synaptic " pruning" was inhibited. Abnormal synaptic pruning is characteristic of schizophrenia. Using the electronic data records of more than 20,000 individuals who had received either minocycline or another antibiotic in adolescence, the study was able to demonstrate a clear protective effect from minocycline treatment in relation to schizophrenia onset. It is unlikely this would have been discovered without the BBB-on-Chip model.

The last example concerns unpublished work from the Mummery group (ORCHID coordinator) regarding a disease called hereditary hemorrhagic telangiectasia (HHT). HHT patients have weak blood vessels and suffer from severe hemorrhages particular of the nose which causes extreme anaemia and poor quality of life. Mutant mice with the HHT gene defect only show mild symptoms though and are only suitable for testing drugs when the gene is deleted entirely, not as heterozygotes as in the case of patients (Lebrin et al., 2010). The coordinator's lab generated hiPSC from HHT patients but unexpectedly found no phenotype showing defective endothelial cells in 2D models of the vasculature. However, using 3D models of the HHT hiPSC endothelial cells under fluid flow (enabled by AIMBIOTECH chips), features of the disease as observed in patients were clearly evident. These included reduced endothelial cell proliferation and poor interaction of the hiPSC-derived vascular smooth muscle cells with the endothelial cells. This would be sufficient to cause weak vessel walls in patients. Using this model, two candidate drugs for repurposing were identified and a grant obtained for a clinical trial (Orlova, Lebrin, Mager and Mummery, unpublished).

Of note, all of these articles are very recent and thus represent state-of-the-art OoC technology. A reflective review on OoC is presently under review at Nature Reviews on Drug Discovery. These and multiple other examples (Maass et al., 2019; Edington et al., 2018; Sarkar et al., 2017) are at the stage of validation/qualification, which in general means that compounds and drugs already demonstrated as toxic or effective in treating disease in animals or patients show similar effects in OoC models. This is thus

expected to encourage their adoption by industry, their acceptance by regulatory bodies and their development as animal alternatives but this is not yet wide reality.

Next steps for qualification include performing intra- and inter-laboratory assays to assess reproducibility and accuracy and assess stability and robustness of the device/method. The CiPA initiative has been using this qualification route as well and is close to setting a novel international guideline to update ICH S7B and E14 (Millard et al. 2018). Independent testing centres such as the NCATS in the USA act as a third party ensuring an independent characterization and are key to this process for OoC[4].

Moving forward

The existing OoC community that was built partially by EUROoCS and ORCHID partners is an extremely valuable ecosystem. EUROoCS is a growing community that already has 167 members from academia, industry, regulatory agencies, patient associations and other organizations in more than 20 countries worldwide. This community should reach out globally and initiate dialogues with all stakeholders to define the OoC roadmap in Europe. The EUROoCS society can take the task to set up working groups around a specific topic, such as safety screening, for example, to stimulate the right interactions and enable the road to international harmonization. Other use cases can follow that model.

Currently, with the support and in the context of EUROoCS a European OoC Infrastructure is being designed that will bridge the gap between Developers and End User applications and support widespread implementation and acceptance of the OoC models: from experimental model to standard product. This infrastructure will select and qualify models based on criteria defined and established jointly by Developer and End User stakeholders that include the European OoC Society (EUROoCS), the Netherlands Organ-on-Chip Consortium (hDMT), regulatory agencies such as the European Medicines Agency (EMA) and the Food and Drug Administration (FDA; USA) and representatives of pharma. Models that comply with initial requirements will be taken forward to in depth testing and qualification leading to fully characterized models with guidelines and standard operating procedures on use and applications. All results will be available in a database based on FAIR guidelines, and will help end users to choose the right model for their applications. In addition, complex *in silico* modelling (PK/PD modelling) based on clinical data available through the regulatory agencies and pharma will support *in vitro* to *in vivo* translation.

EUROoCS can also act as a communication channel to promote specific topics, organize theme-based meetings and leverage the website to broaden the OoC network and share access to data[5]. The society should also help to develop training programs and stimulate communication and dissemination (e.g. through a journal). Policy makers can take this opportunity to actively help promote and even guide this process. In Europe, there are several calls in work programmes available today to promote research on OoC, but there is also a need for logistical and communication actions.

In addition to safety screening purposes, these OoC systems can also be used for efficacy testing. There are examples of OoC systems being used for PD/PK modelling (Prantil-Baun et al. 2018). For the

[4] https://ncats.nih.gov/tissuechip/projects/centers
[5] https://euroocs.eu/

development of new disease models, organ-level functions are required that recapitulate key phenotypic features of human disease in a cell or tissue. Animal models have been used to try model this complexity, but with limited success. The use of the relative unlimited source of cells with defined phenotypes and genotypes, such as induced pluripotent stem cells, is a promising path forward. These can then be differentiated into the desired somatic cells for the target organ. Introducing further complexity by culturing in three dimensions and adding flow, chemical gradients and mechanical strain to the cell/tissues would display more physiologically relevant attributes. The use of patient cell material opens the avenue to true personalized medicine approaches, in which a 'patient-on-chip' can be developed, that might replace or complement Phase I and Phase 2 clinical trials.

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