Organ on Chip in Development (ORCHID)

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1 Contents

2 Executive Summary

Organ-on-Chip is a range of emerging technologies constituting artificial micro-environments for cells and tissue constructs with possible applications ranging from more efficient drug discovery, animal-free toxicology testing, personalized treatment and cell therapy to organ regeneration. The development of technological solutions is strongly related to specific application goals. There is a stretch between custom solutions and broadly applicable platforms, requiring some degree of standardization. Regulatory conditions and legal implications differ significantly from application to application. The novel capabilities of this emerging technology ask for a re-consideration of ethical standpoints.

Purpose: This study sketches a landscape view on the current regulatory framework, efforts on standardization, and the ethical debate concerning Organ-on-Chip technology. Standardization, regulation, and ethics put a framework around technological solutions, connecting them to the interests of the different stakeholders. Since the ORCHID project aims at developing a technology & application roadmap for organ-on-chip, a study solely limited to a classical Ethical, Legal, Social Implications (ELSI) analysis is too static and does not allow a clear definition and allocation of innovation tasks to stakeholders. Instead, we will identify initial elements that impact Responsible (Research) Innovation, positively and negatively, and link them to the relevant stakeholders. The results of this study are the starting point for further engagement with stakeholders to discuss and develop guidelines for a roadmap on standardization, regulatory aspects, and an ethical debate in sync with the technological roadmap.

Methodology: For each perspective (standardization, regulation, ethics), a landscape analysis is performed based on scientific and (public) business sources. This is followed by an analysis of strengths, weaknesses, opportunities, and threats (SWOT) with respect to the development of an innovation roadmap. The major elements with impact on the innovation roadmap will be identified. Inter-dependencies will be initially identified and worked out in the next phase.

Conclusions:

Today there is no OoC system used in any regulatory approval path. However, there are clear examples of public and private-public partnerships heading this direction. This will open the field for this emerging market and aid adoption in regulatory approvals. Because of strong (international) presence of legislators in OOC development and qualification, this process can be accelerated.

OoC system standardization can build further on existing standards or standardization efforts of its subcomponents and sub-processes. Although there are no golden standards defined today, keeping in mind previous standards and guidelines and stimulating a close collaboration with relevant stakeholders will determine successful introduction of OoC's in drug development.

The ethical discourse has a largely favourable, positive bias today, indicating OoC as a possible solution reducing cost, need, and ethical burden of animal studies, both for drug discovery and even more for toxicology studies. OoC as a broad platform technology has the benefit of being able to adapt to evolutions in biological science, e.g. the replacement of controversial human embryonal cells by human induced pluripotent stem cells, which largely silenced the ethical debate. OoC has also the potential to finally allow

for drug discovery and personalized treatment for small or differentiated target groups (rare diseases, children, pregnant women, gender specific, ethnic specific). However, this also poses the question of who indicates priorities. Citizen/patient donors may expect a personal benefit of donation rather than a societal impact. There is an underlying risk that media interest and coverage on new evolutions in OoC overpromise, possibly resulting in a hype cycle. A second risk is to underestimate aspects of informed consent, data ownership, and privacy concerns when using human-donated cell or tissue samples in combination with OoC trials. The ethics impact of a 'personal OOC model' as part of a personal Avatar model, partly in silico, partly on chip and related aspects of data ownership and privacy are still largely unaddressed.

3 Introduction and Overview: Regulation, Standardization, Ethics

The convergence of microfabrication, microfluidics and tissue engineering gave rise to Organ-on-Chip technologies. Their aim is to replicate key aspects of the human physiology that are crucial for understanding the effects of drugs, thereby improving preclinical drug safety and efficacy testing. 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. These showcases, including detection of thrombotic risk in vessels-on-chip, discovery of targets for metastases in cancer-on-chip, test for kidney toxicity in kidney-on-chip, drug effects on neurons and glia cells-on-chip, prediction of toxicity of nanoparticles in lung-on-chip and drug discovery in a disease model for ALS have been described in D2.2. Of note, all these results are very recent and thus represent state-of-the-art OOC technology. These and multiple other examples are at the stage of validation/qualification, to prove that compounds and drugs that have already been shown to be toxic or effective in treating disease in animals or in 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.

In order for OoC systems to be adapted in pharmaceutical testing processes, a close collaboration between technology developers and regulatory bodies is necessary. Demonstration that the results of testing using OoC systems parallel those from clinical trials is critical for regulatory approval. As qualification of OoC systems is a first step to take and will require significant effort, standardization of OoC technology is only relevant at a later stage. However, it is important to keep standardization in mind when developing OoC systems and strive towards scalable and reproducible fabrication. For some subcomponents used in OoC systems such as microfluidic devices and human stem cells, there are already efforts towards standardization and internationally accepted guidelines. Finally, the use of human material in the construction of OoC's is crucial to emulate the human physiology. Therefore, ethical aspects to be considered are found in patient consent and data privacy. At the same time, the use of human cell material, by definition, follows the 3R's principle and thus the reduction of the use of animals for testing.

Purpose: This study sketches a landscape view on the current regulatory framework, efforts on standardization, and the ethical debate concerning OoC technology. Standardization, regulation, and ethics put a framework around technological solutions, connecting them to the interests of the different stakeholders. Since the ORCHID project aims at developing a technology & application roadmap for OoC, a study solely limited to a classical Ethical, Legal, Social Implications (ELSI) analysis is too static and does not allow a clear definition and allocation of innovation tasks to stakeholders. Instead, we will identify initial elements that impact Responsible (Research) Innovation, positively and negatively, and link them to the relevant stakeholders.

- Section 4 addresses the regulatory context. Current regulatory pathways are not yet targeted to adopt the benefits of OoC technology. However, understanding the drivers behind current regulation will help driving the necessary adoption and qualification processes for OoC.

- Section 5 analyzes the current variety of technologies for OoC and identifies opportunities and barriers for improving ease-of-use and re-use by means of standardization efforts. While less relevant during early technology development, standardization of components, interfaces, and protocols is essential to increase the technology readiness/maturity level, guarantee manufacturability (cost, volume, and complexity of the manufacturing process), and gain broad adoption by the user community.
- Section 6 addresses the ethical impact of the introduction of OoC for drug discovery and toxicology as shorter-term applications and, briefly, for longer-term applications building further on first successes.

The results of this study are the starting point for further engagement with stakeholders to discuss and develop guidelines for a roadmap on standardization, regulatory aspects, and an ethical debate in sync with the technological roadmap.

Methodology: For each perspective (standardization, regulation, ethics), a landscape analysis is performed based on scientific and business sources, capturing the specific OoC context but also dependencies and learnings from adjacent fields on which OoC can draw. Examples are standardization of microfluidic components, regulatory and legal aspects around the use of human induced pluripotent stem cells, or ethical implications of replacing animal studies by human cell-based OoC testing.

This analysis by perspective is followed by an evaluation of strengths, weaknesses, opportunities, and threats (SWOT) with respect to the development of an innovation roadmap. The major elements with impact on the innovation roadmap as well as the link with relevant stakeholders will be identified. Inter-dependencies between the three perspectives will already be highlighted.

This will translate into a set of concrete follow-up actions to be further worked out with the identified stakeholders during the ORCHID project.

4 Regulatory context

4.1 Current regulatory framework for drug approval

Regulation of the development and dissemination of medical drugs involves competing interests: ensuring that agents are both safe and effective, while facilitating the movement of innovative therapies as rapidly as possible through the investigative process to public use. Balancing these goals is globally in large measure a task of the Federal Food and Drug Administration (FDA) in the United States, and of regional and centralized regulatory bodies in the European Union (EU)(Van Norman 2016).

In the drug development process, safety and efficacy testing is an important phase before the drug is tested in human subjects (clinical phase). The guidelines for safety testing of new drugs are harmonised between Europe, Japan and the USA by the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The safety guidelines include guidelines for carcinogenicity studies (S1A-S1C), genotoxicity studies (S2), toxicokinetics and pharmacokinetics (S3A-S3B), toxicity testing (S4), reproductive toxicology (S5), biotechnological products

(S6), pharmacology studies (S7A-S7B), immunotoxicology studies (S8), nonclinical evaluation for anticancer pharmaceuticals (S9), photosafety evaluation (S10) and nonclinical paediatric safety (S11)[1].

Both the FDA and the European Medicines Agency (EMA) are implementing efforts to execute the so-called 3R's principle in regulatory research, product review practices and policy. The principles of the 3R's were first defined by Russell and Burch (1959). Meanwhile, these principles have been further expanded and now encompass(Russell 1995):

• *Replacement*: testing approaches that avoid or replace the use of live animals in an experiment where they would have otherwise been used. Replacement could include the use of established animal and human cell lines, or primary cells and tissues or mathematical and computer models or physicochemical methods.

• *Reduction*: approaches that minimise the number of animals used per experiment or study, either by enabling researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals, thereby avoiding further animal use. Examples include improved experimental design and statistical analysis, combination of studies, international harmonisation of testing requirements (e.g. (V)ICH) to avoid duplicate testing and the use of technologies, such as imaging, to enable longitudinal studies in the same animals.

• *Refinement*: approaches that minimise the pain, suffering, distress or lasting harm that may be experienced by the animals. Refinement applies to all aspects of animal use, from the housing and husbandry used to the scientific procedures performed on them. An example of refinement is the use of appropriate anaesthetics and analgesics.

4.1.1 European framework

The European commission synchronized the regulations of 28 different countries for its drug approval process. Before that, drug approvals were assessed by EC Directive 65/65/EEC (1965) and was (and still is) very similar to its American counterpart. The EMA was formed in 1965 with funding from the EU, pharmaceutical industry and member states. The EMA was responsible for harmonization of the processes in the member state regulatory agencies, in order to reduce the costs to drug companies and to eliminate competition-restricting regulation. The difference with its American counterpart is that the EMA does not oversee all drug approvals; rather, there are 4 routes by which a drug can be approved, which depends on the drug class and manufacturer preference:

1) Centralized process: controlled by EMA. This route is mandatory for some classes of drugs such as treatments for HIV/AIDS, oncology, etc.
2) National process: drugs that fall outside the former; each EU state can have its own procedure.
3) Mutual recognition: drugs approved in one state can obtain authorization in another state.
4) Decentralized procedure: simultaneous approval in more than 1 EU state.

[1] http://www.ich.org/home.html

Animal models are used in safety and efficacy testing of new drugs to measure how much a drug is absorbed in the blood, how the drug is broken down chemically, what the toxicity is of the product and its breakdown components and how quickly the product and its metabolites are excreted from the body. Current regulatory framework for animal testing approaches of human and veterinary medicinal products need to consider the Directive 2010/63/EU, which is the principle of the 3R's. Testing is typically carried out to support clinical trials, marketing authorisation, in-process quality control and at the end of the product process i.e. final product batch testing. In scope of the current project we will only consider human medicinal products for safety/toxicity testing.

As stated in the EMA/CHMP/CVMP/JEG-3Rs/450091/2012 guideline: "Ethical and animal welfare considerations demand that animal use is limited, and preferably avoided, as much as possible. In this respect, Directive 2010/63/EU on the protection of animals used for scientific purposes, which is fully applicable to regulatory testing of human and veterinary medicinal products, unambiguously fosters the application of the principle of the 3R's when considering the choice of methods to be used". [2]

In the United Kingdom, the NC3Rs[3] was set up to act as a focal point for all research related to the 3R's.

4.1.2 Framework in the United States

The Public Health Service (PHS) and thereby FDA's Center for Biologics Evaluation and Research (CBER) and the Center for Devices and Radiological Health (CDRH) adopt the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (the Principles) for preclinical studies that involve animals. Further, the Center for Drug Evaluation and Research (CDER) is the watchdog to ensure evaluation of drugs before they can get sold. CDER makes sure that the drug works correctly, that their health benefits outweigh their known risks, and provides doctors and patients the information they need to use medicines wisely. Testing evidence of safety and efficacy of a particular drug is sent to the CDER for review by the organization of interest. This process is independent and unbiased and performed by the team of specialists within the CDER. The drug approval process takes place within a structured framework including[4]:

- Analysis of the target condition and available treatments: weighing of the drug's risks and benefits.
- Assessment of benefits and risks from clinical data: FDA takes the data into account from the drug maker.
- Strategies for managing risks: risk management including an FDA-approved drug label.

[2] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Official Journal L 276/33).
[3] https://www.nc3rs.org.uk/
[4] https://www.fda.gov/drugs/developmentapprovalprocess/default.htm

In particular, during the pre-clinical stage, drug safety needs to be assessed. Safety pharmacology and pharmacodynamic studies are defined in ICH guidelines. The core battery of safety pharmacology studies includes the assessment of effects on cardiovascular, central nervous and respiratory systems in accordance with ICH S7A and S7B[5].

The FDA also supports efforts to reduce animal testing. In addition, FDA has research and development efforts underway to reduce the need for animal testing and to work toward replacement of animal testing. When animal testing is performed, manufacturers and sponsors are required to follow FDA's regulation "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58). FDA also supports the use of independent animal care and committees (IACUC) for laboratory studies involving animals.

4.2 Regulatory acceptance of 3R's testing approaches

The following criteria should be used before consideration of a 3R's testing approach for regulatory acceptance:

• **Availability of defined test methodology** including standard protocols with clear defined/scientifically sound endpoints.

• **Relevance**, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method[6].

• **Context of use** includes a description of the circumstances under which the 3R's testing approach is applicable in the assessment of human medicinal products and the limitations within which the available data adequately support use of the 3R's testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods.

• **Reliability/robustness**; a measure of the extent that a test method can be performed reproducibly over time and in different laboratories when using the same protocol.

While replacement of animal studies remains the ultimate goal, approaches aiming at reducing or refining animal studies are routinely implemented in regulatory guidelines, where applicable. The European Pharmacopoeia also encourages animal-free approaches to be used by manufacturers including, for example, through the proof of consistency to avoid unnecessary tests in animals on intermediate stages of production or on the final product. Alternative methods need to fulfill the 'fit-for-purpose' criteria for

[5] https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073246.pdf
[6] ICH S1 Regulatory Notice Document (http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2012/12/WC500136405.pdf).

regulatory acceptance (Griesinger et al. 2016). The above-mentioned criteria can be achieved by (1) qualification of the equipment according to standard installation, operation and performance; (2) meeting the rules of Good Cell Culture Practice and use of qualified cell and tissue sources thus assuring reproducible data; (3) improvement of current assays towards closer resemblance of *in vivo* physiology (Griesinger et al. 2016)(Horvath et al. 2016).

4.3 Validation processes for new 3R's test methods

Real barriers for the validation of new methodologies, such as OoC systems, can be lifted through the guidelines as described above. Further, examples of formal validation processes for 3R's test methods are described by the **European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM)** and by the **European Directorate for the Quality of Medicines (EDQM)**. Formal validation generally directly implies the intention to seek regulatory acceptance. EURL ECVAM's validation criteria are comparable to the criteria subsequently defined by the (US) **Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)** and the **OECD**[7,8] (Balls and Fentem 1999). The evolution of a regulatory test is subdivided in five stages that reflect the sequence of steps to be performed for a prospective validation exercise:

(1) Evaluation of candidate method (to see if suitable/ready for validation)
(2) Pre-validation (protocol refinement, transfer and performance)
(3) Validation
(4) Independent peer review
(5) Recommendation for consideration in a regulatory context (e.g. development of new or updated OECD test guidelines).

In a prospective validation study, an inter-laboratory blind trial (involving at least three laboratories) is conducted to assess whether tests can be shown to be relevant and reliable/robust for one or more specific purposes. This inter-laboratory trial is followed by data analyses and an evaluation of the outcome of the study in comparison with predefined performance criteria[9]. The modular approach to the EURL ECVAM principles on test validity allows for flexibility by breaking down the various stages in validation into independent modules and defining for each module the information needed for assessing test validity. This allows for retrospective validation studies to be conducted or for a combination of retrospective and prospective studies (Hartung et al. 2004). At the level of the EDQM, the Biological Standardisation programme (BSP) aims at validating new methods for the quality control of biological

[7] OECD (2005). Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. OECD Testing Series and Assessment Number 34. ENV/JM/MONO(2005)14, pp 96, Paris, France: OECD.
[8] Balls M, Blaauboer BJ, Fentem JH, Bruner L, Combes RD, Ekwall B, Fielder RJ, Guillouzo A, Lewis RW, Lovell DP, Reinhardt CA, Repetto G, Sladowski D, Spielmann H and Zucco, F (1995) Practical aspects of the validation of toxicity test procedures. The report and recommendations
of ECVAM workshop 5. ATLA 23: 129-147.
[9] General Notices, Section 1.1, Demonstration of Compliance with Pharmacopoeia, European Pharmacopoeia, Edition 8.7, 2015.

medicinal products with the goal of including them in European Pharmacopoeia monographs. It is overseen by a steering committee consisting of the chairs of the relevant European Pharmacopoeia groups of experts, representatives from the relevant EMA working parties, co-opted scientific experts and an observer from the World Health Organization (WHO). The programme takes methods of interest which have been validated on a local scale (single laboratory/limited products) and proceeds with a wider generic validation to demonstrate the potential applicability in other laboratories and with other similar products on the market. Similar to the EURL ECVAM procedure the process involves multiple phases including preparatory method refinement, small scale transfer studies and finally large scale international collaborative studies with manufacturers and national control laboratories. The study reports are presented to the relevant European Pharmacopoeia expert group for consideration for inclusion of the method in the European Pharmacopoeia and are made publicly available[10].

3R's testing approaches that have sufficient demonstration of scientific validity but have not been assessed in a formal validation process can however also be accepted in a regulatory submission and/or included in regulatory guidelines/documents wherever possible. In this case, the data are evaluated on a case–by-case basis by the competent authorities (e.g. EMA and National Competent Authorities [NCAs]). For the US landscape, short term goals for validation can be defined to initiate regulatory consideration of the OoC technology. During further refinement of the OoC's, input and guidance of the FDA will be needed (see also 4.4). Systems aiming for safety and efficacy testing during drug development might be qualified through the US FDA drug development tool (DDT) Qualification program.

The pharmaceutical industry also needs to play an important role to help to determine specifications and identify biomarkers. Promotion of the OoC platforms will lead to integration of the technology in FDA applications, which leads to more understanding and confidence. Complementarity to data from other models might be most effective in this aspect. Further, as part of its Critical Path program, the FDA developed the Biomarker Qualification program. Validation of individual biomarkers on individual chips could be an important step towards the qualification and acceptance of OoC systems. Other procedures where OoC's could be included are the Investigational New Drug Application or New Drug Application (Livingston, Fabre, and Tagle 2016).

4.4 Examples of validation of OOC and in vitro assays guided by regulatory bodies

In the USA, the National Center for Advancing Translational Sciences (NCATS), a subsidiary of the National Institutes of Health (NIH), is leading efforts towards replacing animals for toxicity testing, disease modelling and efficacy testing. Together with other NIH Institutes and Centers, the Defense Advanced Research Projects Agency (DARPA) and the FDA have set up a program to develop human tissue chips to

[10] Guideline on the Principles of Regulatory Acceptance of 3Rs (Replacement, Reduction, Refinement) Testing Approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012).

model the structure and function of human organs[11]. In the first phase, the development of 3D cellular microsystems was funded (2012-2014). Systems that showed physiological function successfully were then further refined (2015-2017). The project involves Tissue Chip Testing Centers, which are independent institutions and provide a way to test and qualify tissue chip platforms and promote adoption by the broader research community. The Tissue Chips for Disease Modelling initiative (1) supports development of *in vitro* disease models using primary tissue or induced pluripotent stem cell (iPSC)-derived cells from patient sources on tissue/OoC platforms, (2) determines the disease relevance of these models by preliminary testing of key experimental features and (3) tests the effectiveness of candidate drugs.

Further, there is the Tox21 initiative, using robotics and other testing approaches to more efficiently predict how chemicals may affect human health (Schmidt 2009). Tox21 is developing cell-based tests and biochemical approaches, which measure chemical substances produced in living organisms. Ultimately, these new strategies will help to quickly evaluate thousands of chemicals and support regulatory decisions about the safety of chemicals. Federal partners involved are the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), the NCATS, the FDA and the National Center for Computational Toxicology. The use of robots for handling of chemicals and cells drastically reduces the workload. Part of the data of the project was published in 2015 as computational efforts to predict toxicities of environmental compounds (Eduati et al. 2015).

The EU-ToxRisk project is an integrated European Flagship programme aiming to drive mechanism-based toxicity and risk assessment for the 21st century, thereby shifting paradigm from black box animal testing towards toxicological assessment based on human cell responses[12]. More efforts towards development of innovative tools and methodology for safety assessment are found in programmes such as SEURAT-1[13], eTox[14], ESNATS[15], Chemscreen[16], NOTOX[17], and SCR&Tox[18].

In particular for the assessment of cardiotoxicity, one of the most commonly occurring adverse effects of drug compounds, alternative test methodologies are being investigated. This is a direct response to the lack of predictive power of current paradigms, mostly relating on hERG blocking screening assays and QT prolongation (Fermini et al. 2016)(Servick 2016). The Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative started following a workshop in July 2013 at the US FDA. The objective is to engineer an assay for the assessment of the proarrythmic potential of new drugs that has improved specificity compared with the hERG assay plus thorough QT study[19]. The initiative is headed by a steering team consisting of members of the FDA, Health and Environmental Science Institute (HESI), Cardiac Safety Research Consortium (CSRC), Safety Pharmacology Society (SPS), EMA, Health Canada, Japan National Institute of Health Science and Pharmaceuticals and Medical Devices Agency (PMDA). CiPA workstreams consist of 4

[11] Tissue Chip; https://ncats.nih.gov/tissuechip/about/operations
[12] http://www.eu-toxrisk.eu/
[13] http://www.seurat-1.eu/
[14] http://www.e-tox.net/
[15] http://www.esnats.eu/
[16] http://www.chemscreen.eu/
[17] http://notox-sb.eu/
[18] http://www.scrtox.eu/
[19] http://cipaproject.org/

aspects: develop voltage clamp protocols for multiple human cardiac currents, *in silico* reconstruction of human ventricular cellular electrophysiology, *in vitro* effects of human stem-cell derived ventricular myocytes, unanticipated clinical evaluation in Phase 1 studies. The *in vitro* assay based on human stem cell-derived cardiomyocytes was tested in a blinded pilot study. Ten out of eighteen studies demonstrated sensitivity to potassium and calcium channel block (Millard et al. 2018).

FDA also recently engaged in a collaborative agreement with the US O0C startup Emulate, Inc., to use OoC technology as a toxicology testing platform, for understanding how products affect human health and safety[20]. In the multi-year agreement FDA and Emulate will collaborate to evaluate and qualify the use of Emulate's OoC technology as testing platform to meet regulatory evaluation criteria. Specifically, various cross-species Liver-on-Chip models will be compared to assess toxicology.

4.5 SWOT analysis

Strengths: It is clear that OoC systems hold great potential to complement existing workflows and methodologies in safety screening, disease modelling and drug development. As OoC systems aim to closer mimic the human *in vivo* physiology, it is expected that these systems will have a distinct impact on workflows in regulatory validation, qualification and acceptance processes. Today there are several examples where all stakeholders are organized in consortia to drive the regulatory pathway for O0C systems. The presence and even leading role of regulatory bodies herein is key.

Weaknesses: Today, none of the numerously developed OoC systems is already part of a regulatory process. As the technology is still in its infancy, more time is required to mature the field. The inherent complexity of OoC systems makes this maturation process slow and resource-hungry.

Opportunities: The expectations of O0C systems in risk assessment are high, and the demand is even higher. With the European Cosmetics Directive, the European REACH regulation and the new amendment to the US Toxic Substances Control Act, animal alternatives are being promoted even by legislators. The progress and increasing availability of human-based cellular material is a strong driver for OoC development.

Threats: Validation of OoC systems is crucial to its adoption to regulatory processes. However, comparison with animal studies, which are not 100% reliable on itself, might represent a threat. Comparison to human data would be ideal, but those are not readily available or difficult to interpret. Inter-laboratory reproducibility of novel test methods is also essential and should be given high priority when developing and testing OoC systems for risk assessment.

[20] https://emulatebio.com/press/fda-collab-agreement-emulate/

4.6 Conclusions

Today there is no OoC system used in any regulatory approval path. However, there are clear examples of public and private-public partnerships heading this direction. This will open the field for this emerging market and aid adoption in regulatory approvals. Because of the strong (international) involvement of legislators in OOC development and qualification, this process can be accelerated.

5 Standardization of operational processes and technologies

5.1 Standardization of operational processes

In the following section we discuss different key operational processes involved in the development and use of OoC devices and systems, being microfluidic flow, mechanical actuation, cell culture, sensing, and process automation, and its relation to standardization efforts.

5.1.1 Microfluidic flow

Microfluidics is defined as the science and technology of fluid manipulation in small channels with at least one-dimension less than 1mm. Microfluidic cell culture platforms can better mimic the dynamics of cellular environments *in vivo* compared to standard static cell culture formats. In these devices, cells are cultured in microscopic chambers, while the chambers are refreshed with cell medium by perfusion. Continuous flow is advantageous for tissues because supply of nutrients is more constant and helps removal of waste accumulation of harmful products.

Fluid flow in microfluidic channels is laminar, which can be used to sustain microfluidic gradients for a long time (Takayama et al. 2001). Moreover, more relevant cellular microenvironments are created by exposing cells to physiological levels of fluid shear stress and other mechanical forces, such as cyclic strain and compression (Bhatia and Ingber 2014). The introduction of microfluidic channels in bioreactors offers the ability to construct miniaturized, complex devices with multiple compartments and allows for automation of cell culture processes (Nisisako and Torii 2008). Other advantages are reduced sample/reagent consumption and the potential for high throughput analysis. Most platforms use passive gravity-based flow, but also active pumping has been implemented because the former requires a large volume of cell medium and dilution of important soluble factors is an undesired consequence. Further, external tubing and pumps add to the complexity of the use of the system.

Challenges related to microfluidic cell culture are development of procedures for proper cell seeding, avoiding bubble formation and evaporation, small culture volume, perfusion rate and shear stress. There are various ways to load cells into microfluidic channels, for instance syringe injection or gravity-based flow. The typically high number of cells can lead to clogging or fast nutrient depletion and waste accumulation. Air bubbles can be detrimental to cells as their bursting can rupture the cell membrane. They can also block microchannels and thus impede fluid flow.

Microfluidic flow is used in OoC's to replenish culture medium to the cells, remove waste, add drugs or other molecules of interest, or add shear stress to the cells under investigation. Therefore, it is a crucial parameter to consider in increasing reproducibility of OoC systems and to enable true comparison between systems. Further, microfluidic flow allows automation of cell culture maintenance.

5.1.2 Mechanical actuation

The extracellular environment is an essential mediator of cell function and provides not only biochemical but also mechanical cues to influence cell phenotype and behavior. Among various mechanical cues, matrix elasticity has a crucial role in the induction of cellular responses and fate including proliferation,

differentiation, migration, adhesion, and maturation. Mechanical properties determine the tissue's capacity to resist deformation induced by stress, such as compression, elongation, or shear force (Ahadian et al. 2018). Cells and tissues in the body experience varying degrees of mechanical forces, ranging from tensile to compressive forces (Peyton et al. 2007)(Kurth et al. 2012). However, careful consideration regarding the duration, frequency, and amplitude of mechanical forces is important to mimic physiological mechanical forces experienced by different cells and tissues in the body. In addition to applied stimuli, cells naturally experience varying degrees of mechanical strain due to their interactions with the extracellular matrix (ECM) proteins.

Mechanical actuation can be induced by (indirect) electromechanical stimulation of cells or direct mechanical stimulation. For example, electrical stimulation yields a mechanical contraction in cardiac cells or tissues (Nunes et al. 2013)(Donnelly et al. 2010). Direct mechanical stimulation is performed by applying stretch (Zimmermann et al. 2000). Some implementations include both functionalities (Rangarajan, Madden, and Bursac 2014). Careful consideration regarding duration, frequency and amplitude should be applied when mimicking physiological mechanical forces to cells. Hence, in addition to applied stimuli, cells experience a varying degree of mechanical strain due to interactions with the ECM. In this aspect, spatial cues are equally important. Cells have been shown to respond to sensing surface properties on the nano- and microscale which result in phenotypic changes (Micholt et al. 2013). Engineering tissues for OOC applications requires directing cell growth, orientation and interactions.

Mechanical actuation seems to represent a key parameter in the standardization of technologies used in OoC's, but its implementation is, in analogy with the cells in the body, highly variable. Mechanical strain in the human body ranges typically between a few percent in the tendons and ligaments (2-5%) and in the lung alveoli (4-12%) and can reach up to tens of percent when muscles or skin are stretched (Schleifenbaum et al. 2016). In skin, the general consensus is that mechanical tension promotes tissue formation. In the lung, a number of physical forces are transmitted across different length scales, from the organ to the microscale level (Fredberg and Kamm 2006). These forces include mechanical strain from breathing, shear stress from the blood and surface tension in the alveoli (Chatterjee et al. 2015). The use of PDMS-based soft lithography fuelled the development of cost-efficient microsystems. Besides mechanical strain also shear stress and barrier functions with flexible and porous membranes have been introduced (Xia and Whitesides 1998).

Types of mechanical strain introduced by OoC devices cover a wide range: unidirectional, bidirectional and three-dimensional. Most devices have been used to generate a cyclic mechanical strain of relatively small amplitude. A large variety of strains makes the cross-comparison between experimental results obtained with OoC devices very difficult, although several attempts have been made (Wall et al. 2007)(Roan and Waters 2011). From the collected literature today is clear that we cannot answer yet how accurately the *in vivo* mechanical strain needs to be reproduced *in vitro* to trigger an *in vivo*-like mechanoresponse (Guenat and Berthiaume 2018).

5.1.3 Cell (co-)culture

Standardization of cell material is a critical aspect in achieving enough performance and reproducibility of the OoC system. In order to widely and effectively utilize the OoC systems in the drug development process, discussions on how to establish cell quality standards guaranteeing their reproducibility and robustness is indispensable. With the rapid recognition of such problem, "Good Cell Culture Practice (GCCP)" guidelines have been discussed and introduced (Pamies and Hartung 2017). There are several drivers for this guidance, being the fact that the Good Laboratory Practice (GLP) does not address in detail life science tools and, moreover does not address the relevance of a certain test. The aim of the GCCP is to reduce uncertainty in the development and application of *in vitro* procedures by encouraging the establishment of principles for better international harmonization, standardization and rational implementation of laboratory practices, nomenclature, quality control systems, safety procedures, etc. (Pamies and Hartung 2017). Other initiatives are the Good In vitro Method Practice (GIVIMP) by ECVAM and the OECD[21].

The advent of human (embryonic and induced pluripotent) stem cells has significantly widened the application of human cell culture models beyond primary cells and immortalized cell lines. Although the stem cell field is rapidly growing, optimal protocols to achieve fully functional differentiation and maturation are still not achieved and costs of the cells are high. Protocols are also often very complex, which also increases the risk of infection of the culture. OoC's add another layer of complexity related to cell culture as device designs are not standardized and the cell cultures are more complex and often involve multi-cellular constructs. To meet these increasing demands, the GCCP guideline needs to be updated taken the newest developments in cell culture protocols in mind.

Pluripotent stem cells deserve special attention as they are dynamic cells that can change phenotype by differentiation into different cell types. Controlling differentiation is a challenge, as is reliable maintenance of cells in their undifferentiated state. Moreover, epigenetic markers from the somatic cell of origin might be retained, affecting the cell's behaviour in terms of tumorigenicity and spontaneous differentiation (Polouliakh 2013). Proper authentication of cells is also an important marker for good practice. Cell surface markers such as SSEA-3, SSEA-4, TRA-1-180, etc. and expression of self-renewal genes such as Oct-4, Nanog, Sox-2 can be used to confirm undifferentiated pluripotent stem cells. Genetic variation between donors can also lead to functional differences between iPSC lines, and changes can be induced in the reprogramming process (Liang and Zhang 2013). Further, long-term cultures tend to acquire chromosomal changes, but passage numbers are not precisely defined. The WHO guidance states that passage levels should be minimized to no more than 15[22]. Viability and growth rate measurements can be important tools to standardize iPSC cultures, though the assay should be selected carefully because different factors such as pH, medium type, temperature can be of influence (Hartung 2007).

Culture media for *in vitro* cell cultures try to mimic the cellular *in vivo* environment and thus have a crucial impact on cell culture conditions. Serum-containing media are commonly used in cell culture. However, there are batch-to-batch variations and its composition is very complex, so increasingly more serum-free

[21] http://www.oecd.org/env/ehs/testing/OECD_Draft_GIVIMP_in_Human_Safety_Assessment.pdf
[22] World Health Organisation (WHO) 2013.

media are being used, especially for stem cell cultures. In that case, the medium is supplemented with the additives necessary for obtaining satisfactory cell proliferation and production, or for maintaining a desired differentiation status.

The exhaustion or inactivation of essential nutrients in cell culture media and rising levels of acidic metabolites will inhibit cell growth and cell function and will ultimately cause cell death. Therefore, replenishment of cell culture medium needs to be planned accordingly. Cell cultures can be performed in batch or in perfusion. As discussed above, OoC systems use perfusion-based cell culturing. In principle this should allow for long-term cell culturing in controlled conditions, however, the frequency and amount of cell medium to be replenished is shown to largely affect cell condition and differentiation (Luni et al. 2016).

It is well documented how to create patient-specific iPSC lines by using different reprogramming methods and different starting cell types. However, there are pronounced differences in differentiation potential among iPSC lines. Therefore, there is a high need for establishment of QC standards and control of iPSC lines worldwide to ensure both reproducibility and consistency. For OoC systems, the following cell culture aspects need to be considered for standardization: 1) QC testing standards, 2) validation of iPSC culture media and reagents, 3) establishment of reference iPSC lines, 4) standards for use of microfluidics, 5) co-culture of cells, 6) single perfusion medium for multi-OoC; 7) established cell differentiation protocols (Pamies and Hartung 2017).

5.1.4 Sensors

Biosensors contain the following three components: a detector that can bind analytes , a transducing element that converts those events to an output signal, and a signal processor which amplifies and/or converts the output signal into a readable signal. Most reported biosensors for OoC systems make use of external or offline means of monitoring and/or sensing cell culture or environmental parameters. Important parameters to assess are cell behaviour, mechanical stimulation, chemical gradients, electrical stimulation, metabolites, cell secretes and physical parameters. The advantages of using established methodologies to acquire data from OoC's are the ease of use of commercially available sensors, reproducibility of the data and the cost. Current OoC systems mostly consist of transparent microfluidic channel-based cell culture chambers. Hence, (fluorescence) microscopy is an important tool to study cellular parameters in these systems (Kilic et al. 2018).

Metabolites are important for cellular function. Glucose is the main energy source of the cells and under anaerobic conditions, lactate is formed. Measuring both molecules is interesting in models for myocardial ischemia, congestive heart failure, or to detect metabolic changes in cancerous cells (Mathupala et al. 2007). There are various types of glucose and lactate sensors, of which the transducing element is mainly optical or electrochemical. Electrochemical sensors incorporate enzymes such as glucose oxidase, lactate oxidase or lactate dehydrogenase to metabolise the corresponding target and produce H_2O_2 which is then measured via voltammetric or amperometric methods (Bavli et al. 2016)(Ges and Baudenbacher 2010). Optical sensors have advantages over electrochemical biosensors due to the fact that no labels are used,

and measurements can be run real-time and continuous for a long time. To enable detection, these sensors make use of fluorescent dyes, quantum dots, or plasmonic nanoantennas (Nichols et al. 2013).

Sensing of molecules, secreted by cells, is very important to detect changes in cells/organs after a particular stimulus. Cytokines are small signalling proteins secreted by cells and are involved in immune responses such as proliferation, migration or activation of cells, but also in cancer and cell-cell signalling (Stenken and Poschenrieder 2015). The most used cytokine sensors are based on ELISA assays, antibodies, bead-based and aptamer-based assays (Liu et al. 2015)(Matharu et al. 2014)(Chokkalingam et al. 2013). Sensors for detection of IFN-gamma, TNF-alpha, TFG-beta have been developed for liver signalling during injury, detection of activated T-cells or mitogenic stimulation (Zhou et al. 2015). Other molecules of interest include proteins, microRNAs and exosomes.

The cell's environmental conditions can be measured by monitoring physical parameters. Oxygen, pH, and temperature sensors are the most commonly investigated sensor implementations (Wikswo et al. 2013)(Bavli et al. 2016)(Krommenhoek et al. 2008). Oxygen sensors are also implemented using either optical or electrochemical transduction principles (Oomen, Skolimowski, and Verpoorte 2016). Ion-sensitive field effect transistors (ISFETs) are the preferred sensors to determine pH in a solution (Parizi et al. 2017)(Lehmann et al. 2001). As these sensors are based on integrated circuitry they allow for *in situ* measurements (see 5.2.3).

Electrical sensing of cells is achieved by microelectrode arrays (MEA). These sensors are used for detection and stimulation of electrical activity from electrogenic cells (cardiomyocytes, neurons) (Johnstone et al. 2010)(Jans et al. 2017)(Hierlemann et al. 2011), but can also be used to perform electrochemical impedance spectroscopy (EIS) (Goikoetxea et al. 2018)(C.M. Lopez et al. 2018). EIS sensors can detect changes in cell motility, cell adhesion, ion channel activity and gene expression (Asphahani et al. 2011). These sensors can also be used under flow conditions, or as cytometer implementation (Daoud et al. 2015).

Besides the numerous examples of sensors in literature, there are few examples of commercially available sensors that could be used in OoC systems. The advantage of using a particular commercially available sensor is the prospect of direct comparison of the measured data between different OoC systems. In this way, they can enablestandardization of OoC sensor implementation. Sensors are available for measurement of pH (made by Cellasys GmbH), glucose (GLU.K.OMETERpro) and lactase (LACpro) by BST, and for pH, oxygen and carbon dioxide by PreSens. Disadvantages of external sensor implementations are the need for additional hardware, the indirect measurement methodology, the lack of *in situ* measurement, the difficult integration, and the use of (cell toxic) labels.

5.2 Standardization of OoC technology

In 2010, Huh *et al.* reported the very first Lung-on-Chip model in Science, a biomimetic microsystem that reconstituted functional alveolar-capillary interface of the human lung (Huh et al. 2010). The system, comprised of a permeable membrane sandwiched between two microfluidic channels, is a mechanically active system which mimic lung functionality. It is an example for the currently fast-paced field of OoCs. In the following, we will approach the different types of technology used in OoC systems from a standardization point of view and attempt to categorize the myriad of examples in literature according to the type of technology used.

5.2.1 Microfluidic chip based OoC's

The design of microfluidic OoCs enables control over the cellular microenvironment to create more physiologically and relevant cell models. This is in contrast with the traditional way of static cell culture systems and bioreactors. The development of microfluidic OoC systems has mainly relied on the interdisciplinary cooperation between the engineering community and biologists. Although these interactions have been widely successful, there have been issues and a mismatch between materials that engineers can easily prototype and materials that biologists are familiar with (Berthier, Young, and Beebe 2012).

Microfluidic fabrication is most commonly carried out with elastomers. Polydimethylsiloxane (PDMS) is the exponent material used for the creation of complex microfluidic devices due to both its ease in prototyping as well as its material properties, such as transparency and oxygen permeability. Microfabrication methods are used to generate soft, flexible and micropatterned structures using soft lithography processes. However, PDMS may influence the experimental results, as it is prone to adsorbing hydrophobic compounds from the solutions into the bulk PDMS (Nianzhen Li, Schwartz, and Ionescu-Zanetti 2009). Biologists have predominantly used glass and polystyrene (PS) to build substrates and for this reason, they do not have much experience with PDMS. The use of PS has dominated cell culture applications since the 1960s due to its low cost, optical clarity, durability, and mechanical strength. Even though glass and PS are harder and more brittle than PDMS, devices from these materials do not have the same limitations as PDMS, including hydrophobic adsorption, evaporation, and leaching of uncrosslinked polymers. Unfortunately, the production of microfluidic devices based on thermoplastic polymers and glass is more complex than when PDMS is used. These devices can be produced at low-cost by large-scale fabrication but prototyping is more costly. Therefore, thermoplastic polymer based OoC's are currently less widely investigated than PDMS-based.

The complexity of microfluidic OoC depends on the cell type and architecture of the microfluidic chip. The simplest OoC models contain only a single cell type in a straight microfluidic channel. The microchannel surfaces may be coated with hydrogels to improve cell adhesion and proliferation. Despite the simplicity of the microenvironment, such devices have interesting applications for studies on vascular (S. Kim et al. 2017), cardiac (Agarwal et al. 2013) and liver tissue (Moraes et al. 2012). To improve the relevance of single-cell type OoC models, a variety of microfluidic devices was microfabricated to engineer the cellular microenvironment. The most common example contains a porous membrane which is sandwiched

between two microfluidic channels for models of the kidney (Jang and Suh 2010) and gut (H. J. Kim et al. 2012). These membranes allow the adhesion of cells, transport of molecules, and the formation of molecular gradients. A second device contains cells cultured in a central chamber, that is connected through microchannels to separate side channels. Medium is perfused through the latter channels(Nakao et al. 2011)(Mathur et al. 2015) and nutrients and waste are diffusively transported to/from the cell culture chamber, this mimicking the vascular system. As the requirements for an engineered microenvironment are highly dependent on the organ model and not yet fully understood, further research is required for standardized device architectures.

More complex OoC systems have been established by creating microfluidic devices that contain multiple cell types. The different cell types can be injected simultaneously into the microfluidic device (H. J. Kim et al. 2012) or physically separated by a barrier. In the case of microfluidic channels separated by a flexible membrane (as described previously), different cell types can be grown on opposite sides of the porous membrane. The co-cultures may consist of endothelium and epithelium cells for applications such as Lung-on-Chip (Huh et al. 2010) or endothelium and astrocytes or pericytes in the Blood-Brain-Barrier-on-Chip (Phan et al. 2017). The thickness of the porous membrane and pore sizes also varies between applications.

Microfluidic cell culturing platforms are also commercially available. For example, Micronit and Elveflow offer a wide range of products of varying OoC complexity levels, but both require specific platforms and peripheral equipment to control and perfuse the devices. Microfluidic design and manufacturing parameters are important considerations in standardizing OoC's. Today, it is extremely difficult to compare the vast amount of literature reports making use of microfluidics and distill common denominators. An important step towards standardization (and manufacturability) of microfluidic devices is made in the European MFManufacturing project (ENIAC Joint Undertaking)[23]. The objective of this project is to bring the manufacturing of microfluidic devices to the same level of maturity and industrialisation as electronic devices. The project aims to gain maturity in microfluidic functions and processes through a distributed pilot line, novel hybrid integration processes and commercialization perspectives.

5.2.2 Well-plate based OOC's

A typical R&D track for the development of a single drug requires the screening of hundreds of lead and back-up compounds. To achieve this, low-cost and high-throughput assays are required to evaluate all these compounds. The automation of analytical tests is crucial to the development of *in vitro* drug screening and development. Pharmaceutical industries currently use high throughput screening systems, relying on robotics, data processing/control software, liquid handling devices, and sensitive detectors, to speed up drug research. The screening tests are carried out on microtiter plates containing small wells to carry out tests.

To make automated systems from different suppliers inter-operable, the Society for Biomolecular Screening (SBS) first defined a standard definition for a microtiter plate. The standards of microtiter plates

[23] http://mf-manufacturing.eu/

were further defined by the Society for Laboratory Automation and Screening (SLAS) and the American National Standards Institute (ANSI). These well plates require a standard footprint, height, and bottom outside flange dimensions, well positions and well bottom elevation. These standards have been adopted by pharmaceutical industries, contract research organizations, and academia.

The microfluidic OoC technologies described above (Section 5.2.1) were not designed according to those standards or based on microtiter plates. Moreover, the whole infrastructure required for either lab automation and HTS may be incompatible with these devices. Often, these devices require custom operating systems which cannot control all types of microfluidic OoC devices. As a result, it remains challenging to implement these systems in standard laboratories based on traditional cell culture procedures.

As a result, there have been efforts to design OoC devices according to well plate standards. The presence of OoCs in separate wells increases the throughput of assays and tests. Furthermore, the open-well interface with the user allows for easier handling and compatibility with automated pipette robots. Open well plate-based systems include 24 well plates with an integrated strain gauge (Lind et al. 2017) or "Biowire" (Tara Biosystems) (Nunes et al. 2013) to assess contractility of cardiac cells. Other examples are the commercially available Organoplate from Mimetas which has been used for several applications, including a high-throughput Gut-on-Chip (Trietsch et al. 2017). 4D Cell is offering multi-well plates with integrated micropatterns and perfusion systems (Maiuri et al. 2015)(Vargas et al. 2017). Well plates can also contain integrated microfluidic pumps, combining the standardized polystyrene well plate with microfluidic flow (Park et al. 2014)(Pauwelyn et al., n.d.).

5.2.3 Integrated sensors/actuators

Besides various methods to monitor cellular and microenvironment parameters in OoC systems using external equipment such as microscopes, ELISA, peripheral sensor systems etc., there has been efforts to integrate sensors into OoC devices. Integration of sensors is the way forward for OoC systems as real-time, *in situ* and quantitatively obtained information is an important aspect for the monitoring of cellular processes. Also, integrated sensing has the advantage that multiple types of sensors can be implemented, and thus multiple analytes can be detected simultaneously. At the same time, integration of biosensors into OoC's is very challenging because of the complexity of sample preparation, biocompatibility of the used materials, system integration, sensor saturation and regeneration, and challenges related to sensor biofouling (Kilic et al. 2018). Examples of sensor integration are oxygen, pH and cell morphology (impedance) and electrical activity sensors (Moya et al. 2018)(Shaegh et al. 2016) (Alexander, Eggert, and Wiest 2018).

A multi-sensor integration effort was recently presented by Zhang et al., in which the authors built a microfluidic breadboard for routing of fluids, physical sensors for measurement of extracellular microenvironment parameters (pH, O_2, temperature), electrochemical sensors for measuring soluble protein biomarkers and miniature microscopes for observation of cells (Y. S. Zhang et al. 2017). The system also includes a miniature microscope mounted under the microfluidic chambers. This platform is a big

step forward towards multi-sensor readouts for OoC's, although some of the sensors were not yet integrated completely.

Another level of sensor integration can be achieved by integrating readout and actuation electronics. Integrated circuitry (IC) offers miniaturization, scalability and increased complexity of sensor designs. Complementary metaloxide semiconductor (CMOS) technology has enabled very high density and high-performance IC's for consumer electronics. In recent years, this technology is also adopted for interfacing with cells and tissues (Carolina Mora Lopez et al. 2018)(Hierlemann et al. 2011). The advantage of IC's is that external readout systems can now be built inside the silicon substrate, and because multiplexing can be used, the number of available sensing sites is often dramatically larger than when no IC's are used. Besides this, multiple types of sensors can be implemented, and because of the technology standard, chips can be produced in large numbers at low cost.

5.2.4 Modular approaches

OoC systems can be classified in single-organ and multi-organ systems. Multiple organs can be connected and thus a set of 'organ' building blocks can be used for each specific disease model. Multi-organ systems can also investigate how metabolites pass through the body as a whole.

Researchers from the Vanderbilt University designed stackable and modular microfluidic devices that gives the users more freedom in designing their systems[24]. Cells can be cultured in individual, injection-moulded components before being assembled into multi-layer devices with multiple cell models. In these devices, the flow travels in parallel over interconnected microfluidic systems. Alternatively, OoC models may also be connected into series through tubing to better model the flow of liquid inside an organ. Maoz *et al.* connected three separate OoC modules to create a more advance Brain-on-Chip model (Maoz et al. 2018). Its separate modules were required to simulate (1) the influx of molecules over the blood-brain barrier, (2) interactions with the brain tissue, and (3) the efflux of the compound over the blood brain barrier.

OoC systems with interconnected sub-organ modules can also be used to better understand the interactions between multiple organs. The organ modules may be designed in a single device with a fixed architecture. These devices link organ compartments in one fluid stream, but flexibility is limited by its definite geometry (C. Zhang et al. 2009)(Sung and Shuler 2009)(Esch et al. 2014). However, the maturation of different organ models may require specific procedures. Therefore, the OoC modules must first remain flexible and customizable. Once these models have reached a certain maturation level, they can be connected to a multi-organ microphysiological system. In one approach, standard Transwell® supports are placed in devices with a fixed architecture (Wagner et al. 2013)(Maschmeyer et al. 2015)(Schimek et al. 2013)(Ma et al. 2012). Devices are already commercially available through TissUse GmbH. Multiple chambers containing OoC models cultured with different cell types are connected by a fixed series of microfluidic pumps and sensors to a multi-OoC.

A more flexible alternative is connecting separate modular OoCs. Devices may be connected through a series of connectors and tubing (Skardal et al. 2017). To further facilitate user handling, Loskill *et al.*

[24] https://cttc.co/technologies/modular-and-stackable-microfluidic-devices.

developed a Lego®-like system with plug & play connectors to connect adjacent OOC devices (Loskill et al. 2015). A mix-and-match toolbox enabled tailored OoC systems with a standardized protocol to developm into multi-organ systems (Rogal, Probst, and Loskill 2017). Standardized connectors would offer interoperability of OoC developed in different companies and research groups. Sensor modules could also be plugged in when required.

However, the adequate design of a generalized Human-on-Chip model by coupling OoC modules is challenging. Current multi-compartment organ systems may be used for applications of generalized drug discovery. To detect physiologically relevant inter-organ relationships, the many organ-compartments must be scaled appropriately to better mimic physiological interactions (Moraes et al. 2013). The development of modular OoCs for generalized Body-on-Chip will thus require further advances in design to achieve this goal. Another challenge for multi-compartment organ systems is the development of a common cell culture medium suitable for all organ models (Wikswo et al. 2013). In addition, the use of whole blood as the single perfusion medium needs to be considered.

5.3 SWOT analysis

Strengths: A large collection of different flavours of OoC systems serving many application domains have been reported in the last decade. The demand from pharmaceutical industries and regulatory bodies to adapt OoC devices in drug development and safety assessment is clearly increasing. OoC's represent a fast-growing, multidisciplinary field built on progress obtained in the last decades in lab-on-chip technology, stem cell technology, microfluidics and (bio)sensors development. Standardization efforts already obtained in these fields can lay the foundation for standardization and qualification of OoC systems.

Weaknesses: There is no good science in bad models: the best available model is the one that gives the best result – which is the most reliable and most relevant one. OoC systems are complex multi-parametric implementations and aim to answer complex networked responses of the biological model at hand. For some of the technologies or processes such as mechanical actuation and sensor technology there is currently no existing or ongoing standardization effort.

Opportunities: Standardization of OoC devices could build upon several already established standards: well plate formats for cell culture, microfluidic design and fabrication processes established in the field, guidelines for (stem) cell use and tissue biobanks, and standard microfabrication technologies for sensor design. Modular individual OoC's are an interesting path towards more flexibility.

Threats: Non-trivial comparison of data obtained in the OoC models compared to current animal models, complex multi-organ systems based on prototyping technologies and resource-hungry technological integration might hamper fast adaptation of OoC's by stakeholders, and thus slow down standardization.

5.4 Conclusions

OoC system standardization can build further on existing standards or standardization efforts of its subcomponents and sub-processes. Although there are no golden standards defined today, keeping in mind previous standards and guidelines and a close collaboration with relevant stakeholders will determine successful introduction of OoC's in drug development.

6 Ethical dimension

Technological innovation cannot be seen without an application purpose. Standardization of technology goes hand in hand with expectations on efficient production, quality control, reliability, and ease-of-use for the end user. But OoC is about a technology enabling improvements on healthcare for humans on the one hand yet requiring the use of cell and/or tissue samples donated by humans as a *conditio sine qua non*.

Still, we have already come a long way from the absence of any ethical concern regarding animal studies to the prevalence of 'value free' and 'ethics free' science. New ethics have been introduced on social ethics for humans, *mutatis mutandis* (Rollin 2012). The introduction of the Animal Welfare Act and equivalents has been a major step forward to come to a serious assessment of ethical concerns such as balancing burdens and benefits; yet, awareness and proper management of ethical issues around animal studies remains an investment: "'Animal research is not a moral issue; it is a scientific necessity' - as if it could not be both." Legal and regulatory efforts have instrumentalized the 3R principles on replacement, reduction and refinement. OoC is expected to contribute here and relieve part of this burden on animal studies, replacing them by *in vitro* techniques using human cells, expecting more relevant results to human health.

As the case may be, **relaxation of concerns on animal studies may be outweighed by properly addressing new concerns regarding the use of human cell samples,** such as consent, privacy, data ownership, trust. Inherent with the shift to human biomaterials is the fact that the human cell donor is now actively involved or may expect to be *somehow* actively involved along the trajectory his or her cells may go.

This section aims at charting the ethical discourse on OoC technology and related application areas that may be strongly affected by it or affecting it.

6.1 Current ethical discourse

There is little ethical discourse *specifically* on OoC, certainly when compared to other emerging bio-technologies such as next-generation sequencing or genome editing (Biesecker et al. 2012). On the other hand, the ethical debate on the use of human embryonal cells in a lab-on-chip context has had its effect. With the availability of hiPSC, the ethical debate has rather shifted to handling informed consent from the human cell donor.

We primarily investigate here the ethical implications when using OoC for applications in drug discovery and toxicology. However, we elaborate further on implications that would emerge on a longer-term perspective but find their roots already in today's application scenarios.

A dilemma – where are the alternatives to animal studies?

For decennia, scientists have been looking into alternatives for animal studies, where are they?[25]. The need persists, and expectations have been created, but truly viable alternatives are still scarce.

OoC contributes to the 3Rs, reducing today's ethical burden on animal studies

OoC is considered as a replacement alternative for some animal studies in drug discovery and may contribute to a large reduction of animal studies for toxicology assessment.

There is already a legal and regulatory framework for phasing out animal testing for cosmetic purposes[26]. A testing ban on finished cosmetic products is active since 2004, a testing ban on ingredients or combination of ingredients since 2009, followed by a two-stage marketing ban in 2009 and 2013, irrespective of the availability of alternative non-animal tests. From a societal point-of-view, a consensus has been reached on lowering the burden of animal testing by applying and promoting 3R principles.

The European regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) from 2016 has, on the counterside, vastly increased the need for alternative toxicity test methods to meet the required provisioning of safety data on a large set of insufficiently investigated substances.

This is a clear opportunity for OoC to fill the gap on missing compliant test methods, but also a request to provide test methods that scale to the needs of multiple sectors such as cosmetics, chemistry, or pharmaceutical industry.

Animal model and human show bad correlation in studies – towards more truth

Bad correlation between studies on animal models and human are legacy (Hartung 2017)(Olson et al. 2000). They are very dependent on disease and often show low reproducibility across studies, platforms, or laboratories. Nevertheless, the purpose of animal experiment is prediction. It is intrinsically an ethical concern that researchers continue working with the expectation of such low correlation, but in many cases, better methods are truly unavailable or unaffordable e.g. in the case or rare diseases.

OoC may lower the ethical concern by offering an alternative to arrive at **better reproducibility of experiments, and hence a more stable, trustable drug discovery process**.

According to Hartung *et al.*, "Half of the results are wrong […] - we only don't know which half … but the statement 'half are wrong' is probably rather optimistic (Hartung 2017)." Unfortunately, a large contributor to low reproducibility seems to be low adoption of best practices on reporting of animal

[25] Die Zeit, Ersatz gesucht: "Seit Jahrzehnten prüfen Forscher Alternativen zum Tierversuch – wo bleiben sie"; Jan 9, 2016.
[26] EC Cosmetics Directive

experiments. Some concerns have been addressed by developing the ARRIVE guidelines for animal experimentation. Such an effort on **guidelines for proper use and reporting may also be needed for OOC experimentation to build trust and reliability**. Starting points for OoC exist such as Good Cell Culture Practises (GCCP), but they need adoption to the specific context of OoC. Note also the interesting concern that best practises **should not lead to a deviation from the 'natural situation' or eliminate 'natural variability' such that the experimental conditions become irrelevant** (such concerns *e.g.* exist regarding the use of cancer cell lines due to their high robustness withstanding inadequate culturing conditions but also the use of genetically modified animal clones).

More targeted, more efficient drug discovery – room for ethically-defendable choices

OoC aims at offering *in vitro* models with an unprecedented richness of information, customized to the experimental need during the process of drug discovery, preferably re-using already developed modules and techniques. This shall allow more targeted, more efficient drug discovery which, ultimately, turns into a better spending of development resources. That may in return lead to a lower attrition rate (due to earlier abandoning of non-promising compound or drug candidates) or to a faster development process (due to re-focusing not-spend effort on the remaining promising candidates)(Jekunen 2014). But the personalization of experiments based on samples of human origin will also allow addressing specific disease or population niches; in classical drug discovery, this is often considered very hard due to lack of population statistics (*e.g.*, for rare diseases, for ethnicity, for gender- or age-specific risks, etc) or insufficient access to relevant patients/volunteers, e.g. due to ethical concerns on trials involving children[27].

A case triggering interest is the effort around EVATAR, collaborative work between several US universities to build an OoC comprising the biological representation of the female reproductive organs (Xiao et al. 2017)[28]. OOC may be well suited to analyze differences in drug metabolism between males and females. OoC may offer unprecedented possibilities for gender-specific therapy development with much enhanced outcome.

Responsible experimentation guidelines such as described in the European Code of Conduct for Research Integrity[29] demand

- "Researchers to handle research subjects, be they human, animal, etc. with respect and care, and in accordance with legal and ethical provisions." and
- "Research protocols take account of, and are sensitive to relevant differences in age, gender, culture, religion, ethnic origin and social class."

[27] FDA; https://www.fda.gov/downloads/ScienceResearch/SpecialTopics/PediatricTherapeuticsResearch/UCM346996.pdf
[28] https://www.techtimes.com/articles/203454/20170329/organ-on-a-chip-scientists-recreate-menstrual-cycle-in-3d-to-aid-in-drug-testing.htm
[29] Allea, The European Code of Conduct for Research Integrity, 2017.

Note, however, that there is also a counter-side to this. If OoC lives up to its promise of becoming a fairly generic platform technique, some stakeholders may want to call in ethical consultation to weigh on which categories a therapy should be fully developed. In current practice, these are usually governmental institutions in the case of epidemic risks, e.g. with respect to vaccine development and deployment. We also see charities becoming more active in this space, e.g. to address severe diseases in less-developed countries. With the advent of OoC, expectations may also rise from other stakeholders such as patient associations and advocacy groups and, ultimately, the individual person donating cells. Prioritization may become less an issue of sheer 'market size' (addressable patients) but rather **an issue of managing interests and consents of a complex stakeholder group**.

Responsibility for organizing & granting support across stakeholders

Individual initiatives and projects promoting 3Rs methods often receive initial financial support, given a positive ethical inclination, but suffer from a financing gap between feasibility and product prototyping.

The reason for this is the complex benefit relationship of stakeholders involved. It is important to clarify this investment-benefit relationship in commitment and time horizon for all stakeholders involved to prevent such a gap, essentially leading to an unethical waste of resources. A proper ethical debate with all stakeholders is required to develop a Responsible Innovation roadmap that also incorporates the economical aspect and a cost-benefit rationale.

Several examples stress the importance of broadly clustering efforts and diversifying financial and non-financial support:

- **Dutch example**, foundation of hDMT (Institute for human Organ and Disease Model technologies) in 2015, a precompetitive non-profit technological R&D institute. Obtained substantial Dutch government support e.g. NWO project "NOCI" (2017; 18.8 M Euro for 10 years) and works with industry partners.

- **UK example**, illustrating scientific, mission-oriented hub backed by government, charity, industry funding sources: National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs), founded 2014: Collaboration between government, industry, academic research labs, and regulatory agencies to accelerate adoption of 3Rs (Holmes, Creton, and Chapman 2010): Strategic roadmap for developing 3R methods, boost commercialization, publishing methods (besides research results), support institutions to promote 3Rs, offer training, invest into global harmonization of regulatory aspects/method validation[30].

- **US example**, illustrating concerted support from US government public funding, health-programmatic, and regulatory agencies: Prior to 2012, biomedical applications of lab-on-a-chip and organ-on-a-chip "resided mainly within academic research laboratories". Partnerships between NIH, DARPA and FDA started and resulted in a major acceleration (initial investment of $13M as part of a $70M 5-year funding effort). "Principal aim was to provide new models that

[30] NC3Rs, Our Strategy 2017-2019.

could be used for screening drugs in a cost-effective manner and thereby reducing the high drop-out and overall drug pipeline costs" (Greenman 2017).

Can OoC go beyond safety and address efficacy? Can it truly offer a better model?

"Currently, more than half of the drugs that reach later-stage (phase II and III trials) human testing fails because they do not demonstrate efficacy" (Kimmelman and Federico 2017). Safety is evaluated by regulators before human trials can proceed, but not evidence for potential efficacy. "The battery of animal-toxicity and dosage tests that regulators require before allowing human trials do not provide this evidence". **Can OoC address the desire to go beyond safety only?**

Integration of physiologically relevant in vitro assays at the earliest stage of drug discovery could improve the likelihood of successfully translating preclinical discoveries to the clinic (Engle and Puppala 2013). In classical drug discovery processes, high-throughput screening is used to identify e.g. biologically active small molecules for further optimization into candidate drugs. There is a risk that this "reduction" leads to **assays that do not capture the full diversity of regulation seen in native cells. Can OoC reintroduce such variety in its experimental portfolio?**

OoC as facilitator of other techniques - inheriting their ethical concerns

OOC depends on a mix of technologies and methods. Hence, **OoC is rather a modulator of ongoing ethical debates on other technologies**. It may be versatile enough to replace controversial approaches on the biological side whenever an alternative becomes available. An example is the switch from human embryonic cells (embryos or oocytes) to human induced pluripotent stem cells when they became available. iPS cells avoid the heated debates about the ethics of embryonic stem cell research because embryos or oocytes are not used. Facilitating the use of hiPSC ceased also any ethical concern around eligible manipulations and experiments instantaneously[31]. This is a positive aspect of OoC as a platform.

On the other hand, OoC may leverage other techniques under debate, such as embedding genome editing or cell reprogramming, and hence import the ethical issues into its own ethical discourse[32]. Note that this is a minor issue for shorter-term goals such as drug discovery and toxicology but becomes a serious concern when closing the cycle between patient sample and patient, e.g. by re-applying modified cells in the case of cell therapy or (re-)generation of tissue/organ parts for transplantation.

[31] The President's Council on Bioethics, Alternative Sources of Human Pluripotent Stem Cells, Washington D.C.
[32] GermanStemCellNetwork, Applied Stem Cell Research in Germany, Annual Magazine 2015/2016, pp. 39ff and 49ff.

OoC using human cells needs to outperform existing methods based on animal studies – a *paradox*

Regulatory institutions such as FDA in the US or EMA in Europe encompass as a major item of their mission "protecting human health". Validation of methods is rigorous, time-consuming and costly. Adoption and implementation of such methods in industry is a significant investment into training and quality control. As a consequence, approved test methods tend to persist, while novel alternatives may struggle to find support for re-executing the entire validation procedure. This is not unique to OoC. However, results from novel OoC methods using human cell samples would be compared to match well-known (not necessarily always fully understood) results from animal study methods. Clearly, a *paradox* since OoC is not just expected to generate more accurate date but primarily more relevant data; deviations from animal studies should be expected. This raises an ethical discourse on how to structure a trusted process that handles the dilemma of advancing and adopting a new truth, endorsed by all stakeholders.

Affordability of OoC - from multi-national to individual citizen?

Generally speaking, the use of OOC is expected to lower overall investment and cost as compared to animal studies for the same purpose; primarily through improved efficiency and improved relevance of results. However, similar to the use of human gene sequencing, cost of OoC and willingness to pay a higher price to use it for specific purposes, may pose a challenge in which settings OoC technology will actually be deployed. Predominant economic viability may be a first, natural driver for companies to invest, but OoC may have the potential to attract other stakeholders to set priorities that are more oriented towards societal or even personal needs (*cfr.* discussion on niches and evolution on human gene sequencing).

Appropriate citizen or patient consent

The starting point to use OoC is the availability of donor biomaterials. In contrast to animal studies, human cell or tissue samples will be used to arrive at better relevance or even person-specific results and recommendations.

Consent between the donor and the *receiver* of the donated materials on the rights and purposes to use donated materials is essential. This is an act of trust since the donated material potentially reveals very intimate health information of the donor. The donor must be able to make an informed and voluntary consent, knowing about the use of the samples, potential impact on his/her interests by the research, future storage and use. With the advent of OoC enabling complex in vitro models, **describing *all* purposes of experimentation upfront is non-trivial**. Guidelines addressing "experiments of concern" may be beneficial e.g. to exclude dual-use or otherwise undesired use (Miller and Selgelid 2008).

Broad and enduring consent ('generic consent'), i.e. broad in scope and time, is the primary choice since it allows efficient use of samples and trust with donors, but it requires transparent governance procedures. Developing an appropriate, broad and enduring consent process for tissue donors is complex given immortality of cells, intimate connection to the health information of specific individuals, and their

unprecedented scientific potential (a.o. enabled by OoC). Note also that donor incentives (e.g. payment for donation) may be illegal in many countries. Alternatives to convince and motivate donors may be at hand, e.g. sharing clinically relevant findings, but overshooting expectations must be prevented (Bredenoord, Clevers, and Knoblich 2017). The challenge in the context of OoC is the sheer wealth of possible applications of the platform and the longevity of the donated samples.

Operational and ethical guidelines are largely available to address procedures for biosample handling, informed consent, data access, and privacy. A.o. MRC features a guide on "Human Tissue and Biological Samples for Use in Research: Operational and Ethical Guidelines"[33]. MRC aims at ensuring that biosamples and data are supported with multiple use in mind.

"Personalized drug testing may close the gap between preclinical drug development and clinical trials" but it also "**blurs the line between research and care**". Proper consent is essential here as well as governance regarding access to personal health data derived from OoC experiments. Such experiments could identify personal health risk factors that could lead to reclassification e.g. by insurance companies if such data would be accessible to them or to changes in reimbursement (Bredenoord, Clevers, and Knoblich 2017).

Donation and ownership on biological material – mine, yours or a common good?

A human donating biological cell or tissue material lies at the base of OoC. Donation comes with an informed consent that should match the journey of the biological material.

However, as opposed to organ donation[34,35] with a well-established ethical and regulatory context and the purpose of transplantation, hiPSC material can be preserved or transformed/processed (modified, differentiated) many times for many different purposes.

Questions arising that lead to ethical and legal concerns:

- Do journey and initial consent for the donation match?
- Preservation allows transportation world-wide, hence an extension covering multiple regulations across nations may apply.
- Who adopts stewardship for donated cells and tissues?
- Can we ensure fair procurement of cells and tissues?
- Can we apply changes to the consent?
- Can we provide feedback, motivation or personal benefits to the donor?
- Can we guarantee fair distribution of processed cells and tissues?
- A distributed network of brokers, processors, preservers and distributors emerges, ultimately

[33] MRC Ethics Series, Nov 2014

[34] A. Schulz-Baldes, N. Biller-Andorno, A.M. Capron, International Perspectives on the Ethics and Regulation of Human Cell and Tissue Transplantation, WHO, 2007.

[35] J.-P. Pirnay, A. Vanderkelen, M. Zizi, D. De Vos, T. Rose, G. Laire, N. Ectors, G. Verbeken, Human cells and tissue: the need for a global ethical framework, Bulletin of the WHO 2010;88:870-872. DOI: 10.2471/BLT.09.074542

creating a marketplace – can we track the journey? Can we positively influence the 'marketplace' dynamics? Can they be disturbed?
- Can we balance non-profit vs profit?
- Who owns what? Which rights can you enforce? Is this creating risks?

These questions are not necessarily unique for OoC but OoC enables a distributed and massive use of such resources. Local or centralized management may need to be replaced by a framework that allows trusted, distributed collaboration. Today, a jungle of sample and cell collections through a variety of research projects and clinical studies exists. Efforts are undertaken to organize access to such sample and data collections through the set-up of biobanks and tissue banks. However, much information is scattered. The H2020/IMI StemBANCC project collects skin tissue samples from 500 patients with the ambition of creating 1500 iPS cell lines[32] in order to create a broad, common ground to build on further with clear, legitimate consent and well-known start conditions. From an ethical point-of-view, we have to consider the balance between the right to first and exclusively exploit results, sharing of results, or the possible re-creation of experiments and experimental data due to a competition-induced lack of access to certain samples or data.

Note that, in particular, a multitude of derivatives can be created from initial human cell samples with a broad set of ethical implications. Many of them also relate to privacy concerns, which need to be addressed (in Europe, for European donors) according to the GDPR legislation (Morrison et al. 2017):

- Immortal cell lines => end of consent? Purpose of consent?
- Organoids carrying personal, possibly identifiable information of the donor => privacy?
- Hybrid artefacts consisting of organoids and e.g. non-biological circuitry => privacy?
- In silico models derived from observational data, capturing organoid *behavior* or even feeding complex personal Avatar models => privacy?

Expectation management

OoC requires the engagement of many stakeholders contributing to the success of a single use case or experiment, e.g. generating a set of high-quality data for drug discovery. Each stakeholder may have different expectations on contribution, responsibility but also benefit. Some stakeholders may expect a return on their investment (donation of samples, time, data, expertise, …) and in a timely manner.

Much of the expectation management towards donors can be addressed in a proper informed consent
Quoting Morrison *et al.*: "Researchers need to communicate the distinction between the long-term hope for effective treatments and the uncertainty inherent in any phase I trial. Participants in phase I studies need to understand that the intervention has never been tried before in humans for the specific condition, that researchers do not know whether it will work as hoped, and that the great majority of participants in phase I studies do not receive a direct benefit. Researchers need to verify that participants have a realistic understanding of what their donation to an OoC trial means. In practice, it is observed that participants

in phase 1 stem cell-based clinical trials might overestimate their (personal) benefits and underestimate the risks" (Morrison et al. 2017).

Towards the wider public, dissemination of what OoC may deliver by researchers themselves or by media needs to be critically reviewed. Examples are announcements of FDA collaborating with Emulate Inc on testing of effectiveness, still lacking a time horizon (FDA, April 11, 2017) while initial research started in 2012 with promising announcements, or the announcement of a Lung-on-Chip system replacing a lab animal test[36] still waiting. There is often a lack of communicating or misinterpreting the technology readiness/maturity level and the time to reach an effective product. In drug discovery and toxicology, OoC is even only one tool supporting the R&D pipeline. **Wrong expectations are easily created, possibly resulting in**

- disengagement of donors (with potential implications on the use of samples or data),
- withdrawal of sponsors (e.g. underestimating time to return-of-investment, underestimating total investment, underestimating late phases),
- halt of research collaborations (e.g. due to different interpretation of TRL between technology and biology research partners)

Avoid the hype cycle. Compare to the failure of the use of genomics for personalized medicine; it was predicted earlier and with more momentum, but circumstances were not yet ready.

6.2 SWOT analysis

The detailed analysis of the ethical debate is briefly summarized in major terms, expressing strengths, weaknesses, opportunities, and threats:

Strengths: Drawing on technologies and approaches also used in other fields, enables to start the ethical debate at an advanced level and allows comparisons. Exploit existing know-how from other debates.

Weaknesses: OoC as a set of technologies and approaches has the potential to serve a multitude of applications. This multitude very much widens the ethical discussion over the complete health range from prevention to early diagnostics, therapy development and personalized therapy follow-up, with the risk of either losing depth or arriving at too narrow conclusions. Connect with other debates in the same field; separate technology/processes and processes/purpose.

Opportunities: A balanced discourse on OoC is still possible since OoC by itself is not yet at the centerpoint of the discourse, while some technologies and approaches used in OoC have been widely discussed. While the use of OoC as an alternative to animal tests in drug toxicity testing and drug discovery would likely lower the sheer amount of animal tests, thus reducing ethical burdens there, OoC's more advanced applications in personalized treatment or regenerative medicine may require new ethical concerns. Start this discussion now. The debate on longer-term applications has not yet affected OoC so much. Neutral

[36] NRC, 2012.

ground and time are necessary to progress from first applications such as drug discovery or toxicology into more advanced topic such as cell therapy or regenerative medicine early enough with different stakeholders.

Threats: There is a risk that OoC does not deliver its maximum impact and one or more stakeholders may rate it a hype at a given moment, which may lead to a withdrawal of support and undermine its further evolution. Other risks are disengagement; not bringing up an alternative, so that ethical burdens remain; Use of even more animal resources (ROI issue) for development of the techniques. In addition, OoC may not exploit its full potential, going beyond animal studies replacement. Not addressing the human informed consent & data ownership & privacy concerns properly may create distrust and disengagement, basically depriving the OoC approach from its access to crucial human cell and tissue donors.

This initial SWOT analysis will be further refined in collaboration with identified stakeholders and serve as a starting point for drawing up action plans for a roadmap on ethics in sync with standardization and regulatory efforts and supporting the technology innovation roadmap. The goal is to shape the ethical debate in such a way that gaps or disagreements are properly addressed. To this end, existing, prior methodological work will be considered.

Use of existing Ethical frameworks for further investigation

Learnings from **3D bioprinting assessment**: "3D bioprinting has the potential to be a 'game-changer', printing human organs on demand, no longer necessitating the need for living or deceased human donation or animal transplantation (Vermeulen et al. 2017)". The authors create a socio-ethical view on 3D bioprinting which has several touchpoints with OoC (e.g. 3D bioprinting can be a technique employed for OoC). The authors stress the importance of identifying expectations at all stakeholders (and, in particular, public expectations). Properly driving through the hype cycle is essential. They recommend **Responsible Research Innovation** [EC, Rome declaration on responsible research and innovation in Europe, 2015] as an oversight model that links intentions, expectations, and roles of all stakeholders; this should balance technology push vs application pull and prevent non-engagement of silent stakeholders. They advocate to consider upfront how far pricing and affordability would play a role on adoption or application scope. They identify the hype cycle as a risk. The authors segregate between short term benefits and long-term benefits, adopting a two-tier approach.

Lessons learned will be retrieved from and contacts will be sought with recently finished and running projects on **ethical impact assessment** in similar areas, amongst others

- SIENNA (H2020), www.sienna-project.eu on human genomics, started Oct 1, 2017. Classical ethical impact assessment (EIA) in four steps: technological conceptualization and foresight analysis; socio-economic impact assessment and foresight analysis; ethical impact analysis; ethical evaluation and recommendations *but* combined with legal and human rights analyses. Addressing a broad stakeholder group and the strict regulatory context are similar and will add value.

- SATORI (FP7), www.satoriproject.eu, finished. Proposes a framework for ethics assessment and recommendation for roll-out of Ethical Impact Assessment (EIA) in a scalable way. Scalability and dynamic roll-out will add practical value to our guidelines.

Responsible Innovation has been developed and applied using constructive technology assessment in domains close to OoC such as nanotechnology and synthetic biology[37]. Limitations of the Responsible Innovation approach have been identified, asking for further modifications of the methodology (Kerr, Hill, and Till 2018). They are linked to aspects of care and vulnerability, stakeholder/user experiences and dynamics of innovation processes and require attention to:

- Balanced view on 'growth as a driver'
- More attention to how innovation is produced or marketed (link with expectation management and ethics)
- Consideration of the impact of politics and policy setting on 'power distribution' across stakeholders
- Encompassing a full view on engagement, also on absent or passive stakeholders

6.3 Conclusions

The ethical discourse has a largely favourable, positive bias today, indicating OoC as a possible solution reducing cost, need, and ethical burden of animal studies, both for drug discovery and even more for toxicology studies. OoC as a broad platform technology has the benefit of being able to adapt to evolutions in biological science, e.g. the replacement of controversial human embryonal cells by human induced pluripotent stem cells, which largely silenced the ethical debate. OoC has also the potential to finally allow for drug discovery and personalized treatment for small or differentiated target groups (rare diseases, children, pregnant women, gender specific, ethnic specific).

However, this also poses the question of who indicates priorities. Citizen/patient donors may expect a personal benefit of donation rather than a societal impact. There is an underlying risk that media interest and coverage on new evolutions in OoC overpromises, possibly resulting in a hype cycle. A second risk is to underestimate aspects of informed consent, data ownership, and privacy concerns when using human-donated cell or tissue samples in combination with OoC trials. The ethics impact of a 'personal OoC model' as part of a personal Avatar model, partly in silico, partly on chip and related aspects of data ownership and privacy are still largely unaddressed.

[37] A. Rip, Fashions in Science Policy, past and present, Fred Jevons Lecture, 2014.

7 Conclusion

Regulatory context: Today there is no OoC system used in any regulatory approval path. However, there are clear examples of public and private-public partnerships heading this direction. This will open the field for this emerging market and support adoption in regulatory approvals. Because of strong (international) presence of legislators in OoC development and qualification, this process can be accelerated.

Standardization: OoC system standardization can build further on existing standards or standardization efforts of its subcomponents and sub-processes. Although there are no golden standards defined today, keeping in mind previous standards and guidelines and a close collaboration with relevant stakeholders will determine successful introduction of OoC's in drug development.

Ethical dimension: The ethical discourse has a largely favourable, positive bias today, indicating OoC as a possible solution reducing cost, need, and ethical burden of animal studies, both for drug discovery and even more for toxicology studies. OoC as a broad platform technology has the benefit of being able to adapt to evolutions in biological science, e.g. the replacement of controversial human embryonal cells by human induced pluripotent stem cells, which largely silenced the ethical debate. OoC has also the potential to finally allow for drug discovery and personalized treatment for small or differentiated target groups (rare diseases, children, pregnant women, gender specific, ethnic specific).

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Next steps: The findings of this landscape study will be used to address relevant stakeholders and work out possible actions and guidelines that drive a synchronous evolution of the ethical debate, the regulatory transformations, and the standardization efforts with the technological progress on OoC.

8 References

Agarwal, Ashutosh, Josue Adrian Goss, Alexander Cho, Megan Laura McCain, and Kevin Kit Parker. 2013. “Microfluidic Heart on a Chip for Higher Throughput Pharmacological Studies.” *Lab on a Chip*. https://doi.org/10.1039/c3lc50350j.

Ahadian, Samad, Robert Civitarese, Dawn Bannerman, Mohammad Hossein Mohammadi, Rick Lu, Erika Wang, Locke Davenport-Huyer, et al. 2018. “Correction to: Organ-On-A-Chip Platforms: A Convergence of Advanced Materials, Cells, and Microscale Technologies (Advanced Healthcare Materials, (2018), 7, 2, (1700506), 10.1002/Adhm.201700506).” *Advanced Healthcare Materials*. https://doi.org/10.1002/adhm.201800734.

Alexander, Frank A., Sebastian Eggert, and Joachim Wiest. 2018. “Skin-on-a-Chip: Transepithelial Electrical Resistance and Extracellular Acidification Measurements through an Automated Air-Liquid Interface.” *Genes*. https://doi.org/10.3390/genes9020114.

Asphahani, F, K Wang, M Thein, O Veiseh, S Yung, J Xu, and M Zhang. 2011. “Single-Cell Bioelectrical Impedance Platform for Monitoring Cellular Response to Drug Treatment.” *Phys Biol*. https://doi.org/S1478-3975(11)64689-4 [pii]\r10.1088/1478-3975/8/1/015006.

Balls, M., and J. H. Fentem. 1999. “The Validation and Acceptance of Alternatives to Animal Testing.” In *Toxicology in Vitro*. https://doi.org/10.1016/S0887-2333(99)00067-3.

Bavli, Danny, Sebastian Prill, Elishai Ezra, Gahl Levy, Merav Cohen, Mathieu Vinken, Jan Vanfleteren, Magnus Jaeger, and Yaakov Nahmias. 2016. “Real-Time Monitoring of Metabolic Function in Liver-on-Chip Microdevices Tracks the Dynamics of Mitochondrial Dysfunction.” *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1522556113.

Berthier, Erwin, Edmond W.K. Young, and David Beebe. 2012. “Engineers Are from PDMS-Land, Biologists Are from Polystyrenia.” *Lab on a Chip*. https://doi.org/10.1039/c2lc20982a.

Bhatia, Sangeeta N., and Donald E. Ingber. 2014. “Microfluidic Organs-on-Chips.” *Nature Biotechnology*. https://doi.org/10.1038/nbt.2989.

Biesecker, Leslie G., Wylie Burke, Isaac Kohane, Sharon E. Plon, and Ron Zimmern. 2012. “Next-Generation Sequencing in the Clinic: Are We Ready?” *Nature Reviews Genetics*. https://doi.org/10.1038/nrg3357.

Bredenoord, Annelien L., Hans Clevers, and Juergen A. Knoblich. 2017. “Human Tissues in a Dish: The Research and Ethical Implications of Organoid Technology.” *Science*. https://doi.org/10.1126/science.aaf9414.

Chatterjee, Shampa, Keigi Fujiwara, Néstor Gustavo Pérez, Masuko Ushio-Fukai, and Aron B. Fisher. 2015. “Mechanosignaling in the Vasculature: Emerging Concepts in Sensing, Transduction and Physiological Responses.” *American Journal of Physiology-Heart and Circulatory Physiology*. https://doi.org/10.1152/ajpheart.00105.2015.

Chokkalingam, Venkatachalam, Jurjen Tel, Florian Wimmers, Xin Liu, Sergey Semenov, Julian Thiele, Carl G. Figdor, and Wilhelm T.S. Huck. 2013. “Probing Cellular Heterogeneity in Cytokine-Secreting Immune Cells Using Droplet-Based Microfluidics.” *Lab on a Chip*. https://doi.org/10.1039/c3lc50945a.

Daoud, J., K. Heileman, S. Shapka, L. Rosenberg, and M. Tabrizian. 2015. “Dielectric Spectroscopy for Monitoring Human Pancreatic Islet Differentiation within Cell-Seeded Scaffolds in a Perfusion Bioreactor System.” *The Analyst*. https://doi.org/10.1039/C5AN00525F.

Donnelly, Kenneth, Alastair Khodabukus, Andrew Philp, Louise Deldicque, Robert G. Dennis, and Keith Baar. 2010. “A Novel Bioreactor for Stimulating Skeletal Muscle *In Vitro*.” *Tissue Engineering Part C: Methods*. https://doi.org/10.1089/ten.tec.2009.0125.

Eduati, F., L.M. Mangravite, T. Wang, H. Tang, J.C. Bare, R. Huang, T. Norman, et al. 2015. “Prediction of Human Population Responses to Toxic Compounds by a Collaborative Competition.” *Nature Biotechnology*. https://doi.org/10.1038/nbt.3299.

Engle, Sandra J., and Dinesh Puppala. 2013. “Integrating Human Pluripotent Stem Cells into Drug Development.” *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2013.05.011.

Esch, Mandy B., Gretchen J. Mahler, Tracy Stokol, and Michael L. Shuler. 2014. "Body-on-a-Chip Simulation with Gastrointestinal Tract and Liver Tissues Suggests That Ingested Nanoparticles Have the Potential to Cause Liver Injury." *Lab on a Chip*. https://doi.org/10.1039/c4lc00371c.

Fermini, Bernard, Jules C. Hancox, Najah Abi-Gerges, Matthew Bridgland-Taylor, Khuram W. Chaudhary, Thomas Colatsky, Krystle Correll, et al. 2016. "A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive in Vitro Proarrhythmia Assay Paradigm." *Journal of Biomolecular Screening*. https://doi.org/10.1177/1087057115594589.

Fredberg, Jeffrey J., and Roger D. Kamm. 2006. "STRESS TRANSMISSION IN THE LUNG: Pathways from Organ to Molecule." *Annual Review of Physiology*. https://doi.org/10.1146/annurev.physiol.68.072304.114110.

Ges, Igor A., and Franz Baudenbacher. 2010. "Enzyme-Coated Microelectrodes to Monitor Lactate Production in a Nanoliter Microfluidic Cell Culture Device." *Biosensors and Bioelectronics*. https://doi.org/10.1016/j.bios.2010.05.030.

Goikoetxea, Erkuden, Denis Routkevitch, Ami de Weerdt, Jordan J. Green, Hans Steenackers, and Dries Braeken. 2018. "Impedimetric Fingerprinting and Structural Analysis of Isogenic E. Coli Biofilms Using Multielectrode Arrays." *Sensors and Actuators, B: Chemical*. https://doi.org/10.1016/j.snb.2018.01.188.

Greenman, John. 2017. "Looking to the Future of Organs-on-Chip." *Future Science OA*. https://doi.org/10.4155/fsoa-2017-0040.

Griesinger, Claudius, Bertrand Desprez, Sandra Coecke, Warren Casey, and Valérie Zuang. 2016. "Validation of Alternative In Vitro Methods to Animal Testing: Concepts, Challenges, Processes and Tools." In *Advances in Experimental Medicine and Biology*. https://doi.org/10.1007/978-3-319-33826-2_4.

Guenat, Olivier T., and François Berthiaume. 2018. "Incorporating Mechanical Strain in Organs-on-a-Chip: Lung and Skin." *Biomicrofluidics*. https://doi.org/10.1063/1.5024895.

Hartung, Thomas. 2007. "Food for Thought ... on Cell Culture." *ALTEX*. https://doi.org/10.14573/altex.2007.3.143.

———. 2017. "Opinion versus Evidence for the Need to Move Away from Animal Testing." *ALTEX*. https://doi.org/10.14573/altex.1703291.

Hartung, Thomas, Susanne Bremer, Silvia Casati, Sandra Coecke, Raffaella Corvi, Salvador Fortaner, Laura Gribaldo, et al. 2004. "A Modular Approach to the ECVAM Principles on Test Validity." *ATLA Alternatives to Laboratory Animals*.

Hierlemann, By Andreas, Urs Frey, Sadik Hafizovic, and Flavio Heer. 2011. "Growing Cells Atop Microelectronic Chips : Interfacing Electrogenic Cells In Vitro With CMOS-Based Microelectrode Arrays." *Proceedings of the IEEE* 99 (2).

Holmes, Anthony M., Stuart Creton, and Kathryn Chapman. 2010. "Working in Partnership to Advance the 3Rs in Toxicity Testing." *Toxicology*. https://doi.org/10.1016/j.tox.2009.11.006.

Horvath, Peter, Nathalie Aulner, Marc Bickle, Anthony M. Davies, Elaine Del Nery, Daniel Ebner, Maria C. Montoya, et al. 2016. "Screening out Irrelevant Cell-Based Models of Disease." *Nature Reviews Drug Discovery*. https://doi.org/10.1038/nrd.2016.175.

Huh, Dongeun, Benjamin D. Matthews, Akiko Mammoto, Martin Montoya-Zavala, Hong Yuan Hsin, and Donald E. Ingber. 2010. "Reconstituting Organ-Level Lung Functions on a Chip." *Science*. https://doi.org/10.1126/science.1188302.

Jang, Kyung-Jin, and Kahp-Yang Suh. 2010. "A Multi-Layer Microfluidic Device for Efficient Culture and Analysis of Renal Tubular Cells." *Lab on a Chip*. https://doi.org/10.1039/B907515A.

Jans, D., G. Callewaert, O. Krylychkina, L. Hoffman, F. Gullo, D. Prodanov, and D. Braeken. 2017. "Action Potential-Based MEA Platform for in Vitro Screening of Drug-Induced Cardiotoxicity Using Human IPSCs and Rat Neonatal Myocytes." *Journal of Pharmacological and Toxicological Methods* 87. https://doi.org/10.1016/j.vascn.2017.05.003.

Jekunen, Antti. 2014. "Decision-Making in Product Portfolios of Pharmaceutical Research and Development – Managing Streams of Innovation in Highly Regulated Markets." *Drug Design, Development and Therapy*. https://doi.org/10.2147/DDDT.S68579.

Johnstone, Andrew F M, Guenter W Gross, Dieter G Weiss, Olaf H-U Schroeder, Alexandra Gramowski, and Timothy J Shafer.

2010. “Microelectrode Arrays: A Physiologically Based Neurotoxicity Testing Platform for the 21st Century.” *Neurotoxicology* 31 (4): 331–50. https://doi.org/10.1016/j.neuro.2010.04.001.

Kerr, Anne, Rosemary L. Hill, and Christopher Till. 2018. “The Limits of Responsible Innovation: Exploring Care, Vulnerability and Precision Medicine.” *Technology in Society*. https://doi.org/10.1016/j.techsoc.2017.03.004.

Kilic, Tugba, Fatemeh Navaee, Francesca Stradolini, Philippe Renaud, and Sandro Carrara. 2018. “Organs-on-Chip Monitoring: Sensors and Other Strategies.” *Microphysiological Systems*. https://doi.org/10.21037/mps.2018.01.01.

Kim, Hyun Jung, Dongeun Huh, Geraldine Hamilton, and Donald E. Ingber. 2012. “Human Gut-on-a-Chip Inhabited by Microbial Flora That Experiences Intestinal Peristalsis-like Motions and Flow.” *Lab on a Chip*. https://doi.org/10.1039/c2lc40074j.

Kim, Seunggyu, Wanho Kim, Seongjin Lim, and Jessie Jeon. 2017. “Vasculature-On-A-Chip for In Vitro Disease Models.” *Bioengineering*. https://doi.org/10.3390/bioengineering4010008.

Kimmelman, Jonathan, and Carole Federico. 2017. “Consider Drug Efficacy before First-in-Human Trials.” *Nature*. https://doi.org/10.1038/542025a.

Krommenhoek, Erik E., Michiel Van Leeuwen, Han Gardeniers, Walter M. Van Gulik, Albert Van Den Berg, Xiaonan Li, Marcel Ottens, Luuk A.M. Van Der Wielen, and Joseph J. Heijnen. 2008. “Lab-Scale Fermentation Tests of Microchip with Integrated Electrochemical Sensors for PH, Temperature, Dissolved Oxygen and Viable Biomass Concentration.” *Biotechnology and Bioengineering*. https://doi.org/10.1002/bit.21661.

Kurth, Felix, Klaus Eyer, Alfredo Franco-Obregón, and Petra S. Dittrich. 2012. “A New Mechanobiological Era: Microfluidic Pathways to Apply and Sense Forces at the Cellular Level.” *Current Opinion in Chemical Biology*. https://doi.org/10.1016/j.cbpa.2012.03.014.

Lehmann, Mirko, Werner Baumann, Martin Brischwein, Hans Jürgen Gahle, Ingo Freund, Ralf Ehret, Sabine Drechsler, et al. 2001. “Simultaneous Measurement of Cellular Respiration and Acidification with a Single CMOS ISFET.” *Biosensors and Bioelectronics*. https://doi.org/10.1016/S0956-5663(01)00123-3.

Liang, Gaoyang, and Yi Zhang. 2013. “Genetic and Epigenetic Variations in IPSCs: Potential Causes and Implications for Application.” *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2013.07.001.

Lind, Johan U., Moran Yadid, Ian Perkins, Blakely B. O’Connor, Feyisayo Eweje, Christophe O. Chantre, Matthew A. Hemphill, et al. 2017. “Cardiac Microphysiological Devices with Flexible Thin-Film Sensors for Higher-Throughput Drug Screening.” *Lab on a Chip*. https://doi.org/10.1039/c7lc00740j.

Liu, Ying, Ying Liu, Zimple Matharu, Ali Rahimian, and Alexander Revzin. 2015. “Detecting Multiple Cell-Secreted Cytokines from the Same Aptamer-Functionalized Electrode.” *Biosensors and Bioelectronics*. https://doi.org/10.1016/j.bios.2014.08.034.

Livingston, Christine A., Kristin M. Fabre, and Danilo A. Tagle. 2016. “Facilitating the Commercialization and Use of Organ Platforms Generated by the Microphysiological Systems (Tissue Chip) Program through Public-Private Partnerships.” *Computational and Structural Biotechnology Journal*. https://doi.org/10.1016/j.csbj.2016.04.003.

Lopez, C.M., H.S. Chun, L. Berti, S. Wang, J. Putzeys, C. Van Den Bulcke, J.-W. Weijers, et al. 2018. “A 16384-Electrode 1024-Channel Multimodal CMOS MEA for High-Throughput Intracellular Action Potential Measurements and Impedance Spectroscopy in Drug-Screening Applications.” In *Digest of Technical Papers - IEEE International Solid-State Circuits Conference*. Vol. 61. https://doi.org/10.1109/ISSCC.2018.8310385.

Lopez, Carolina Mora, Ho Sung Chun, Laurent Berti, Shiwei Wang, Jan Putzeys, Carl Van Den Bulcke, Jan Willem Weijers, et al. 2018. “A 16384-Electrode 1024-Channel Multimodal CMOS MEA for High-Throughput Intracellular Action Potential Measurements and Impedance Spectroscopy in Drug-Screening Applications.” In *Digest of Technical Papers - IEEE International Solid-State Circuits Conference*. https://doi.org/10.1109/ISSCC.2018.8310385.

Loskill, Peter, Sivan G. Marcus, Anurag Mathur, Willie Mae Reese, and Kevin E. Healy. 2015. “MOrgano: A Lego®-Like Plug & Play System for Modular Multi-Organ-Chips.” Edited by Raghavan Raju. *PLOS ONE* 10 (10): e0139587. https://doi.org/10.1371/journal.pone.0139587.

Luni, Camilla, Stefano Giulitti, Elena Serena, Luca Ferrari, Alessandro Zambon, Onelia Gagliano, Giovanni G. Giobbe, et al. 2016.

"High-Efficiency Cellular Reprogramming with Microfluidics." *Nature Methods*. https://doi.org/10.1038/nmeth.3832.

Ma, Liang, Jeremy Barker, Changchun Zhou, Wei Li, Jing Zhang, Biaoyang Lin, Gregory Foltz, Jenni Küblbeck, and Paavo Honkakoski. 2012. "Towards Personalized Medicine with a Three-Dimensional Micro-Scale Perfusion-Based Two-Chamber Tissue Model System." *Biomaterials*. https://doi.org/10.1016/j.biomaterials.2012.02.054.

Maiuri, Paolo, Jean François Rupprecht, Stefan Wieser, Verena Ruprecht, Olivier Bénichou, Nicolas Carpi, Mathieu Coppey, et al. 2015. "Actin Flows Mediate a Universal Coupling between Cell Speed and Cell Persistence." *Cell*. https://doi.org/10.1016/j.cell.2015.01.056.

Maoz, Ben M, Anna Herland, Edward A FitzGerald, Thomas Grevesse, Charles Vidoudez, Alan R Pacheco, Sean P Sheehy, et al. 2018. "A Linked Organ-on-Chip Model of the Human Neurovascular Unit Reveals the Metabolic Coupling of Endothelial and Neuronal Cells." *Nature Biotechnology*. https://doi.org/10.1038/nbt.4226.

Maschmeyer, Ilka, Alexandra K. Lorenz, Katharina Schimek, Tobias Hasenberg, Anja P. Ramme, Juliane Hübner, Marcus Lindner, et al. 2015. "A Four-Organ-Chip for Interconnected Long-Term Co-Culture of Human Intestine, Liver, Skin and Kidney Equivalents." *Lab on a Chip*. https://doi.org/10.1039/c5lc00392j.

Matharu, Zimple, Dipali Patel, Yandong Gao, Amranul Haque, Qing Zhou, and Alexander Revzin. 2014. "Detecting Transforming Growth Factor-β Release from Liver Cells Using an Aptasensor Integrated with Microfluidics." *Analytical Chemistry*. https://doi.org/10.1021/ac502383e.

Mathupala, Saroj P., Chaim B. Colen, Prahlad Parajuli, and Andrew E. Sloan. 2007. "Lactate and Malignant Tumors: A Therapeutic Target at the End Stage of Glycolysis." *Journal of Bioenergetics and Biomembranes*. https://doi.org/10.1007/s10863-006-9062-x.

Mathur, Anurag, Peter Loskill, Kaifeng Shao, Nathaniel Huebsch, Soon Gweon Hong, Sivan G. Marcus, Natalie Marks, et al. 2015. "Human IPSC-Based Cardiac Microphysiological System for Drug Screening Applications." *Scientific Reports*. https://doi.org/10.1038/srep08883.

Micholt, L., A. Gärtner, D. Prodanov, D. Braeken, C.G. Dotti, and C. Bartic. 2013. "Substrate Topography Determines Neuronal Polarization and Growth In Vitro." *PLoS ONE* 8 (6). https://doi.org/10.1371/journal.pone.0066170.

Millard, Daniel, Qianyu Dang, Hong Shi, Xiaou Zhang, Chris Strock, Udo Kraushaar, Haoyu Zeng, et al. 2018. "Cross-Site Reliability of Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Based Safety Assays Using Microelectrode Arrays: Results from a Blinded CiPA Pilot Study." *Toxicological Sciences*. https://doi.org/10.1093/toxsci/kfy110.

Miller, Seumas, and Michael J. Selgelid. 2008. *Ethical and Philosophical Consideration of the Dual-Use Dilemma in the Biological Sciences*. *Ethical and Philosophical Consideration of the Dual-Use Dilemma in the Biological Sciences*. https://doi.org/10.1007/978-1-4020-8312-9.

Moraes, Christopher, Joseph M. Labuz, Brendan M. Leung, Mayumi Inoue, Tae Hwa Chun, and Shuichi Takayama. 2013. "On Being the Right Size: Scaling Effects in Designing a Human-on-a-Chip." *Integrative Biology (United Kingdom)*. https://doi.org/10.1039/c3ib40040a.

Moraes, Christopher, Geeta Mehta, Sasha Cai Lesher-Perez, and Shuichi Takayama. 2012. "Organs-on-a-Chip: A Focus on Compartmentalized Microdevices." *Annals of Biomedical Engineering*. https://doi.org/10.1007/s10439-011-0455-6.

Morrison, Michael, Jessica Bell, Carol George, Shawn Harmon, Megan Munsie, and Jane Kaye. 2017. "The European General Data Protection Regulation: Challenges and Considerations for IPSC Researchers and Biobanks." *Regenerative Medicine*. https://doi.org/10.2217/rme-2017-0068.

Moya, A., M. Ortega-Ribera, X. Guimerà, E. Sowade, M. Zea, X. Illa, E. Ramon, R. Villa, J. Gracia-Sancho, and G. Gabriel. 2018. "Online Oxygen Monitoring Using Integrated Inkjet-Printed Sensors in a Liver-on-a-Chip System." *Lab on a Chip*. https://doi.org/10.1039/c8lc00456k.

Nakao, Yosuke, Hiroshi Kimura, Yasuyuki Sakai, and Teruo Fujii. 2011. "Bile Canaliculi Formation by Aligning Rat Primary Hepatocytes in a Microfluidic Device." *Biomicrofluidics*. https://doi.org/10.1063/1.3580753.

Nianzhen Li, Michael Schwartz, and Cristian Ionescu-Zanetti. 2009. "PDMS Compound Adsorption in Context." *Journal of*

Biomolecular Screening. https://doi.org/10.1177/1087057108327326.

Nichols, Scott P., Ahyeon Koh, Wesley L. Storm, Jae Ho Shin, and Mark H. Schoenfisch. 2013. "Biocompatible Materials for Continuous Glucose Monitoring Devices." *Chemical Reviews*. https://doi.org/10.1021/cr300387j.

Nisisako, Takasi, and T. Torii. 2008. "Microfluidic Large-Scale Integration on a Chip for Mass Production of Monodisperse Droplets and Particles." *Lab on a Chip*. https://doi.org/10.1039/b713141k.

Norman, Gail A. Van. 2016. "Drugs and Devices: Comparison of European and U.S. Approval Processes." *JACC: Basic to Translational Science*. https://doi.org/10.1016/j.jacbts.2016.06.003.

Nunes, Sara S., Jason W. Miklas, Jie Liu, Roozbeh Aschar-Sobbi, Yun Xiao, Boyang Zhang, Jiahua Jiang, et al. 2013. "Biowire: A Platform for Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes." *Nat Methods*. https://doi.org/10.1167/iovs.07-1072.Complement-Associated.

Olson, Harry, Graham Betton, Denise Robinson, Karluss Thomas, Alastair Monro, Gerald Kolaja, Patrick Lilly, et al. 2000. "Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals." *Regulatory Toxicology and Pharmacology*. https://doi.org/10.1006/rtph.2000.1399.

Oomen, Pieter Edmond, Maciej Skolimowski, and Sabeth Verpoorte. 2016. "Implementing Oxygen Control in Chip-Based Cell and Tissue Culture Systems." *Lab Chip*. https://doi.org/10.1039/C6LC00772D.

Pamies, David, and Thomas Hartung. 2017. "21st Century Cell Culture for 21st Century Toxicology." *Chemical Research in Toxicology*. https://doi.org/10.1021/acs.chemrestox.6b00269.

Parizi, Kokab B., Xiaoqing Xu, Ashish Pal, Xiaolin Hu, and H. S. Philip Wong. 2017. "ISFET PH Sensitivity: Counter-Ions Play a Key Role." *Scientific Reports*. https://doi.org/10.1038/srep41305.

Park, J. S., B. Rhau, A. Hermann, K. A. McNally, C. Zhou, D. Gong, O. D. Weiner, B. R. Conklin, J. Onuffer, and W. A. Lim. 2014. "Synthetic Control of Mammalian-Cell Motility by Engineering Chemotaxis to an Orthogonal Bioinert Chemical Signal." *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1402087111.

Pauwelyn, Thomas, Beatrice Miccoli, Aravinthan Velnayagam, Robert Jan Boom, Maciej Skolimowski, Elwin Vrouwe, Dries Braeken, and Veerle Reumers. n.d. "High-Throughput CMOS MEA System with Integrated Microfluidics for Cardiotoxicity Studies." *Frontiers in Cellular Neuroscience*, no. 9. https://doi.org/10.3389/conf.fncel.2018.38.00009.

Peyton, Shelly R., Cyrus M. Ghajar, Chirag B. Khatiwala, and Andrew J. Putnam. 2007. "The Emergence of ECM Mechanics and Cytoskeletal Tension as Important Regulators of Cell Function." *Cell Biochemistry and Biophysics*. https://doi.org/10.1007/s12013-007-0004-y.

Phan, Duc T.T., R. Hugh F. Bender, Jillian W. Andrejecsk, Agua Sobrino, Stephanie J. Hachey, Steven C. George, and Christopher C.W. Hughes. 2017. "Blood–Brain Barrier-on-a-Chip: Microphysiological Systems That Capture the Complexity of the Blood–Central Nervous System Interface." *Experimental Biology and Medicine*. https://doi.org/10.1177/1535370217694100.

Polouliakh, Natalia. 2013. "Reprogramming Resistant Genes: In-Depth Comparison of Gene Expressions among IPS, ES, and Somatic Cells." *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2013.00007.

Rangarajan, Swathi, Lauran Madden, and Nenad Bursac. 2014. "Use of Flow, Electrical, and Mechanical Stimulation to Promote Engineering of Striated Muscles." *Annals of Biomedical Engineering*. https://doi.org/10.1007/s10439-013-0966-4.

Roan, Esra, and Christopher M. Waters. 2011. "What Do We Know about Mechanical Strain in Lung Alveoli?" *American Journal of Physiology - Lung Cellular and Molecular Physiology*. https://doi.org/10.1152/ajplung.00105.2011.

Rogal, Julia, Christopher Probst, and Peter Loskill. 2017. "Integration Concepts for Multi-Organ Chips: How to Maintain Flexibility?!" *Future Science OA*. https://doi.org/10.4155/fsoa-2016-0092.

Rollin, Bernard E. 2012. "The Moral Status of Invasive Animal Research." *Hastings Center Report*. https://doi.org/10.1002/hast.99.

Russell, W. M. 1995. “The Development of the Three Rs Concept.” *Alternatives to Laboratory Animals : ATLA*.

Schimek, Katharina, Mathias Busek, Sven Brincker, Benjamin Groth, Silke Hoffmann, Roland Lauster, Gerd Lindner, et al. 2013. “Integrating Biological Vasculature into a Multi-Organ-Chip Microsystem.” *Lab on a Chip*. https://doi.org/10.1039/c3lc50217a.

Schleifenbaum, Stefan, Michael Schmidt, Robert Möbius, Thomas Wolfskämpf, Christian Schröder, Ronny Grunert, Niels Hammer, and Torsten Prietzel. 2016. “Load and Failure Behavior of Human Muscle Samples in the Context of Proximal Femur Replacement.” *BMC Musculoskeletal Disorders*. https://doi.org/10.1186/s12891-016-0998-7.

Schmidt, Charles W. 2009. “Tox21: New Dimensions of Toxicity Testing.” *Environmental Health Perspectives*. https://doi.org/10.1289/ehp.117-a348.

Servick, K. 2016. “A Painstaking Overhaul for Cardiac Safety Testing.” *Science*. https://doi.org/10.1126/science.353.6303.976.

Shaegh, Seyed Ali Mousavi, Fabio De Ferrari, Yu Shrike Zhang, Mahboubeh Nabavinia, Niema Binth Mohammad, John Ryan, Adel Pourmand, et al. 2016. “A Microfluidic Optical Platform for Real-Time Monitoring of PH and Oxygen in Microfluidic Bioreactors and Organ-on-Chip Devices.” *Biomicrofluidics*. https://doi.org/10.1063/1.4955155.

Skardal, Aleksander, Sean V. Murphy, Mahesh Devarasetty, Ivy Mead, Hyun Wook Kang, Young Joon Seol, Yu Shrike Zhang, et al. 2017. “Multi-Tissue Interactions in an Integrated Three-Tissue Organ-on-a-Chip Platform.” *Scientific Reports*. https://doi.org/10.1038/s41598-017-08879-x.

Stenken, Julie A., and Andreas J. Poschenrieder. 2015. “Bioanalytical Chemistry of Cytokines - A Review.” *Analytica Chimica Acta*. https://doi.org/10.1016/j.aca.2014.10.009.

Sung, Jong Hwan, and Michael L. Shuler. 2009. “A Micro Cell Culture Analog (CCA) with 3-D Hydrogel Culture of Multiple Cell Lines to Assess Metabolism-Dependent Cytotoxicity of Anti-Cancer Drugs.” *Lab on a Chip*. https://doi.org/10.1039/b901377f.

Takayama, Shuichi, Emanuele Ostuni, Philip LeDuc, Keiji Naruse, Donald E. Ingber, and George M. Whitesides. 2001. “Subcellular Positioning of Small Molecules.” *Nature*. https://doi.org/10.1038/35082637.

Trietsch, Sebastiaan J., Elena Naumovska, Dorota Kurek, Meily C. Setyawati, Marianne K. Vormann, Karlijn J. Wilschut, Henriëtte L. Lanz, et al. 2017. “Membrane-Free Culture and Real-Time Barrier Integrity Assessment of Perfused Intestinal Epithelium Tubes.” *Nature Communications*. https://doi.org/10.1038/s41467-017-00259-3.

Vargas, Pablo, Lucie Barbier, Pablo José Sáez, and Matthieu Piel. 2017. “Mechanisms for Fast Cell Migration in Complex Environments.” *Current Opinion in Cell Biology*. https://doi.org/10.1016/j.ceb.2017.04.007.

Vermeulen, Niki, Gill Haddow, Tirion Seymour, Alan Faulkner-Jones, and Wenmiao Shu. 2017. “3D Bioprint Me: A Socioethical View of Bioprinting Human Organs and Tissues.” *Journal of Medical Ethics*. https://doi.org/10.1136/medethics-2015-103347.

Wagner, Ilka, Eva Maria Materne, Sven Brincker, Ute Süßbier, Caroline Frädrich, Mathias Busek, Frank Sonntag, et al. 2013. “A Dynamic Multi-Organ-Chip for Long-Term Cultivation and Substance Testing Proven by 3D Human Liver and Skin Tissue Co-Culture.” *Lab on a Chip*. https://doi.org/10.1039/c3lc50234a.

Wall, Michelle E., Paul S. Weinhold, Tung Siu, Thomas D. Brown, and Albert J. Banes. 2007. “Comparison of Cellular Strain with Applied Substrate Strain in Vitro.” *Journal of Biomechanics*. https://doi.org/10.1016/j.jbiomech.2005.10.032.

Wikswo, John P., Frank E. Block, David E. Cliffel, Cody R. Goodwin, Christina C. Marasco, Dmitry A. Markov, David L. McLean, et al. 2013. “Engineering Challenges for Instrumenting and Controlling Integrated Organ-on-Chip Systems.” *IEEE Transactions on Biomedical Engineering*. https://doi.org/10.1109/TBME.2013.2244891.

Xia, Younan, and George M. Whitesides. 1998. “SOFT LITHOGRAPHY.” *Annual Review of Materials Science*. https://doi.org/10.1146/annurev.matsci.28.1.153.

Xiao, Shuo, Jonathan R. Coppeta, Hunter B. Rogers, Brett C. Isenberg, Jie Zhu, Susan A. Olalekan, Kelly E. McKinnon, et al. 2017. “A Microfluidic Culture Model of the Human Reproductive Tract and 28-Day Menstrual Cycle.” *Nature Communications*.

https://doi.org/10.1038/ncomms14584.

Zhang, Chi, Ziqing Zhao, Nur Aida Abdul Rahim, Danny Van Noort, and Hanry Yu. 2009. “Towards a Human-on-Chip: Culturing Multiple Cell Types on a Chip with Compartmentalized Microenvironments.” *Lab on a Chip*. https://doi.org/10.1039/b915147h.

Zhang, Yu Shrike, Julio Aleman, Su Ryon Shin, Tugba Kilic, Duckjin Kim, Seyed Ali Mousavi Shaegh, Solange Massa, et al. 2017. “Multisensor-Integrated Organs-on-Chips Platform for Automated and Continual in Situ Monitoring of Organoid Behaviors.” *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1612906114.

Zhou, Qing, Dipali Patel, Timothy Kwa, Amranul Haque, Zimple Matharu, Gulnaz Stybayeva, Yandong Gao, Anna Mae Diehl, and Alexander Revzin. 2015. “Liver Injury-on-a-Chip: Microfluidic Co-Cultures with Integrated Biosensors for Monitoring Liver Cell Signaling during Injury.” *Lab on a Chip*. https://doi.org/10.1039/c5lc00874c.

Zimmermann, W H, C Fink, D Kralisch, U Remmers, J Weil, and T Eschenhagen. 2000. “Three-Dimensional Engineered Heart Tissue from Neonatal Rat Cardiac Myocytes.” *Biotechnology and Bioengineering*. https://doi.org/10.1002/(SICI)1097-0290(20000405)6.