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Organ-on-Chip In Development: Towards a roadmap for Organs-on-Chip

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Abstract

Organ-on-Chip is a game-changing technology born from the convergence of tissue engineering and microfluidic technology. Organ-on-Chip devices (OoCs) are expected to offer effective solutions to persisting problems in drug development and personalized disease treatments. This opinion paper surveys the current landscape in research, development, application and market opportunities for OoCs to help establishing a global and multi-stakeholder OoC ecosystem. Based on a bibliometric study, a market analysis, expert interviews, and panel discussions held at the *ORCHID Vision Workshop* (Stuttgart, 23 May 2018), we outline presently unmet needs, key challenges, barriers and perspectives of the field, and finally propose recommendations towards the definition of a comprehensive roadmap that could render OoCs realistic models of human (patho)physiology in the near future.

1. Introduction: the healthcare challenge and the Organ-on-Chip roadmap

A critical problem in the development of effective disease treatments is the lack of adequate model systems to identify drug targets, screen toxicity, and predict clinical drug efficacy and the effects of active substances in humans [1]. Traditional animal models [2] or conventional cell cultures [3] often do not accurately mimic human physiology, and thus tend to poorly recapitulate human disease pathophysiology or accurately predict *in vivo* responses to medical treatments. This is a major cause of late drug failures in clinical trials, expensiveness of new drugs, and lack of medication for some diseases [1]. In addition, translational issues [2] and ethical questions raised by animal use increase the pressure to minimize animal experimentation. For these reasons, the pharmaceutical industry is looking for new ways to improve the drug development process [4], drug toxicity assessment [5], and to identify effective and personalized treatments; biomedical researchers require better systems to model diseases for a better understanding of their mechanism and aetiology; and cosmetics, chemical, food and other industries are in need of physiologically relevant human models to test toxicological hazards and assess the risk of substances under increasingly stringent regulatory requirements [1].

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Organ-on-Chip (OoC) is a game-changing technology expected to offer effective solutions for these problems [6] and meet the needs of different stakeholders [7]. In spite of its allure [8], pharma has thus far remained cautious to invest in this new technology, presently awaiting evidence of its added cost-benefit value, and whether it could represent a feasible route to precision medicine and improved patient stratification. It is thus necessary to bridge the gap between the potential of OoC systems (OoCs) and their worldwide acceptance. Defining the putative benefits of OoCs and how these can be proven and achieved is the preamble for an OoC roadmap – which was one of the aims of the ORCHID project.

The Horizon 2020 FET-Open project *Organ-on-Chip In Development* (ORCHID) started in 2017 with the goal of creating a roadmap for OoC technology and of building a network of academic, research, industrial and regulatory institutions to move OoCs from laboratories into the reality of the citizens of Europe and the rest of the world. The ORCHID Consortium is a collaboration between 7 partner organisations from 6 European countries[*], and engages an international advisory board of world-renown experts involved in the OoC field (www.h2020-orchid.eu). As part of the project, these and other experts have been asked to share their view on the state-of-the-art, unmet needs, challenges and barriers of the field. The results of the expert interviews[†], together with the results of bibliographical, bibliometric and market analyses of the OoC technology carried out within the ORCHID project (see the Appendix for a description of the analytical methodology), formed the basis of the *ORCHID Vision Workshop*, held in Stuttgart on 23 May 2018. The goal of the workshop was to define the pillars of a European OoC roadmap, including the definition of desired and feasible goals for a concrete deployment of the OoC technology.

This paper outlines the state-of-the-art, results, expert discussions, conclusions and recommendations that emerged from the ORCHID's analyses and workshop, in the perspective of fostering the establishment of a thriving global OoC ecosystem.

2. Organ-on-Chip: Definition, key features and value chain

According to the interviewed experts, and as confirmed during the ORCHID workshop, an OoC can be defined as "*a fit-for-purpose microfluidic device, containing living engineered organ substructures in a controlled microenvironment, that recapitulates one or more aspects of the organ's dynamics, functionality and (patho)physiological response* in vivo *under real-time monitoring"*.

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[†] Refer to the section A.2 of Appendix for the full list of interviewees and experts that contributed to the ORCHID.

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OoCs can be classified into two types with complementary goals and distinct complexity: (i) *single-organ systems*, emulating key functions of single tissues or organs, and (ii) *multi-organ platforms*, combining multiple OoCs to reproduce the systemic interaction and response of several organ models within a single system. Multi-OoCs link individual OoCs through microfluidic, preferably vascularized channels that mimic *in vivo* physiological coupling, and enforce cell-to-fluid volume ratios and flow distributions to create *in vitro* models of subsystems of the human body [9]. Further along this line, Human(-body)-on-Chip (HoC) systems are aiming to emulate whole organismal physiology by integrating many relevant single-organ models [1].

As implied by these definitions, the OoC technology stands on converging advances in tissue engineering, semiconductor and polymer microfabrication, and human cell sourcing. The associated value chain emerging from the ORCHID analyses (Fig. 1) highlights the need of a multidisciplinary approach to implement OoCs, and of a facilitating dialogue between developers, both academic and industrial, and stakeholders such as clinicians, patients, regulators and different end-users. All these disciplines are represented in the ORCHID Consortium.

3. State-of-the-art

A worldwide growing field of research

The OoC field emerged from the convergence of microfluidics and tissue engineering research. Whereas in early 2000's patents and publications mainly focused on microfluidics and associated fabrication techniques, more recent advances in stem cell biology, combined with decades of fundamental biological studies in cell signalling and biomechanics, accelerated the development of OoCs. The ORCHID bibliometric approach reflected this increasing activity by evidencing a rise in both dedicated patents and publications with a compound annual growth rate (CAGR) of +46% over the last ten years (123 patents and publications in 2007, compared to at least 390 in 2017) (Fig. 2a). Such continuous growth, driven by the huge market potential, was made possible by the concomitant diffusion of multidisciplinary approaches supported by an improved dialogue between developers such as biologists (cell culture, physiology), engineers (microfluidics, biosensing systems), material scientists (microenvironment, substrates), but also regulators, patients, clinicians and end users from both academia and industry. Originating from the USA, which pioneered the development of customized cellular microenvironments to capture the structural complexity of organs, this worldwide interest is rapidly engaging Europe, which has a strong track record in tissue engineering and microfluidics, and for which OoCs could represent a new growth opportunity (Fig. 2b-c). The Asia-Pacific region (APAC) contributes also substantially to the field with both established players and newcomers, supported by an exponential growth in technological and biological research, especially in Korea, China and Japan.

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OoCs hold great promises as avatars for native functional tissues

Very dynamic R&D activities have been translated into technological advances in both microfluidics and tissue engineering, providing OoCs with key added values towards a more accurate view of what happens in humans compared to other existing models (Fig. 3). OoC technology allows reconstitution of the microarchitecture of the organ supported by the design of a dedicated mechanical context matching the shape, surface pattern and stiffness of organ-specific microenvironments. Precise microfluidic flow control enables optimal oxygenation and nutrition that not only afford long-term viability of healthy tissues, but also an efficient circulation of immune system cells, antibodies, biochemical signalling molecules and metabolites, and the ability to collect tiny secretion volumes for analyses. Continuous perfusion and mechanical stress help to build dynamic tissue models, supposedly far more relevant than conventional static cell cultures, and enable the control of spatiotemporal chemical gradients and mechanical cues to study the influence of the microenvironment on the cells. Moreover, OoCs allow the precise investigation of specific tissue-tissue interfaces and biological events that cannot be monitored in animals or human patients. Their minute size, the control of the microenvironment, the facilitated optical access at high spatial resolution, and the integration of biosensors for real-time data collection bring OoCs large advantages over other models [1]. The tightly monitored regulation of the cellular environment and homeostasis should facilitate long-term cell culture, possibly over months. In addition, the great diversity and range of complexity of OoCs offer the opportunity to optimize or even customize the design of targeted studies, paving the way to personalized medicine.

According to their expected benefits, the main applications of OoCs range from toxicity test to drug discovery, and target users from various fields, such as biomedical researchers, modelling diseases for improved understanding of their mechanisms and aetiology, and industrials from different profiles – pharmaceutics, biotechnology, cosmetics, chemistry and environment – especially interested in predicting efficacy and safety of compounds for humans. Some players are even going further, introducing in their scientific publications the more long-term potential of OoCs for regenerative tissues and medicine. Existing models and proposed applications are comprehensively reviewed in [1, 8-10].

Nevertheless, OoCs still need scientific evidence of clinical correlation to physiological human behaviour. In particular, the metrics to be considered and their corresponding readouts largely remain to be defined. They are a prerequisite to demonstrate the relevance of the use of OoC compared to other traditional models, and to envision how they could influence the decision-making process.

The rapid development of OoC is driven by academics supplying multifaceted industrial companies

The OoC market gathers many players from different horizons, and evolves continuously due to rapid technological advances and the field's strong multidisciplinary attractiveness. The ORCHID bibliometric analysis identified at least 650 players (the top 30 are shown in Fig. 4). Most of the well-known academic

teams are concentrated within pioneering American hubs such as the ecosystema of Boston (Wyss Institute, MIT, Harvard, Brigham Women Hospital) and California (Berkeley, UCLA, UCI, Stanford), and the universities of New York (Cornell, Columbia) and Pennsylvania (Drexel, Pittsburgh). They tend to work especially on brain, lung and heart to address toxicological issues, but also on muscle, vasculature and bone marrow. The APAC region shows historical players like the University of Seoul (Korea), the Chinese Academy of Science (Beijing, China) and the University of Tokyo (Japan), leading chemical, physical and biological engineering approaches to emulate in particular brain, liver and lung tissues. The attractive market drives also the repositioning of key European players in microfluidics and cell culture, such as the Fraunhofer Institute (Germany), the University of Twente (The Netherlands), the CEA (France), the Technological University of Compiègne (part of the CNRS, France), Jena University (Germany), and the Swiss Federal Institute of Technology (EPFL, Switzerland), which appear to focus their research on the modelling of brain, liver, kidney and skin for toxicological concerns.

This academic ecosystem supplies industrials from various domains, which may coexist to play a dedicated role in the OoC value chain (Figs. 5 and 6): *i.* pharmaceutical companies; *ii.* companies specialized in microfabrication, imaging and electronics/robotics; *iii.* lab-on-chip manufacturing companies; and *iv.* OoC companies (*i.e.* commercializing fully operational OoC). Indeed, the development of OoCs has been supported over the past several years by pharmaceutical companies (*e.g.* GSK, Roche, AstraZeneca) searching for alternative predictive models, especially for the lung, the liver, the digestive and the nervous systems. OoC manufacturing and peripheral instrumentation require the concomitant involvement of supplying companies specialized in microfabrication, imaging, electronics and robotics (*e.g.* Seiko, Philips, Carl Zeiss; Fig. 6), in particular for OoC characterization and monitoring. Historical lab-on-chip manufacturing companies (*e.g.* Aline, Micronit, Microfluidic Chipshop, Minifab) consider OoCs as a promising growth opportunity. They are starting to partner with OoC companies and propose tailored approaches to support hardware development to scale up and standardize chip production.

OoC companies are mostly start-ups founded by ex-academic teams (*e.g.* Draper, CN Bio, TissUse, Emulate, Mimetas; Fig. 6) [8]. According to the Yole market report, only 18 private OoC companies were found on the market in 2017 [11]. Among the most active companies, TissUse and Nortis are characterized by a multi-tissue R&D activity, whereas the others seem to prioritize single-tissue development. Very few of them are already in the production and commercialization phase, the others being engaged in an iterative process with end users to test different OoC solutions, *i.e.* manufacturing prototypes and producing small series in-house. For instance, Emulate formed various strategic partnerships with pharmaceutical industries (Roche, Takeda Pharmaceuticals, Merck, Janssen) to improve its solutions, but also with the Food and Drug Administration (FDA) to evaluate and qualify the use of its technology for toxicology testing. The latest collaborative partnership, led with AstraZeneca's Innovative Medicines and Early Development Unit

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(IMED), aims to integrate Emulates' OoCs within the IMED drug safety laboratories and to co-locate Emulate's scientists within AstraZeneca's labs to accelerate the development of OoCs[‡]. In addition to its successful collaboration with AstraZeneca on a comprehensive type 2 diabetes-on-a-chip model, TissUse recently announced a cooperative agreement with Roche to develop applications for its multi-OoC platform in drug R&D. The aim of this three-year project is to establish *in vitro* assays for the assessment of lineage-specific hematopoietic toxicity and the evaluation of pharmacokinetics of therapeutic antibodies[§]. OoC companies need both external partners and high financial investments to go through the prototyping phase and to scale up the production. Impressive growth is also possible by a switch towards the small-scale stage, as shown by Mimetas which is mass-producing its OrganoPlate® after only 3 years of existence, and is supplying nearly all major pharmaceutical companies.

The current market positioning of OoC companies is tightly associated with the type of devices developed and their level of maturity. Three different business models can be distinguished: *i.* ready-for-culture microfluidic devices (chips and/or plates); *ii.* fully operational OoCs, including the microfluidic device and cell culture integration; and/or *iii.* a full-service offering to perform in-house tests requested by end users. Companies may switch between these business models; and according to the Yole market report, the large majority of OoC companies are even not sure whether they should sell the devices or offer testing services [11]. Starting with a service-based business model may help to build strong relationships with customers, whereas getting direct input and better understanding of customers' needs enables the joint development of effective OoC platforms. From an end-user point of view, the service-based offer could be of great value for early stage screening, when there is a large amount of compounds to be tested, especially in terms of logistic organization. It is mostly more convenient to ship a drug or a chemical compound than cell cultures. To understand a complex biological interaction, though, some customers, particularly pharmaceutical, may be more interested in buying the OoC to do their experiments in-house.

Whatever the business model of choice, the OoC companies know that minimizing the operational complexity is crucial for commercialization and market penetration. They need to entertain tight relationships with academia to ensure a continuous technological sourcing and to remain competitive.

Public and private investments are required to move OoC from bench to bedside

Both public and private sources have substantially funded OoC start-ups and research groups during the last few years. For instance, USA's National Center for Advancing Translational Sciences (NCATS), in conjunction with the National Institutes of Health (NIH) Common Fund, has invested $70M over a 5-year period to launch the Microphysiological Systems or Organs-on-Chips Program (2012) [12]. The aim of this

[‡] Emulate Inc., available at https://emulatebio.com/collaborations/ (accessed: February 25th, 2019)
[§] TissUse Gmbh, available at https://www.tissuse.com/en/news/press-releases/ (accessed: February 25th, 2019)

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program, part of a coordinated effort between the NIH, the Defense Advanced Research Projects Agency (DARPA) and the FDA, was to accelerate the development of human OoCs that "*will improve the reliability to identify human drug toxicities and predict the potential efficacy of a drug in a human population prior to use of the drug in late-stage clinical studies"* (NCATS website).

In the meantime, private investors have gained confidence in OoC technology. In particular, 2018 was a prolific year in fundraising, with $36M and $20.5M secured by Emulate and Mimetas respectively. According to the Yole market report, the most efficient company in fundraising is Emulate with 4 rounds in 4 years for a total of $93M [11]. Other US companies have also raised several millions of dollars, such as Hμrel Corporation ($9.2M in 2013), Nortis (more than $8M in total, with a first round of $2.65M in 2014), and Tara Biosystems (fundraisings in 2014, 2016 and 2017, for a total of $11.75M; source: Tara Biosystems website). In Europe, 2 companies have also succeeded to raise funds: Mimetas (The Netherlands) with a total of $27.65M (3 rounds in 2014, 2015 and 2018), and TissUse (Germany) with $4.6M in 2015. The Netherlands have funded a national OoC initiative (NOCI) with €18.8M for 10 years; a H2020 Innovative Training Network project on OoC (EUROoC), coordinated by Fraunhofer IGB, was recently granted with €3.94M for 4 years. Globally, American companies are way ahead with regard to fundraising: the US government is willing to support the OoC field, especially through the DARPA and the NIH, and lately the NASA and CASIS, with an investment of more than $200M over the past 5 years.

Market forecasts: Potential development from an emerging market towards an exponential growth

The Yole market report estimates the combined sales of OoC devices and services at no more than $7,5M in 2016, with the potential to undergo an impressive growth and become a multi-billion dollar market in the mid- to long-term, in view of the fact that OoCs help the industry to save billions of dollars every year, especially by bridging the translational gap between preclinical and clinical studies required for the drug development process [11]. As already indicated, very few players are currently in the production and commercialization phase; and while pharmaceutical and cosmetics companies are engaged in an iterative process to test different OoC solutions, they are conservative and might need time to widely adopt the technology. This overall context led Yole Développement to detail both a realistic and optimistic scenario, in which the market could grow at a CAGR from 2017-2022 of 38% to 57% to reach $60M to $117M, respectively in 2022 (Fig. 7; for further market data, see [11]).

In the optimistic scenario, based on the companies' forecasted revenues (Fig. 7a), the Yole report considers that if all conditions are met, *i.e.* industrial adoption is speeding up, OoC companies are able to overcome technical challenges and to upscale production, production of 858k units will be reached in 2022, corresponding to an overall market of $117M [11]. The service-based market is expected to remain much smaller than the device-sales market, and may not be sustainable on the long-term.

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In the realistic scenario (Fig. 7b), the Yole report forecasts that the majority of OoC developers will face issues when the demand will grow [11]. Especially, it considers that scaling up the production will likely slow down the growth of OoC companies during the period 2018-2021. Most of them will have to switch from PDMS prototyping and small-series to large-scale production in other materials (glass, polymer injection moulding), requiring redesign steps that will be very expensive for the relatively small companies involved. In this scenario, the OoC market will grow from $7,5M in 2016 to $59,7M in 2022 because only few companies have already managed to scale up their production. The revenue fraction deriving from services is higher than for the optimistic scenario because OoC companies will focus on customized services to offset losses due to the costs for production upscaling.

4. Challenges

Unmet needs

Evidence of added value

A notable unmet need that emerged from the experts' interviews is data-supported evidence of actual advantages of the OoC technology compared to existing conventional models or well-established approaches to tissue engineering. Particularly, while the superiority of 3D over 2D cell cultures for *e.g.* phenotypic expression is attested in recent scientific literature [13], the advantages deriving from the inclusion of dynamic perfusion and *in situ* stimulation in OoCs arguably need more substantial support.

Organs-on-Chip can pave the way to personalized medicine and de-risking drug development

The failure of preclinical cell cultures and animal models to reliably predict drug efficacy and safety at the human clinical trial stage ends up wasting billions of dollars each year and slows down the development of medical treatments. Whereas spending on drug development increased over the past 20 years, the number of drugs approved annually declined [8]. Many compounds with high potential health benefit are eliminated early in development due to the poor predictability of preclinical models. Drugs could even be withdrawn shortly after entering the market, mainly because of cardiac, liver and kidney toxicities. Late-stage failures cause catastrophic losses while significantly driving up cumulative costs and patient risk. Positioning OoCs as powerful predictive tools for preclinical screening might represent the ideal solution for the pharmaceutical industry to eliminate ineffective drug candidates as early as possible and curb the costs of drug development [8].

As expected, the OoC application most cited in scientific literature and in the ORCHID experts' interviews lies in the pharmaceutical drug development process, including safety assessment and efficacy testing. The main idea shared by all players is that OoCs may enable more rapid, accurate, cost-effective and clinically relevant testing of drugs. According to a recent scenario-based cost analysis conducted in the

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context of the ORCHID project, experts expect a positive budget impact, reaching a reduction up to 26% in the total R&D costs for drug development [14]. While all cost drivers may be impacted, savings would mostly be achieved by improving the success rates. The R&D phases in which experts expect the most benefits are lead optimization and preclinical phases.

Data from animal studies are often poorly indicative of the human situation [2, 15], since animal preclinical models have limitations in mimicking the complex processes specifically occurring within the human body [1]. OoC models may potentially bridge this translational gap by fostering the implementation of 3Rs, *i.e.* Reduction, Refinement, and Replacement of animal testing. However, all interviewed experts agreed that OoCs are currently far away from replacing all animal models, and should be used mostly as a complementary approach to animal testing. Specifically, and as evidenced in the ORCHID impact analysis [14], high-throughput plate-based microphysiological systems may find use in target identification, lead selection and lead optimization at assay scale; chip-based, low-to-medium throughput devices may find most fitting use in pre-clinical, single-organ toxicity or efficacy tests; and multi-organ systems may target the replacement of animal models for toxicity screening, and phase I / phase II clinical testing in patients – which is being prefigured as (pre)clinical-trials-on-chip.

Replacing (pre)clinical human trials and advancing towards the implementation of personalized medicine are the grand unmet needs targeted by multi-OoC and HoC systems. OoCs pave the way to personalized medicine approaches by enabling the use of patient-specific, primary and stem cells to capture important differences due to genetic diversity, origin, gender or age. For instance, OoCs can enable the development of *in vitro* clinical trials for patient populations unfit for standard clinical trial designs (*i.e.* rare and/or paediatric diseases) or to develop drug regimens that are optimized for specific patient biology [9]. Along this path, OoCs may represent models not only capable of capturing prior organ(ismal) knowledge and recapitulating known physiological responses, but also, and even more importantly, unique tools for unprecedented investigations and new discoveries for the further advancement of physiology and medical science.

OoC can benefit toxicology testing

The interviewed experts share the opinion that industries developing cosmetic, chemical and agro-food consumer products can find a huge potential in OoCs regarding toxicological hazard and risk assessment of substances for the study of *e.g.* metabolism, effect of toxicants including nanomaterials, collective responses and allergies (see also [8]). Regulatory requirements for chemicals and cosmetics do not accept any significant hazard potential of substances used for humans. Moreover, there is rising interest in alternative models allowing skin sensitization assessment and systemic toxicity testing for regulatory use, because animal testing has been banned in 2013 for cosmetic and fragrance products marketed in Europe. China is

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meanwhile changing regulations about animal testing for cosmetics, has approved in 2016 a first non-animal tested cosmetics, and is pushing the authorities for reducing animal use; and the USA has included a ban on animal testing in their 2007 roadmap for all industries, including pharmaceutical, chemical and cosmetics industry [11]. The chemical industry is also actively working on such strategies, especially on *in vitro* skin sensitization testing, to avoid unnecessary reassessment of chemicals due to the Registration, Evaluation, Authorization and Restrictions of Chemicals (REACH) regulation [1]. Nevertheless, more formal validation efforts will be needed prior to full industrial adoption of OoCs in chemical and cosmetics industries.

Though not mentioned by any ORCHID interviewee, the tobacco industry could represent another OoC targeted segment in the near future due to the high pressure to increase the safety of this industry's product portfolio [1]. Ethical considerations lead the tobacco industry to develop new approaches and tools to assess smoke-related adverse effects on the human respiratory system, primarily airways and alveoli. OoCs could represent ideal tools to investigate the onset of lung diseases, such as the smoking-induced chronic obstructive pulmonary disease and its malignant transformation.

The future of OoCs lies in automation, robustness and integration of complexity

Automation and ease of use

Present OoCs are typically complicated pieces of engineering, whereby the microfluidic chip hosting the cell co-cultures is connected to external control and supply peripherals. For this reason, ease of use of the systems and increased automation in setting up cell cultures and keeping them viable throughout study times are commonly perceived unmet needs in laboratory practice. The ideal OoCs would be simple devices displaying user-friendly interfaces that facilitate the work of users with even limited training on *e.g.* cell cultures and instrumentation. These devices should be easily transferrable from developers to end users by means of standardized guidelines concerning biomarkers and endpoints.

Improving single OoCs

Among the many different tissue types that can be currently emulated [1], the outcome of the experts' interviews and of the ORCHID bibliometric analysis shows a strong preference for ADME pathways (Absorption, Distribution, Metabolism, Excretion) including metabolic (liver, kidney) and digestive organs [16], along with cardiac tissue, lung and the central nervous systems coupled to a blood-brain barrier [17]. Organ-specific needs to be met include, among others: for the liver, the development of models making use of iPSC-derived hepatocytes; for the heart, efficient maturation protocols for iPSC-derived cardiomyocytes to avoid expression of immature phenotypes; for skin, establishing methods to derive all skin cells from iPSCs, including *e.g.* dermal papilla cells needed for hair generation. OoC devices can perfuse vessels, thereby including the vitally important flow of a blood surrogate to feed nutrients and remove excretion products

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through vasculature, and the accompanying shear stress. The experts emphasized that, while immunogenicity testing of drug candidates in animals is obsolete, immune and endocrine systems are missing components required to improve the physiological relevance of OoCs [18]. Different academic players are indeed developing bone marrow-on-chip to integrate aspects of immunocompetence in OoC-based drug safety and efficacy testing [1]. Moreover, predictive models for gametogenesis and testicular toxicity, an infrequent but severe cause of arrest of drug development, and for other specific toxicities for which no satisfactory preclinical model exists are presumed to be valuable as a niche market for OoCs.

The ORCHID bibliometric analysis highlights a very high interest in OoCs as cancer modelling systems, which could help to better understand tumour progression (Fig. 8). The exponential growth of publications dedicated to OoC cancer models may correlate with the fact that OoCs are the only systems to model tumour cell intravasation into a surrogate blood stream or immune cell extravasation into the tumour by combining human micro-perfused 3D tumour models with human vasculature [1]. Such strong interest is also supported by epidemiology data and the high market potential for cancer treatments.

The expert interviews also indicated that, from a patient perspective, stringent medical unmet needs are represented by ways to improve the treatment of diseases – such as *e.g.* cancer, dementia, kidney and rare diseases – for which there is currently no treatment, insufficient treatment [19], or only treatments which are too expensive.

Integrating more complexity within multi-OoCs.

As mentioned, the development of multi-organ OoCs is envisioned in the perspective of recapitulating the complexity of the human physiology at organism level, which is lost in moving away from animal models. A recent eminent example of a multi-OoC is the Evatar system, which couples Fallopian tubes, uterus, cervix and liver made from human tissues with ovaries from mouse tissue to recapitulate 28-day female hormonal cycles [20].

The original claim of a forthcoming full HoC system appears to be presently more realistically modulated by a technical assessment of the formidable difficulties facing its achievement. The latter include: (1) the onset and maintenance of self-contained homeostasis; (2) the inclusion of the influence of missing organs, particularly the hormone background (*e.g.* gender and reproductive hormones) and immunocompetent cells; and (3) the identification of (alternative solutions to) a single perfusion medium shared by the multiple cell types comprised within the system. Other challenges for multi-OoC and HoC systems lie in scaling the relative volumes of the individual OoCs [21], the interconnection topology, and perfusion rates approximating physiological flow configurations [9]. Organs involved in the ADME are considered the basis of a HoC, since they are responsible for significant homeostatic effects and are foreseen in comprehensive organ models for compound testing, for instance in skin and lung models to evaluate compound concentration in urine

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and blood. Modelling of ADME is considered central also because drug distribution is a main cause of unwanted drug side effects – a global leading cause of death. To this purpose, efforts should focus on combining a set of organs which can match the critical functions needed for drug study with a functional endothelium serving as selective barrier for transport of drugs and bioactive factors [9].

Technical challenges

Structural materials: alternatives to PDMS?

Polydimethylsiloxane (PDMS) supplanted glass and ceramic as the most commonly used substrate material for the fabrication of OoCs. The very wide adoption of this type of silicone ensues from a convenient combination of advantages, such as biocompatibility and oxygen permeation (crucial for cell viability), optical transparency (suiting real-time optical inspection), softness (which supports mechanical actuation), wide availability, and general ease of processing (no strict need for specific cleanroom facilities) – including deposition by casting and spin-coating, functionalisation, metallisation, and patterning by soft lithography and moulding [10]. The almost-unanimously remarked disadvantage of PDMS – though common to most polymers, including the plastics of interconnecting tubes – lies in its lipophilicity and ensuing unselective absorption of hydrophobic drugs, particularly of small molecular weight [22]. Unselective absorption complicates the localization of target compounds in the devices and, in absence of models predicting drug absorption rates in substrates, can drastically affect the interpretation of analytical results. Moreover, drug absorption directly affects the choice of the method used to deliver drugs to OoCs. Drug delivery in OoCs should generally replicate the physiological application in humans, *e.g.* direct exposure to cells, inhalation, skin absorption, oral delivery, or intravascular delivery by perfusion in vessels. The limitations of PDMS may be tolerated during device prototyping and characterisation, but may hinder future large-scale integration. Unselective compound absorption in PDMS is being faced mainly by (1) the search for application-specific formulations of PDMS or alternative polymers, most of which – based on *e.g.* polycarbonate, polystyrene, Styrene Ethylene Butylene Styrene (SEBS), polyimide, polyurethane, hydrogels – represent intellectual property covered by patents; or (2) the coating of polymer surfaces with layers of endothelial cells, providing a barrier to drug absorption into the substrate.

Optimal cell sourcing and shared culture media

Access to abundant, good quality human cell sources represents an essential technical aspect of OoCs. Multiple cell sources are currently available, and each cell type presents advantages and issues. The highest clinical relevance for humans is guaranteed by the use in OoC models of adult primary cells obtained directly from patients. However, genetic variance between individuals may be present when the cells cannot be obtained from the same donor; and these cells cannot be obtained in large quantities from all tissues, *e.g.* from the brain and the heart. Adult stem cells on the contrary are obtained from almost all endodermal

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organs, *e.g.* the organs of the gastro-intestinal tract and the lungs, and they can be genetically modified and grown in culture in large quantities; but they are not available for all tissues. Pluripotent stem cells (PSCs), derived from reprogramming of adult somatic cells (human induced PSCs, hiPSCs in short) can give rise to almost all types of cells in the human body, and can be obtained from any individual [23]. On the other hand, they are phenotypically immature, similar to foetal cells; hence it is a challenge to develop culture conditions for the maturation of such cells, and to use them to model diseases occurring after birth [24]. Spheroids and organoids [25] derived from human biopsies and pluripotent stem cells are also being envisioned as sources of pre-organized, higher-order cell tissues to be included in OoCs [26].

While the dynamic microenvironment of OoCs, mimicking the human physiology, can support cell differentiation and maturation better than static cell culture conditions, the co-presence of multiple cell types in the same microphysiological device sharing a common conditioning medium may be an issue. According to the ORCHID experts, a solution for the formulation of a single perfusion medium may come from a strict biochemical approach, rather than from simple media mixing; and whereas media such as high concentration glucose are standard, many application-specific media are proprietary. Serum, widely used at the beginning of the century, is currently disfavoured, and ethical issues are implied with foetal serum particularly. Blood is being used for media as well, and should be on the roadmap in spite of increasing constraints on its usage. Finally, whereas the choice of cell types is generally dependent on the target organs and applications, a related challenge concerns the appropriate representation of the diversity inherent in the human population in terms of *e.g.* gender, origin and age.

Long-term viability and multimodal real-time characterisation can revolutionize cell cultures and analysis

With typical sizes on the order of a few millimetres, OoCs afford a distinct advantage in terms of non-invasive organ monitoring and tissue investigation compared to the actual inspection of human patients. The small volumes of tissues and culture media confined within the closed, controlled environment of the devices limit the dilution of xenobiotics and metabolites. Small chip volumes additionally help preserving sterile culture conditions, though they are also responsible for reduced throughput. A related advantage concerns the extended viability of the cells in OoC models, enabled by continuous microfluidic nutrient feeding and removal of waste products. Required model viability is considered by the interviewed experts to be sensibly dependent on the application and specific question at hand: it can range from a few hours to a few days (*e.g.*, 3 to 8 days) for repeated dose/exposure and acute toxicity testing; up to a few weeks for safety and efficacy testing (*e.g.*, 28 days for systemic testing, in accord with the Organisation for Economic Co-operation and Development (OECD)'s guidelines for animal tests) [16]; and up to several months for chronic toxicity testing or disease course modelling, in analogy with phase II clinical trials. Tissue viability

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is supposed to extend indefinitely in the presence of self-contained homeostasis, particularly when sustained by vascularized interconnections among several organ modules.

Online characterisation of organ- or tissue-specific phenotypes, readouts and endpoints in OoCs – *e.g.* vascular contraction, cell migration, genetic reporters, metabolic pathways – is largely dominated by optical and fluorescence microscopy, expected to remain prevalent also in the future. A challenge for assay imaging systems is translation into high-throughput industrial settings. Incubators are commonly used to host OoCs and regulate cell culture parameters, such as temperature and CO_2 levels. Composition of perfusion media, fluid flow rates and gas pressure for pneumatic actuation of flexible membranes are mostly controlled by external, partly automated equipment. Connection of fluidic peripherals to chip or plate devices typically involves silicone tubes, which may cause bubble formation and non-selective drug absorption. Proper utilization of devices and peripherals typically requires specific training of users and is expected to be regulated by standardized guidelines. Including such peripherals as integral components of actual, self-contained OoC devices is a standing challenge. According to the ORCHID experts, OoCs should be consumables whereby real-time sensor readout and system control are simultaneous and aligned. The experts recognized that real-time monitoring of bioreactions in OoCs may revolutionize *in vivo* studies by increasing the throughput of analyses, and acknowledged the current existence of a gap between hardware development and the needed absolute quantification of biological response, partly because the physical, chemical or electrical responses to bioreactions in OoCs are not fully understood. Therefore, the integration of biocompatible electrical, chemical or physical sensors and actuators is highly sought after and widely investigated [26]. For instance, trans-epithelial electrical resistance (TEER) is being measured for real-time readout of tissue barrier functions [27]; and micro-impedance tomography is used to quantify the deflection of membranes induced by breathing motion [28].

Qualification for contexts of use should be preferred to validation

Some form of validation of OoC systems is critical for their broader acceptance and uptake in industrial and clinical settings, particularly in relation to supporting decision-making in a regulatory context. In the Guidance Document 34, the OECD defines *validation* as "the process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose". Although this definition is in principle applicable to OoC devices used for a particular purpose, the context of OECD guidance typically relate to the validation of methods intended to form the basis of internationally recognised test guidelines that can be used in any of the 36 OECD member countries, implemented within a Good Laboratory Practice (GLP) quality system (as exemplified in *e.g.* the OECD's Guidance Document on Good *In Vitro* Method Practices (GIVIMP)), and satisfying the conditions of Mutual Acceptance of Data (MAD) between jurisdictions and regulatory agencies. Therefore the validation processes foreseen in this context may be more appropriate for highly standardised and widely applicable methods, and probably

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unsuitable for emerging OoC devices in the near future. Moreover, validation per se is not considered by some experts an appropriate nor meaningful concept, because it implies the existence of an accepted standard to be used to confirm or measure validity. In fact, neither animal models nor human clinical effects can be univocally used as golden standard for validity. Besides, a universal approach to validation is probably unrealistic, and a harmonisation across contexts and cultures is expected to take too long, if possible at all.

Considering that the application of OoC devices in the short/medium term is most likely to be in the pharmaceutical sector, and will primarily target specific and well-defined contexts of use, focus should be on the qualification of devices, rather than on the validation in the broader sense. *Context of use* refers here to a clearly articulated description delineating the manner and purpose of use of a tool, while *qualification* is understood as arriving at a conclusion that the results of an assessment using a model or assay can be relied on to have a specific interpretation and application in product development and regulatory decision-making. Aiming for qualification of OoC devices was in fact the approach proposed in a recent series of workshops devoted to this important topic – held both in the US by the FDA and in Europe by the European Medicines Agency (EMA)'s Safety Working Party – and it was confirmed in the ORCHID workshop. The typical qualification process applied to OoC devices would foresee a comparison between OoC data and corresponding data derived from conventional preclinical drug development models (*e.g.*, animal models, cell suspensions) or human clinical data if available. If feasible and appropriate, this will inform the assessment of whether the OoC response corresponds to the expected behaviour, and thus can be used as a reliable predictor of human response.

More generally, it is desired and expected that OoCs reproduce salient physiological features and functional aspects of organs which recapitulate the human condition and responses to exogenous stimuli such as drug molecules. A challenge in qualification practice is therefore the establishment of relevant sets of reference or benchmarking compounds with well-known and properly documented pharmacological action and *in vivo* effects (*e.g.* paracetamol, beta blockers). Initiatives exist – such as led by the NIH in the USA, or the SEURAT-1 program[**] funded by the European Union – which aim to establish a public database to collect readout data in a very specific and platform-independent format, useful for both pharmaceutical companies and regulatory agencies. The ORCHID experts suggested that such initiatives may inform an open platform shared by partners in pre-competitive settings, tapping crowdsourcing approaches to *e.g.* data analysis and device testing, and crowdfunding to share costs. They may be inspired by examples of shared technology planning and development, such as the former International Technology Roadmap for Semiconductors (ITRS) and the International Roadmap for Devices and Systems (IRDS)[††].

[**] Available at: http://www.seurat-1.eu/ (accessed: February 25th, 2019)
[††] Available at: https://irds.ieee.org/ (accessed: February 25th, 2019)

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The current scenario is nonetheless fragmented, as most pharmaceutical companies intend qualification as an internal recognition process that does not need to be translatable to other companies, let alone the whole sector. Accordingly, OoCs need only comply with company-specific qualification protocols using company-specific test sets of compounds, established or under development. Though such compounds are typically proprietary and not easily accessible to academic research groups, the IQ Consortium, a not-for-profit organization of pharmaceutical and biotechnology companies[‡‡], is compiling a list of reference compounds, that might be shared for qualification purposes.

Standardization: when is the right time?

Standardization of OoC devices will likely contribute to ensuring reproducibility and robustness of results both within a single lab and across different labs; uniformity or compatibility of cell or tissue types and sources; and compatibility among chips or modules fabricated by different developers upon interconnection into a multi-organ system. Moreover, standardization will aid in the practical incorporation of OoC-based studies into analysis workflows and the utilization of results within decision-making contexts. Thus a move towards standardization is desirable from an industrial as well as from a regulatory point of view. This objective is however not compatible with the current, early stage of academic and commercial development of the majority of devices. The exponential growth of the number of publications on OoCs, whose dynamics is even superior to that which accompanied the development of the Human Genome Project, testifies to the continuous introduction, at least in the academic domain, of novel or ever updated OoC models which contemplate variations in the substrate technology and/or in cell sources and types. The interviewed experts mostly share the opinion that such wide exploration of solutions is welcome and typical of early stages of technology development. To bring the most benefit to the field, it should currently not be hindered, at least in academic research, by the strict imposition of standards or constraints deriving from intellectual property, even though this would defy the perceived interest of pharmaceutical companies and industrial end users. Additionally, it would be premature and counterproductive to converge towards robustly qualified or standardized manufacturing processes prior to gathering substantial evidence and characterization of the capacity of OoC models to host the intended target biology and recapitulate the *in vivo* human physiology and response of interest. Standardization of OoC devices and readouts may appear at some later stage, as in the case of other technologies, due to the convergence towards solutions generally considered most convenient or optimal, issues related to intellectual property, reorganization of companies, market conditions and end-user demands. Finally, standardization may alter the landscape of players in the field by constraining the numbers that can afford to comply with both its financial and engineering aspects.

[‡‡] Available at: https://iqconsortium.org/ (accessed: February 25th, 2019)

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5. Barriers and perspectives

From industrial hesitance towards acceptance

OoC developers expect a large market to take off in the next 2-3 years. This optimism is supported by major recent technical breakthroughs [10], which make OoCs a credible option to provide a variety of end users with more predictive testing models. Once OoC models are available, demand is expected to increase and smoothen the road to applications. On the other hand, focusing on lesser applications may provide an additional point of market entry for OoCs.

Nevertheless, a more realistic view of the situation shows that there are heavy barriers to be crossed. Industry is still testing OoCs in comparison with alternatives (*e.g.*, 3D cell cultures, spheroids, tumoroids, organoids) to determine the models most suited for their needs. Evidence-based convenience of the models is required to induce industrials, especially pharmaceutical, to integrate OoCs into near-future routine processes. Although OoCs may be more relevant and accurate than animal models or cell cultures, they are still expensive and need costly redesign to suit mass production. In addition, compatibility problems may occur due to the current lack of standardization (*e.g.*, different shapes, interfaces, sizes, tissue types and quality, ways of use); and it may take years before OoCs are accepted by regulatory agencies. Hence a pragmatic attitude prevails in the industrial context – which, as for any available alternative, evaluates OoCs either on a fit-for-purpose or on a cost-per-data point benefit they may afford. Accordingly, the new technology may be accepted if it provides simpler or cheaper alternatives to established models while reproducing the same results, or if it affords models for which no alternatives currently exist. An example of the latter is provided by a joint work of the Wyss Institute with Janssen to model the toxicity of a monoclonal antibody therapeutic which caused death in patients due to pulmonary embolism in Phase I clinical trials: Janssen had not observed any toxicity in preclinical animal models, whereas the Wyss Institute's OoC could replicate the thrombotic behaviour [29].

According to the Yole market report, in the most likely scenario OoCs will be increasingly adopted by the industry, due to their significant advantages over existing solutions in terms of *e.g.* predictivity, cost saving, alternative to animal testing [11]. Newcomers, mostly spin-off from academia, will appear in the OoC developers market, but in the meantime some companies will fail. It would not be surprising to see biotech companies and instrument developers, who are providing tools to the pharmaceutical industry, acquiring OoC developers, because of the good synergies between these organizations.

Early dialogue to ease regulatory hurdles

Regulatory aspects can represent another significant barrier towards the broader adoption of OoCs. There is a generic and non-OoC specific need to speed up regulatory validation processes. Examples of prior technologies attest to a resistance in the adoption and regulation of new medical options. The decisions

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of regulatory authorities on the efficacy of novel treatments can have significant repercussions in delaying the acceptance and implementation of those treatments, in making the treatment costs liable to reimbursements to patients, and generally on the societal costs of healthcare. In addition, the costs of the certification procedure, involving an extensive series of tests, may escape the possibilities of academic research groups. This conversely should incentivize the involvement of companies and funding agencies in the development of OoCs as well as in early dialogue with regulatory authorities to ensure effective and transparent communication and to avoid procedural obstacles at later stages of development. Industrial acceptance typically precedes and pushes regulation [1]. Still, there may be a catch in the transfer of the device development process from initial developers to companies. On one hand, the developers may be in immediate need for funding to advance or characterize their models to a degree sufficient to attract the interest of the companies. On the other hand, the companies may hesitate to provide funding until the developers have demonstrated that their models fit their specific purposes or that they can overcome the companies' fear to be the first to introduce a new model on the market – a model that later might eventually reveal ineffective in humans, or not compliant to future regulations.

Keeping expectations realistic

Industry and government agencies have placed huge expectations on a few developers of OoC technology, in particular academic centres like the Wyss Institute and the MIT as well as companies such as Emulate Inc. and CN Bio. These developers were repeatedly awarded funding for several tens of millions of dollars, and are the most visible in the field. The Yole market report suggests that the success of these well-funded organizations might be critical for the future of the OoC area [11]. Some industry players believe these organizations cannot fail, whereas some think the gap between prototyping and mass commercialization is too significant. The consequence of a potential failure of such field pioneers would be devastating for the OoC field: besides the high visibility of the failure (media coverage, top-rank universities), a loss of confidence from both industry and investors would disrupt many small companies.

The interviewed experts echoed similar concerns, and expressed almost unanimously the need to keep realistically optimistic about the OoC technology. They generally expect OoC to become a key technology in the coming years, yet prescribe to remain very cautious in stating the promises and the potential of the OoC technology to media and general public to avoid the pitfalls of hype psychology. While the experts do not agree whether the OoC technology is currently the object of a hype, the Human Genome Project, stem cells for regenerative medicine, microarray technology, proteomics and gene therapy represent significant examples of programs, initiatives or technologies that may risk to fail to deliver to the high expectations they raised at the outset.

A further analogy with these precedents is represented by the size of funding considered necessary to achieve the field's purported goals, expectedly in the order of hundreds of millions to billions of dollars. A

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large fraction of the costs concerns the translation of proof-of-concept devices from laboratories to market entry, and the tests prescribed for regulatory approval. OoC-related companies are expected to drive such advances, eventually in partnership with governmental agencies, and they find themselves in a critical position in this respect. Attracting investors and considerable funding is critical to gather the multidisciplinary technical expertise and build the layered infrastructure that can support the development of prototypes into mass-fabrication to meet the industrial demands of quality control and reproducibility. To achieve this, the companies need to rely on advertising, which is susceptible to involve overstatements. The latter may raise premature or unmatched societal expectations about a technology, which conversely is generally considered to be still in an early development stage.

A common perception of the interviewed experts is that the lay public easily grasps the core concept of OoC technology, and tends to get quickly excited about the potential to decrease or even replace animal testing. However, *Organ-on-Chip* as technology-defining name may confuse the public by association with other technologies such as artificial organ prostheses, organ replacement or regenerative medicine. The public may not understand how OoC technology actually works and what its limitations are, unless browsing specialized journals. On the other hand, the more popular media may tend to cover sensational stories and thus feed the false impression that the technology is already achieving all the advertised promises. An antidote to such perceived distortions may be a synergistic outreach activity by key players and stakeholders based on careful communication of recent achievements and ongoing developments that could inform about actual trends and realistic perspectives.

Collaboration and education must be encouraged early on

The interviewed experts remarked the strong need for integrative programs, collaborative projects, consortia and international funding strategies for the development of OoC technology and applications. Lack of international coordination may be caused by the heterogeneity of the global legislative, regulatory, financial and ethical scenario. Coordination efforts should be supported by a widespread outreach activity, targeting all levels of audience, and by an improved dialogue between regulators, industries, clinicians and patient groups since the early stages of development. In this respect, a digital platform is now being developed within the ORCHID to bring together players (*e.g.*, researchers, laboratories, companies, patient associations, regulators) and to create a community in the OoC field. The platform will evolve over time through the addition of publications, collaborative projects and additional information supplied by the members who will progressively become part of it.

Finally, besides topical and field-specific workshops and conferences, incubators of talents are elicited as prime accelerators for the progress of the technology. This should be the pivotal role of institutes, inspired by analogous initiatives of successful information technology companies, where diverse experts could

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freely interact to traverse linguistic and departmental boundaries and leverage ample resources to develop new ideas and conduct multi-disciplinary research.

6. Summary and recommendations

The synergistic convergence of microfabrication technologies and tissue engineering renders OoCs as promising tools for the realistic modelling of human physiology and pathology. The aim of an OoC is not to replicate a whole living organ, but rather to sustain a minimal functional (sub)unit of an organ or tissue that can controllably recapitulate salient aspects of human physiology. For this purpose, as argued in this paper, the main and desired features of an OoC can be divided into three categories:

(1) Tissue architecture

- Integrated long-term cell culture in defined spatial organizations
- Tissue-tissue interfaces/cell-cell contacts/cellular heterogeneity
- Miniaturization

(2) Conditions

- Controlled microenvironment (topology, biochemistry, physics)
- Controlled dynamics (fluid flow, electro-mechanical stimuli)
- Continuous automated perfusion
- Real-time monitoring of multiple physical, bio- and electro-chemical parameters
- Automated reproducible multi-sample analysis, comparable with R&D robotics
- Large-scale manufacturability

(3) Functions

- Physio- and pathological relevance
- Recapitulation of organ structure and function
- Recapitulation of dynamic mechano-biological properties and stimuli response of organs

In view of this and of the unmet needs and challenges, the ORCHID elicited the following recommendations (summarised in Fig. 9) to foster further progress in this rapidly establishing field:

Characterization, qualification and standardization

1. *Do not aim for the whole human: start mimicking single organs first.*
2. *Demonstrate the benefits and prove with reliable data OoCs' added value compared to other models.*
3. *Describe the value of OoCs on a fit-for-purpose or cost-per-data point scenario.*
4. *Develop guidelines for qualification of OoCs through a collaborative process involving developers, end-users and regulatory bodies.*

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5. *Create a list of reference compounds and related annotations for specific organ effects to qualify OoCs for a specific context of use, and share the data through a public database.*
6. *Compare OoC's tissue architectures and cellular phenotypes with* in vivo *tissue histology or histopathology using existing technologies.*
7. *Only consider standardization of OoCs once there is substantial evidence for OoC platforms to recapitulate the* in vivo *human physiology and response of interest.*

Technology

8. *Integrate real-time, bio-compatible electrical, chemical or physical sensing and analysis, and define and measure the salient parameters that can be predictive.*
9. *Address the issue of unselective compound absorption in OoC substrate materials.*
10. *Minimize operational complexity: develop automation for speeding up OoC assays, and improve ease of use to enhance reproducibility, robustness, and ease of transfer from developer to end user.*
11. *Integrate OoC and computational (open source) models, starting with pharmacokinetics.*

Biology

12. *Define strategies to tackle issues on cell-to-cell variation, maturation and stability of cells.*
13. *Standardize the culture medium and explore the use of blood in single perfusion of multi-OoC.*
14. *Include immune and endocrine system components in OoC to improve physiological relevance.*
15. *Include gender-, origin- and age-related aspects in OoCs for representation of human diversity.*

Applications

16. *Define the type of throughput required for the application of OoCs.*
17. *Address personalized medicine (*you-on-a-chip*), clinical trials-on-chip and environmental toxicological assays as highest priority applications.*
18. *Involve the food industry to raise their interest for OoC applications.*
19. *Focus on toxicities or diseases for which no satisfactory pre-clinical models and treatment exist as a niche market for OoCs.*
20. *Combine a set of organs involved in ADME pathways (Absorption, Distribution, Metabolism, Excretion) that can match the critical functions needed for compound testing and studying transport of drugs and bioactive factors.*

Dissemination, communication and collaboration

21. *Keep expectations about the technology realistic when communicating with media and the public.*
22. *Develop a synergistic outreach based on careful communication of achievements and new developments by developers and stakeholders.*

23. *Involve patients, clinicians, companies and regulatory authorities early in the development of OoCs to ensure effective dialogue and avoid procedural obstacles for implementation.*
24. *Attract investors and funding agencies to support the development of prototypes into mass fabrication to meet the industrial demands of quality and reproducibility.*
25. *Build the network, and realize integrative programs, collaborative projects, consortia, a digital platform and international funding strategies for the development of OoC technology.*
26. *Foster next generation researchers: organize exchanges among institutes, trainings, workshops and conferences to nurture talents as prime accelerators for the progress of OoC technology.*

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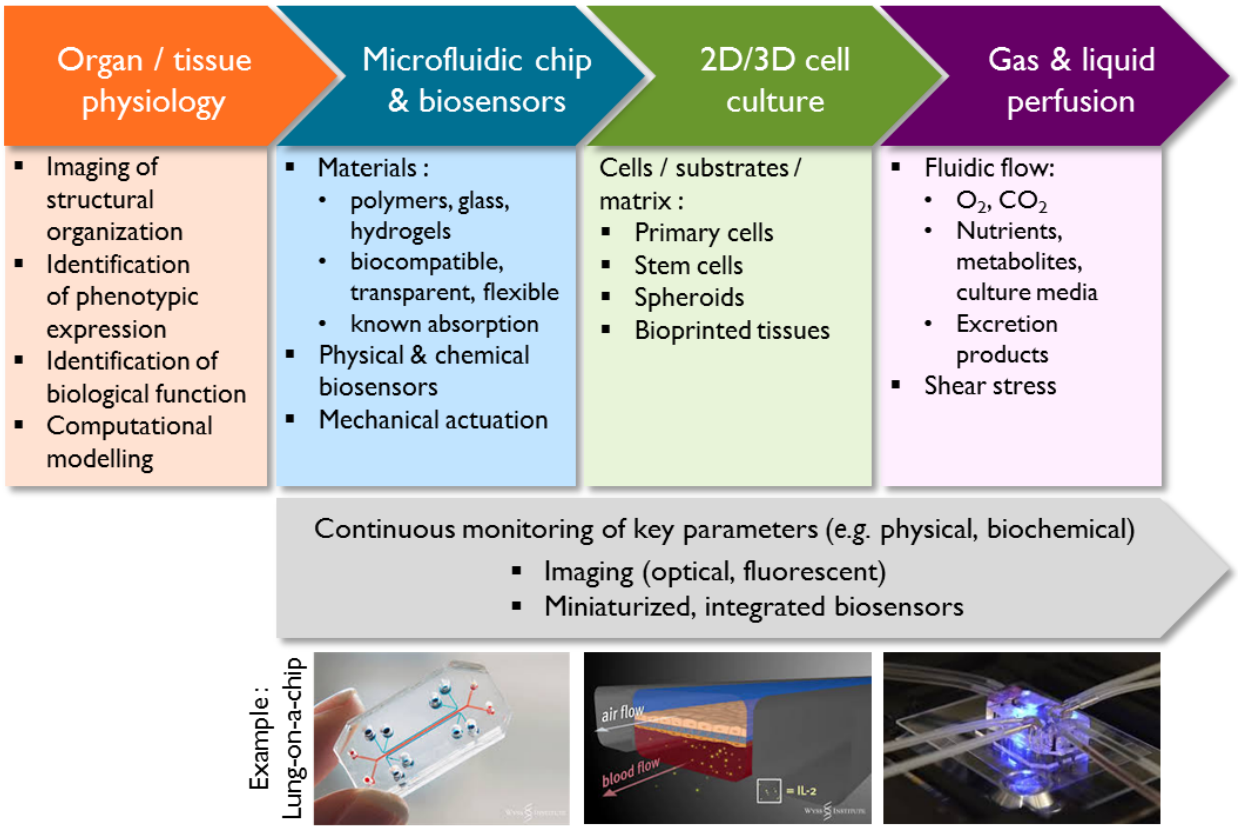

Figure 1. Simplified synopsis of the OoC value chain, according to the ORCHID analyses (pictures: courtesy of the Wyss Institute at Harvard University).

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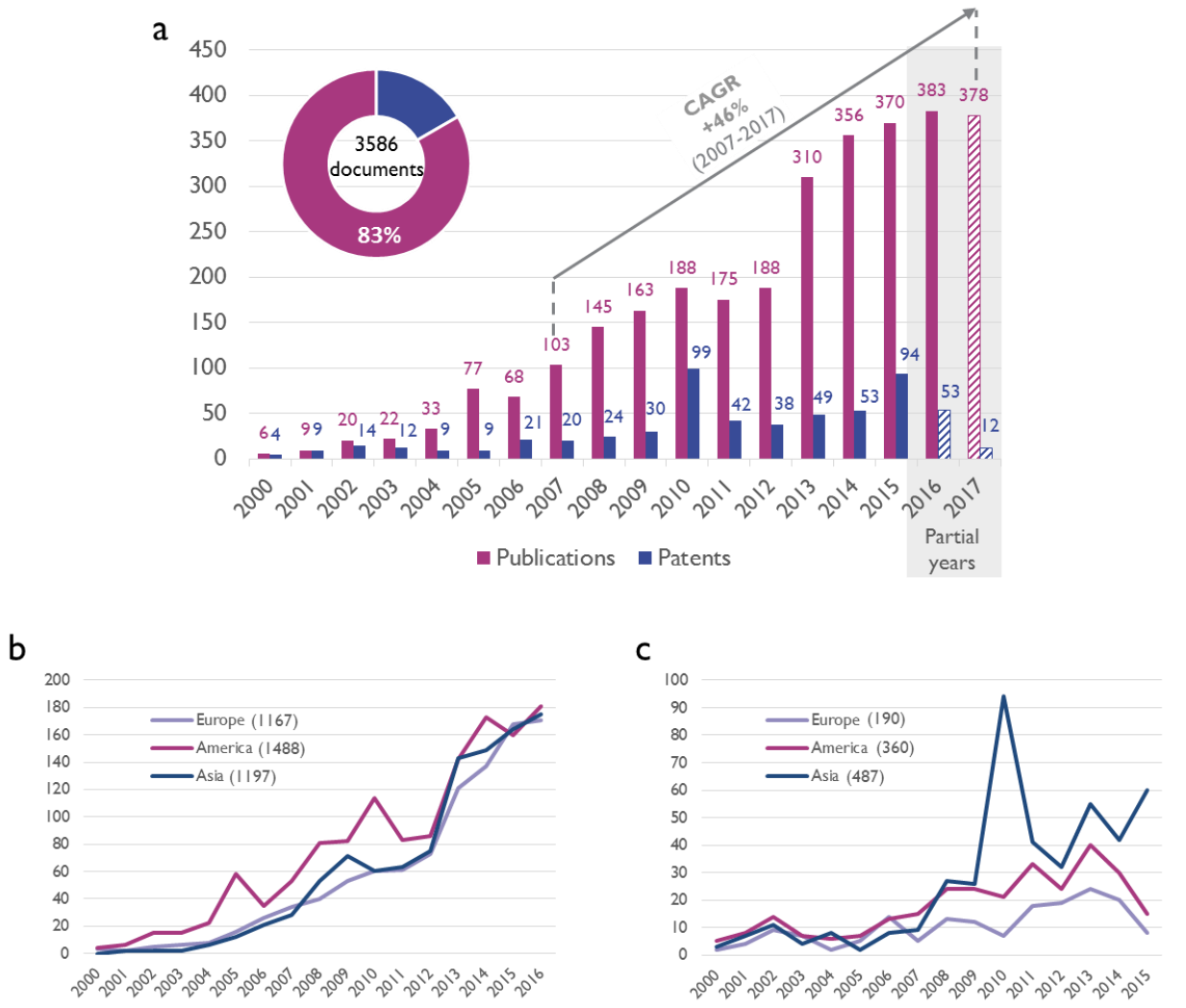

Figure 2. The ORCHID bibliographic analysis. **a)** Temporal evolution of the number of OoC-related patents and scientific publications over the 2000-2017 period. The imported database includes 3586 documents, mostly publications (83% *versus* 17% of patents). The 592 identified patents include granted (38.9%), pending (25.8%) and fallen or revoked (35.3%) patents. Year 2015: latest complete year for patents (18 months are needed to publish the patent application). Year 2016: latest complete year for publications (documents were imported in November 2017). CAGR: Compound Annual Growth Rate. **b)** Temporal evolution of the number of publications per region. **c)** Temporal evolution of the number of patents per region.

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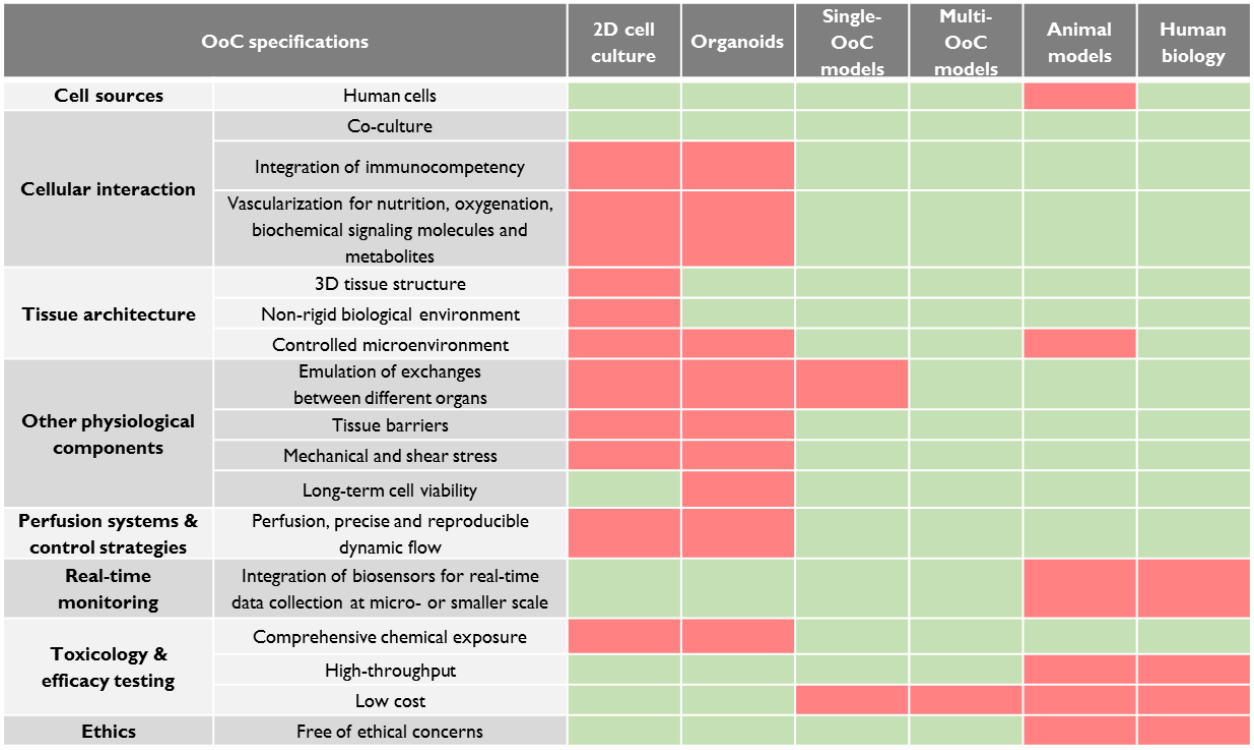

Figure 3. Comparison of key specifications and features across available biological models. *Red boxes*: not feasible; *green boxes*: already technically available, or envisioned in the near future.

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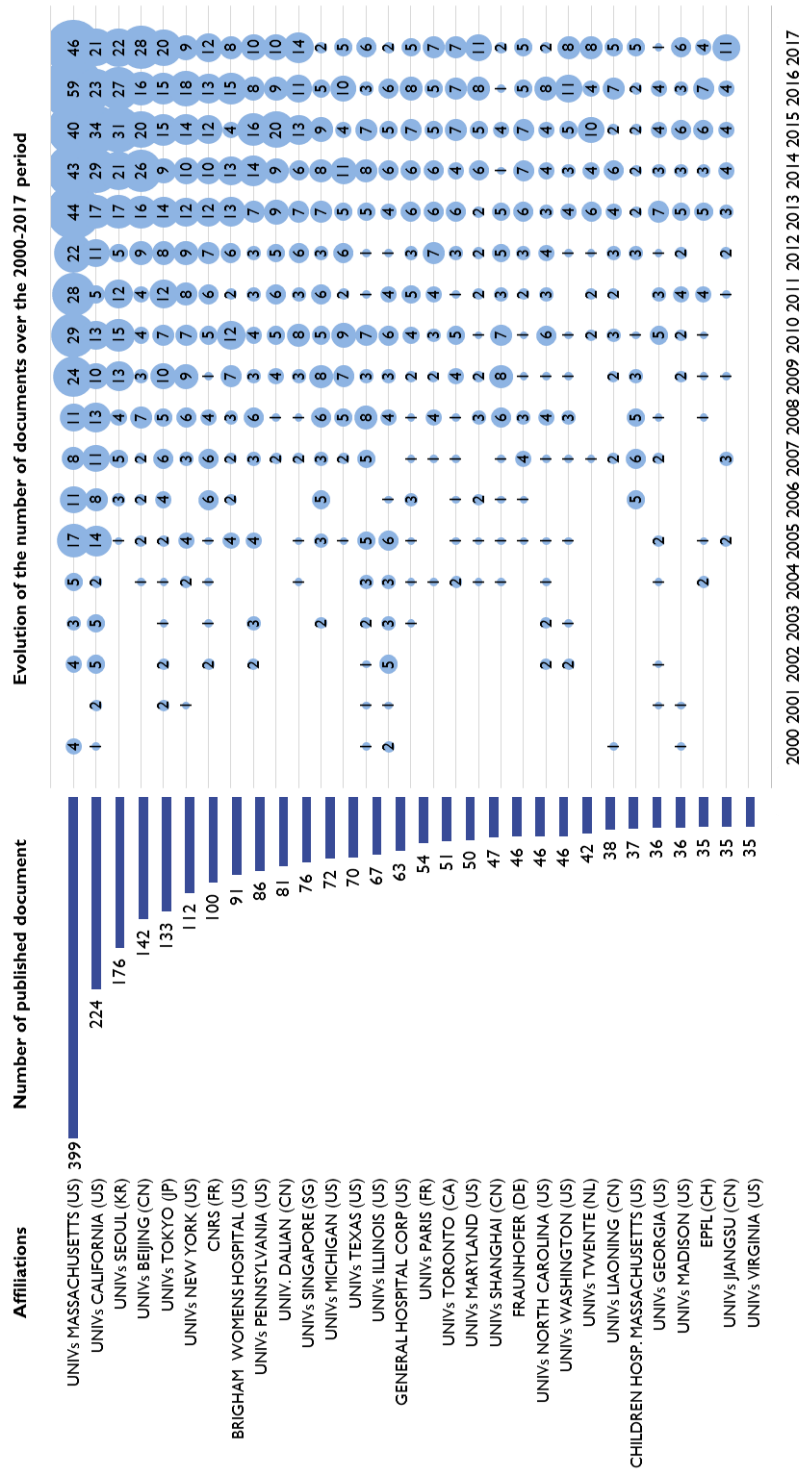

Figure 4. The top 30 academic players in the OoC field according to the ORCHID bibliographic analysis. These, out of 650 identified academic players, published more than 34 documents over the 2000-2017 period. *Blue bars*: number of patents and publications. The size of the circles is directly proportional to the number of identified documents published each year.

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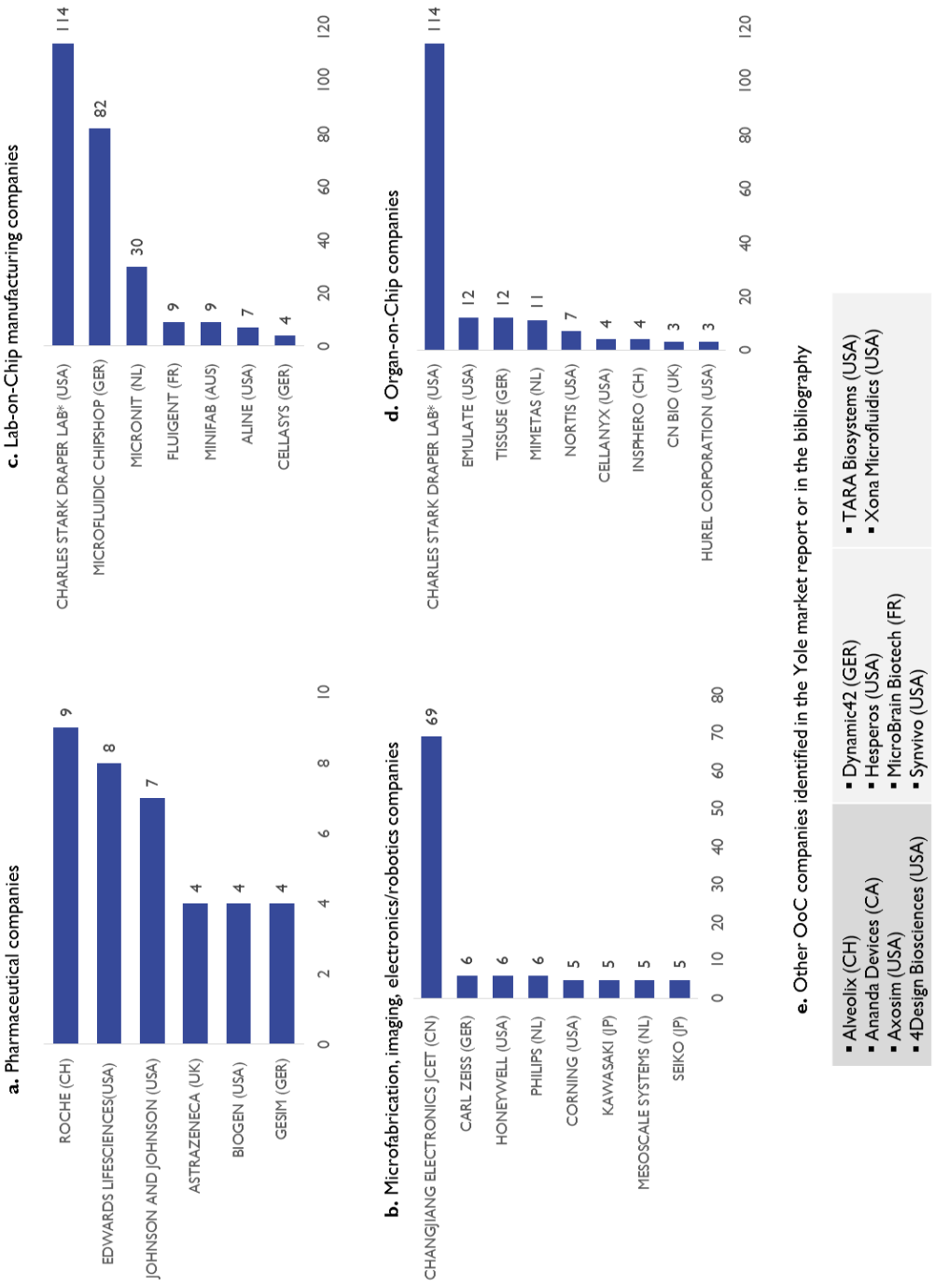

Figure 5. Overview of the top 30 industrial players in the OoC field, which published more than 2 documents over the 2000-2017 period. (Note: Charles Draper Lab is a non-profit and non-stockholding organization with a strong R&D activity. For this reason, it is listed here among companies.)

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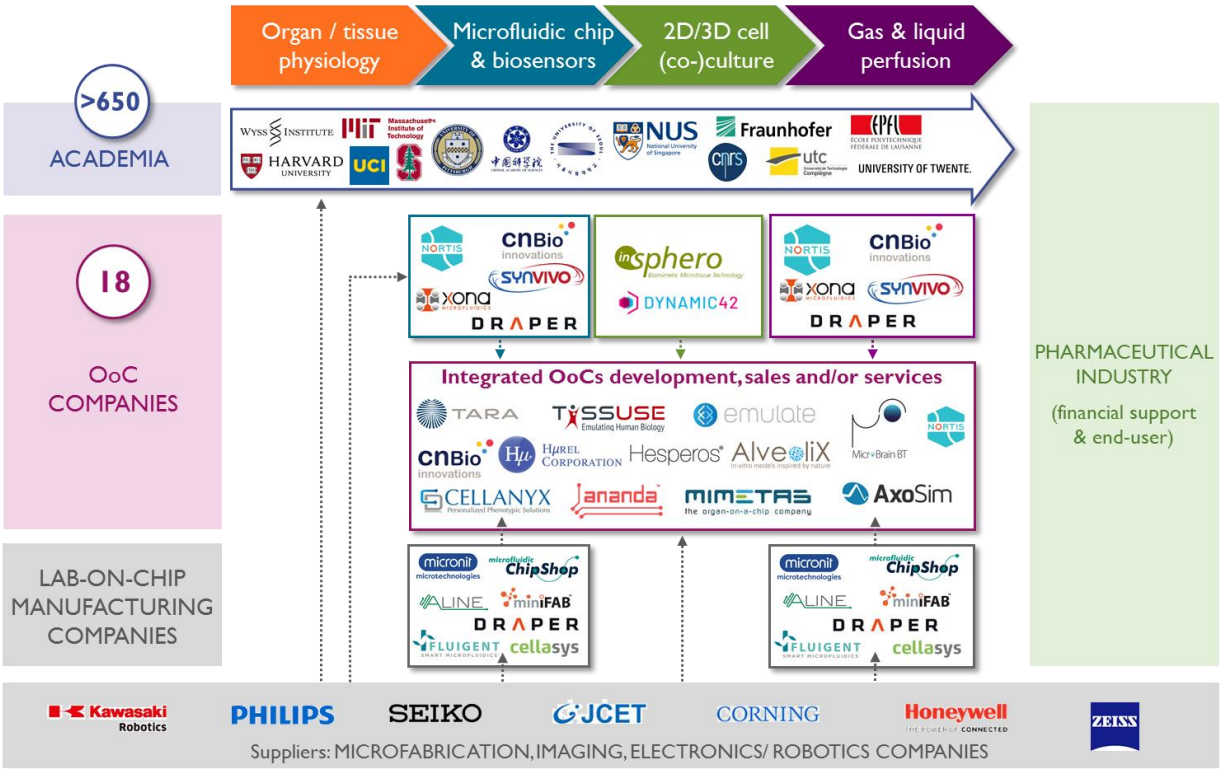

Figure 6. OoC players' positioning along the value chain (see Fig. 1). The figures represent the number of academic players (blue circle) and OoC companies (purple circle) identified *via* the bibliometric approach. Academics and OoC companies show a dedicated expertise for the development of fully operational OoCs, whereas the other industrials are supporting their development technological expertise, financing and/or partnerships. For each profile, only the top players are shown.

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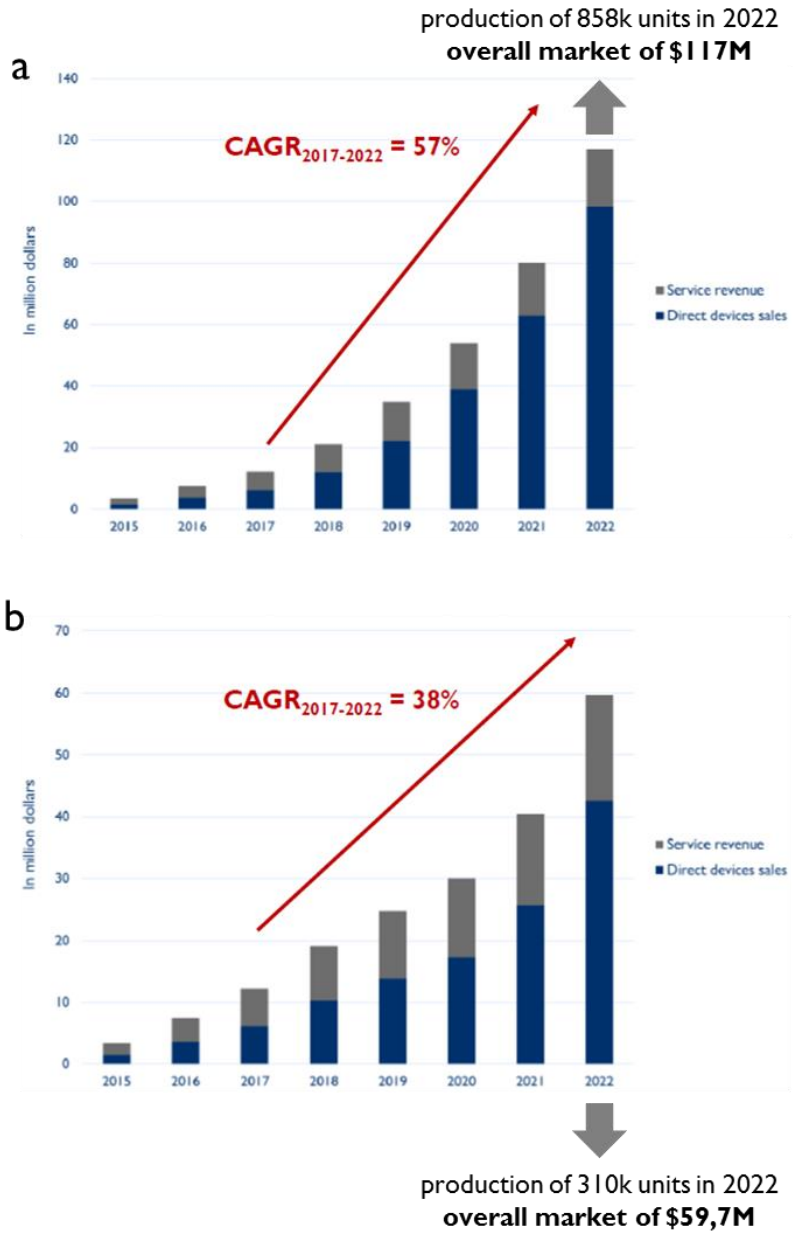

Figure 7. The optimistic (a) and realistic (b) OoC market scenario proposed by the Yole market report [11]. The estimated revenues are in million dollars and split between direct devices sales (ready-for-culture devices and fully operational OoC; blue bars) and full service sales (tests performed in-house; grey bars). CAGR: Compound Annual Growth Rate.

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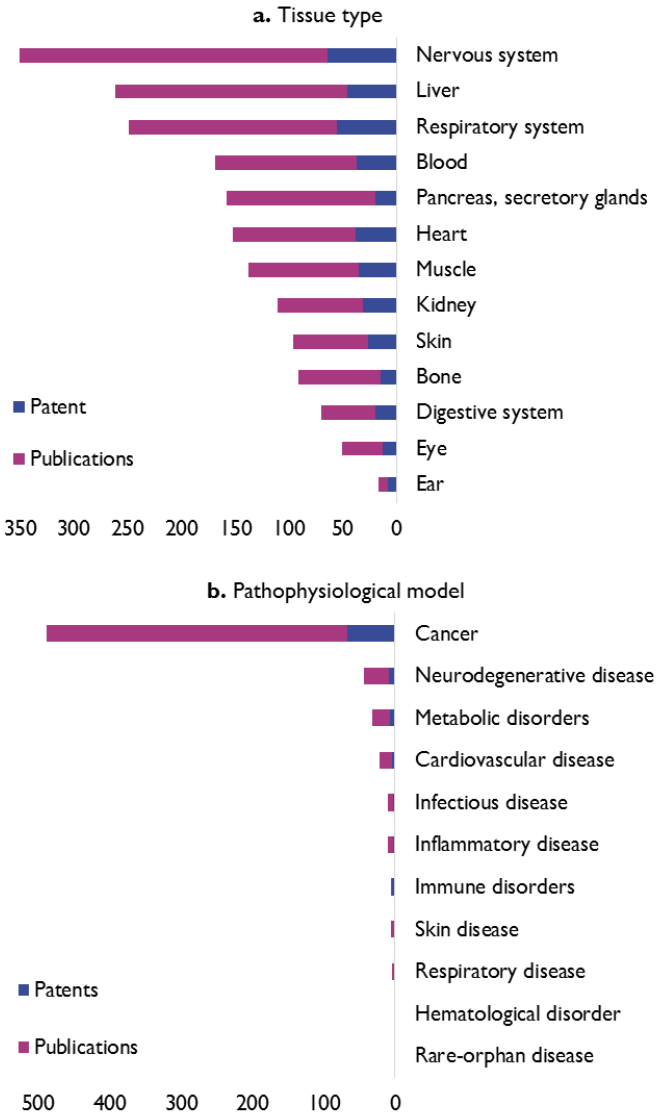

Figure 8. Global trends in pathophysiological models and tissue types. The imported database of patents and scientific publications was segmented with regards to emulated tissue types (a) and pathophysiological models (b) on a worldwide scale.

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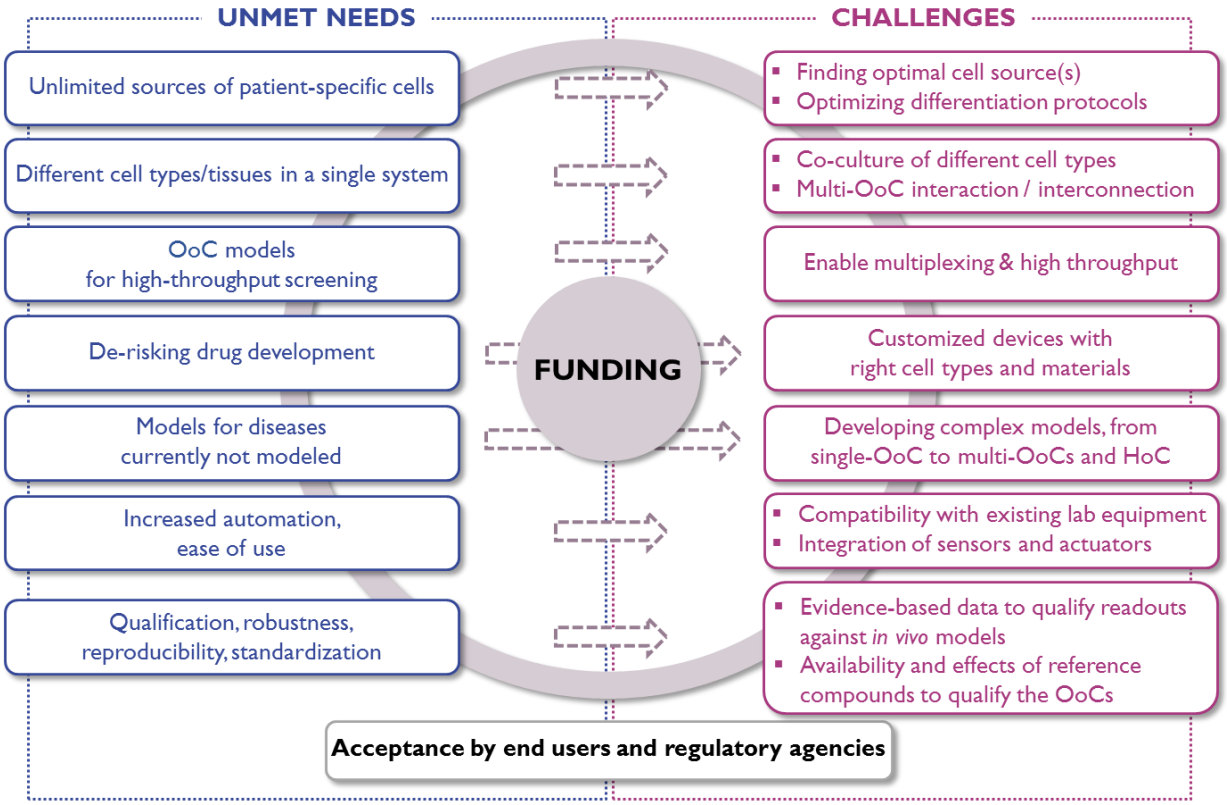

Figure 9. Key unmet needs and challenges for OoC development.

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Appendix: Methodology overview

The studies presented in this paper draw on (1) a documentary approach, combining both a bibliometric and a market analysis, and (2) interviews with field experts, with the aim of assessing the existing and perspective OoC landscape. The methodology and results of the studies were reviewed and discussed by a committee of experts during the *ORCHID Vision Workshop*, held on May 23, 2018 in Stuttgart (DE).

The methodology is summarized in the Fig. A1 and detailed in the following sections.

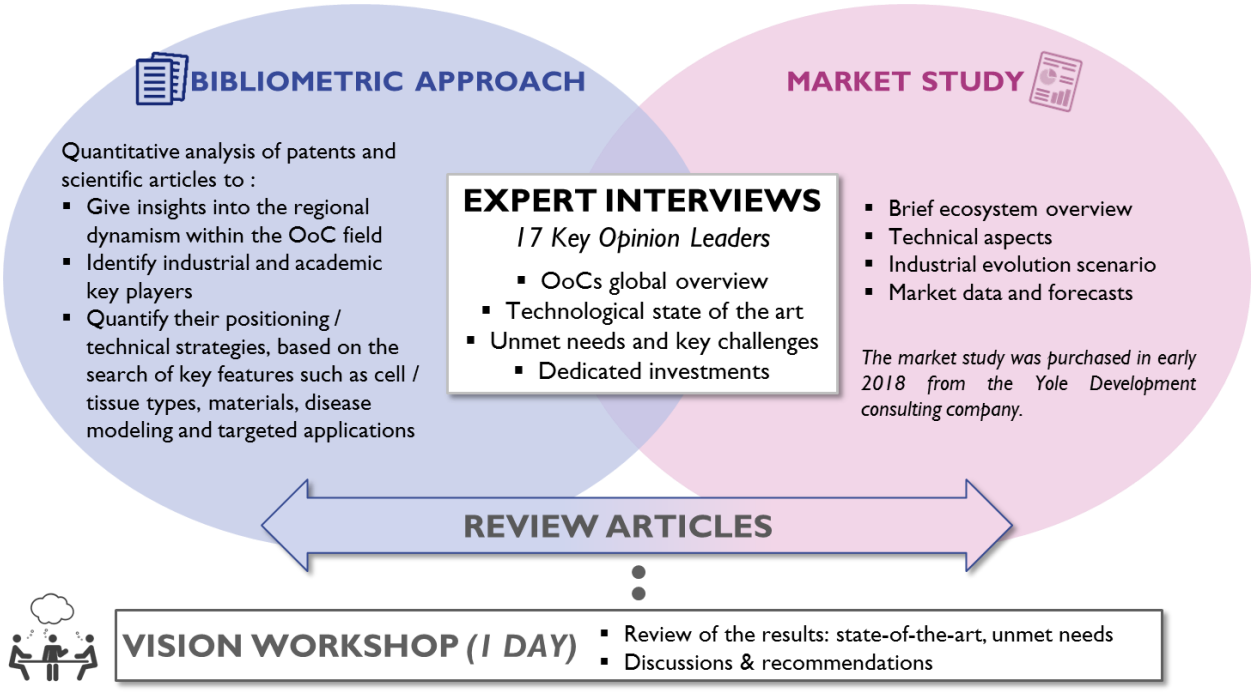

Figure A1. The methodology of the ORCHID OoC analyses presented in this paper.

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A1. Documentary approach

A.1.1 Bibliometric study

A bibliometric study is a quantitative analysis of scientific and technical information in a given area to provide a global overview of the associated ecosystem. The ORCHID bibliometric study was meant to give insights into the OoC's regional R&D dynamism, to identify the key players, and to quantify their positioning and technical strategies. The study was based on the search and classification of key features – including cell/tissue types, materials, disease modeling and targeted applications – in peer-reviewed articles and patents.

The bibliometric study followed a five key steps methodology: a. the definition of the keywords; b. the validation of the associated corpus of documents; and c. its associated segmentation, followed by d. a semi-manual normalization of the affiliations and e. the final analysis.

a. The keywords definition is related to the technological scope specified through discussions with experts in the OoC field. The keyword definition led to the implementation of six search strategies characterized by a complex combination of keywords with Boolean operators which were used to build a dedicated corpus of documents: Organ on chip / Specific terms (artificial organs, organoids, spheroids, 3D cultures, etc.) / Human tissues & cells / 3D bioprinting / Microsystems / OoC Companies.
b. The associated relevant documents were collected from the specific databases *Orbit* (patents) and *Scopus* (scientific articles) over the 2000-2017 period. An additional expert's validation of the imported documents was required to ensure that the extracted documents were reasonably exhaustive and relevant.
c. A customized segmentation of the documents was used to sort articles and patents within the database. The four main identified segments were in turn divided into sub-segments to facilitate the analysis:
    1. Human cell technologies: *i.* environment substrate & scaffolds; *ii.* tissue types; *iii.* cell types.
    2. Chip technologies: *i.* material; *ii.* microsystem.
    3. Monitoring & analysis technologies (sensors, imaging, computational modelling, TEER, OoC characterization)
    4. Market: *i.* applications; *ii.* targeted areas (pharmaceutical, cosmetics, chemical & environment).
d. The normalization of the identified organizations' affiliations was required to perform
e. an efficient quantitative analysis of the collected documents. This was performed using the Intellixir statistical analysis tool (Questel).

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This overall process enabled us to extract a database of 3497 relevant documents for further analysis.

Nevertheless, such bibliometric approach has limitations:

i. it inherently provides a screenshot of the OoC ecosystem at the time of the documents' extraction from the databases (namely, November 2017). Patents and scientific articles published since that date were not included within the analysis;

ii. the search strategies were limited to the title, abstract or keywords of the documents, so that some documents might have been excluded or overseen although potentially relevant;

iii. the approach gives mainly quantitative information. Such information helps to identify key players as well as key used technologies on the basis of the number of patents and scientific articles found; on the other hand, the quantitative aspect of such approach may assign poor visibility to recent organizations with limited track record, despite the eventual quality of their contributions to the field.

A.1.2 Market analysis

Since market reports are already available from consulting firms, a customized market report dedicated to OoCs was purchased in early 2018 from Yole Développement [11]. This report, published early 2017, provided the ORCHID consortium with market data and forecasts as well as a focus on OoC's technological aspects and challenges.

Yole Développement's methodology for building market forecasts is, reportedly, to lay out a model where all the data from product shipments, average selling price of the devices, and player market share, are aggregated and processed with detailed assumptions. Data were collected from several sources, including primary data from direct interviews and visits with key players, direct contact and surveys with equipment & materials suppliers, and comparisons across publicly-available secondary data. A market tracker on the OoC field was implemented to update the market information presented in the report on a regular basis. As a result, the report presents synthetic market metrics intrinsic to the OoC specific industry. The main advantage of this approach is the delivery of homogeneous data, ranging from unit shipments and system sales to player market share.

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A2. The ORCHID partners: interviewees and other contributors

17 interviews with recognized experts and key opinion leaders in the OoC field were conducted within the ORCHID WP2 over a 4 months period, namely from January until April 2018, to support the state-of-the-art analysis described in the previous sections. A list of about 50 questions, spanning all aspects of the field mentioned in the main text, formed the base of the interviews. Each expert was interviewed once, and the duration of each interview was set to about 1 hour.

The expert selection process proceeded through 1) the identification of eminent expert profiles with different backgrounds (academic, industrial, clinicians, patients, regulatory), and 2) a subsequent prioritization within the candidate set, which ensured a relatively consistent profile distribution (Fig. A2).

The experts that participated in the interviews are[§§] (in alphabetical order): Sofia Batista Leite (EC-JRC, Italy), Anthony Bahinski[1,3] (GlaxoSmithKline, USA), Lorna Ewart[1,3] (IMED Biotech Unit Drug Safety and Metabolism, AstraZeneca, UK), Suzanne Fitzpatrick (FDA, USA), Marie Fortin (Jazz Pharmaceuticals, USA), Olivier Guenat[1] (University of Bern & AlveoliX AG, Switzerland), Donald E. Ingber (Wyss Institute at Harvard University, USA), Jochen Kuehnl[1] (Beiersdorf AG, Germany), Cecile Legallais (CNRS, France), Uwe Marx[3] (TissUse GmbH, Germany), Alexander Mosig (Universitaetsklinikum Jena & Dynamic42 GmbH, Germany), Christine Mummery[1,2] (Leiden University Medical Center, the Netherlands), Kevin Parker (Harvard University, USA), Adrian Roth (Hoffmann-La Roche, Switzerland), Cees Smit[3] (EGAN, the Netherlands), Danilo Tagle (NIH, USA), Jan Willem van der Laan[1] (MEB, the Netherlands).

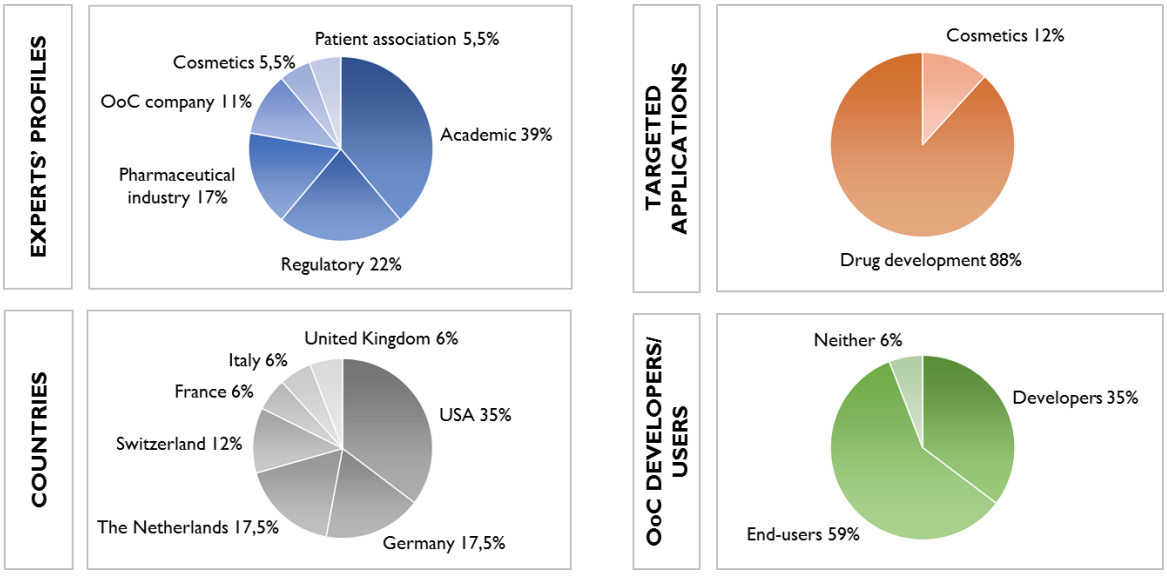

Figure A2. Profile of the 17 interviewed experts.

[§§] In the reminder, the following description applies: [1] Participant of the ORCHID Vision Workshop, [2] ORCHID partner, [3] Member of the ORCHID Advisory Board.

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Within the profile distribution of the interviewed experts (Fig. A2), a bias is observed regarding the applications targeted by the interviewees, since they mostly develop or use OoCs in the drug development process. Other clinicians and experts from food industries and cosmetics were also contacted, but they did not answer to our inquiry within the period dedicated to the interviews.

In addition to experts' interviews, a short survey composed by a selection of the interview's questions was submitted to 9 experts within the ORCHID consortium to additionally benefit also from their technical expertise and point of view.

The other experts involved in the ORCHID were (in alphabetical order): Dries Braeken[1,2] (imec, Belgium), Wolfgang Eberle[1,2] (imec, Belgium), Thomas Eschenhagen[3] (University Medical Center Hamburg, Germany), Luis Fernandez[2] (University of Zaragoza, Spain), Lino Ferreira[1,3] (Biocant & University of Coimbra, Portugal), Xavier Gidrol[1,2] (CEA, France), Mart Graef[1,2] (TU Delft, the Netherlands), Anna Herland[1], (KTH & Karolinska Institutet, Sweden), Reyk Horland[1] (TissUse GmbH, Germany), Steven Kushner (Erasmus MC, the Netherlands), Shannon Layland[1] (Fraunhofer IGB, Germany), Peter Loskill[1,2] (Fraunhofer IGB, Germany), John Martens (Erasmus MC, the Netherlands), Torsten Mayr[1,3] (University of Graz, Austria), Ignacio Ochoa[1,2] (University of Zaragoza, Spain), Nathalie Picollet-d'Hahan[1,2] (CEA, France), Mieke Schutte[1,2] (hDMT, the Netherlands), Jens Schwamborn[1,3] (University of Luxembourg, Luxembourg), Pietro Siciliano[1,3] (IMM, Italy), Maria Tenje[1,3] (Uppsala University, Sweden), Andries van der Meer (University of Twente, the Netherlands), Anja van de Stolpe (Philips Research (Philips Group Innovation) & Clinical Advisory Board hDMT, the Netherlands), Jacqueline van Engelen[1,3] (RIVM, the Netherlands), Maurice Whelan[1] (EC-JRC, Italy), Ioanna Zergioti[1,3] (NTUA, Greece).