Building blocks for a European Organ-on-Chip roadmap

Mastrangeli, Massimo; Millet, Sylvie; Mummery, Christine; Loskill, Peter; Braeken, Dries; Eberle, Wolfgang; Cipriano, Madalena; Fernandez, Luis; Graef, Mart; Gidrol, Xavier

DOI
10.14573/altex.1905221

Publication date
2019

Document Version
Final published version

Published in
ALTEX - Alternatives to Animal Experimentation

Citation (APA)
Mastrangeli, M., Millet, S., Mummery, C., Loskill, P., Braeken, D., Eberle, W., Cipriano, M., Fernandez, L., Graef, M., Gidrol, X., Picollet-D'Hahan, N., van Meer, B., Ochoa, I., Schutte, M., & van den Eijnden-van Raaij, J. (2019). Building blocks for a European Organ-on-Chip roadmap. *ALTEX - Alternatives to Animal Experimentation*, *36*(3), 481-492. https://doi.org/10.14573/altex.1905221

Workshop Report

Building Blocks for a European Organ-on-Chip Roadmap

doi:10.14573/altex.1905221

1 Introduction

This paper summarizes the outcome of the Organ-on-Chip (OoC) ORCHID Strategy workshop (Leiden, The Netherlands, January 17, 2019) intended to establish a European OoC roadmap through expert discussions, conclusions, and recommendations. The workshop identified six specific building blocks for the OoC roadmap: (1) application, (2) specification, (3) qualification, (4) standardization, (5) production and upscaling, and (6) adoption. Complementary aspects relating to ethics and communication were also addressed. Priorities, methods and targets for the roadmap were proposed for each building block. General consensus was reached on the potential contribution of the newly founded European Organ-on-Chip Society (EUROoCS), which could facilitate deployment of each block. EUROoCS would ideally initiate and catalyze dialogue between OoC developers, end users and regulators. The dialogue should address qualification, open technology platforms, standardization and implementation of OoC technology, as well as ethical aspects of human tissue mimics, training the next generation of OoC researchers, dissemination and communication.

1.1 The current landscape of Organ-on-Chip technology

Born at the confluence of tissue engineering and microfluidics, OoC technology is widely postulated as a promising approach to better model systems for healthcare research. OoC models aim to recapitulate aspects of human physiology and pathology for use in drug discovery, efficacy and toxicology testing, and personalized (or precision) medicine, with the goal to improve upon existing bioassays and provide insights into the mechanisms underlying the development and progression of diseases. In addition, OoCs are considered relevant to reduce the need, cost, and ethical burden of animal studies.

Though the OoC field is still in its infancy, several showcases of OoC models have already provided insight into disease etiology and supported identification of drug target pathways. These showcases include detection of thrombotic risk in vessels-on-chip (Barrile et al., 2018), discovery of targets for metastases in cancer-on-chip (Song et al., 2018), a test for kidney toxicity in kidney-on-chip (Vormann et al., 2018), drug effects on neurons and glia cells-on-chip (Wevers et al., 2016), prediction of toxicity of nanoparticles in lung-on-chip (Zhang et al., 2018), and drug discovery in a disease model for amyotrophic lateral sclerosis (Osaki et al., 2018). These and multiple other examples are at the stage of validation/qualification, which in general means that compounds and drugs already demonstrated as toxic or effective in treating disease in animals or patients show similar effects in OoC models. This is expected to encourage OoC adoption by industry, acceptance by regulatory bodies, and further development as animal alternatives. However, this outcome is still pending growth in confidence on OoC predictivity and utility.

A comprehensive survey of the current OoC landscape in research, development, applications, and market opportunities was recently carried out by the Horizon 2020 FET-Open project *Organ-on-Chip In Development* (ORCHID[1]). The goal of ORCHID is to create a roadmap for OoC technology, identify potential roadblocks and corresponding solutions, raise awareness and build ecosystems conducive to wide implementation and use of OoCs in science, R&D, and regulatory guidelines in Europe and elsewhere. ORCHID recently published a report (Mastrangeli et al., 2019) based on a bibliometric study, market analysis, interviews, and panel discussions with 31 experts at the ORCHID Vision workshop (Stuttgart, Germany, May 23, 2018). The report described current unmet needs (including evidence of added value, methods for automation and robustness), key challenges (structural materials, cell sourcing and culture media, long-term cell viability, real-time characterization, increasing complexity, qualification), barriers and perspectives (industrial acceptance, appropriate and timely dialogue among players) of this technology, as well as recommendations for defining a European OoC roadmap. The present document builds on this preliminary assessment and identifies potential solutions.

1.2 The future strategy for Organ-on-Chip technology

Following up on the ORCHID Vision workshop, the ORCHID Strategy workshop was held in Leiden on January 17, 2019. 32 experts (see Appendix B)[2] from academia, innovation hubs, pharmaceutical and cosmetic industry, patient organizations, ethics schools, biotech companies, and regulatory agencies attended. They represented OoC developers, end users, and regulators in Europe. The aim of the workshop was to sketch an OoC landscape for future development of the technology by defining concrete goals and milestones that would form the roadmap strategy for moving forward. During two brainstorm sessions, expert groups focused on four application domains: personalized medicine, drug efficacy, drug toxicity, and disease mechanisms. The groups addressed domain-specific issues from the perspective of both developers and end users/regulators.

[1] ORCHID partners are: Leiden University Medical Center (coordinator; The Netherlands), Institute for Human Organ and Disease Model Technologies (hDMT) and Delft University of Technology (Delft, The Netherlands), CEA (France), imec (Belgium), Fraunhofer Institute for Interfacial Engineering and Biotechnology (Fraunhofer IGB, Germany), and University of Zaragoza (Spain).

[2] doi:10.14573/altex.1905221s

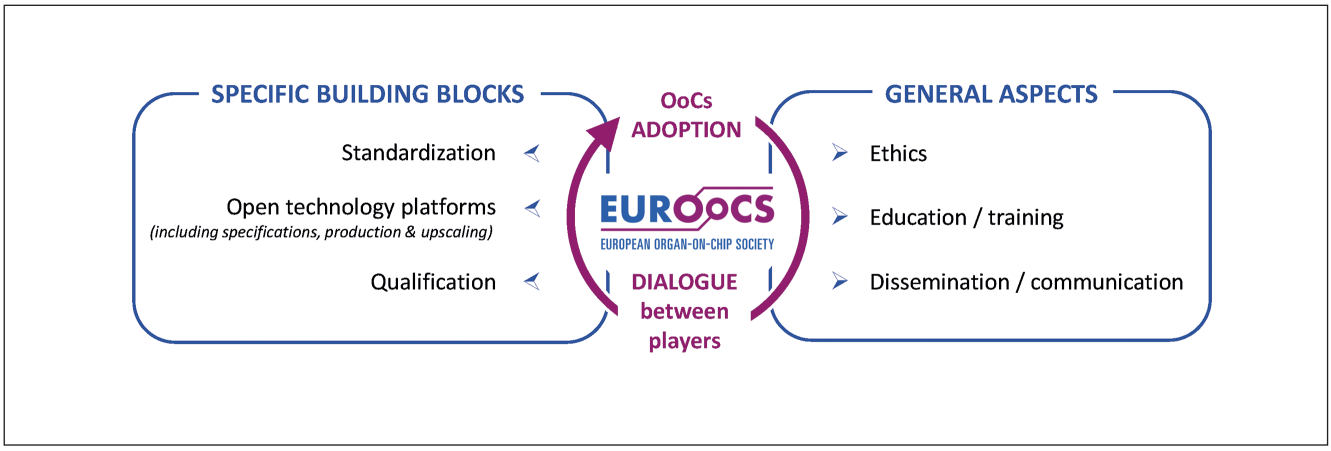

Fig. 1: The facilitating role of the European Organ-on-Chip Society (EUROoCS) in the context of the proposed OoC roadmap

This report summarizes the expert discussions, conclusions, and recommendations that emerged from the ORCHID Strategy workshop. Six building blocks of the OoC roadmap were identified: (1) application, (2) specification, (3) qualification, (4) standardization, (5) production and upscaling, and (6) adoption. These are discussed individually in depth in Section 4. Potential ethical issues were also considered, as well as training of the next generation of OoC researchers, and dissemination and communication of the technology. These issues are addressed in Sections 2 and 3, which set the context for the core building blocks. We take as given the prominent role that the existing OoC community will play in this endeavor.

2 The European Organ-on-Chip Society (EUROoCS[3])

2.1 The role of EUROoCS in community building

As articulated at the ORCHID Strategy workshop and detailed below, collaboration between all stakeholders is key to further acceptance, development and implementation of OoC technology. In Europe, an OoC network of more than 28 partners in 17 countries has recently been formed. Many countries, including the United Kingdom, Scandinavia, Belgium, and Israel, have drawn inspiration from the Dutch OoC consortium hDMT[4] and are starting to link OoC players in their own countries in similar ways. This paves the way for a European Center of Excellence on human OoC by creating strong research collaborations throughout Europe and beyond. The European Organ-on-Chip Society (EUROoCS), an outcome of ORCHID, aims to facilitate and stimulate further growth of OoC networks.

EUROoCS was launched during the International OoC Symposium (IOOCS18) at the Eindhoven University of Technology in the Netherlands on 8 November 2018. Its purpose is to encourage and develop OoC research, and provide opportunities for sharing and advancing knowledge and expertise in this field towards a better health for all (Fig. 1). Membership is open to individual researchers and others with an interest in OoC technology worldwide. Benefits include access to the digital OoC platform, where members can present their expertise and research projects, interact with others in the OoC field, and find new collaborators and tailored information (see Section 3.3). EUROoCS will stimulate and provide a platform for dialogue and interaction between all parties involved in the implementation of the OoC roadmap strategy. This is captured in the EUROoCS logo, which symbolizes both technology and community building in Europe and beyond (Fig. 1).

3 General aspects of an Organ-on-Chip roadmap

3.1 Ethics

An ethics roadmap was regarded as important to identify emerging ethical issues and address them in a timely way as the technology progresses. Such a roadmap is a sensible way to balance short-term issues related to the use of human cells, donor procedures, ownership, and present work in the laboratory with longer-term discussions about future "human-on-chip" models that would include but not be limited to brain-on-chip, reproduction system-on-chip, and the right to know/not to know regarding unexpected phenotypes revealed by chip technology.

Communication to the public warrants care

The ethics roadmap will help the discussion of ethical and societal issues with the general public, as well as the conversation on possible future societal impacts and controversies. To avoid misunderstanding, communication about OoC technology should carefully consider the language used and how it is understood by the public and different stakeholder groups. The workshop participants noted a discrepancy between expansive ambitions (e.g., "human-on-chip") and actual work in the laboratory (technical innovation). Discussions overly focusing on the ambitions may create unjustified expectations and ensuing disappointment (also

[3] https://www.euroocs.eu
[4] https://www.hdmt.technology

known as the hype cycle), or lead to controversy over issues not related to the research actually undertaken. Cautious language use is all the more important with regards to patients potentially waiting for these technological developments to become healthcare reality. The expert group therefore recommended to carefully plan a fine-tuned dissemination strategy that might also manage possible associated risks.

Privacy and ownership issues ask for clear management
There is a strong awareness of bioethics issues among researchers, and experience on how to manage them properly. However, privacy and informed consent procedures may have to be revisited to address issues related to personalized medicine, especially if rare diseases are targeted.

In addition, standardization of technology platforms raises issues related to the ownership of results. There is an underlying dilemma between the ambition to contribute to societal goals (e.g., new therapies, replacement, reduction, and refinement (3Rs) of animal testing) and the need for economic viability of the solutions. Though there is no best solution, these questions should be addressed further.

Interdisciplinary research and global dialogue raise awareness
OoC research requires interdisciplinary collaboration between physicists, biologists, engineers, chemists, and researchers from other disciplines. Researchers who are active across disciplines may be incorrectly presumed to have correspondingly adequate skills, whereas different laboratories often require specific training. Therefore, care must be taken so that personnel are offered appropriate training, including regarding safety (see next subsection).

Another dilemma to be addressed relates to the organization of collaborative research communities on complex high-tech platforms and ensuing requirements for data sharing. By lowering the entry point threshold, new entrants may bring in innovative ideas, though they may also be less aware of safety issues.

The European OoC strategy explicitly addresses ethical issues; however, this may not *per se* prevent the emergence of new products or systems in other parts of the world that conflict with European values. Therefore, a role for EUROoCS might be to engage in a global dialogue on ethical issues and develop a common understanding of how these can be addressed.

3.2 Training

Tailored training programs for next generation OoC professionals
The multidisciplinary character of the OoC technology raises challenges to develop appropriate training programs, as working in this field requires experts with a broad skillset covering various aspects of bioengineering, cell biology, materials science, and other disciplines. Specialized training and expertise is of utmost importance for anyone involved in the development and utilization of OoC systems as well as in the assessment of the systems and results they yield. The recommended training programs shall prepare scientists and technicians for new types of employment that will arise, but also provide industry and academia with professionals who are able to keep up with innovation in the field. Training programs will thus need to cover a wide range of topics including biomaterials, microfabrication and manufacturing technologies, and microfluidic principles, but also cell culture, stem cell technology, biobanking, data management and protection, monitoring and analysis (molecular biology/omics, sensors, imaging), pharmacology and toxicology principles, pharmacokinetics/pharmacodynamics (PK/PD) modelling, quality assurance, science communication, regulatory affairs and ethics. Each of these have differing degrees of relevance depending on end-user or developer requirements. Tailored training programs will be necessary for scientists in academia and industry, for decision-makers (regulators, grant evaluators or peer reviewers), as well as for technicians and research clinicians.

The level of complexity and depth of the training could range from introductory knowledge (awareness) and basic competence (theoretical or practical skills) to deep knowledge (theoretical and practical skills). The programs could be implemented at various stages of education (bachelor-, master-, doctorate studies, or post-doctoral training) and could include either specific postgraduate courses (1 to 2 years), seminars/courses integrated in a broader training program (1 semester), or only individual training sessions (theoretical or practical). Besides specific training programs, focused seminars/courses/workshops on aspects of OoC could be incorporated into traditional educational programs such as engineering (mechanic, materials, chemical), bioengineering (biotechnology, biomedical), physics, chemistry, biochemistry/biology, medicine, and pharmacology/toxicology. Input into curriculum development will be essential.

In the process of European roadmap development for OoC, comprehensive stakeholder consultation is currently taking place to assess training needs and skillset requirements and to provide recommendations for specific training programs[5].

3.3 Dissemination and communication

The EUROoCS digital platform as a market place: Organ-on-Chip for all
A dissemination and communication strategy for OoC research and activities is essential if the EU scientific knowledge base is to be implemented optimally and to foster innovation, economic growth, and jobs. EUROoCS, its support website, including a digital platform for members, and its annual conference can contribute to forming a central coordination point, ensuring good visibility for European research teams that will promote their leadership in the OoC field. The EUROoCS platform will gather information centrally and promote OoC implementation, development and community building at European and international levels (Fig. 2).

EUROoCS board and members will ensure that sector-specific boundaries and language barriers are crossed to facilitate effective dialogue and collaboration between the scientific community, regulators, industry, clinicians, and patient groups from the earliest stages of development, and to catalyze OoC adoption by

[5] Questionnaire for stakeholders: Training needs of the next generation of researchers and technicians in Organ-on-a-Chip, available at: https://ec.europa.eu/eusurvey/runner/Orchid_Questionnaire_Training_needs_Organ-on-a-chip (accessed 29.09.2019).

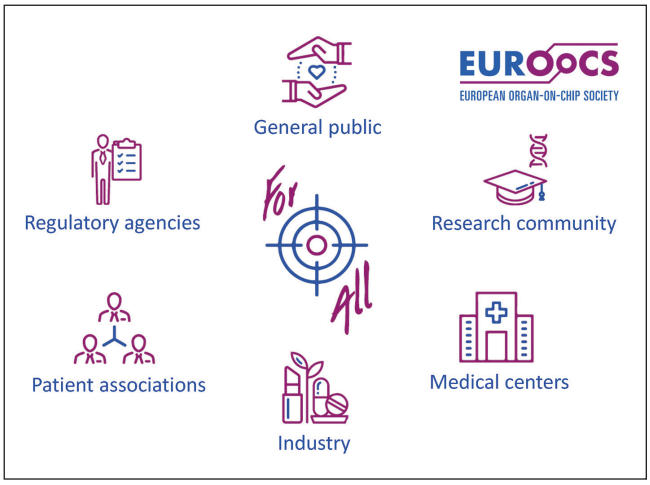

Fig. 2: The EUROoCS website/digital platform as a "market place" for all

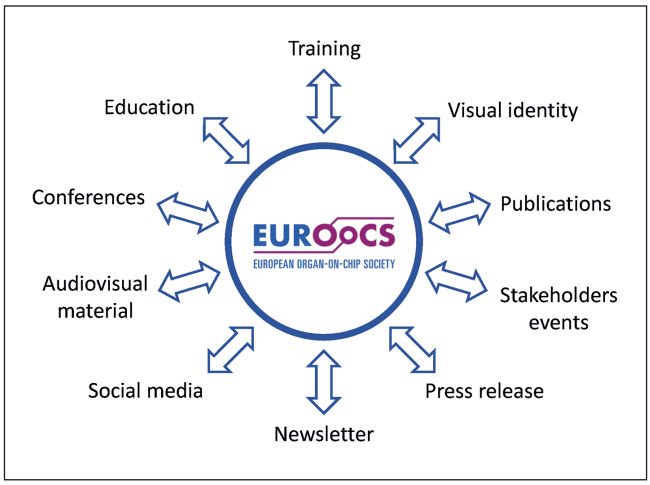

Fig. 3: Tools of dissemination

end-users. Working together as a community and becoming visible through the EUROoCS website/digital platform could also help establish standards in the field that would provide industry with confidence regarding implementation. As a standardization catalyst, the EUROoCS digital platform will also collect expert insights from different stakeholder groups to provide an indirect roadmap through building guidelines for OoC implementation.

The EUROoCS website[3] was conceived by ORCHID members and validated by a test group of selected OoC experts to provide key information efficiently. The majority of tabs (library, latest news and recent events, announcements of trainings, workshops, OoC timeline, reports and reviews) are available with a free subscription to allow the users to gain a better understanding of OoC and keep them informed about new scientific and technological developments. It is updated regularly by members and will continue beyond the lifetime of ORCHID through EUROoCS. Specific links to general brochures are included in the website. Moreover, the website was designed to be inviting for students looking for jobs, funding opportunities and informative newsletters, and accessing social networks, whereby EUROoCS will host different groups interested in OoC technology. Information on key publications, patents, and discoveries will thus be distributed to the dissemination target groups besides being accessible directly from the website. The EUROoCS website will evolve over time through the continuing addition of publications, latest news, event announcements, collaborative projects, and member-supplied content. Finally, by providing the opportunity to industry for exposure of their technology and by being attractive for investors and funding agencies to support the development of prototypes, the EUROoCS website will also contribute to increase innovation and competitiveness for European health-related industries and services.

The ORCHID expert community unanimously perceived the EUROoCS digital platform as a means to build a network, realize integrative programs and collaborative projects or consortia, and to find new academic/industrial partners or individuals involved in regulation or patient associations. In this respect, a reserved area, accessible to EUROoCS members, provides expert contacts, project descriptions, and mapping. This detailed information as well as the opportunity to debate specific topics through a discussion forum aim at encouraging membership and joining the community. This member-restricted area of the digital platform is expected to be a market place for OoC-stakeholders, with individuals from a wide range of backgrounds benefitting from the availability of OoC ideas and expertise, initiating specific working groups, or discussing current topics on OoC.

Building dedicated communication tools to continuously raise awareness

Having established a communication strategy, it is important to determine whether the expected impact is actually achieved. To this end, online dissemination tools (e.g., website, newsletters, social media) will be regularly monitored and some key performance indicators (number of visitors, downloads/open clicks, statistics on the impact of the newsletter, number of articles viewed, user feedback on the newsletter's content) will be periodically checked for quantitative and qualitative evaluation (Fig. 3). Qualitative evaluation will also be conducted on the basis of questionnaires following special events. Finally, the impact, strengths, and weaknesses of the dissemination strategy on target groups will be examined regularly as well as the quality of communication on OoC.

Participating in conferences and organizing training and dedicated workshops on OoC will raise awareness of the community and capture young researcher attention on OoC activities. This is essential if the full potential of OoC is to be realized. To that end, EUROoCS members are being encouraged to establish connections with press, media, politicians, general public, and schools, to act as focal points for local dissemination of information, to use connections to other academics working in related domains at local and international levels, and to generate active social media content to introduce European citizens to OoC technology as a concept. A visual identity of OoC in Europe was developed to ensure consistency and higher visibility of the OoC field. Commu-

Tab. 1: Overview of the applications and associated models discussed during the ORCHID Strategy workshop
ADME, absorption, distribution, metabolism, excretion; BBB, blood brain barrier

Context of use	Disease area	Key tissue model	End user
Disease mechanisms	Cancer	Tumor models	Biomedical researchers Clinicians Pharmaceutical industry
	Neurodegenerative diseases	Brain, BBB, neurons, retina	
	Cardiometabolic disorders	Heart, lung, liver, pancreas, vessels, adipose	
	Autoimmune diseases	Immune system, gut, pancreas, neurons, skin	
	Fibrosis	Connective tissues, lung, liver, kidney	
Drug efficacy	Cancer	All types	Industry: pharmaceutical, cosmetics Biomedical researchers
	Neurodegenerative diseases	Brain, BBB, neurons	
	Cardiometabolic disorders	Heart, lung, liver, pancreas, vessels	
	Autoimmune diseases	Immune system, gut	
	Fibrosis	Connective tissues, lung, liver, kidney	
Drug toxicity	All types	ADME pathway (liver, kidney), barrier systems (gut, lung, BBB), heart, brain, immune system	Industry: pharmaceutical, cosmetics Biomedical researchers
Personalized medicine: – Patient stratification (adverse effects, dynamics/resistance, identification of vulnerable population) – Companion diagnostics (responders, disease progression)	Cancer	All types	Pharmaceutical industry Hospitals/clinicians
	Rare diseases	All types	
	Systemic diseases	Multi-organs	
	Autoimmune diseases	Immune system, gut	

nication tools (e.g., social media, brochures, newsletters, press releases) will be adjusted to the type of information and to the specific targeted group. Establishing a proper platform for critical dialogue and dissemination, monitoring interest, and acceptance at every level are essential features of the roadmap that will determine rate of progress and ultimate outcome beyond promise.

4 Specific building blocks of the Organ-on-Chip roadmap

4.1 Application

Priority for disease mechanisms, drug efficacy and toxicity, and personalized (or precision) medicine

An important domain to which OoCs can contribute is the drug screening and development process[6], which ranges from fundamental research to personalized medicine and targets users such as biomedical researchers, (research) hospitals, and pharmaceutical industries. According to the consulted OoC experts, there are four main contexts of use:

- improving understanding of human disease mechanisms and etiology;
- predicting drug efficacy in humans;
- predicting drug toxicity in humans;
- paving the way to personalized (or precision) medicine.

The associated priority (patho)physiological areas are driven by the need for new therapeutics discovery, including emerging drug modalities (e.g., large molecules/biologicals like monoclonal antibodies, antibody-drug conjugates, protein therapeutics), especially for diseases for which few or no effective drugs are available due to poor knowledge of pathophysiological mechanisms in humans combined with a paucity of predictive disease models (see Tab. 1). Among the most cited public health priorities are cancer, neurodegenerative conditions like Alzheimer's disease, cardio/metabolic disorders, autoimmune diseases, fibrosis, and rare diseases, such as hemophilia and metabolic syndromes. Each requires its own panel of cell/tissue models that may include single- to multi-OoCs. Primary or stem cell-derived cells and tissues can be a first approach of choice, but in some cases cells will not respond to drugs or reveal disease mechanisms as expected

[6] Other relevant applications include toxicity screening of compounds for cosmetic and chemical industries and for environmental agencies, and countermeasures against chemical and biological warfare – for a comprehensive overview, see the ORCHID Vision workshop report (Mastrangeli et al., 2019).

outside a 3D environment or without biophysical stress/flow. For toxicity testing and safety assessment, there may be a strong preference for OoCs mimicking ADME pathways (absorption, distribution, metabolism, excretion) including metabolic organs (liver, kidney), barrier/digestive systems (blood-brain barrier and gut for physiological absorption), and the presence of the immune component of the disease.

OoCs also can be personalized with patient samples to mimic key aspects of a (patho)physiological state, including specific disease-related parameters. By capturing a higher level of physiological complexity from particular individuals, OoCs are predicted to be of value as companion diagnostic tools to differentiate patients that are "responders" from "non-responders" to medication, to refine doses for individual patients (exposure-response relationships by PK/PD modeling), to define combination therapies and personalized drug delivery, or evaluate disease progression, and to predict specific adverse events (patients at risk), thereby tailoring treatment strategies to improve the benefit-risk ratio. OoCs might also contribute to patient pre-stratification for clinical trials, leading to protocol optimization and supporting clinical decision-making processes. As importantly, patients often become tolerant to drugs which are then no longer effective. Identification of "back-up" drugs that have similar effects to the original but via a different pathway in the OoC model can offer an alternative in the clinic, which is then highly relevant to treatment benefit. In the future, the implementation of health-related data in OoCs (lifestyle, epigenetics) may lead to even more predictive personalized devices and to position OoCs as physical avatars for individuals or groups of individuals (e.g., of different ethnic backgrounds, gender, age).

4.2 Specification

Customizable platforms for fit-for-purpose modular OoCs

It was generally agreed that OoCs should be conceived and proposed as robust tools tailored to fit a purpose by the end users. End users should accordingly be provided with flexible solutions ranging from ready-for-use devices to open customizable platforms. Existing ready-to-use OoCs offer a certain level of customization, which can be further extended towards models of increasing complexity via acquired laboratory experience and with customizable platforms. In this respect, the group and collective discussions of the ORCHID Strategy workshop highlighted the following technical and functional recommendations for the development of OoC models:

a) *Modularity*. It is unlikely that a single OoC device would be able to satisfy the requirements to serve all conceivable functions or applications within the reach of the OoC technology. For instance, and even though certain molecular pathways are repeatedly defective in multiple diseases (e.g., SMAD, WNT signaling), a comprehensive list of disease mechanisms is hardly feasible. Besides, the requirements may be mutually incompatible, and a single device may easily turn out to be overwhelmingly complex, economically non-viable, and ultimately not effective for potential end users. Instead, a reductionist, flexible integration approach is suggested whereby OoC devices are assembled over platforms out of sets of basic and standardized modules according to user needs. Standardized interfaces and well-defined assembly of modules are essential for the success of such a modular approach. Both technical and biological modules are considered:
   1. *Technical modules:* mechanical actuators and stimuli (forces, stresses, strains), electrical stimuli, perfusion (microfluidics, pumps, valves), reporter systems and sensors (for, e.g., oxygen, carbon dioxide, pH, glucose, metabolites, flow rates, impedance);
   2. *Biological modules ("functions"):* 3D scaffolds, cell-cell interactions, neuron/neurite outgrowth, barrier mechanisms, vascularization, air/liquid interface.

   The modular composition of OoCs within standard platforms shortcuts the issue of defining the end use of the devices *a priori*, instead leaving it open and suitable to a variety of different users and applications. This approach is envisioned to afford or be compatible with:
   − simplicity and ease of use;
   − possibility to stack levels of complexity in 4 dimensions (space + time);
   − customization and user-defined fit-for-purpose;
   − standardization of modules (see also Section 4.4).
b) *Increased throughput/scalability* to reduce cost-per-data point and extend the range of OoC use in pre-clinical screening. Required throughput depends on applications, research questions, and drug development stage (Probst et al., 2018; see also Section 4.5).
c) *Automation* to limit errors and improve reproducibility, repeatability, and cost-effectiveness.
d) *Multi-parametric assays* to benefit from various data points in a single test system, thereby improving efficiency and limiting heterogeneity. The selected parameters should provide sufficient data to support study conclusions, allow regulatory acceptance, and they should be linked to clinical expectations to prove the added value of the technology.
e) *Use of genetically & phenotypically characterized human cells* to mimic human (patho)physiology, with eventual dedicated benchmarking (not based on animal use).
f) Sustain *long-term measurements* to evaluate (chronic effects of) compounds, perceived as preferred or complementary to short kinetic and metabolic measurements.
g) Generic and standardized *open technology platforms*, both in hardware and software, for full public availability and compatibility across platforms (see Section 4.4).

Modular as well as flexible, ready-to-use OoCs align with the belief that the ultimate purpose of the devices may only emerge from the end users themselves once the devices are in their hands.

4.3 Qualification

Qualification processes for context of use need independent testing centers

The ORCHID Vision workshop highlighted the need to focus on the qualification or characterization of the OoCs rather than on their validation *per se*. The latter was considered by most experts neither an appropriate nor a meaningful concept for highly complex models such as OoCs, because it implies the existence of an accepted standard or reference to measure validity. The ORCHID Strategy workshop confirmed that while regulatory acceptance

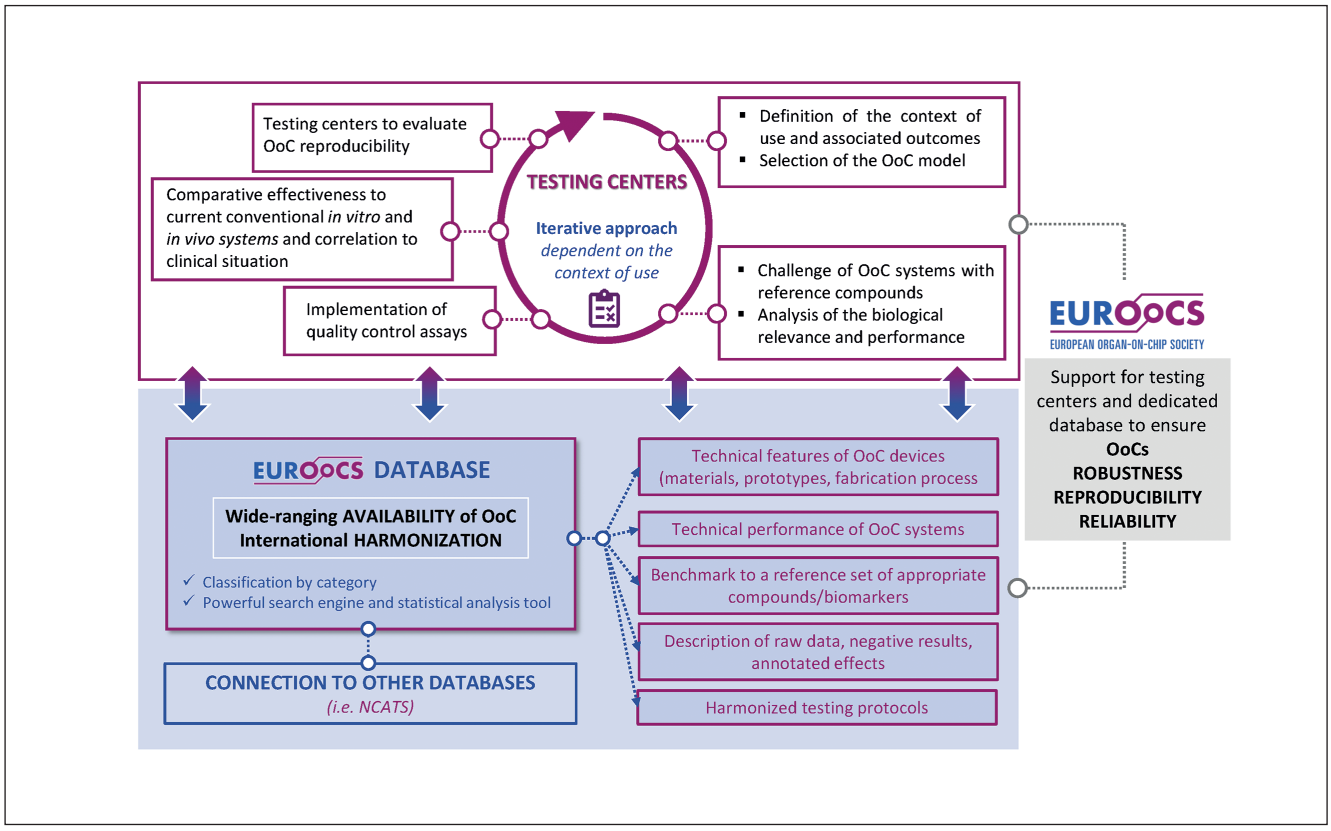

Fig. 4: Synopsis of OoC qualification processes and interfacing with a centralized database

is beneficial for the commercialization of OoC devices, this acceptance should not hinder the development process. Indeed, regulatory agencies should be considered key players, involved in the early stages of OoC development along with end users, in order to better understand the potential of the technology and its applications. Industry needs confidence in the robustness of the data retrieved through the devices, whereas regulators typically require a case-by-case analysis. Therefore, while considered necessary, the qualification of a device does not necessarily prelude to its regulatory acceptance nor to user adoption.

For drug screening and development, OoCs should recapitulate human tissue physiology but also disease-related parameters to be used as predictive models for assessing safety and efficacy of promising therapies that are preferably better than current cell and animal models. The characterization and qualification of such devices should be based on a generic study design including the following key aspects:

a) Defining the context of use and its associated outcomes to select the most relevant OoC model;
b) Challenging OoC systems with reference compounds insofar as they are classified regarding context of use and specific parameters. This aspect will help determine the biological relevance of the devices and will allow qualification of their performance. It will by definition rely on known outcomes of animal experiments and preferably their discrepancies with clinical/patient data or safety/feasibility in phase I trials on healthy human individuals.
c) Implementing quality control assays ensuring the functional characterization of cell cultures to fit with human (patho)physiological responses but also expressed in the form of material qualification (drug-biomaterial interaction), manufacturability, and availability of devices.
d) Evaluating effectiveness of OoC compared to current *in vivo* profiles, through the analysis of the corresponding data derived from conventional drug development models, and correlation with clinical data or manifestations.
e) Performing intra- and inter-laboratory assays (ring trials) to assess the reproducibility and accuracy of OoC devices as well as monitoring technological performances (stability and robustness).

These last aspects should be considered as iterative approaches, supported by ongoing pharmaceutical projects to bring added value, aiming at investigating the correlation between OoCs and *in vivo* data, if relevant, and to establish the critical link to clinical expectations. Ideally, all qualification studies should be performed by a third party, as proposed by the testing center initiatives in the US funded by NCATS, to ensure an independent analytical characterization (Fig. 4).

Towards a centralized database information sharing and promoting OoC development

To achieve optimal results from the qualification studies, a key challenge is to establish an evolutive database, clustering all available data on a reference set of the most appropriate com-

pounds and biomarkers, together with the results on the performance and accuracy of the specific OoC systems under test and the context of use for the target tissue(s). The aim of this centralized and publicly accessible database would be to provide the scientific community with in-depth information (including raw data and negative results) on well-characterized pharmacological and toxic compounds to demonstrate *in vivo*-like responses in OoC devices and to go beyond a simple and linear annotation of the compounds' effects. Providing relative data from reference compounds/biomarkers and allowing the community (including stakeholders and regulators) to use it wisely, may help both developers and end users to challenge OoC systems and may influence the decision-making process.

However, the quest for qualification of OoC devices entails abundant human data sets being available as well as multi-parameter readouts without bias (achievable via proper automated statistical data analysis). In addition, a relevant list of test compounds/biomarkers is not readily available but may be in the making thanks to several independent initiatives (e.g., the NIH in the US and the Crack it! Program in the UK). Beyond having a list of compounds for which the outcome and mechanisms of action are agreed, the compounds may not necessarily be available to academic research groups since they may be proprietary or may no longer be synthesized. A solution would be to work closely with structures like the IQ Consortium[7], a not-for-profit organization of pharmaceutical and biotechnology companies, which is compiling a list of reference compounds that may be shared for qualification purposes. NCATS is also currently working with well-known pharmaceutical companies like AstraZeneca, GSK, Pfizer, Roche and Sanofi, and has for that purpose already established a material transfer agreement to ensure that compounds/biomarkers provided for characterization are cleared for inclusion in academic work (Ewart et al., 2017). Alternatively, medicinal chemists may be contacted to synthesize specific compounds or variants of those.

EUROoCs may play a catalyzing role in collecting the information with the necessary infrastructure, data management and statistical capabilities to ensure an extended dissemination among the OoC community and beyond (Fig. 4). This publicly accessible database could also be a promising tool to promote OoC adoption supported by early engagement of academic, industrial, and regulatory players. Finally, the coordination of a EUROoCS-supported database with other international existing ones should reinforce multi-partner task forces and contribute to international harmonization.

4.4 Standardization

A task for the OoC community that should be internationally harmonized

Standardization is an overloaded concept with multiple interpretations across different sectors and markets. Standardization of OoCs is very challenging since OoC technology is inherently interdisciplinary. In recent history, technological standards have usually arisen either from dominant commercial players or from collective entities such as regulatory authorities or roadmaps jointly established among field competitors. While the former appear to be the current case in the US, where several larger groups are trying to define OoC standards, standardization emerging from a collective dialogue among developers and end users and from ensuing cross-constraints is ultimately expected to prevail. Successful examples of standardizations in electronics (e.g., data communication protocols, interfaces, and peripheral cross-compatibility) can be capitalized as important learning experiences. In particular, lack of standardization for lab-on-chip approaches may be the reason for the problems that technology currently has in getting into the market.

To avoid this risk, OoC standardization should be addressed very early in development to enhance the prospect of being competitive with its alternatives. On the other hand, standardization cannot be promoted by most of the current stakeholders, as these are mostly small (biotech or start-up) companies without sufficient financial flexibility to support a standardization strategy. OoC standardization is therefore considered a task for the OoC community. The role of the community is in fact central, because the purpose of standards is foremost to enable the OoC community to cooperate towards developing prototypes. Community-driven standardization may also ensure that standardization addresses sufficiently common issues, benefitting a large set of users and thus becoming a means to accelerate innovation. EUROoCS can play an important role in bringing developers, stakeholders, regulators, and end users together into a community, as well as in serving as a collective expert group to advise on OoC standards, protocols, methods, and guidelines, similarly to prior experiences in, e.g., stem cell research and toxicology, whereby protocols were defined by panels of experts.

Different standardization layers should be identified

Layers of standardization can be envisioned, ranging across multiple levels of abstraction and user experience. They include: materials, dimensions, cell input and content, perfusion media, flow rates, interconnections and interfaces, optical access, platforms, cross-compatibility among modules, back-compatibility with existing substrate standards (e.g., multi-well plates, microscope slides, multi-electrode arrays) and laboratory instrumentation, cell sources and lines, cell phenotypic and genotypic characterization and protocols for cell differentiation, cell handling, use of devices, and quality control. Additional layers should be further considered. Standards for commercialization could eventually emerge from research prototypes, though this should not be the primary aim of the community. Commercial standardization should be internationally harmonized, avoiding competing groups particularly between the US and Europe.

Towards standardization: open technology platforms for OoC

One approach that experts recommended to encourage the OoC community to converge towards standardization was the realization of open technology platforms. These can be seen as shared technology platforms created to gather knowledge and expertise into a centralized database, in which potential users could contribute by developing and sharing building blocks of modular sys-

[7] https://iqconsortium.org/

tems to enable customized solutions for specific applications. The open technology platform concept is in line with the modular approach suggested for the development of OoCs. It will stimulate further innovation rather than restrain it. These platforms would reduce barriers to expensive manufacturing of devices, because they could generate the production volumes needed for sustained technology development. The freedom to develop demonstrators in parallel may, moreover, lead to quick learning cycles and broad uptake of successful innovations in the community. However, the implementation of an open technology platform raises crucial questions concerning the co-existence of, on the one hand, open interfaces, open standards, and the freedom to exploit open source content together with, on the other, patenting and licensing of intellectual property as sources of commercial drive and market penetration. These and similar issues related to the co-existence of private profit and public availability are well-known from prior standardization attempts in other fields, and they evidently represent an important aspect of the proposed roadmap that needs to be resolved.

Further information on the benefits and pitfalls of standardization in the field of OoC devices and systems is described in the *ORCHID whitepaper on standardization*. The whitepaper was written by ORCHID partners in the context of the ORCHID project in addition to the Strategy workshop, and is provided in Appendix A[2]. The whitepaper in particular identifies ongoing standardization efforts which address certain aspects of technology and operational processes in the OoC field.

4.5 Production and upscaling

Industrialization requires choices in early development of OoC devices

OoC production perspectives will be determined by the type and scale of use of OoCs – whether for, e.g., drug screening or replacement of animal tests or personalized medicine – such that a 96-well plate format or similar may need to be developed for applications requiring high throughput, whereas in other cases a 2-well plate or single-chip format may be sufficient if examining, for example, efficacy. Clear and standard guidelines for quality control of technology and biology should be introduced in all cases to ensure and maintain robustness. The type of use will also determine the allocation of resources. In this respect, drug development prioritizes rate of success and time-to-market, and hence time-saving rather than cost-saving.

It is important to remark that upscaling of OoCs inherently involves both technological and biological components. This respectively implies mass production of chips or microfluidic devices and generation of large batches of differentiated cells that are quality-controlled prior to use in OoCs. As demonstrated by the success of microelectronics, high volume production of chips typically coincides with decreased manufacturing costs and variability and leads to highly reproducible devices. However, there is a caveat here. On the one hand, device-level reproducibility would be required, especially in early stage OoC development and qualification (see Section 4.3), since the inherently variable biology introduces another potential layer of variability and cannot otherwise be properly assessed. On the other hand, setting up mass production of devices requires large investments, which are not likely for non-qualified devices. Solving this issue might require specific, public-private funding calls.

Depending on applications (see Tab. 1), at least three different upscaling strategies could be envisioned:

1) *Drug efficacy and toxicity* in pharmaceutical industry. In this case, the SBS well-plate format will likely be the preferred and target format, with highly characterized, robust, and reproducible OoC enabling relative comparison of hundreds of drugs (Probst et al., 2018);
2) *Personalized medicine*, possibly in a hospital setting or dedicated SMEs. This will entail robust and reproducible OoCs with (patient-derived or genetically-modified) disease bearing cells, and upscaling to test tens (i.e., 10 to 50) of potential drugs and find the right concentration of the right drug for specific patients or disease states;
3) In the longer term, *clinical trials*. To date, there are no clinical trials on, e.g., children, pregnant women, or specific or unique ethnic groups. OoC could enable a better representation of human phenotypic diversity in clinical trials. The upscaling requirement to perform clinical-trials-on-chip by contract research organizations will be dependent on the trial.

An industrial-level fabrication volume puts manufacturing constraints on the design, dimension and structural materials of the devices. These choices should be considered as early as possible in device development along with back-compatibility with established laboratory tools and cross-compatibility among platforms. At the same time, the use of standard cell lines might not match such extended device requests, though standard cell handling protocols could still be helpful, and banks hosting cells for different population subgroups might need to be established.

4.6 Adoption

Adoption of OoC technology requires well-documented showcases

The OoC technology may be accepted and thus adopted if it provides simpler, cheaper, and more relevant alternatives to established models (even those based on multi-cell type cultures) while reproducing at least the same results supported by convincing and reliable metrics, or if it affords models for which no alternative approaches currently exist (such as rare diseases in which microfluidics is required to reveal the phenotype; see Mastrangeli et al., 2019). In addition, OoC adoption implies the satisfaction of many other conditions, including ease of use, back-compatibility with established laboratory processes, cross-laboratory reliability, along with a supporting and organized user's community (Fig. 5).

In this respect, experts deemed the identification and stratification of end users, stakeholders, and sectors as critical. It should be part of a primary action conducted by dedicated methodologies and teams, and a prerequisite to organize a global community centered around open technology and allowing a smooth transfer of expertise among disciplines and domains (e.g., including regulatory bodies). To build confidence in the value of OoCs and facilitate engagement of all players, structuring of (a) neutral organization(s) capable of testing and qualifying devices has been proposed, in line with OECD guidance. The adoption process will emerge from collective end users of OoCs and will rely on

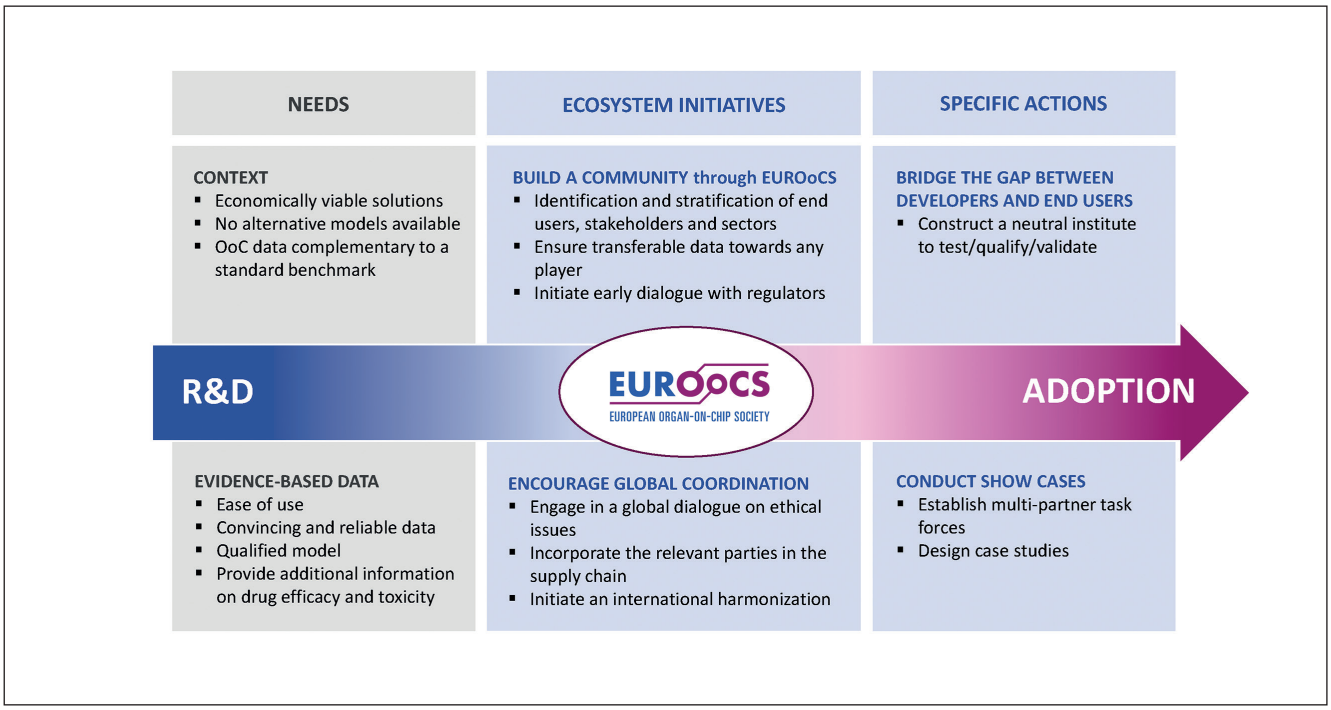

Fig. 5: Overview of the context needs, initiatives and specific actions to promote OoC adoption

the data obtained with OoCs included in a regulatory approval and perceived by regulatory bodies as complementary data to the standard benchmark. The applicability of these guidelines to rapidly developing technologies like OoCs remains a key challenge. A convincing way to circumvent these issues would be to encourage multi-partner task forces to conduct showcases capable of fostering end user adoption. Such showcases may be represented by case studies, such as the Crack it! challenges, could be non-optimized or finalized, and should involve end users along with regulators and developers to start and sustain a fruitful and timely dialogue. As pointed out by the regulatory experts, the final decision will be up to developers, since currently there are no restrictions or requirements from regulatory bodies for the use of specific cells.

Finally, community organization and subsequent adoption of OoCs by end users will need sustained dialogue and collaboration to move across the limitations of sector-specific cultures and languages: EUROoCS is expected to play a leading and catalyzing role in that respect.

5 Discussion and recommendations

The ORCHID Strategy workshop converged on the proposition of the roadmap for OoC development represented in Figure 6. The roadmap emphasizes a dual path, involving proprietary, ready-to-use devices and open technology platforms running in parallel and interacting along the way. OoCs are expected to serve three major markets: 1) industry (mainly pharma, cosmetics, and chemicals), 2) hospitals, and 3) academic research. Industry and hospitals might mostly require ready-to-use devices, whereas the latter would strongly benefit from open technology platforms. As described in the previous sections, to move forward from the current status to applications of OoCs, the roadmap is envisioned as making use of a set of main building blocks. Some building blocks are specific to the present state of the art, whereas others correspond to common development stages for which prior experience in collateral roadmaps can be capitalized. Along with the concept of the OoC roadmap, the experts agreed on the catalyzing role that EUROoCS could and should play in the deployment and actualization of each building block.

It should be noted that the entries in Table 1 are not exhaustive but are rather priority lists. This holds in particular for known disease mechanisms, for which a complete list is hardly conceivable, and for OoC applications, whereby the long-term list may even turn out to be different from the one proposed, depending on the future needs of actual end users and how their drug discovery priorities change. Whereas the application domains were chosen mainly to guide the discussions, the roadmap and its building blocks are expected to apply, irrespective of the directed *versus* emergent nature of the final applications.

Alongside flexible, ready-to-use devices, the modular approach to OoCs based on customizable platforms was recommended as a solution to enable both user-defined and specific fit-for-purpose applications and at the same time to align or facilitate qualification, standardization, and large-scale production of OoCs. Device qualification, *per se* not corresponding to device adoption or regulation, should pass through independent testing centers, preferably established in Europe following the US lead. A single, worldwide database, eventually emerging from the harmonization and interconnection of local databases, would be ideal to collect and share all related data, following the example of shared compound

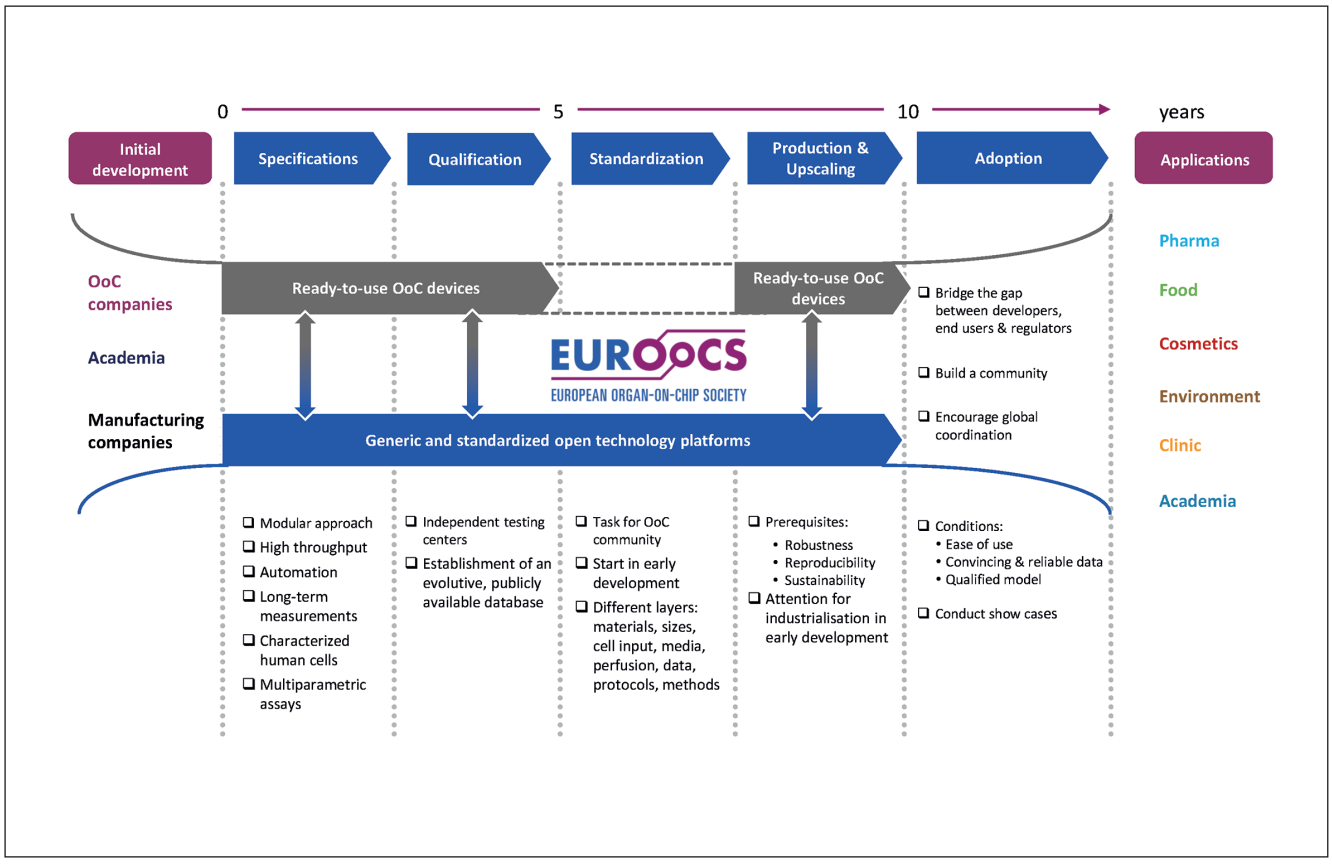

Fig. 6: The ORCHID roadmap for OoC development

lists of, e.g., the IQ consortium. Standardization should address multiple levels of OoC technology and should start by forming task forces of expert groups and learning from existing standards (see Appendix A2). It is worth emphasizing once again the advantages of open technology platforms, along with the need to manage the coexistence of, at times, divergent needs related to intellectual property and technology sharing. Successful handling of these aspects additionally highlights the key role of a large, open, and interconnected OoC community, not least for its role in innovation. In this respect, while supporting the roadmap, the experts at the workshop at the same time acknowledged the possible dualism and coexistence of a more directed and linear approach to OoC development, as indeed embodied in a roadmap, and of an emergent and more non-linear approach building upon crowdsourcing and user-generated targets and solutions. Both approaches have earlier examples in recent history, and can certainly interact for mutual benefit. Irrespectively, it is clear that to achieve the envisaged long-term goals of globally improved healthcare and personalized medicine, OoCs will need to be suitable for large-scale production, an aspect that should consider the choice of biological and synthetic materials and of manufacturing technologies from early stages of development. Along this line, compatibility of the devices with established laboratory practice and standards as well as successful showcases of complete OoC platforms and applications should favor OoC penetration and speed up worldwide adoption.

Finally, the roadmap that this report has introduced – and of which the final structure will be released in October 2019 – necessarily addresses related ethical questions, cautiously optimistic communicational strategies, and information of laypeople as well as in-depth training and education of the next generation of experts. For all of the above tasks, EUROoCS, supported by its digital platform, publications, and regular meetings, should be ready to play a central role.

Recommendations

1. Focus on selected pathophysiological areas in the context of OoC models for disease mechanisms, drug efficacy and toxicity, and personalized medicine: cancer, neurodegenerative diseases, cardio-metabolic disorders, autoimmune diseases, fibrosis, and rare diseases.
2. Provide end users with customizable platforms for fit-for-purpose OoC models.
3. Explore a modular approach for the OoC models, with technical and biological modules that can be assembled within standard open technology platforms.
4. Converge towards standardization of components, methods, and data via collective dialogue among experts, facilitated by EUROoCS, and starting very early in the development.

5. Establish independent testing centers for the qualification and characterization of OoC models for a specific context of use.
6. Develop a publicly accessible evolutive database, supported by EUROoCS, that clusters all available data from reference compounds and OoC test data, and interfaces with other databases to contribute to international harmonization.
7. Resolve issues related to the co-existence of private profit and public availability of technology in the OoC field.
8. Address upscaling requirements and constraints on design, dimension, and structural materials as early as possible in device development to make the right choices for industrial-level fabrication.
9. Encourage multi-partner task forces to come up with well-documented showcases, based on case studies, to stimulate adoption of the OoC technology. EUROoCs should catalyze this process.
10. Engage as EUROoCS in a global dialogue on ethical issues, and address them in a timely manner as the technology progresses.
11. Develop tailored training programs for the next generation OoC researchers.
12. Build the OoC community further and bridge the gap between end users, developers, and regulators with the support of EUROoCS.
13. Stimulate information, communication, and interaction via the digital platform and targeted meetings of EUROoCS and measure the impact on OoC ecosystem development.
14. Plan a careful dissemination strategy for the general public based on realistic expectations and ambitions.

References

Barrile, R., van der Meer, A. D., Park, H. et al. (2018). Organ-on-chip recapitulates thrombosis induced by an anti-CD154 antibody: Translational potential of advanced microengineered systems. *Clin Pharmacol Ther 104*, 1240-1248. doi:10.1002/cpt.1054

Ewart, L., Fabre, K., Chakilam, A. et al. (2017). Navigating tissue chips from development to dissemination: A pharmaceutical industry perspective. *Exp Biol Med 242*, 1579-1585. doi:10.1177/1535370217715441

Mastrangeli, M., Millet, S., Partners, T. O. and van den Eijnden-van Raaij, J. (2019). Organ-on-chip in development: Towards a roadmap for organs-on-chip. *Preprints*, 2019030031. doi:10.20944/preprints201903.0031.v1

Osaki, T., Uzel, S. G. M. and Kamm, R. D. (2018). Microphysiological 3D model of amyotrophic lateral sclerosis (ALS) from human iPS-derived muscle cells and optogenetic motor neurons. *Sci Adv 4*, eaat5847. doi:10.1126/sciadv.aat5847

Probst, C., Schneider, S. and Loskill, P. (2018). High-throughput organ-on-a-chip systems: Current status and remaining challenges. *Curr Opin Biomed Eng 6*, 33-41. doi:10.1016/j.cobme.2018.02.004

Song, J., Miermont, A., Lim, C. T. and Kamm, R. D. (2018). A 3D microvascular network model to study the impact of hypoxia on the extravasation potential of breast cell lines. *Sci Rep 8*, 17949. doi:10.1038/s41598-018-36381-5.

Vormann, M. K., Gijzen, L., Hutter, S. et al. (2018). Nephrotoxicity and kidney transport assessment on 3D perfused proximal tubules. *AAPS J 20*, 90. doi:10.1208/s12248-018-0248-z

Wevers, N. R., van Vught, R., Wilschut, K. J. et al. (2016). High-throughput compound evaluation on 3D networks of neurons and glia in a microfluidic platform. *Sci Rep 6*, 38856. doi:10.1038/srep38856

Zhang, B., Korolj, A., Lai, B. F. L. and Radisic, M. (2018). Advances in organ-on-a-chip engineering. *Nat Rev Mater 3*, 257-278. doi:10.1038/s41578-018-0034-7

Acknowledgements

The authors would like to thank all experts who took part in the ORCHID Strategy workshop (see Appendix B[2]) for their valuable contributions. This work was funded by the European Union's Horizon 2020 Research and Innovation program under grant agreement No. 766884, and the Netherlands Organ-on-Chip Initiative (NOCI) Gravitation grant of the Netherlands Organization for Scientific Research (NWO).

Massimo Mastrangeli[1], Sylvie Millet[2], Christine Mummery[3], Peter Loskill[4], Dries Braeken[5], Wolfgang Eberle[5], Madalena Cipriano[4], Luis Fernandez[6], Mart Graef[1], Xavier Gidrol[2], Nathalie Picollet-D'Hahan[2], Berend van Meer[3], Ignacio Ochoa[6], Mieke Schutte[7] and Janny van den Eijnden-van Raaij[7]

[1]Delft University of Technology Delft, The Netherlands; [2]CEA, Grenoble, France; [3]Leiden University Medical Center, Leiden, The Netherlands; [4]Fraunhofer IGB, Stuttgart, Germany; [5]imec, Leuven, Belgium; [6]University of Zaragoza, Zaragoza, Spain; [7]hDMT, Eindhoven, The Netherlands