CEN/CENELEC FGOoC

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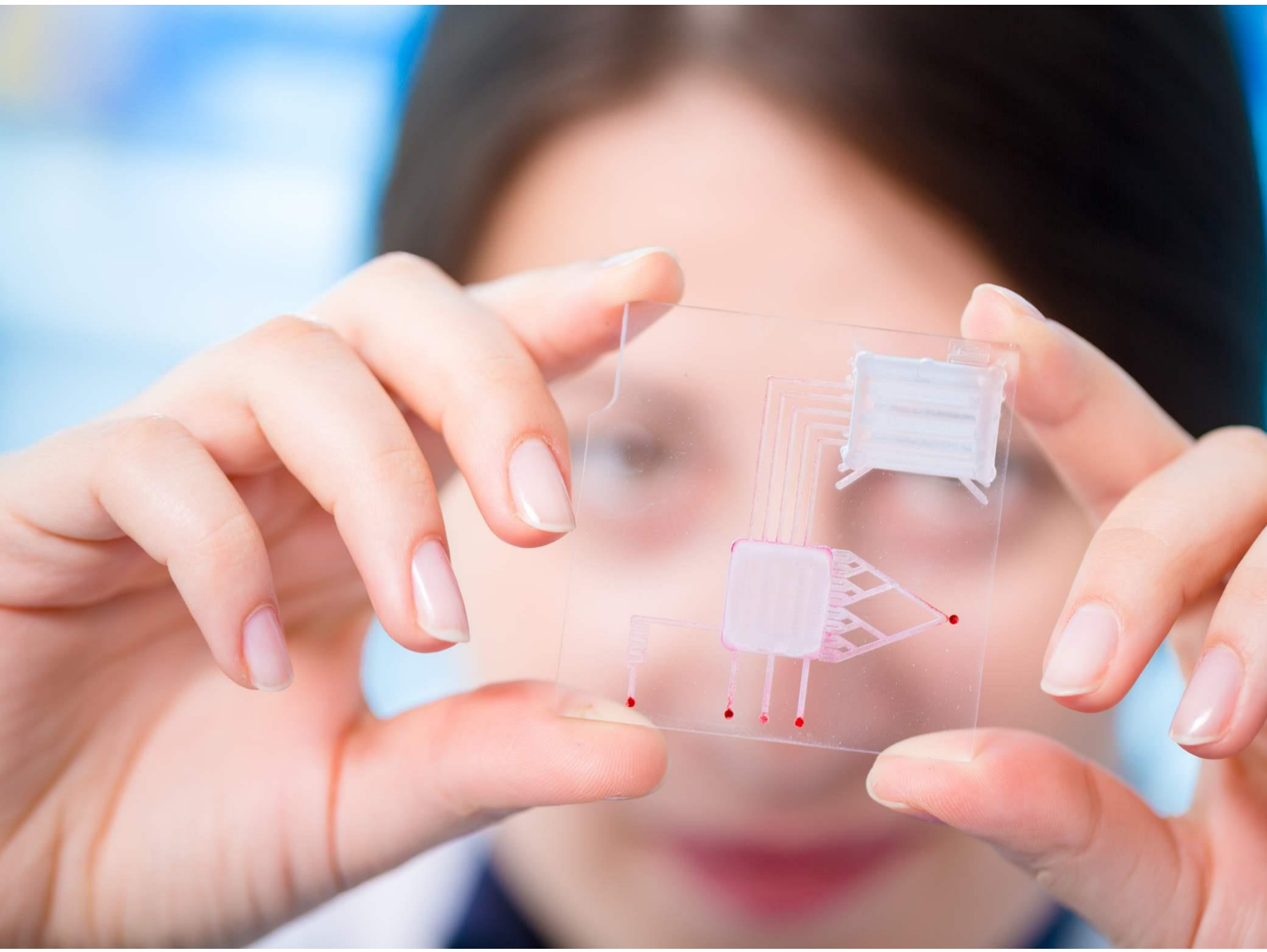
Focus Group Organ-on-Chip Standardization Roadmap



Contents

1 Recommendations

This roadmap document is the results of two years of work, 10 Focus Group meetings, numerous Working Group (WG) meetings and the active participation of around 120 experts of the CEN/CENELEC Focus Group Organ-on-Chip (FGOoC).

The following sections provide an overview of the recommendations for topics for standardisation. This overview is based on the detailed analyses in the respective sections outlined in the document, as well as on a centralized FGOoC survey for prioritisation. For the survey, each WG was asked to assign an urgency level to 117 items, using a five-point scoring system (see table in Annex C for the scoring). A pondered calculation was made to account for consensus among WGs and level of importance of each item. The items were prioritized and grouped into 10 different areas of interest for OoC standardisation (see Annex C). The three areas of interest with the highest urgency for standardisation were 1) qualification of materials, 2) sterilization, and 3) cell integrity, identity, function, all of which are clearly represented in the respective sections below.

Recommendation 1: Terminology, ecosystem, interdependencies

Summary: WG1 was involved in the identification of terms in the OoC field that need harmonization and uniform definition. In the context of the roadmap, a majority of the FGOoC members agreed to use the definitions of the terms Organ-on-Chip and Microphysiological Systems according to the ASTM F3570 – 22 - Standard Terminology Relating to Microphysiological Systems. However, a more extensive discussion is required to reach full consensus about these definitions. A list of 38 relevant terms related to OoC/MPS technology and systems was created based on inquiries performed among the FGOoC members during the development process of the roadmap.

Rationale: The field of OoC technology currently lacks a standardized set of terminologies and symbols for elements of OoC systems. Terms such as 'Organ-on-Chip', 'Microphysiological Systems', 'complex in vitro models', 'NAMs', and 'context-of-use' are defined differently across various studies and discussions. OoC systems are currently not described with a uniform technical-symbolic language. Standardized and consensus-based terminology and definitions, complemented by uniform symbols, will facilitate clearer communication and collaboration within the international OoC community, thereby accelerating progress in the field.

Recommendation: Develop standards documents that provide harmonized terminology and definitions for key items and symbols in the OoC domain, thereby considering in particular the relevant terms described in the roadmap.

Type	Scope
Standard[1]	Terminology and definitions
Standard	Symbols

[1] An International Standard provides rules, guidelines or characteristics for activities or for their results, aimed at achieving the optimum degree of order in a given context. It can take many forms. Apart from product standards, other examples include: test methods, codes of practice, guideline standards and management systems standards [https://www.iso.org/deliverables-all.html#IS]

Recommendation 2: Biosciences

Summary: WG2 concludes that cell biology and biomaterials in OoC devices lack standards, but at the same time it would not be feasible to define standards or specifications in an evolving area. Therefore the WG advices to focus more on reporting guidelines.

Rationale: The field is rapidly evolving, there are numerous protocols available. There is no way to determine what would be the optimized approach. Establishing standards at this stage would be very restrictive for the organic development of the field. Having minimum reporting guidelines, will lead to more consistent and reproducible studies.

Recommendation: Work towards defining minimum reporting requirements for cells and biomaterials used in OoC systems. This should be done in alignment with existing initiatives in this domain.

Type	Scope
Technical Specification[2]	Minimum reporting requirements for bioscience

Recommendation 3: Engineering

Summary: WG3 was involved in analysis of all engineering aspects of OoC. The main problem to be solved creating an OoC system is the difficulty associated with the selection of appropriate hardware, installing, and operating it. For this it might help if the components and instruments were designed in such a way that plug and play installation is possible. Therefore, there should be compatibility between components and instruments.

Rationale: The engineering of OoC systems encompasses a wide range of aspects, from sterilization of components and systems, integration with existing workflows, documentation of materials used, to modular integration of components and operation in specific environments. Currently, these aspects lack standardisation, leading to inconsistencies and inefficiencies in the field. Standardizing these aspects will streamline the design, fabrication, and operation of OoC systems, facilitating reproducibility and comparability across different studies and platforms.

Recommendation: Develop comprehensive standards documents that address the key engineering aspects of OoC systems.

Type	Scope
Technical Specification	Measurement and qualification of materials
Technical Specification	Flow control
Technical Specification	Compatibility with existing lab infrastructure (microtiterplate workflow)
Standard	Microfluidic connection
Technical Specification	Reliability related aspects like leakage, material – liquid interaction, sterilization (method and control)

[2] A Technical Specification addresses work still under technical development, or where it is believed that there will be a future, but not immediate, possibility of agreement on an International Standard. A Technical Specification is published for immediate use, but it also provides a means to obtain feedback. The aim is that it will eventually be transformed and republished as an International Standard. [https://www.iso.org/deliverables-all.html#IS]

Recommendation 4: Hardware parameters, experimental design and data management

Summary: A number of standards and/or guidelines already exist for aspects of experimental design and data management, but for the most part these have not been developed specifically for OoC.

Rationale: Standardized experimental design and data management practices will ensure the reliability and reproducibility of OoC studies. It will also facilitate the integration of OoC data with other computational modelling studies, enabling in vitro-in vivo extrapolation and the application of machine learning algorithms. Moreover, for OoC data to be used in decision making (including for regulatory science), it will be important to build a clear framework for defining the qualification of OoC models, including the context-of-use of the data being generated.

Recommendation: Evaluate how OoC is already covered in other laboratory practices and legal framework, find where specific standardisation approach is needed. Where these gaps are identified, develop documentation that outlines the specific requirements for experimental design and data management in OoC studies, as well as the framework towards qualification of OoC models and their data for specific contexts of use. This includes standards for aspects of experimental design including positive and negative controls, sample size and randomisation, and for data management the use of software and programming languages, documentation verifying the use of FAIR principles, guidelines for using statistical software tools and tests as well as data analyses, and reporting practices.

Type	Scope
Technical Specification	Study design – factors to be taken into account such as: positive and negative controls, sample size, randomisation, operators etc.
Technical Specification	The experimental protocol should be completely described such that it can be reproduced
Technical Specification	A standardised method to acquire and store data is crucial for subsequent data analysis and publication of results

Recommendation 5: User perspective and regulatory, legal and ethical aspects

Summary: OoC can be applied in various scientific fields, being used in various risk assessment and decision making scenarios, intersecting with numerous regulations. The scientific community must be transparent to the public as more understanding is gained of the broader impact, benefits and risks of OoC within personalised medicine, toxicology and other applications, so that the field may continue to move forward in a meaningful, and potentially transformational, way.

Rationale: The current state of OoC development does not completely align with existing regulations for medical devices and medicinal products. Furthermore, OoC devices are used for regulatory toxicology, necessitating validation and scientific assessment to comply with current requirements for test methods. Ethical considerations arise when using human and non-human animal models as defined in EU regulations. Lastly, pharmaceutical companies are increasingly using OoC-based methods for internal decision making during drug development and as human-based tools to support drug repurposing. However, data generated with OoC devices are very rarely included in dossiers submitted to agencies, limiting the impact of these technologies in the regulatory arena. Efforts will be needed to facilitate alignment, acceptance and integration into regulatory frameworks.

Recommendation: Develop documentation that outlines the specific requirements for the use of OoC devices in various application domains, and facilitate and enable the use of OoC-based methods for specific applications. This includes considerations for their application in medical decision-making, regulatory toxicology, and ethical implications. The involvement of regulatory

and policy experts is key to complement the expertise by developers and end-users in scientifically assessing OoC technologies for specific uses.

Type	Scope
Guidelines/guidance documents	Regulatory guidance for application of OoC in testing and repurposing medicines
Guidelines/guidance documents	Define a framework for regulations for OoC with diagnostic applicability
Guidelines/guidance documents	Define the framework for use of OoC models in regulatory toxicology of chemicals, biocidal products, cosmetic products or veterinary medicinal products
Guidelines/guidance documents	Defining a framework for fully protecting autonomy of patients or other donors of cells and tissues for OoC
	Consultation with the Medical Device Coordination Group (MDCG) to define the applicability of the IVDR to OoC and enable use and commercialisation in medical settings (e.g. as a diagnostic tool).
Guidelines/guidance documents	Provide guidance to industry on how to use use OoC-based methods to generate data for drug repurposing claims
Guidelines/guidance documents	Provide guidance to medical doctors and hospitals on the use of OoC-based methods to generate data for personalised medicine and definition of patient-specific drug treatment

2 Introduction and scope

2.1 Organ-on-Chip Technologies

Organ-on-Chip (OoC) is a research field that focuses on advanced tissue culture models. The history of OoC can be traced back to the development of micro-electro-mechanical systems (MEMS) in the 1960s and 1970s, which enabled the fabrication of miniaturized sensors and actuators on silicon chips. In the 1980s and 1990s, the similar fabrication technologies were used to develop microfluidic 'lab-on-a-chip' devices that could perform various analytical functions on small volumes of fluids. As lab-on-a-chip technology matured, scientists began exploring the possibility of culturing living cells on these chips. The 2000s witnessed significant progress in cell culture in microfluidic chips, and the devices were used to produce microenvironments that mimic physiological conditions (e.g. in terms of gradients, strain and stress). In the mid-2000s, a perceptual shift occurred in the field of microfluidic cell culture. Scientists recognized that the functional integration of engineered devices and living tissues yielded models with an unprecedented complexity. Since some of these models even recapitulated organ-level functionality, the term 'Organ-on-Chip' was coined to describe them.(Huh et al., 2010)

OoC technology has opened new avenues for understanding human biology and disease, discovering new drugs, testing drug safety and efficacy, evaluating health and food products like cosmetics, and developing personalised medicine. OoC devices have also been used to test the effects of environmental factors, such as air pollution, radiation and microgravity, on human health. OoC technology is expected to contribute to the principle of 'Refinement, Reduction, Replacement' ('3R') in animal-based research by offering an alternative avenue as well as opportunities to improve the human relevance of research in human (patho)physiology, toxicology and pharmacology.

2.2 OoC as a Growing Field

The field of OoC grew rapidly after 2010, not only with many academic research groups, but also with established companies, which is reflected in a compound annual growth rate (CAGR) of 70% from 2015 to 2020, and a projected CAGR of 31% from 2020 until 2030 to a total of 1.6 billion USD.(Business Wire, 2016) The research activities have grown into a worldwide endeavour, with research groups and companies on all continents. The companies in the field commercialized OoC models by developing their own microfluidic systems, cell cultures and read-outs. The main market for commercial OoC models is in disease modelling and pharmaceutical drug development, with large pharmaceutical companies as end-users or customers.

Much research in academia and industry is also devoted to further developing and extending the concept of OoC. Currently, development of the next generation of OoC technology focuses on e.g. advanced disease modelling, personalisation, multiplexing, and combining multiple devices to generate 'Body-on-Chip' systems. Many research groups worldwide are contributing to these developments.

2.3 The growing need for standardisation in the field of OoC

As the field of OoC keeps growing, it becomes increasingly clear that the relative lack of standards is impeding both the implementation and innovation of the technology. A lack of standards negatively affects reproducibility and comparability of results, making it more difficult to promote active use of this data by companies and regulatory bodies. Moreover, the lack of standards also hampers interoperability of different OoC components and systems, thereby slowing down innovation and scalable manufacturing.

Several consortia recognized this issue and developed position papers outlining the collective vision of numerous stakeholders regarding the need for standardisation to advance the OoC field.(Piergiovanni, Leite, et al., 2021) Notably, the EU H2020 Organ-on-Chip In Development

("ORCHID") project identified standardisation as a crucial element for the progression of OoC technologies at the European level.(Mastrangeli et al., 2019) Similarly, the transatlantic think tank for toxicology (t4) summarized the views of 46 international stakeholders on the challenges faced by the OoC community, identifying standards as tools to support qualification and achieve regulatory acceptance.(Marx et al., 2020)

Based on this groundwork, the OoC community begun to actively involve organizations for standards development in their work to discuss collaborative actions. Notably, the European Commission's Joint Research Centre (JRC) together with the European Committee for Standardization and the European Committee for Electrotechnical Standardization (CEN and CENELEC) made orchestrated efforts to push towards concrete actions for standardisation in OoC. JRC and CEN and CENELEC, supported by the European Organ-on-Chip Society (EUROoCS), organized the 'Putting Science into Standards' (PSIS) 2021 workshop, which brought together stakeholders from academia, industry, and regulatory agencies,(Piergiovanni, Leite, et al., 2021) thereby taking a key step in the process towards standardisation for OoC.(Piergiovanni, Jenet, et al., 2021)

Standardisation can facilitate the definition of common terminology, specifications, protocols, methods, metrics, and criteria for OoC design, fabrication, characterization, operation, analysis, and reporting. Standardisation can also enable the development of reference materials, quality control procedures, best practices, guidelines, and regulatory frameworks. By establishing a common ground for OoC development, standardisation will not only promote reproducibility, robustness and qualification, it can also stimulate the creation of new models and platforms.

2.4 Scope of this document and the Focus Group OoC

Based on the multi-stakeholder call for action in the aforementioned PSIS workshop, CEN and CENELEC decided to establish a Focus Group on Organ-on-Chip (FGOoC) to (1) systematically plot the landscape of standards for OoC and to (2) define a roadmap on OoC standardisation for the coming years. Starting in March 2022, the goal of the FGOoC was to establish how standards can contribute to designing, developing, fabricating and testing OoC models, in a way to improve their reproducibility, reliability, comparability and validity, finally leading to significant improvements in their development and implementation. In this roadmap document, the FGOoC aims to draft an overview of the landscape of standards that are relevant for the domain of OoC. This includes the identification of existing standards or standardisation initiatives, as well as recommendations on the priorities and opportunities for drafting new standards in the coming years.

The primary audience for this roadmap is the (inter)national community of organizations and stakeholders that will participate in standardisation of OoC. The roadmap will also be of interest to researchers and industry professionals working in the field of OoC, regulatory agencies, funding bodies, and other stakeholders who are interested in the development and application of OoC.

Enabling technologies and topics that are included in the scope of the roadmap include microfluidics, (stem) cell biology, biomaterials, tissue engineering, data management, bioanalytical techniques, and ethical and regulatory aspects. These topics are included because they are integral to the development and application of OoC, and because they can help to define best practices for designing, fabricating, and testing OoC models. In contrast, topics that are excluded from the scope of the roadmap, or only discussed indirectly, include broader topics such as omics, artificial intelligence, drug discovery, clinical diagnostics and regenerative medicine. Although related to the application of OoC, these topics are not directly relevant to the development of best practices for designing, fabricating, and testing OoC models. Moreover, the focus of FGOoC was on identifying standards and providing a roadmap for the overall domain of OoC, rather than on focusing on specific models, organs, or applications.

The following aspects of OoC standardisation will be covered in the roadmap document.

- First of all, the document will discuss the main relevant terms and their definitions (including references) for OoC. This is essential to establish a common language that can be used by the OoC community.

- The roadmap will also give an overview of the OoC ecosystem and address the interdependencies between various stakeholders, including researchers, industry professionals, regulatory agencies, and funding bodies, and how these interdependencies impact the development and implementation of OoC.

- The roadmap will also focus on best practices for bioscience-related aspects of OoC, including the source, selection and culture of cells, the use of stem cells and extracellular matrix proteins, as well as the design and use of culture media.

- In addition, the roadmap will address the engineering aspects of OoC, such as the design and fabrication of microfluidics, laboratory equipment, and microelectronics, including materials selection, fabrication techniques, and quality control.

- The roadmap will identify important aspects of experimental design and data management. This includes the development of standardized protocols for experimental design, as well as repositories for OoC data and the use of reference compounds to facilitate comparison of OoC results across different experiments and laboratories for qualification and validation.

- Finally, the roadmap will take into account the user perspective of OoC, as well as the regulatory, legal, and ethical aspects of their development and use. The regulatory landscape for OoC, including the role of regulatory agencies, in the adoption and use of this innovative technology will be addressed, as well as legal and ethical considerations related to in vitro diagnostics regulations (IVDR), intellectual property (IP), informed consent, and privacy.

2.5 Process and workflows of drafting this roadmap

The FGOoC consists of experts from different fields of activity, including OoC development and use, who contributed from various perspectives and areas of expertise. Main stakeholders involved are related to: 1) research, including fundamental or applied research in university settings and commercial R&D; 2) industry, including both developers from sub-systems or suppliers and end-users; and 3) non-governmental and governmental European or member state organisations, institutions, and research and technology organizations.

The outcome of the FGOoC is this roadmap, which is primarily an advisory document for the CEN and CENELEC Technical Board, but is expected to also provide a reference point for the field of OoC as a whole. The roadmap indicates which standards must be developed and in what structure. The text of the roadmap has been developed in five Working Groups (WGs), focusing on different subtopics: WG1 Terminology, ecosystems, interdependencies, WG2 Biosciences, WG3 Engineering, WG4 Experimental design and data management, and WG5 User perspective and regulatory, legal and ethical aspects. The interaction between the different WGs is illustrated in Figure 1.

The roadmap was drafted in a bottom-up process. The state of the art was investigated for each subtopic; already published standards were listed and opportunities for standardisation were identified by the participating stakeholders in the dedicated WGs. These opportunities were then prioritized according to importance, dependencies, and probability of reaching consensus. The draft text of the roadmap was reviewed multiple times by the FGOoC. Furthermore, input was

received from interested international parties. In total around 120 experts contributed to the roadmap, in ten plenary FG meetings and around 90 monthly meetings in total for the five WGs.

The FGOoC is facilitated by NEN, the Royal Netherlands Standardisation Institute. As a national standardisation institute, NEN plays a pivotal role in European standardisation by facilitating technical committees tasked with developing and maintaining standards crucial for various industries. NEN's primary responsibility lies in coordinating these committees, ensuring that experts from relevant fields collaborate effectively to establish consensus-based standards. This involves organizing meetings, managing communications, and overseeing the drafting and revision processes to align with European and international standards frameworks.

NEN serves as a link between national and European standardisation bodies, facilitating collaboration and harmonization of standards across borders. In connection to NXTGEN Hightech, a Dutch initiative aimed at fostering innovation in next-generation technologies, NEN provides expertise and guidance in the development of standards specific to OoC technologies. These standards are essential for ensuring the reliability, reproducibility, and safety of OoC platforms, thereby accelerating their adoption and facilitating their integration into research, development, and industrial applications across Europe. Through its involvement in NXTGEN Hightech and dedication to advancing standardisation in cutting-edge fields like OoC, NEN reinforces Europe's position as a leader in innovation and promotes the growth of a robust and competitive European market.

The working groups (WG 2-5) are aligned along a conceptual experimental timeline, which is laid out to answer an initial research question of interest (top panel of Figure 1). The WGs defined up to three sub-topics, covering across the WGs all relevant topics from planning to execution of an experiment, data storage, and regulatory aspects.

The WGs are based on a conceptual mapping of the field of OoC (bottom panel of Figure 1). The foundation of all work in the field lies in having a clear understanding of the full ecosystem as well as a well-defined, shared terminology (WG1). Based on this foundation, a functional OoC model integrates biological components, including living cells and tissues, as well as engineered components and subsystems, and both are addressed in their respective WGs (WG2, WG3, resp.). The functional OoC models then form the basis for experimental studies, in which they are operated according to experimental protocols, with defined parameters, to generate relevant data (WG4). The generated data are to be used in multiple contexts, that include legal, ethical regulatory aspects (WG5). Similarly, the biological and technical components of an OoC model

(particularly its human cells) can also be prone to regulation (WG5).

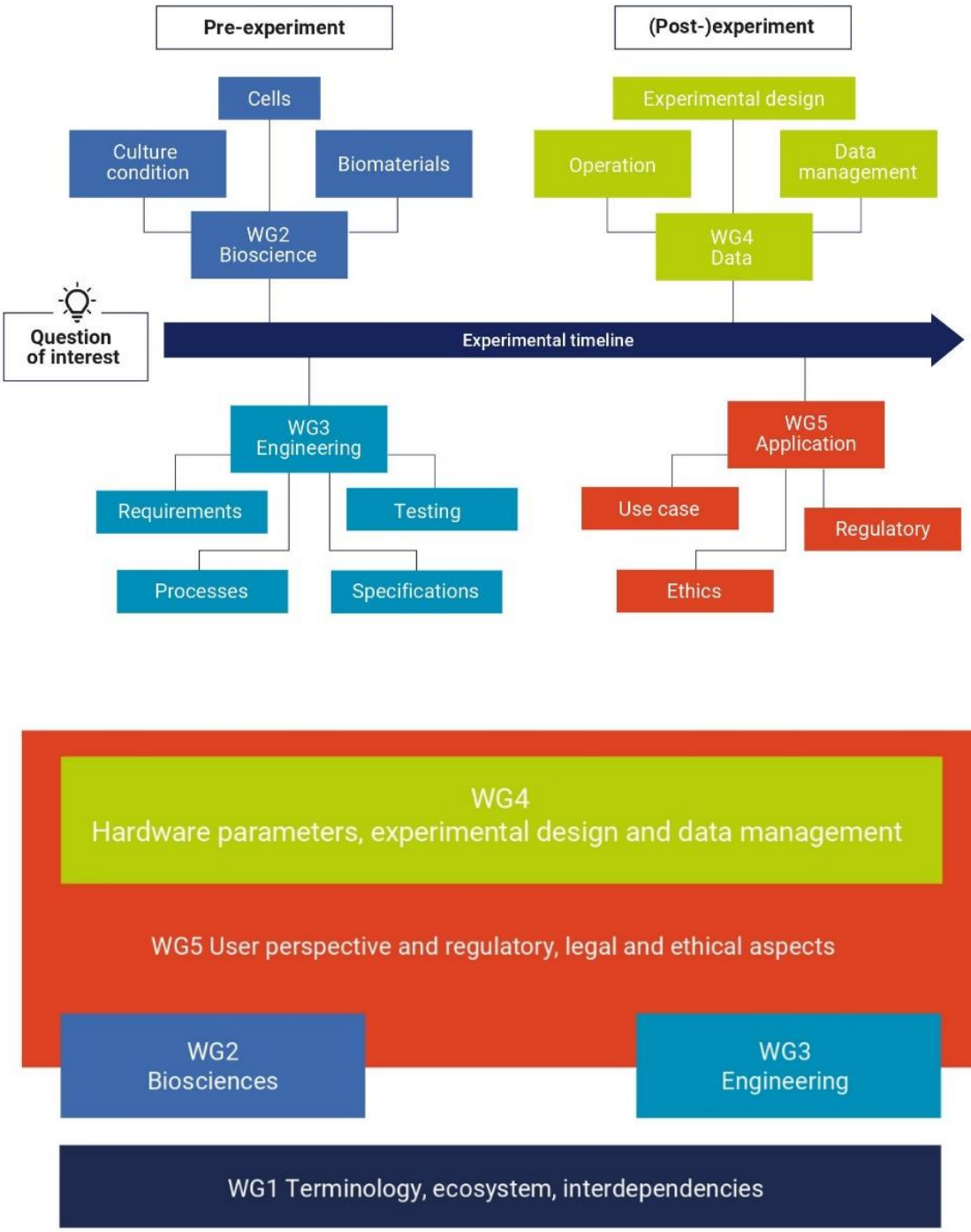


Figure 1: Interplay of Working Groups

2.6 Standardisation

Standardisation plays a crucial role in virtually every aspect of modern society, from ensuring interoperability and safety to facilitating international trade and innovation. At its core, standardisation involves establishing a set of guidelines, specifications, or criteria that products, processes, or practices must meet. This process is vital for harmonizing practices across industries and regions, promoting efficiency, quality, and reliability.

In fields such as technology and manufacturing, standards enable different systems and components to work together seamlessly, fostering compatibility and reducing the risk of incompatibility issues. For example, standards like USB, HDMI, and Wi-Fi ensure that devices from different manufacturers can connect and communicate effectively, regardless of their origins.

The process of creating standards involves various stakeholders, including industry experts, regulators, consumer advocates, academia, and government representatives. Consensus-building is a fundamental aspect of standardisation, as it ensures that standards are widely accepted and implemented. The consensus-building process typically involves extensive collaboration, negotiation, and sometimes compromise.

Standards often have a direct impact on European legislation and the internal market. EU directives and regulations frequently reference standards as a means to ensure compliance with established requirements and to support the free movement of goods and services within the EU. By aligning standards with legislative objectives, European standardisation organizations contribute to the development of a cohesive regulatory framework that fosters innovation, competitiveness, and consumer protection across the EU member states.

The process of developing European Standards involves a structured approach primarily facilitated by CEN and CENELEC, where members, including the National Standardisation Bodies and National Committees, predominantly drive the initiatives. Occasionally, proposals for standards may originate from the European Commission or other stakeholders.

Upon sufficient interest from CEN and/or CENELEC members to participate in the development process, the responsibility is delegated to the respective Technical Committee (TC), focusing on the relevant field. National mirror committees comprising stakeholders determine the national contributions to the standard's development. Additionally, alongside CEN and/or CENELEC members, Technical Committees also accommodate observers, including ISO/IEC members, European Commission/EFTA, European partners such as Annex III organizations, external European industry associations, and other affiliated bodies.

Subsequently, upon the evaluation and approval of the standard proposal, it advances to the drafting stage, characterized by consensus-building. Upon finalizing the draft standard, it undergoes a public enquiry accessible to all interested parties. Following the conclusion of the enquiry, votes and comments on the standard are assessed, determining whether the draft is published or requires further refinement before undergoing formal voting. Alternatively, standards may be developed internationally at ISO, truly establishing a global standard. The process and principles of standardisation remain the same.

3 Terms and Definitions

The following definitions for OoC and microphysiological systems have previously been used in the literature and are used in the context of this roadmap. Other terms requiring formal definition are listed in Annex A.

NOTE The exact definitions are still subject to discussion in the field. Their use in the context of the roadmap does not imply that these represent a consensus definition.

3.1

Organ-on-Chip

a subset of microphysiological systems that replicates one or more aspects of an organ's in vivo dynamics, functionality, structure, and/or (patho)physiological response(s) of multiple cell types integrated within a non-biological platform

3.2

Microphysiological systems

fit-for-purpose devices, containing one or more engineered organ(s), organ substructures, and/or functional organ unit(s) in a controllable microenvironment

Note to entry An MPS represents one or more aspects of the organ or organ system's dynamics, functionality, and/or (patho)physiological response such as responding to biologic, mechanical, electromagnetic (light and/or radiation), or pharmaceutical stimuli in vivo. Ideally, an MPS has the capacity to be monitored under real time. MPS platforms may comprise mono-cultures, cocultures of multiple cell types, maintenance of explants derived from tissues/organs, and/or inclusion of organoid cell formations.

3.3 List of available standards

— ASTM F3570 – 22 - Standard Terminology Relating to Microphysiological Systems

— ISO 10991:2023

4 Terminology, ecosystem, interdependencies

4.1 Introduction

The landscape of standardisation in OoC is highly complex, with multiple domains and a very diverse group of stakeholders with many interrelationships in different application fields. Standardisation can only be achieved by a concerted effort of all stakeholder groups that form the OoC ecosystem. The European OoC roadmap, mentioned earlier, developed in the ORCHID project, defines the building blocks of the processes from initial development to the final application and use of OoC models. These building blocks include specification, qualification, standardisation, production and upscaling, and adoption of these innovative systems. Many different actors are involved in these processes. The interaction and a collective dialogue among all stakeholders in these processes are essential to realize robust, reproducible, easy to use, standardized, qualified and validated fit-for purpose OoCs, that meet the need for better models. EUROoCS, the European Organ-on-Chip Society, acts as a catalyst to build this community further and bridge the gap between the different actors.

4.2 Actors

As shown in Figure 2 six different categories of actors can be identified in the OoC field, each with a specific role and position in the circular workflow from new solutions for unmet needs to the use of standardized and validated end products. The separate groups are described below.

4.2.1 R&D-Scientific Community

The R&D-Scientific Community consists of scientists, researchers and developers from both non-profit and commercial organizations and consortia.

— **Academia and knowledge institutes:** Including (technical) universities, university medical centers and universities of applied sciences develop new ideas and technologies, that form the basis for novel/advanced OoC models. They have a central role as a breeding place for providing and training the next generation of researchers and teaching them the skills needed to design and use OoC

technology. Most devices developed in these settings remain at a proof-of-concept level and do not yet offer the ease-of-use, manufacturability and throughput necessary for widespread application. For this reason, it is important that scientists from academia collaborate with end users to understand their needs and interact with the supplier industry to bridge the valley of death by translating their inventions into marketable products. Academics can also be end users of OoC models, applying them to the biomedical research, where tools able to model complex, mechanistic phenomena are crucial to better understand health and diseases. To advance science and promote an increased use of OoC technology, it is important that not only results, but also methods and protocols, are published in (open access) journals.

— **Scientific societies and consortia:** Among them in the OoC field is EUROoCS, that brings together all actors involved. EUROoCS has partnered with the International Society for Stem Cell Research (ISSCR) to be able to use Stem Cell Reports as the home journal. The ISSCR, in partnership with global stakeholders, is currently developing research standards on stem cells that can be adopted worldwide. In Europe, other consortia/societies on OoC include hDMT (Dutch Organ-on-Chip Consortium), ISOoC (Italian Organ-on-Chip Society) and the Nordic Organ-on-a-Chip Network. On the global level the international MPS Society (iMPSS) has recently been established. During the pandemic the NC3Rs MPS CoRe Working Group was born aiming to help coordinate global efforts to use MPS/OoC for assessing the safety and efficacy of potential novel therapeutics for infectious diseases, starting from COVID-19, through building connections between technology developers and end-users.

— **Commercial actors:** Including companies and CRO/service -based research, such as Small Medium Enterprises (SMEs), start-ups and spin-offs, that are focused on bringing their products to the market via the process of proof-of-concept development, prototyping and testing. These companies are commercial providers and vendors of ready-to-use devices and assays (B2C marketing), and suppliers (biotech, micro/nanotech, high-tech) of different components (B2B marketing) of OoC systems. The latter components can be combined by system integrators into open technology platforms, that can be customized for a specific application. As indicated in this roadmap, both roads for the development of ready-to-use devices and open technology platforms are interconnected. Early interaction with the manufacturing companies in the development process is required for setting up the pilot line and related factory for the production and upscaling of the products. Suppliers of peripheral instrumentation (imaging, electronics, robotics equipment) form another important group of companies, required for the use of OoC in practice and compatibility with laboratory equipment.

— **Industry associations and fora in the MPS field:** Provide a venue for appropriate collaboration and data sharing to facilitate the industry implementation and qualification of MPS models. In particular, the IQ MPS Affiliate is a not-for-profit organization devoted to raising awareness, advancing the science and supporting the implementation of MPS in drug discovery. The North American 3Rs collaboration (NA3RsC MPS Initiative) aims to increase the adoption of MPS technologies through stakeholder engagement. Related organizations include EFPIA (European Federation of Pharmaceutical Industries and Associations) and EPAA (European Partnership for Alternative Approaches

to Animal Testing). Relevant fora for the standardisation of OoC are the SiLA Consortium, Animl (data standards), and SLAS (microplate standards).

4.2.2 End users

End users of OoC models include those industries that adopt the equipment and OoC-based assays to support the development and the regulatory authorization of new medicines or consumer products, such as the pharmaceutical, food, cosmetics and chemical industry. CROs and biotech industry with a fee-for-service model for testing drugs for pharmaceutical companies are also envisioned as end users of test methods based on OoC technologies. Academia use the models for biomedical research, to get insight into the mechanisms of disease or as a basis to propose new therapies. Healthcare providers can use personalized OoC models (patient-on-chip) to define the best treatment for each individual patient.

4.2.3 Governance

Among the governance organizations are the overarching institutions with policy makers, regulatory authorities and standardisation bodies, that have a crucial role in the approval and adoption of OoC for different purposes.

— **Standardisation organizations**: Stimulate and support the development of standards. In the process of OoC standardisation all relevant stakeholders are involved, including developers, suppliers, regulatory bodies and end users. The European committees for standardisation, CEN and CENELEC, established a FGOoC, that is supported by the Dutch Normalization Institute NEN to develop this roadmap for OoC standardisation, followed by the development of the standards defined. The FGOoC and the Organ/Tissue on a Chip (O/ToC) Engineering and Efficacy Standards Working Group at NIST (National Institute of Standards and Technology in the US) are exploring the opportunities to bridge the gap between their efforts in Europe and USA. CEN and CENELEC collaborate with international organizations for standardisation (ISO, IEC, ASTM, ANSI) to align the activities regarding the development of (international) standards.

— **Regulatory or notified bodies and agencies**: Responsible for the authorization of new medicines, devices or consumer products in the respective geographical part of the world for which they are responsible. In Europe, the EMA (European Medicines Agency) is in charge of the evaluation, under the central authorization procedure, and supervision of pharmaceutical products. The European Commission is the authorising body for all centrally authorised product, who takes a legally binding decision based on EMA's recommendation (European Medicines Agency, 2024a). While the majority of new, innovative medicines are evaluated by EMA and authorised by the European Commission in order to be marketed in the EU, most generic medicines and medicines available without a prescription are assessed and authorised at national level in the EU. A medical device may be placed on the market or put into service only if it complies with the Medical Device Regulation or, whenever appropriate, with the In Vitro Diagnostic Medical Device Regulation. The conformity assessment is made, for devices of risk class above the lowest classification class, by notified bodies, i.e. conformity assessment bodies assessed, designated and notified by the Member states (art. 35 MDR), by means of a specific authority ('authority responsible for notified bodies')." Other agencies are responsible for safety of food (EFSA), chemicals (ECHA), food and drugs (FDA (US)), China Food and Drug Administration), or have a broad spectrum of safety management (PMDA - Pharmaceuticals and Medical Devices Agency (Japan)). Some other international organisations (OECD, ICH) are involved in the development of new tools, standards, policy and/or approaches to

assess the safety, quality and performance of regulated products. The FDA Modernization Act and the policy of the EMA 3Rs Working Party emphasize the increasing possibilities for collaboration and interaction between regulators and other stakeholders in the OoC field. A notified body is an organisation designated by an EU country to assess the conformity of certain products before being placed on the market. These bodies carry out tasks related to conformity assessment procedures set out in the applicable legislation, when a third party is required.

— **Qualification and validation centers:** Play an essential role in the independent qualification of OoC to ensure the reproducibility and reliability of a particular model for (regulatory) decision-making. ECVAM and its international partners have an important role in coordinating development, validation and regulatory acceptance of alternative methods and approaches. In Europe, the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) has been established by ECVAM to provide technical support to validation studies, designed to assess the reliability and relevance of alternative methods that have a potential to replace, reduce or refine (3Rs) the use of animals for scientific purposes. In the US, the Texas A&M Tissue Chip validation (TEX-VAL) Consortium have been established to provide a way to test and validate OoC devices, and thereby promote the adoption of this technology by the broader research community.

4.2.4 Information managers

Information managers have a role in creating optimal awareness regarding OoC developments and new results, and in stimulating the use of these models by widespread communication.

— **Editors of scientific journals:** Important actors in the OoC field regarding the publication of research methods and results obtained with animal-free models. A discussion between editors and the scientific community is necessary to explore options to adjust the publication policies, since some journals still require evidence for the research results based on animal experiments in order to justify publication.

— **Repository managers:** Structure the organization of databases containing data about OoC, such as test and qualification results for different applications and context of use, but also specifies on the specific test method and protocol applied. They manage the access and interaction of users with the database and can support in maximizing its value and promoting good reporting and reusing of scientific results.

4.2.5 Funders

Both public and private sources are necessary to fund research groups and start-ups in the OoC field. Among the public funders are the European Commission (EU funding programmes) and national governmental funding agencies (NCATS, research councils), that are becoming increasingly interested in supporting new research approaches. In the private domain charities, health funds, and patient organizations are providing grants for OoC research to advance scientific knowledge and reduce the number of animals used for testing. The necessary capital for technology transfer and production upscaling is provided by Venture Capital firms and Angel investors.

4.2.6 Civil Society

The promise of OoC technology to solve societal challenges, such as a reduction of the number of animal experiments, the improvement of drug development and the identification of effective and personalized treatments has raised the interest of many societal stakeholders. These include animal protection and animal welfare organizations, such as the Humane Society International, but also patient organizations. An important stakeholder is the general lay public that can raise a societal voice to decrease or even replace animal testing and gets quickly excited about the potential of OoC. A careful communication of recent achievements and ongoing developments on OoC models is required to avoid the risk of overselling or overpromising and to keep expectations realistic.

4.3 Interrelationships

In an optimal OoC workflow from idea to use (Figure 2) all actors described above have their specific roles, tasks and responsibilities, and collaborate already from the start to define unmet needs and develop new ideas. End users in particular are essential to prioritize the missing tools and the contexts of their use. Once funding is available, the design, specification, proof of concept and prototype can be performed by researchers from the R&D-Scientific Community. The prototypes can vary from ready-to-use devices to flexible open technology platforms. Alignment between the suppliers of components, system integrators and manufacturers, and collaboration with the suppliers of peripheral equipment, are required to guarantee the scaled production of robust and reproducible fully operational OoCs. The next necessary step is the assessment of the scientific validity of the prototypes. Depending on the context of use, this evaluation will be carried out by the end users themselves, by independent Tissue Chip Testing Centres or by the regulatory authorities. The industrial partners/manufacturers will then start up the pilot production, followed by large-scale production including assembly of the components of the model. The final step is product standardisation and marketing authorization (if applicable), at the regional, national or global level. Feedback from the end user to the developers for improvement or additional functionality of the OoC, or discussion with the other actors about new unmet needs, closes the loop of the circular OoC workflow.

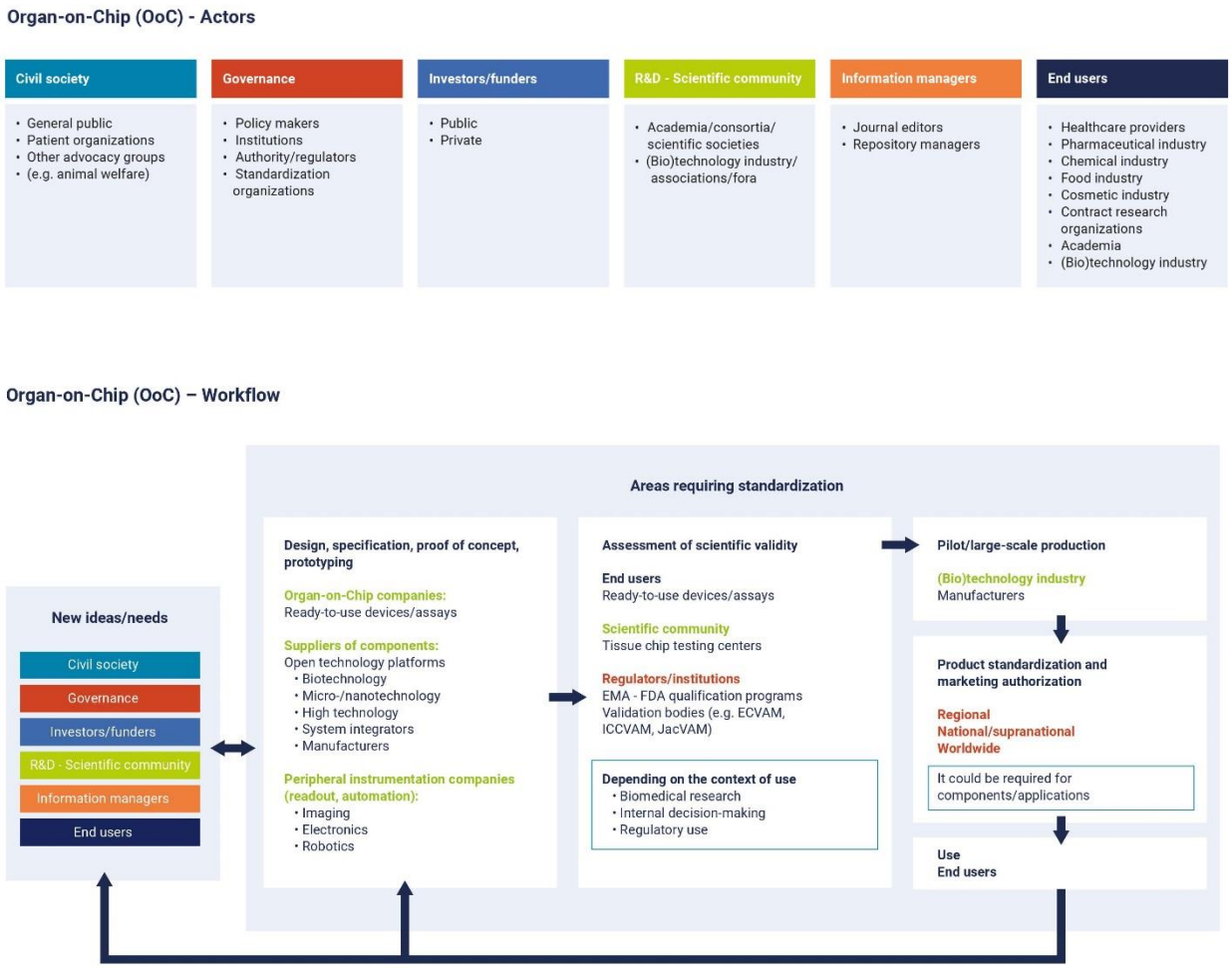


Figure 2 - OoC Actors and Workflow

NOTE The various actors involved in the design, realization and implementation of a successful OoC project are grouped according to their role in the upper part of Figure 2. The lower part of Figure 2 shows a circular workflow where needs and new ideas are proposed and discussed with R&D and the scientific community, leading after a multi-step process to use by end users, who in turn provide feedback to achieve continuous improvement. The realization and implementation of the workflow requires standardisation of the processes involved, which are included here in the light blue area. The extent of standardisation depends on the particular needs and goals of each project.

5 Biosciences

5.1 Introduction

This chapter focuses on the identification of existing standards for biological inputs of OoC models. Biological inputs include aspects related to cell and tissue sources and standards, 3D matrices and 2D coatings to culture cells and biophysical/biochemical cell culture conditions. For all these topics, the WG first gathers ongoing initiatives and/or available standards to be used as starting points, and eventually identify gaps towards the definition of a roadmap. As a general remark, in this chapter the identified topics are approached with an “agnostic” perspective (i.e. not focusing on a specific context of use) in order to provide a broader starting point at the service of other

chapters. The following topics are going to be discussed: cell sources, biomaterials, and cell culture conditions.

5.2 Cell and Tissue sources

The scope of this subtopic is to define and identify possible existing standards for cells used in OoC systems. Topics include, but not necessarily limited to, cell type (primary, pluripotent stem cell, cell line), cell/tissue isolation, cell source (commercial, patient-derived, biobanks etc.), species, quality control (karyotyping, phenotyping, mycoplasma testing), and reporting criteria for the topics. This subgroup primarily focuses on key characteristics of cells that can be determined without knowledge on their use i.e. pre-experimental.

Cells used in OoC devices are a key source of variability in device performance. Initially, OoC devices were used with immortalised cell lines but with advances in stem cell technology, primary cell sources are widely applied for increased physiological relevance along with donor-to-donor differences inherent to human samples. As the use of physiologically relevant cell sources increase, it is imperative that the appropriate quality control criteria and sufficient information on the cells are reported for the appropriate evaluation of results obtained from OoC devices.

Several standards for the use of cells in vitro have been identified. Despite these standards being available, it is often not clear if they are applied when used in OoC systems. Moreover, at present, there is no single official standard covering all cell sources or all aspects of good cell culture practice. Moreover, different cell types will require very specific functional assays for validation. Therefore, an exhaustive guide towards standardisation of cells used in OoC systems is impractical. However, it is highly desirable that appropriate standards are followed, and minimum reporting criteria are incorporated when describing cell used in OoC systems. This will provide reviewers and regulatory authorities the necessary information for appropriately evaluating the biological results obtained from OoC devices.

The key finding is the lack of a unified standard document on the different cells used in OoC devices. The definition of a future set of standards covering all aspects of cell (pre-experimental) including quality control procedures and minimum reporting guidelines are recommended. Some of these recommendations, based on the currently available standards, are described in 5.2.1.

5.2.1 Definitions

Cell sources used in OoC devices include pluripotent stem cells (PSC), multipotent stem cells, tissue slice cultures, primary cells, immortalised cells, and commercial cell lines (Wnorowski et al., 2019).

- **Pluripotent stem cells (PSC)**: PSCs are undifferentiated cells of embryonic or somatic origin that can differentiate into cells of the three embryonic germ layers (ectoderm, mesoderm, and endoderm) and have self renewing capacity (Romito & Cobellis, 2016). There are various states of pluripotency that resemble different states of the embryo, not just one. It is important to demonstrate what qualitative and quantitative assays have been performed along with the markers that are used to demonstrate the state of pluripotency of the cells. Induced pluripotent stem cells (iPSCs) are a particular type of pluripotent stem cells obtained from the conversion of somatic cells (such as skin cells) into embryonic-like cells through a process known as reprogramming.

- **Multipotent stem cells**: (e.g. blood stem cell, mesenchymal stem cell). These are undifferentiated cells considered to be able to self-renew and differentiate into all cell types within one lineage (Khanlarkhani et al., 2016).

- **Tissue slice cultures**: Organotypic tissue slice culture systems represent in vitro cultures of explants of patient- or animal-derived tissues – normal/pathology associated (e.g. tumours) (He & Deng, 2022).

- **Primary cells:** These are cells freshly isolated directly from living organisms and maintained from growth in vitro. They enable researchers to study the morphological, cellular, and functional behaviour of the tissue. Standard protocols have been established to isolate epithelial, endothelial (and endothelial progenitors (EPCs), fibroblasts, and immune cells from human, mouse, and rat tissue. Primary cells should be characterised according to best practice (e.g. For Mesenchymal Stem Cells– International Society for Gene and Cell Therapy (ISGCT) guidelines (Dominici et al., 2006))

- **Immortalised cells (Derived in house)**: They derive from human or animal sources and have been manipulated to proliferate indefinitely in vitro and can thus be cultured for long periods of time. This immortalisation can be induced through genetic engineering or can be because of isolation from sources that are chromosomally abnormal or that carry mutations that enable continuous cell division. For reproducibility, it is important that cell line identity is reported according to best practice (ANSI, 2022).

The following should be considered when using cells:

- A **master cell bank** (an aliquot of a single pool of cells that has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers, and stored under defined conditions) should be generated prior to experimental work.

- When setting up a biobank, **Standard Operating Procedures** (SOPs) should be used in all aspects of the biobanking process, from procurement, shipment and safety to processing and storing. The key aspects to consider when setting up a biobank are discussed in ISO 20387:2018.

- Each cell source has **different requirements for validation** which should be considered (see standards in 5.2.2.).

5.2.2 List of available standards

Several standards are already in place that outline the need and provide guidance for standardisation of cells used for in vitro studies. However, the available standards do not always cover all cell sources described above and are not directly related nor directly referring to OoC. A full list of available standards is presented in Annex B.

- **Good In vitro Method Practices (GIVIMP)**(OECD, 2018)

In the past several decades, there has been a substantial increase in the availability of in vitro test methods for evaluating chemical safety in an international regulatory context. To foster confidence in in vitro alternatives to animal testing, the test methods and conditions under which data are generated must adhere to defined standards to ensure resulting data are rigorous and reproducible. Good In vitro Method Practices (GIVIMP) for the development and implementation of in vitro methods for regulatory use in human safety assessment aims to help reduce the uncertainties in cell and tissue-based in vitro method derived chemical safety predictions. GIVIMP provides guidance for test method developers and end users of resulting data on key elements of in vitro methods. GIVIMP tackles ten important aspects related to in vitro work: (1) Roles and

responsibilities, (2) Quality considerations, (3) Facilities (4) Apparatus, material and reagents, (5) Test systems, (6) Test and reference/control items, (7) SOPs, (8) Performance of the method, (9) Reporting of results, (10) Storage and retention of records and materials.

GIVIMP(OECD, 2018) is a guidance document from the OECD for test method developers and end users of resulting data on key elements of in vitro methods. The cell and cell sources are not the key focus of this guidance document. However, as a part of the Annex, GIVIMP provides guidance on good cell culture practice for cells in general and for stem cells and stem cell-derived models

— **ISSCR guidelines for stem cell research and clinical translation** (International Society for Stem Cell Research, 2023)

The international society for stem cell research identifies quality standards and outlines basic core principles for the use of tissue and pluripotent stem cells. The standards initiative from ISSCR aims to define standards for basic, preclinical, and clinical research. As of August 2023, the ISSCR standards cover basic and preclinical research while the standards for clinical research are under development. These guidelines only cover stem cells and not all cell sources that may be used in OoC devices.

This document identifies quality standards and outlines basic core principles for the laboratory use of both tissue and pluripotent human stem cells and the in vitro model systems that rely on them. Overall, the emphasis of this document is creating a set of recommendations that, when taken together, establish the minimum characterization and reporting criteria for scientists, students, and technicians in basic research laboratories working with human stem cells.

— **Guidance on Good Cell Culture Practice (GCCP)**(Price A & Coecke S, 2011)

The GCCP is a chapter in Cell Culture Techniques that addresses issues related to cell culture including quality assurance; recording and reporting; safety, education, and training; and ethics. This guideline is not from a specific standardisation body but covers broad aspects related to cell culture. The first guideline published in 2011 was then updated (GCCP 2.0)(Pamies et al., 2022) to incorporate recent advances.

The use of various in vitro systems is expanding dramatically not only in basic research, but also to meet regulatory requirements for chemicals and products of various kinds. Further significant developments are certain to result from the use of in vitro systems for high throughput screening in pharmacology and toxicology. Because the maintenance of high standards is fundamental to all good scientific practice, and is essential for maximising the reproducibility, reliability, credibility, acceptance and proper application of any results produced that guidelines has been developed to define minimum standards in cell and tissue culture, to be called GCCP. The scope of the document has been broadly defined, to include systems based on cells and tissues obtained from humans and animals, and issues related to the characterisation and maintenance of essential characteristics, as well as quality assurance; recording and reporting; safety, education and training; and ethics. This GCCP Guidance lists a set of six principles intended to support best practice in all aspects of the use of cells and tissues in vitro, and to complement, but not to replace, any existing guidance, guidelines or regulations.

— **Guidelines for the use of cell lines in biomedical research** (Geraghty et al., 2014)

Like the GCCP above, this guideline is a scientific publication that addresses several aspects of cell culture and provides advice on legal and ethical requirements for cells. However, this guideline is solely aimed at cell lines and does not cover other cell sources.

Cell-line misidentification and contamination with microorganisms, such as mycoplasma, together with instability, both genetic and phenotypic, are among the problems that continue to affect cell culture. Many of these problems are avoidable with the necessary foresight, and these Guidelines have been prepared to provide those new to the field and others engaged in teaching

and instruction with the information necessary to increase their awareness of the problems and to enable them to deal with them effectively. The Guidelines cover areas such as development, acquisition, authentication, cryopreservation, transfer of cell lines between laboratories, microbial contamination, characterisation, instability and misidentification. Advice is also given on complying with current legal and ethical requirements when deriving cell lines from human and animal tissues, the selection and maintenance of equipment and how to deal with problems that may arise.

— **Biobanking**

Scientific research using cell lines has contributed greatly to the understanding of human health. Cell cultures are increasingly used to complement studies using animal models. Although cell lines are important research tools, potential problems have recently been identified. Cell lines have unique characteristics and behaviour that can change as they continue to be passaged. The original phenotype (e.g. expression of specific biomarkers) can be lost or new characteristics or behaviour (e.g. development of tumorigenicity) may develop. It is important to minimize passaging to retain the original characteristics that were present when the cell line was first established. Other problems such as contamination, either with microorganisms or another cell line, and misidentification can also arise. Cultures can become contaminated during cell line establishment or later when cultures are passaged. These problems are often not visible by eye and require specific testing to be detected. To help address these issues, the research community has called for an international effort to create standards for biobanks. The ISO/TC 276 relates to the standardisation in the field of biotechnology processes. The topics include biobanks, bioresources, bioprocessing, analytical methods, and data validation and integration. In this technical committee needs and gaps in standardisation are identified regarding these topics in the field of biotechnology. ISO 20387:2018 was published to provide an overarching standard for biobanks. ISO 21709:2020 provides additional technical specifications for biobanks that handle mammalian cell lines. Such biobanks can demonstrate their competence in biobanking by complying with the specifications within this document, in addition to the requirements prescribed in ISO 20387.

ISO 21709:2020 aims to meet the current demand for standardized PSC procedures of biobanks and builds on international consensus agreed by PSC resource centres. This document specifies the establishment, maintenance, characterization, storage and distribution requirements for mouse and human PSCs, providing a general guideline for both biobanking and fundamental research of PSCs. See also ISO 24603:2022.

5.3 Biomaterials

The scope of this subtopic is to identify the most used **type of biomaterials** for OoC, focusing on both 3D matrices (i.e. scaffolds and hydrogels) and 2D coatings.

For each type of biomaterial, **critical characteristics** (e.g. biocompatibility, fabrication method, mechanical properties, architecture, biochemical properties, compatibility with OoC/substrate) are listed and briefly described. As a deeper level of detail and to provide examples, for each type of biomaterial, **specific examples are listed and briefly described, classified according to biomaterial origin**: i.e. synthetic, natural, hybrid and decellularized or cell/tissue-derived matrices.

There is growing appreciation of the role that the extracellular environment plays in regulating cell behaviour. Mechanical, structural, and compositional cues, either alone or in concert, can drastically alter cell function. Biomaterials have been developed and implemented to present defined subsets of these cues for investigating countless cellular processes as a means of understanding morphogenesis, aging, and disease (Caliari & Burdick, 2016). Extracellular matrices (ECM) not only provide the necessary physical support, tensile strength and scaffolding

for cells, but also serves other functions such as presenting bioactive signals to cells and acting as a reservoir for growth factors and other soluble factors to govern cellular fate processes including adhesion, attachment, proliferation, differentiation and apoptosis. In vivo, cells attach to proteins and carbohydrate moieties present in the ECM. Once cells are isolated from tissue and removed from the native matrix, differentiated cells rapidly lose important characteristics when cultured without an adequate supportive microenvironment such as a substrate coating or a feeder layer. It has been shown that in culture cells growing on ECM demonstrate enhanced proliferation and differentiation potential.

Significant advances in biomaterials have offered great opportunities to facilitate the construction of tissue/organ model systems with higher fidelity by integrating with microscale technology and stem cell biology. A range of biomaterial systems have been developed toward this goal, and among them the most interesting in the OoC field include **hydrogels, scaffold and 2D coatings.**

Main gaps have been identified for hydrogels (mechanical properties, architecture, degradation, crosslinking, chemical composition, sterilization). The possibility to introduce a hydrogel precursor in a OoC setup and to perform its crosslinking in-site is a critical parameter when planning the use of hydrogels for OoC. Also upon crosslinking and integration within the system, stability of the hydrogel may be affected by the microscale and the presence of physical stimuli in the system (e.g. the hydrogel may have to resist to fluid flow). All these parameters should be considered and may require adjustment to standard protocols applied optimized for different applications (e.g. described in ASTM F2150-19 for regenerative medicine applications).

Regarding scaffold, many individual scaffold materials are well described and characterised, with some relevant standards in place. A critical aspect that has not been addressed is the impact of integration into a system with fluidic shear force and mechanical stress. This may also impact absorption of molecules and the ability to test biological function or toxicity of agents.

Functional 2D coating are well characterized in literature. For example, coating of polystyrene is well established as most widely used substrate for 2D cell culture (as reviewed by (Lerman et al., 2018)) (even if we could not find any available standards). Nevertheless, conclusions cannot be drafted regarding the use of coating within OoC without understanding which materials are used to fabricate OoC (indeed protocols to perform functional coating may be or not available and reproducible for different materials). Efforts will need to be addressed in translating available protocols for coating macroscale substrates into miniaturized setup (with the hypothesis that OoC will be mainly fabricated with materials for which functionalization protocols are available).

5.3.1 List of available standards

- ASTM F2739 – 19 Standard Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds
- ASTM F2150-19 Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products
- ASTM F2038-18 Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications Part I & II Formulations and Uncured Materials
- ASTM F2315-18 Standard Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels
- ASTM F748-16 Standard Practice for Selecting Generic Biological Test Methods for Materials and devices
- ASTM F3142-16 Standard Guide for Evaluation of in vitro Release of Biomolecules from Biomaterials Scaffolds for TEMPs

— ASTM F3354-19 Standard Guide for Evaluating Extracellular Matrix Decellularization Processes

5.3.2 Hydrogels

Hydrogels are water-swollen networks of polymers. Hydrogels have emerged as a promising option for cell culture since they mimic salient elements of native extracellular matrices (ECMs), have mechanics similar to those of many soft tissues, and can support cell adhesion and protein sequestration. Hydrogels can be broadly classified as either natural (e.g. collagen, fibrin, alginate, matrigel), synthetic (e.g. polyacrylamide, polyethylene glycol) or hybrid materials (e.g. hyaluronic acid, polypeptides). Of note, a recent review discussed the application of hydrogels in OoC (Liu et al., 2019). In the following section, we reported considerations on the selection of hydrogel for OoC applications, based on analysis performed on the state of the art and available standards. Although characterization of hydrogels requires consideration of the individual composition and application the hydrogel will be used for, there are common generic requirements that can be summarised as follows.

— **Mechanical properties**

Hydrogel mechanical properties are important for the stability of the material in culture and may also influence cellular mechanotransduction, which in turn has consequences for cellular behaviours like spreading, migration, and stem cell differentiation. Comprehensive reviews of hydrogel mechanical characterization techniques are available in literature (Oyen, 2014). While mechanical properties of hydrogels are well described in a research setting, especially for commercially available hydrogels, how mechanical properties of hydrogels are changing within OoC setup is not yet systematically addressed.

— **Architecture**

The mesh size, or molecular porosity, of the hydrogel is typically on the nanometer scale and can influence nutrient flux throughout the matrix. It is correlated to hydrogel swelling behavior and mechanical properties, since lower swelling and higher modulus indicate a smaller mesh size. Details on the characterization of hydrogel swelling ratio and mesh size can be found in several research papers (Peppas et al., 2000). Still characterization of this aspect is rather fragmented and highly dependent on the specific type of hydrogel. A systematic way to characterize architecture, particularly related to the experiment requirements, is missing. Notably, this parameter may be cause of high experimental variability.

— **Degradation**

Hydrogel degradation can lead to changes in mechanics and swelling over time, which in turn affect cell behaviors such as motility, spreading, and traction force generation. Hydrogels typically degrade through either hydrolytic or enzymatic mechanisms, where hydrolysis occurs throughout the entire hydrogel and enzymatic degradation is local to the presented enzyme. It is important to note that even hydrogels that would be considered nondegradable on the time scale of most cell experiments, may eventually degrade. Degradation and relative method to measure it are well described in available standards applied to a different application (e.g. ASTM F2150-19). We anticipate that application in OoC setup may alter this parameter due to the different sizescale and related time course of phenomena. We envision necessity of additional work to fill this gap.

— **Crosslinking method**

Forming hydrogels for cellular experiments typically involves either encapsulation of viable cells within the material or fabrication of substrates using molds that are later seeded with cells (the latter point is covered in the functional coating section). Hydrogel formation involves the

transition of liquid precursor solutions into solid materials, which can be achieved using either physical (noncovalent) or chemical (covalent) crosslinking to assemble the hydrogel components. The chosen crosslinking strategy can have a significant impact on cell viability. It is important that the polymerization time and reagents be designed so that cell encapsulation occurs in a cytocompatible manner. Gelation also needs to occur fast enough to prevent the settling of cells during the encapsulation process. Kinetics of formation and relative methods to characterize it are well described in available standards applied to different applications, i.e. tissue engineering (ASTM F2150-19) and medical devices (ASTM 2038-18 Part I). Also, standards referring to specific hydrogels are available (e.g. ASTM F2315-18 for alginate). However, it is anticipated that application in OoC setup may alter this parameter due to the different size scale and related time course of phenomena. We envision necessity of additional work to fill this gap.

— **Biocompatibility**

Biocompatibility is linked to the kinetics of formation and degradation described above, and not only to the material itself. The ASTM F2739–19 (Standard Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds), describes test methods used to quantify cell viability and related attributes on non-porous or within porous hard or soft 3D synthetic or natural-based biomaterials, such as ceramics, polymers, hydrogels, and decellularized extracellular matrices. The test methods also apply to cells seeded on porous coatings. Thus, this standard covers all the types of biomaterials described in this chapter. It is anticipated that application in an OoC setup would not drastically alter this parameter, thus defining a good starting point.

— **Biological properties**

Some materials interact with cells through integrin–ligand interactions (for example, collagen, fibrin, polypeptides) or other cell surface receptors (for example, HA), while others are considered more inert (for example, PEG, polyacrylamide). Biological properties and relative method to characterize it are well described in available standards applied to a different application (e.g. ASTM F2150-19). We anticipate that application in an OoC setup would not drastically alter this parameter, and application in regenerative medicine is anyway more stringent in terms of biological properties and compatibility thus defining a good starting point.

— **Chemical composition**

Chemical composition of hydrogels is a key parameter that defines previously cited characteristics and strongly influence reproducibility. While natural matrices, like Matrigel, may lead to the low reproducibility of engineered tissues/organs (given to a poorly reproducible and defined composition), chemically defined hydrogels can serve as suitable matrices to improve the reliability of chip-based tissue models by more precisely controlling the matrix composites in cellular microenvironment. This parameter is crucial and poorly described in literature, especially related to OoC applications.

— **Sterilization**

For cell encapsulation, the precursor solutions must be sterilized before hydrogel formation. Attention should be paid in choosing a technique that will not degrade, denature, or otherwise alter hydrogel physical properties. Sterilization protocols and related issues are well described (even if we could not find standards available) for some commercially available hydrogels, which are typically provided pre-sterilized or may include specific sterilization instructions. A review provides insights on conventional and emerging technologies for hydrogels sterilization (Bento et al., 2023). The ASTM standard F2038-18 Part II on Silicone Elastomers, Gels, and Foams Used in Medical Applications partially addresses sterilization of gels, but without a direct focus only related to application in medical device and not including cell-laden hydrogels, which are the most relevant for OoC applications. Overall, description of how to maintain hydrogel sterility also in the

presence of cells (i.e. non impacting biocompatibility) and or to translate sterility protocols to OoC setups is not well covered in the state of the art.

5.3.3 Scaffolds

Scaffolds can be defined as natural or synthetic biomaterials that possess characteristics appropriate for replacement of extracellular matrix (ECM) in 3D, including mechanical and biochemical features that support cell adhesion, polarisation and phenotype (Osório et al., 2021). Surface treatment or cell patterning techniques are employed to influence adhesion/proliferation/differentiation/migration of cells, which can also be spatially segregated where appropriate. Scaffolds can be broadly classified as either natural (e.g. based on collagen, chitosan, alginate, gelatine, decellularized matrix), synthetic (e.g. polycarbonate, ceramic, silicon based organic polymer Polydimethylsiloxane) or hybrid materials. In the following section, considerations of the key characteristics that should be considered when choosing a scaffold for OoC applications (based on analysis performed on the state of the art and available standards) are reported.

— **Biocompatibility**

For general biocompatibility considerations, see section 5.3.2 Hydrogels. Of note, many of the materials used for scaffolds are also used for the fabrication of consumables in research and medical devices, and have been subject to biocompatibility testing and certification, as described in available standard ASTM F748-16. The potential for batch to batch variation, along with presence of poorly defined animal-derived aspects (where relevant) should be considered. Moreover, there is a series of standards setting out critical requirements and associated test methods for materials and matrices used in scaffolds, e.g. alginate, chitosan salts and hyaluronan. For example, ASTM F3142-16 is a Standard Guide for Evaluation of in vitro Release of Biomolecules from Biomaterials Scaffolds for TEMPs.

— **Mechanical properties and architecture**

ASTM published two standards on Silicone Elastomers, Gels, and Foams Used in Medical Applications (ASTM F2038-18 Part I & Part II) to address formulation and the fabrication process. ISO standards are available for all the main plastics used with moulding and extrusion processes e.g. polycarbonate, PMMA, polystyrene, polyethylene, acrylonitrile–butadiene–styrene and polypropylene. Surface treatment or cell patterning techniques can be employed to influence adhesion and phenotype of cells and facilitate spatial segregation where appropriate. While mechanical properties of scaffold are well described in existing standards, how mechanical properties may change within OoC setup is not yet systematically addressed. Elasticity; oxygen permeability, etc must be tuneable to characteristics of the target organ and adapted to the microscale.

— **Biochemical properties**

Absorption of molecules circulating in the medium can compromise accuracy of the results. This has been studied in relation to PDMS, but may apply to several scaffolds of both natural and synthetic origin. To this regard, no standards exist in terms of test methods, suitable measurement units and performance criteria(Piergiovanni, Leite, et al., 2021). This is particularly relevant in toxicity or efficacy studies, where the effective concentration of a test compound is crucial to accuracy of results on the biological response. Small molecules can also be sequestered by coatings or bind to proteins/lipids in the medium.

— **Sterilization**

For general sterilization considerations, see section 5.3.2. Hydrogels.

— **Decellularized based scaffold**

As an additional discussion for scaffolds, specific missing points for decellularized based scaffold (dECM-based scaffold) should be considered. The preparation of dECM-based biomaterials consists of two main steps, including the decellularization of a tissue or organ and terminal sterilization of the dECM, respectively, and both steps are highly effective in obtaining a biomaterial with the desired properties. DECM-based biomaterials encompass mixtures of various biomolecules that regulate cell adhesion, proliferation, migration, and differentiation, such as glycosaminoglycans, adhesion proteins (i.e. laminin, integrin), and structural proteins (i.e. collagen). Therefore, the selected decellularization and sterilization methods should have a minimal negative impact on the biochemical and morphological composition as well as mechanical properties of the decellularized matrix, as described both in literature (Yildiz et al., 2007) and in available standard ASTM F3354-19.

5.3.4 2D Coatings

Functional coatings for cell culture are structural proteins / protein-like substances that have adherent capabilities and increase cell-substrate interactions in a culture dependent environment. Biodegradable synthetic polymers such as poly-L-Lysine have been used to provide coatings that promote the attachment of various anchorage dependent cell types. Natural polypeptide-based cell attachment factors such as collagen and fibronectin have been effectively utilised for culture in certain cell lines and primary cell culture. A guide to the composition and functions of the extracellular matrix is provided by (Karamanos et al., 2021). Of note, an explicit focus on the precise documentation of coating details (i.e. coating components, amount, volume of coating, coating period, washing steps, storage after coating) is essential to ensure reproducibility in general and thus also in OoC applications.

In the context of coatings, it is important to also consider the use of surface treatment to prevent cell attachment like for spheroids formation. Here, examples of **cell-repellent coating** can be found in large scale bioreactors and medical devices. In this context, multiple strategies have been adopted like PEG coatings, Pluronic F-127, certain hydrogels or fluorinated polymers. Note that in the choice of a specific coating will depend on the application, the type of cell used and the length of the expected culture in the system as well as the possibility to affect biomolecular adsorption of the drug tested.

The most important techniques are wet chemical coating, electrospinning, dip-coating and spin-coating as physically coat substrates (Song et al., 2020); plasma treatment to create a range of hydrophilic surface finishes that enhance cell adhesion (North et al., 2010; O'Sullivan et al., 2020); Layer by-layer (LbL) assembly (Fukuda et al., 2018; Matsusaki et al., 2012).

For general biocompatibility considerations, see section 5.3.2 Hydrogels.

5.4 Cell culture conditions

The goal of this subtopic is to identify and share existing standards on the use of media for OoC culture, focusing on media composition, antibiotic and growth factor use. It is intended to be used as a guide for researchers who are willing to deepen their understanding on OoC culture. Regarding the media composition, the level of definition of the media will be considered (i.e. defined, undefined, semi-defined), as well as the use of animal-free reagents, specific growth factors and antibiotics.

Moreover, state of the art on different culture conditions within the OoC field are analysed, with a focus on environmental conditions, physical stimuli, scaling and waste accumulation.

Of note, given the very novel nature of the OoC technology, to date not many standards or guidelines have been published. Hence, this sub-topic has been drafted with the aim of creating a checklist/guideline to be followed when OoC experiments need to be set up.

5.4.1 Cell culture media

Cell culture media for differentiation purposes are not yet standardised and laboratories developing cell culture models generate daily their own "homebrew" formulations based on what was successful in their hands. The result is that multiple different formulations have been utilised and published for the same cell type or organoid model. While multiple medium formulations may support growth of the same model, its phenotype and/or genotype may be altered when changing media. Standardisation on this topic is challenging because of the vast variety of media that are usually designed on the basis of the different cell culture conditions.

OoC may be composed of single or multi-cell cultures. Depending on the number and type of cells present, the culture medium may vary. Critical parameters such as viability and functional phenotype for each cell type should be considered.

Owing to the complexity and requirements of specialised and non-specialised cells there is, as of today, no common medium that suits all culture conditions.

Documentation is important to ensure reproducibility and standardisation in the OoC field.

— Based upon single type or multi cell type cultures, media specific for certain cell type is often combined. The used ratios should be documented.

— Documentation of used commercial media, batch and lot numbers

— If homemade media are used, consistent and detailed documentation is required including batch and lot numbers of individual components, (complete) medium storage, storing temperatures and preparation

While individual components can be well described, the combination of different media is not standardized. Medium standardisation is a challenging issue, as the development of the field will quickly outgrow these standards and might hamper innovation. However it is highly recommended that media compositions are correctly and systematically addressed in the OoC field to maximize innovation.

— **Media composition**

Mammalian cell cultures require specialised media. Culture medium is a liquid nutrient consisting of a mixture of base medium, serum and regulatory factors.

The three basic classes of media are basal medium, reduced-serum medium and serum-free medium, which differ in their requirement for supplementation with serum. Serum, such as foetal bovine serum (FBS), is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. However, using serum in media has several disadvantages including high cost, specificity, variability between suppliers, and unwanted effects such as stimulation or inhibition of growth and/or cellular function on certain cell cultures.

The majority of cell lines grow well in a **basal medium**, supplemented with bovine serum, subject to batch and source variability, or with an alternative chemically defined additive. Liquid and powder forms are available from various suppliers, examples include Minimal Essential Medium (MEM), Dulbecco's MEM (DMEM), Roswell Park Memorial Institute (RPMI) 1640 amongst others. These media contain carbohydrates, salts, vitamins, amino acids and a pH buffer system (Gruber & Jayme, 1994; Ham, 1982; D. W. Jayme & Blackman, 1985).

Many mammalian cell lines can be continuously maintained on a relatively simple medium such as MEM supplemented with serum, and a culture grown in MEM can probably be just as easily grown in DMEM or Medium 199. However, when a specialised function is expressed, a more

complex medium may be required. Information for selecting the appropriate medium for a given cell type is usually available in published literature and may also be obtained from the source of the cells or cell banks.

If there is no information available on the appropriate medium for a specific cell type, it is preferable to choose the growth medium and serum empirically, or test several different media for best results. In general, a good place to start is MEM for adherent cells and RPMI-1640 for cell suspensions.

- **Reduced-serum media** are basal medium formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.

- **Serum-free medium** (SFM) circumvents issues with the use of animal serum by replacing the serum with appropriate nutritional and hormonal formulations. Serum-free medium formulations exist for many primary cultures and cell lines, including recombinant protein producing lines of Chinese Hamster Ovary (CHO), various hybridoma cell lines, the insect lines Sf9 and Sf21 (*Spodoptera frugiperda*), and for cell lines that act as hosts for viral production (i.e. 293, VERO, MDCK, MDBK). One of the major advantages of using serum-free medium is the ability to make the medium selective for specific cell types by choosing the appropriate combination of growth factors (Brunner et al., 2010; D. Jayme et al., 1997). Information for selecting serum-free media is available at www.fcs-free.org (van der Valk, 2022).

5.4.2 Use of antibiotics in OoC cell culture

Contamination from bacteria, fungi, mycoplasma and yeast are a serious and frequent issue in cell culture that has to be avoided. This can lead to false results, and at some stage they can irreversibly and completely destroy the cells in culture. The physiological temperature and humidity in the incubator, as well as the nutrients in the medium, provide excellent conditions for the growth of contaminating microorganisms. This can be prevented by adding antibiotics and anti-mycotics to the cell culture media. However, in order to guarantee reliability and reproducibility of cell culture findings, the use of antimycotic- and antibiotic-free culture media is recommended (Farzaneh, 2021; Hassan & Ahmad, 2020). In vitro properties of cells including their proliferation, genetic stability, differentiation or survival, have been shown to be altered by these compounds.

Moreover, genome-wide analyses have identified antibiotic-induced changes in gene expression and regulation (Figure 3).

Beta-lactam antibiotics (penicillin)	▸ Alteration in protein synthesis ▸ Inhibition of cell proliferation
Aminoglycosides (streptomycin, kanamycin, gentamycin)	▸ Reduction of protein synthesis ▸ Decreased ATP levels ▸ Cytotoxicity
Polyene macrolides (amphotericin B/fungizone, nystatin)	▸ Cell membrane disturbance ▸ Cytotoxicity
*Note: These changes are dosage and cell type dependent.	

Figure 3 - Effects of antibiotics on cell cultures (Kuhlmann, 1995)

Other studies have demonstrated that adding Primocin® to the tissue washing solution of patient-derived organoids, is able to eliminate the risk of microbial contamination in cultures, and that the use of Pen/Strep negatively impacts organoids growth (Marinucci et al., 2021).

Although performing studies with antibiotics may be advantageous in terms of contamination and cell survival, these additional molecules within the medium may affect cell response. Hence, during experiments aimed at determining the effect of a particular drug molecule, it should be evaluated whether the use of antibiotics affects cell response to the therapy. If it does, the study should be performed without antibiotics to obtain the most appropriate and reliable readout from the OoC.

5.4.3 Testing for Contamination

While contamination with microorganisms such as bacteria or fungi is immediately noticeable with a microscope, contamination with other microorganisms such as mycoplasma can remain undiscovered, if not specifically tested for. Mycoplasma-contaminated cell cultures are rather infrequent but may show undesirable functional changes in experiments. For the testing of such microbes, various companies offer kits based on PCR or ELISA.

Cross-contamination, which is the unwanted introduction of foreign cells into an existing culture, and cell line misidentification are also problems that must be taken seriously (Almeida et al., 2016; American Type Culture Collection Standards Development Organization Workgroup ASN-0002, 2010; Cabrera et al., 2006). If it remains undiscovered, a researcher could work with an entirely different cell line than the initial one without noticing, which renders all results of cell culture assays invalid. In several papers it has been shown that the penicillin–streptomycin (Pen/Strep) cocktail inhibits the sphere-forming ability of cancer cells in suspension culture, though it has no impact in monolayer culture. This effect is correlated with a significant decrease of cancer stem cells, which hold self-renewal potential.

Detecting microbial and viral infections, including mycoplasma, in cell cultures is crucial for maintaining the integrity of experiments and ensuring reliable results. No standards have been identified in what concert contamination with microorganisms, however general considerations

have been proposed to ensure a proper evaluation and prevention of contamination in culture media.

- Regular monitoring: Perform routine testing to monitor for contamination regularly.
- Quality control: Utilize positive and negative controls in testing procedures.
- Regular and thorough testing is essential for early detection and mitigation of microbial and viral contaminations.
- Commercial services: Consider outsourcing to specialized laboratories for comprehensive testing. It is crucial to tailor the detection methods based on the specific requirements and characteristics of the cell culture system.

5.4.4 Environmental conditions

Environmental conditions in a culture provide the physical and chemical parameters intended to allow the cells to be cultured in the best and/or more physiologically-relevant conditions, which can be replicated in vitro. Thus, they should be adjusted according to the needs of the cultured cells (primary, immortalised, human or animal cell lines). The parameters under consideration are:

- **Gas Composition**

The design of OoC materials should reflect the varying oxygen permeability seen in different organs. This not only helps replicate the organ's natural environment but also ensures accurate cellular responses. Importantly, foaming of a medium that contains serum or proteins (e.g. albumin) refers to the formation of bubbles or foam in the liquid culture medium. This can occur due to various reasons, such as excessive agitation, introduction of air during handling, or the presence of surfactants. In cell culture, particularly when using serum-containing media, excessive foaming can be problematic as it may introduce air bubbles into the culture, potentially leading to mechanical stress on cells or affecting the reliability of experimental results. Therefore, it will be important to minimize bubbling of gas into the media, especially during OoC experiments, to prevent foaming, air bubble introduction, and potential damage to cells. If needed, it may be necessary to use anti-foaming agents.

- **pH**

Different organs exhibit distinct pH environments. In OoC, it is crucial to dynamically control pH, replicating the diverse pH conditions observed in physiological and pathological states. It will therefore be important to establish dynamic pH control strategies, considering the variations across different organs and disease states. Part of this is to adjust bicarbonate buffer concentration and CO_2 levels accordingly. OoCs cultured outside traditional incubators may lack precise control over CO_2 levels. Using CO_2-independent buffering systems like HEPES will ensure stable and physiologically relevant pH conditions.

- **Temperature**

Physiological relevance is vital for OoC experiments to accurately mimic in vivo conditions. Careful temperature control, whether inside or outside an incubator, is necessary for meaningful results. Therefore, it is essential to ensure that OoCs are kept at 37°C or a physiologically-relevant temperature, either inside an incubator or using auxiliary equipment when cultured outside.

- **Humidity**

The high volume-to-area ratio in OoC systems can lead to rapid evaporation in static cultures. Managing humidity or opting for perfusion helps counteract this effect, ensuring stable culture conditions. Evaporation challenges in static OoC cultures can be addressed by maintaining high humidity environments or considering perfusion to ensure a constant supply of media. The unique microscale environment of OoCs requires special attention to the volume-to-area ratio. Strategies should be employed to maintain consistent humidity levels, particularly in static cultures. It should be recognized that the high volume-to-area ratio in OoC systems could lead to rapid evaporation in static cultures, and measures should be implemented to counteract this effect.

— **Physical stimuli**

In replicating physiological conditions within OoC platforms, standardizing mechanical and electrical stimuli is paramount. Mechanical forces, such as shear stress and compression, crucial for tissue development, present challenges in terms of compatibility with real-time imaging and scalability. Despite various technological solutions, the absence of specific standards hinders result comparison and clinical relevance assessment. To address this, future efforts should prioritize the development of standardized guidelines, facilitating the use of well-defined and clinically relevant mechanical and electrical stimuli in OoC studies.

— **Perfusion circuits**

Perfusion circuits are integral to OoC devices, yet the lack of specific standards poses challenges in implementing and maintaining these systems. Whether unidirectional or bidirectional, recirculating or single-pass, standardisation is crucial for ensuring reproducibility and reliability. Specific guidelines should address key considerations like medium replenishment, waste removal, and challenges associated with distinct perfusion strategies. The absence of comprehensive standards highlights a critical area for future exploration within the OoC community, aiming to enhance the standardisation and reliability of experiments involving perfusion circuits.

— **Scaling**

Extreme miniaturisation of in vitro organ and OoC models, without appropriate scaling, can cause significant structural reorganisation and changes in organ proportions, and this is particularly important for toxicity and drug screening assays, metabolic studies and PK/PD modelling. However, scaling remains a significant challenge: the size of the organ, the flow and shear in each organ module and the total volume of medium must all be scaled to physiological dimensions. Disproportionately scaled multi-OoC devices do not properly replicate organ–organ interplay and affect the residence time of medium in the recirculation, thus introducing bias into the experimental outcome. Currently there are several techniques that have been adopted to determine the best scaling processes: direct, allometric, multifunctional and residence time-based scaling, each with its own advantages and disadvantages. Nevertheless, none of them correctly emulates all the in vivo features in mini-organ models (Leung et al., 2022). Hence, the aim of the experiment will determine the type of scaling to be adopted.

— **Waste accumulation**

In vitro, every cell type needs a narrow pH range (within 0.2-0.4 pH units) of its optimum to grow. The production of lactic acid should not exceed the buffering capacity of the medium, as lowering the pH can inhibit cell growth. High ammonium concentrations as a by-product of glutamine catabolism can be toxic to cells, causing cytosol vacuolisation and subsequent cell death. Exchanging the medium prevents these waste product accumulation effects.

However, after every medium exchange the cell secretome is removed and the cells are stimulated to rebuild their communications network by generating fresh molecules. This effort could negatively influence their behaviour and not represent their natural state. The influence of

medium exchange has for example been investigated by measuring actin microfilament structure directly before and after medium exchange. Such exchange led to a rapid disturbance of stress fibre formation and disconnection of cell-cell contacts. Frequent medium exchange is also economically detrimental, as medium can contain expensive additives such as growth factors and animal serum. Medium exchange cannot however be avoided as lack of nutrients and waste accumulation would lead to cell death (Vis et al., 2020).

5.5 Recommendations

5.5.1 Quality controls

Quality controls steps during the culture and maintenance of cells is a key undertaking and without appropriate quality control measures in place, the derived scientific data from OoC systems may be affected. Three key areas of quality controls are identified and must be considered at different timepoints throughout cell culture.

— **Cell integrity and identity**

Contamination of cells in culture with other widely used cell lines is reported to occur frequently and therefore, regular validation of cell identity is critical. Short tandem repeat (STR) typing is the main method to determine the exact cell type. Several commercially available molecular methods that are used to assessing identity of cell lines is listed in the table below (Table 1).

Species	Assays	Consensus Standard Method	Commercially Available Kit	Commercial Service	Comparative Data
Human	STR	ASN-0002	Yes	Yes	ATCC, DSMZ, JCRB, NCBI**
	SNP	No	Yes	Yes	(Liang-Chu et al., 2015[35]) (Yu et al., 2015[36]) NCBI
Mouse	STR*	No	No	Yes	Unpublished
	SNP	No	Yes	Yes	(Didion et al., 2014[37])
African green monkey	STR*	No	No	No	None
Chinese hamster ovary	STR*	No	No	No	None
Rat	STR*	No	No	No	None
Species-level identification	CO1 DNA barcode	ASN-0003	Yes	Yes	Barcode of Life Data System, NCBI**
	Species-specific primers	No	No	Yes	None needed

Table 1: Current status of SNP, STR, and DNA barcode technologies as standard methods for assessing the identity of cell lines from different species (OECD, 2018)

For pluripotent stem cells, it is also important to validate the genomic integrity of the cells in use. Commonly used method for validating genomic integrity of PSCs is karyotyping that can help monitor chromosomal stability and avoid use of PSCs with genetic abnormalities.

— **Cell function**

As culture conditions may influence the differentiation status and thereby their function, it is important to test if the cellular phenotype is maintained prior to incorporation in the OoC. For instance, the functionality of liver cells in a pharmacological context of use, could be tested by CYP450 activity which is a key player in the metabolism of drugs and xenobiotics. Moreover, when

culturing cells from frozen vials, it is recommended that at least 3 vials are initiated and include quality monitoring on the cell viability, proliferation, and specific functional activity for that cell type.

— **Cell contamination**

In addition to contamination of cells with commonly used cell lines, there is potential for contamination with microorganisms. Contamination with bacterial and fungal sources are usually visible by eye. In addition, unexpected changes in the colour of the culture media or increased turbidity could provide key clues to these contaminants. On the other hand, contamination with mycoplasma is not immediately evident and these contaminants can have a significant effect on cell function. Several commercial assays are available for mycoplasma testing as shown in table below (Table 2). In addition to these sources, some cells may contain endogenous viruses that can result in the secretion of viral particles or antigens. Although these are not normally considered contaminants, they may influence readouts when cells are co-cultured. Regardless of the source of contamination, standard operating procedures must be in place to discard positive samples and clear the laboratories of the potential source.

Method	Sensitivity	Advantages	Disadvantages
Indirect DNA stain (e.g., Hoechst 33258) with indicator cells (e.g., 3T3)	High	Easy to interpret because contamination amplified	Indirect and thus more time-consuming
Broth and agar culture	High	Sensitive	Slow and may require expert interpretation
PCR	High	Rapid	Requires optimisation
Nested PCR	High	Rapid	More sensitive than direct PCR, but more likely to give false positives
Enzyme-Linked Immunosorbent Assay (ELISA)	Moderate	Rapid	Limited range of species detected
Autoradiography	Moderate	Rapid	Can be difficult to interpret if contamination is at low level
Immunostaining	Moderate	Rapid	Can be difficult to interpret if contamination is at low level
Direct DNA stain (e.g., Hoechst 33258)	Low	Rapid, cheap	Can be difficult to interpret

Table 2: Mycoplasma detection methods, their sensitivity, and advantages and disadvantages (OECD, 2018)

5.5.2 Minimum reporting requirements for cells used in OoC systems

Characterisation of the cell origin should be reported using 'State of the art' / best practice approaches to confirm cellular phenotype for primary cells e.g. for mesenchymal stromal cells (Dominici et al., 2006). Some key points of consideration are outlined below.

— When using established cell lines: Undifferentiated status should be monitored by quantitative marker analysis

— When using new cell lines: In depth characterization is recommended (with well documented techniques) where possible. In case of large panels of new lines, take a subset and confirm pluripotency by differentiation assays. For remaining lines, quantitative marker analysis monitoring undifferentiated status is minimally recommended.

- When using new cell lines and non-established methodologies: Confirmation of undifferentiated status and pluripotency should be comprehensive
- Larger panel of undifferentiated status
- Proof of capacity of differentiation into the three germ layers
- Additional multi-parametric analysis recommended
- For nomenclature and unique identification, the use of hiPSCreg is recommended.
- Ensure well documented SOPs should be used throughout the isolation process.
- Commercially available cell lines: product number, company, lot number, authentication certificates and time in culture should be reported. Cell lines should be authenticated routinely by Single Tandem Repeat (STR) analysis.
- Primary, immortalized, or stem cell derived.
- Maintenance conditions (passage number, culture-ware, growth factors, culture medium and coating if applicable)

6 Engineering

6.1 Introduction

One of the core difficulties of creating an OoC system is the challenge associated with the selection of appropriate hardware, installing, and operating it. What are the best pieces of hardware to run OoC experiments on a controlled, reliable, and repeatable way? How to set up a system / experiment as efficiently as possible? For this it might help if the components and instruments were designed in such a way that plug and play installation is possible. Therefore, there should be compatibility between components and instruments. Interfaces (physical and "software/data") between the modules of the system should be standardized to ensure compatibility. Other potential topics for standardisation that would help the engineering of OoCs (i.e. sterilization techniques, material properties, etc.) are also explored here in this Engineering section. These other topics are complementary to interfacing standards, but may also be applied in OoC engineering contexts in which interfacing standards are not applied.

It may be valuable to current standardisation efforts in OoC engineering to consider a "hot spot" of working conditions that has been identified in the adjacent field of microfluidics (ISO 22916:2022). This “hot spot” describes working conditions of a large group of users and includes parameters of note such as: a pressure of 2 bar or less, a temperature of 4-50 °C, flow rates of 1µl/min to 100 µl/min, water-based fluids containing biomolecular matter and, the fact that some parts might also need to withstand harsher conditions during cleaning and / or sterilization. These conditions might be very similar to the working restriction for the hardware used in OoC, but this should be verified. Some additional OoC-specific working conditions will be discussed in the following sections.

As a general note: Please be aware that the standardisation to be defined is not about defining the products, but only describing the requirements the products should meet to ensure compatibility. I.e. hardware standardisation is not so much about describing what the hardware should look like, but how to qualify its performance. Furthermore, due to the diversity in applications, specific requirements may apply only to certain classes.

While this section covers standards relevant to the engineering of OoC systems, section 7.2 covers the related topic of standards relevant to hardware setup processes. Section 7.2 bears mention here as the parameters controlled by hardware setup processes may also inform engineering design decisions and therefore standards.

6.2 Material properties and information to be supplied by the manufacturer

In general, one should be able to rely on relevant material specific information from the supplier. For many material properties this is well covered, for instance optical and mechanical properties, hydrophilicity etc. However, OoC applications have some additional requirements, especially regarding issues related to materials in contact with the tissue or the fluid; for instance the used materials should not exhibit toxicity to cells and should not interfere with their functioning; they should be chemically stable and resistant to biodegradation. Standardisation is preferred in two manners.

Firstly, standardisation of the description of the materials in respect to OoC relevant properties. This means that all these properties need good definitions, methods to measure them and methods to qualify materials. This does not mean that the supplier is expected to supply confidential information about the material itself or the manufacturing process as these are not relevant for the user.

Secondly, standards on how to measure and qualify materials may be of interest. Existing ISO standards can be used as a reference, from which adaptions to OoC could be needed.

For a more in-depth discussion on standardisation of biomaterials applied as cell culture substrates (i.e. hydrogels, scaffolds and functional biocoatings) see section 5.3. In this section we focus more broadly on materials that may be used by engineers in the context of OoC.

6.2.1 List of available standards

OoC requirements might be a mix or compromise between the material requirements for medical devices and those for implantable devices.

A selection of existing standards for medical and implantable devices are listed below. Each cover some combination of: material performance requirements, material testing methods, or information reporting requirements. The listed standards have been loosely categorized based on whether they are applied to a specific class of material, or are more material agnostic.

Material Specific Standards:

- ASTM F2027, Standard Guide for Characterization and Testing of Raw or Starting Biomaterials for Tissue- Engineered Medical Products
- ASTM F2212, Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)
- ISO 3826 (all parts), Plastics collapsible containers for human blood and blood components
- ISO 5832 (all parts), Implants for surgery — Metallic materials
- ISO 5834 (all parts), Implants for surgery — Ultra-high-molecular-weight polyethylene
- ISO 5838 (all parts), Implants for surgery — Metallic skeletal pins and wires

- ISO 6474-1:2019, Implants for surgery — Ceramic materials — Part 1: Ceramic materials based on high purity alumina

- ISO 7153-1:2016, Surgical instruments — Materials — Part 1: Metals

Material Agnostic Standards:

- ISO/TS 23565:2021, Biotechnology — Bioprocessing — General requirements and considerations for equipment systems used in the manufacturing of cells for therapeutic use

- ISO 20417:2012, Medical devices – Information to be supplied by the manufacturer

- ISO 16142-1:2016, - Medical devices — Recognized essential principles of safety and performance of medical devices — Part 1: General essential principles and additional specific essential principles for all non-IVD medical devices and guidance on the selection of standards

- ISO 7405:2018, Dentistry — Evaluation of biocompatibility of medical devices used in dentistry

- ISO 10993 (all parts), Biological evaluation of medical devices

6.2.2 Areas requiring standardisation

Below, we identify areas in which conforming to standards (existing or to be developed) might add immediate value to the field of OoC. The list of existing standards given in section 6.2.1 may already apply to some of the identified areas and could be consulted before developing OoC specific standards.

Firstly, a standard specifying information the manufacturer should supply for a given product.

Secondly, standards on how to measure and qualify materials. Existing ISO standards can be used as a reference, from which adaptions to OoC could be needed.

The following topics should be included:

- Leaching of material, for instance in the case of PDMS un-crosslinked oligomers, can be problematic for cell cultures as it can cause toxicity in cells and alter their behaviour.

- Cleanliness of the surface, for instance residues from the fabrication process.

- (Oxygen) permeability.

- Biocompatibility.

- Absorption.

The topics above are more or less easily defined, with one notable exception. Biocompatibility is defined as the ability of a biomaterial to induce or not induce an appropriate host response in a specific application. It is often a relative quantity appreciated through a comparison of behaviour in relation to reference materials. Generally, it results from a set of interactions at the material-tissue interface (unstable extra-physiological situation). Depending on the nature of the device contact, different biological risks need to be evaluated:

- Cytotoxicity, sensitization, irritation, material mediated pyrogenicity, acute systemic toxicity, subacute/subchronic toxicity
- Chronic toxicity/hemocompatibility/genotoxicity
- Carcinogenicity

6.3 Sensors and actuators in the Organ-on-Chip space

Within the OoC space, actuators and sensors are commonplace and allow, for example: different degrees of stimulation, automated recording of assays, flow generation, and flow measurement. The following sections will present: sensors, actuators, the connection between the sensors and the actuators and the measurement of fluids and flow.

6.3.1 Sensors

As OoCs technologies progress, the need for increased information output as well as better quality control processes is leading to the integration of more sensors. This comes with increased cost and complexity of OoC systems.

There is a clear need for OoC-specific sensors, or sensors specifically adapted for OoC from other fields. This can only happen when the supply chain is aware of this need and receives guidance from the OoC community in the form of whitepapers, guidelines and standards.

Important aspects for sensors to be used in OoC systems are:

Specification	**Aspects to consider**		
Measurement Accuracy:	Signal to noise ratio	Resolution	Repeatability and reproducibility
Calibration:	Method and frequency	Drift rate	Dependence on conditions
Measurement deviations:	Temperature and pressure dependence	Cross-sensitivities	Electronic interferences
Access to analyte	Transparency and sizing of optical window	Fouling of sensor surfaces	Membranes or coatings

6.3.2 Actuators

Mechanical actuation is one of the key aspects and advantage of OoCs with respect to standard in vitro models. The type of stimuli can range from shear stress, compression, shear strain, stretch or a combination. To mimic this actuation, specialized equipment is being used. Note that the current equipment is only a selection of the capabilities of mechanical stimulation, but this could change due to the advancement in OoCs. The main technologies used are systems to generate shear stress using a liquid or to apply active (compression, stretch, shear strain) mechanical forces to the cells or 3D – cell structures. This aspect is covered by the section of this roadmap in section 6.3.4. Other equipment relies on parts that mechanically move onto the cells generating similar type of stimuli as by the pressure systems. For both types of equipment, it is key to have a control on the accuracy and the stability of the mechanical forces generated in the model. The technology

to apply these forces and the requirements are very application specific, and less suitable for standardisation, especially while this field is so much in development.

Some materials are stiffer than others, which might have an impact on the mechanical behaviour of the system. Specification of stiffness should be supplied by the material supplier. This aspect is covered further in section 6.2.

One specific type of actuator that might need a bit more attention as it is currently probably the most used actuator by the OoC community are pumps. In OoC pumps have been used to apply well controlled flow to mimic blood circulation. Again, due to the diversity in OoC it might not be feasible to standardize these pumps, but it makes sense to standardize the information that a pump supplier should supply to the user, i.e. a product datasheet. The relevant technical information from the datasheet will enable end-users to compare the performance of the microfluidic pumps and to choose the most fitting pump for their application. Without a standardized datasheet, the comparison of the performance of different microfluidic pumps is often not possible due to the lack of similar information or divergent definitions of similar performance characteristics.

It is also important to note that although the pumps may give an accurate output under ideal conditions it is difficult to determine whether the same type of flow rate is achieved after integration into a platform. This would need to be further investigated by the company supplying the platforms. Here, for example, the supplier should determine whether the flow measured after integration is expected to be consistent with the ideal output flow, or they should provide a datasheet which correlates the actual flow through given fluidic resistances with the one reported under ideal conditions. For the related topic of measuring flows, see section 6.3.4 below. The same process as for the flow would have to be performed for pressure-based actuators.

6.3.3 Connection of sensors and actuators to instrumentation

It is a challenge to effectively deploy sensors in complex applications like OoC, given the interoperability issues that may arise when attempting to integrate sensors from multiple vendors. Hardware compatibility, wired/wireless connectivity, and security are among the issues that need standardisation. In general, the sensors and instruments are selected based on their individual requirements and not necessarily guaranteed to work with each other smoothly. For this hardware and software standards are needed. The application specific operational requirements should be generalized to derive standard application layer interfaces between sensors and instruments.

6.3.4 Measurement of flows and fluids

Flow rate control is critical for most microfluidic applications and is often accomplished by external flow generators connected to the microfluidic chip. Four of the most common types of flow generators used are: peristaltic pumps; syringe pumps; pressure-driven flow generators and piezo electric pumps.

Live flow monitoring can be achieved using flow sensors. An immense variety of flow sensors using different fields of physics are available. Not all of them are suitable for microchannel flows. Choosing the right microfluidic flow meter adapted to the flow regime and fluid is critical for accurate measurements. Apart from mechanical technology, there are many non-thermal flow measurement solutions available. Some of them involve optics, acoustics or electrochemical phenomena.

In order to ensure the control of flow in an OoC device, it is necessary to have appropriately calibrated flow generators traceable to SI units. There are several flow generator measurement methods standardised for macro application which may be used for OoC as well.

There are several factors that can influence the accuracy and stability in flow control, mainly: the chosen flow generator, the fluidic circuit, the liquid properties, and leakage. The flow rate can be considerably affected by leakage in the system, often this happens in the connecting points. Leakage can also occur in case of delamination of the chip, or when cracks appear due to overpressure or destructive modification of the chip material (due to over-heat for example).

6.3.5 List of available standards

- **Sensors**

There are no standards for OoC specific sensors. There are, however, multiple standards for good measurement practices, and standards exist for the application of sensors for physical-, chemical- or biochemical parameters in adjacent application domains. Examples include:

- ISO 14511:2019, Measurement of fluid flow in closed conduits – thermal mass flowmeter
- ISO/TS 23367-1:2022, Nanotechnologies — Performance characteristics of nanosensors for chemical and biomolecule detection
- ISO 14511:2019, Measurement of fluid flow in closed conduits – thermal mass flowmeters

- **Connection of sensors and actuators to instrumentation**

At this point there are no OoC specific standards available yet, inspiration might come from:

CEN ISO/IEEE 11073 is an internationally adopted family of standards developed to enable complete connectivity between medical, healthcare, and wellness devices.

- **Measurement of flows and fluids**

There are some existing standards for flow control and leakage which can be used for OoC. However, it should be investigated if these are good enough or should be adapted

Standards found by this group include:

- IEC 60601-2-24:2012: Medical electrical equipment - Part 2-24: Particular requirements for the basic safety and essential performance of infusion pumps and controllers
- AAMI TIR 101: Fluid Delivery Performance Testing For Infusion Pumps
- ISO 4185:1980, Measurement of liquid flow in closed conduits - Weighing method

As the number of non-OoC applications to which this topic is relevant is large, discovery of other standards is likely still needed

6.3.6 Areas requiring standardisation

- **Sensors**

Standardisation of sensors should include:

- Dead volume

- Flow rates
- Standard interface to enable easy and reliable integration of sensors in OoC systems, either tube based of tube less integration
- Definition of sensor specifications
- Reporting of accuracy and limitations of employed sensors

Furthermore, the OoC community should specify what development in the area of sensors is needed to be better equipped for use in particular applications. This should be stimulated through white papers or guidelines.

- **Actuators**

Standardisation of actuators should include:

- Naming,
- Schematics/symbols,
- Technology characteristics,
- General working conditions,
- Electronical characteristics,
- Mechanical characteristics, and
- Flow characteristics.

Some of the topics are already being approached by the technical Committee ISO/TC 48 Laboratory equipment. However, the specific requirements, especially in regard to flow characteristics, from the OoC community should also be explored.

- **Connection of sensors and actuators to instrumentation**

Introducing standards to ensure interoperability of sensors and actuators may be of value to both users and producers. Users could benefit from a wider selection of compatible products to choose from while producers may find unexpected markets for their products. Potential targets for sensor and actuator interoperability standards include:

- Physical hardware used for interfacing
- Software
- Wired/wireless interfacing
- Security
- Application layer interfaces

- **Measurement of flows and fluids**

Standardisation should focus on the measurement methods of flow generators. The following elements should be included:

- Flow generator
- The microchip
- The liquid properties
- Leakage

6.4 Modular integration of a microfluidic system

Enforcing a standard set of OoC devices, or even a standard set of OoC fabrication pipelines seems counterproductive given the current state of the art. Devices are highly application specific, production volumes are relatively low, and the fabrication is often done in house by end users with all processes and materials chosen as seems fit.

In this design paradigm, an often-taken practical way forward is to create the systems from a combination of inhouse specialized parts and off the shelf parts from external suppliers. Those suppliers cannot create different products for the many different applications, unless at high cost. Standardisation that facilitates the usage of components and subsystems for different applications might therefore be useful. Such standards come down to standards describing interfaces between (off the shelf) components and OoC system. Currently, there are two major approaches for integration of microfluidic based devices: 1) Connecting the components with tubes. 2) Placing the components on a chip or manifold.

A tube-based concept offers maximal flexibility in terms of configuration, component selection and relative low investment, disadvantages are:

- Large dead volume.
- It takes some time for assembly.
- Reliability concerns related to the high number of handmade connections.

Often this approach is used in the development stage, while it is a relative low cost and flexible approach. Advancing from this to an industrialised concept often requires designing a manifold based construction, requiring a complete redesign and often needing another selection of components.

The tube-based set up is especially complicated due to the diversity of tube connection systems. Connection systems in use are designed for other applications and requirements (for instance Luer for medical instruments and flat-bottom fittings for high pressure applications) and are, as a rule, either not reliable enough, have high internal volume, or are too expensive.

The other approach, mounting components on a manifold, offers high reliability, low dead volume and, when a top-down approach is used, potentially a straightforward assembly. The disadvantages are inflexibility and the high start-up cost.

The Microfluidics Association is currently discussing with a group of companies a modular fabrication process where the same base components can be used in a tube based and in a manifold-based variant. This would facilitate the transfer from research to industry, shortening the commercialization time. It offers flexibility in the design phase, while enduring seamless transfer to a more industrial concept.

The tube-based system is based on a top-down connection system where each component is placed on an adapter and tubes are used between the adapters. The adapters have threaded holes for flat-bottom fittings for 1/16 inch tubes. This is a proven technology, making it less of a barrier to entry for the microfluidics community (as opposed to developing another connector) and the

parts can be made at low cost. The fact that the tubing approach still uses top-down connections offers a path towards further integration by the manifold approach, in which the components are fixed on a manifold, without tubing (see below). For both approaches the same components can be used, only the adapters and tubes are replaced by a manifold (Figure 4).

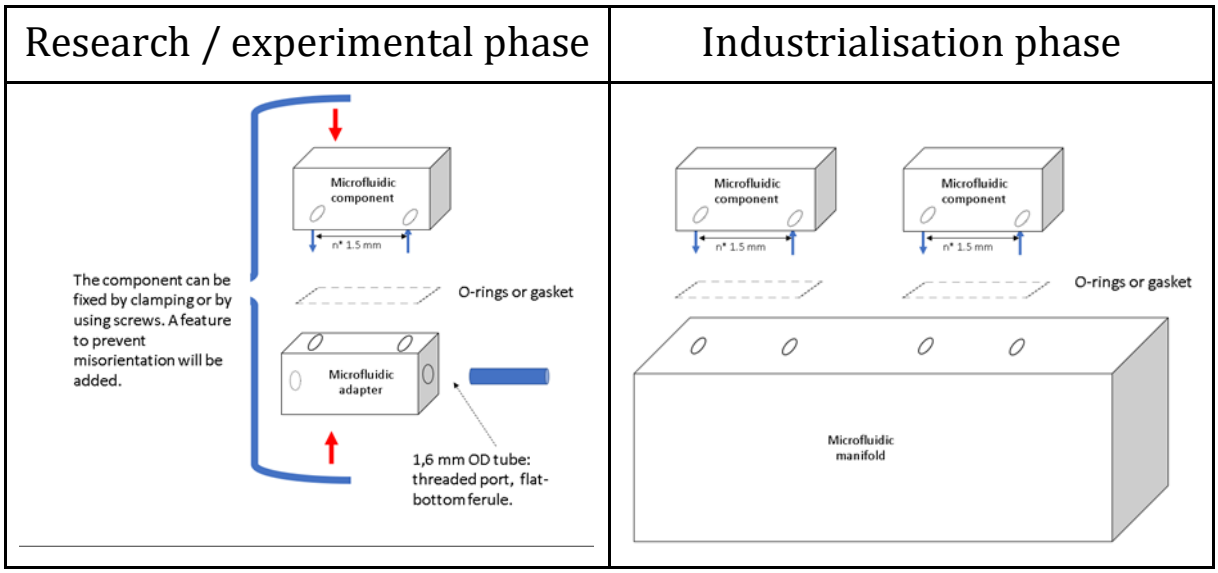


Figure 4. Microfluidic interfacing for components in different phases of product development

The concept also allows for integration with microfluidic chips or sensors and adheres to ISO 22916:2022.

Note that while OoCs are not necessarily microfluidic, microfluidic perfusion is very widely used, in particular for systems designed towards coupling of several tissue models in series. Therefore, whatever workflow is selected, microfluidics is likely to be an essential element of it.

6.4.1 List of available standards

— ISO 22916:2022, Microfluidic devices — Interoperability requirements for dimensions, connections and initial device classification.

6.4.2 Areas requiring standardisation

Potential targets for standardized interfacing include:

— Heterogenous integration: interfacing between tubing-based and manifold-based systems

— Tube based integration: Tube dimensions, type of connection of tubing to component.

— Manifold based integration: footprint of the component, position of microfluidic ports, clamping system and exclusion zone.

6.5 Hardware and Techniques from Existing Cell Culture Pipelines

In this section we cover areas in which the existing hardware and techniques used in cell culture are relevant to OoC. The identified areas are either currently in use in the field of OoC or are expected to become relevant. Many of them may benefit from OoC specific standardisation.

6.5.1 Sterilization quality

Sterilization is important for OoC devices to avoid contamination in the biological cultures. The suppliers of systems and materials are responsible for this but need guidelines from the user community. Sometimes the part needs to be sterilized by the user, for instance when the part is reused. In that case the user should be able to follow the instructions for sterilization from the supplier. When the chip is expected to be used without an extra sterilization step after fabrication, the materials and fabrication tools should be sterilized to ensure aseptic cell culture conditions. It is an obligation for the OoC community to set rules for sterilization quality. This should include specifying appropriate sterilization techniques. Each of them has its own pros or cons and might have an influence on the part's materials. A few examples of techniques that can be used:

- Sterilization by radiation: Radiation such as UV for 30 minutes can damage DNA and therefore be a good tool to sterilize objects.
- 70% ethanol: Metal tools such as triceps can be sterilized by submerging in 70% ethanol, followed by airdrying.
- Autoclavation: Moist heat sterilization is a procedure in which heated, high-pressure steam is used to sterilize an object (at 121-degree °C, 1.03 bar pressure for 15-20 minutes). You can sterilize labware, glass articles, pipettes, and culture media with the help of an autoclave. PDMS chips, tubing and connectors for OoC can be sterilized by autoclavation, however material properties will likely change.
- Filter sterilization: (heat sensitive) liquids can be filter sterilized by the use of a syringe and filter membrane. Common filter sizes are 0.22µm and 0.45µm.
- Gamma irradiation
- Ethylene oxide treatments

Suppliers and users should be aware that sterilization might have an influence on characteristics of the device; think of dimensions and surface quality of the device. It could also leave toxic residues. As OoC devices will be used in an aseptic environment, it is important to keep the material properties and sterilization possibilities in mind when engineering the devices.

6.5.2 Incubators

Control of physical cell culture parameters (pH, temperature, etc.) is essential to support the living material inside the OoC system. Large scale incubators that can encompass an entire OoC system or setup are a common way to achieve this. Alternative incubators to the common MRC incubator could be heating blocks or custom-made devices that control cell culture parameters or control parameters locally in the OoC devices.

Regardless of the type of incubator, control over all the prescribed conditions for air, CO2, temperature and humidity required for maintaining cells and tissues is essential. Secondly, connection with the peripheral equipment is an essential point to consider, i.e. the communication of pumps, sensors etc. Generally, incubators do not have integrated flow control facilities. This

might have disadvantages and should be in the Roadmap as a potential issue. Another aspect to consider is the option of having multiple OoCs inside the incubator to facilitate parallel experiments to increase flexibility and throughput. Furthermore, ideally one would like to be able to do all possible experiments while the OoC device stays in the incubator. That might mean providing access to retrieve the biological material for further analysis and / or an optical window for visual inspection; sometimes even a microscope is inside the enclosure. It remains to be seen if the incubator should adhere to standard dimensions and tolerances already being used for common lab ware, such as multi-well plates, glass slides, coverslips and petri dishes.

At this moment there are no specific standards for the incubators, however manufacturers do provide certificates in which they outline calibration, operating windows of temperatures, gas, etc.

6.5.3 Integration of microfluidics and microplate workflow

Most cell culture workflows are based on the use of microplates, also known as micro-well or microtiter plates. These microplates are highly standardized and system suppliers take well care that their instruments adhere to these standards. Different steps in the workflow run on different instruments (for instance incubators, readers etc.) Microplates offer a high throughput solution, based on massive parallelization of experiments and highly automated workflow that can be adapted to specific demands. OoC experiments, on the other hand, are typically lower throughput, but produce high content information about organ response to stimuli over long periods of time. Comparable to the way a well is part of a larger microplate, in OoCs, a micro-reaction-chamber (MRC) containing a model of the organ is a part of a larger fluidic system. In practice there are many mixed concepts, in particular microplates with microfluidics integrated.

Common to both the microfluidic chips and the standard microplates used in the OoC field is that the cellular model (or the organ) is physically in a MRC. This MRC is usually connected to a fluidic system which can be supported by microfluidic connectors, pipetting robots or others.

The lack of a standardized description of the MRC separates it from the used fluidic system (e.g. static, continuous flow, periodic flow) and the technology used for it (manual pipetting, microfluidic channels, pipetting robots, pumps, etc.).

6.5.4 Microplate limitations

Many systems in use rely on the existing ANSI SLAS 4-2004 (R2012) SBS micro plate format standard. This defines plate dimensions and allows inter-operability between common laboratory tools much as liquid handlers, imagers etc. However, other important aspects are undefined (such as orientation, numbering, plate flatness, plate nest, labelling, etc) which leads to inter-operability challenges. In addition, there is interest in evolving this standard to be more digital and smarter and this could better support the OoC community. Therefore, the integration of electronic connections in the plates to pumps and sensors should be considered. In case flow is required in a OoC system, requirements for the microplate to apply flow should be considered.

6.5.5 List of available standards

- **Sterilization**

In the health care sector and for medical devices several standards for sterilization are available. The OoC community should study existing guidelines and standards for medical devices to check if these are applicable or should be adapted. These standards might be used as reference material or input for standards on OoC sterilization.

- ISO/TS 22421:2021, Sterilization of health care products — Common requirements for sterilizers for terminal sterilization of medical devices in health care facilities

- ISO 22441:2022, Sterilization of health care products — Common requirements for sterilizers for terminal sterilization of medical devices in health care facilities
- ISO 11137, part 1-4 Sterilization of health care products — Radiation
- ISO/TS 21387:2020, Sterilization of medical devices — Guidance on the requirements for the validation and routine processing of ethylene oxide sterilization processes using parametric release
- ISO 11135 (all parts), Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices
- ISO 11138 (all parts), Sterilization of health care products — Biological indicators
- ISO 11140 (all parts), Sterilization of health care products — Chemical indicators
- ISO 7886 (all parts), Sterile hypodermic syringes for single use
- ISO 8536 (all parts), Infusion equipment for medical use
- ISO 8537:2016, Sterile single-use syringes, with or without needle, for insulin
- ISO 13408 (all parts), Aseptic processing of health care products
- ISO 17665-2:2006, Sterilization of health care products , Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices
- ISO 17665-2:2006, Sterilization of health care products, Part 2: Guidance on the application of ISO 17665-1
- ISO 17665-3:2006, Sterilization of health care products, Part 3: Guidance on the designation of a medical device to a product family and processing category for steam sterilization

- **Microplates**

The following existing standards on microplates were identified

- ANSI SLAS 1-2004 (R2012): Footprint Dimensions
- ANSI SLAS 2-2004 (R2012): Height Dimensions
- ANSI SLAS 3-2004 (R2012): Bottom Outside Flange Dimensions
- ANSI SLAS 4-2004 (R2012): Well Positions
- ANSI SLAS 6-2012: Well Bottom Elevation
- ANSI SLAS 4-2004 (R2012) SBS micro plate format

6.5.6 Areas requiring standardisation

— **Sterilization**

Useful standards for OoC work could include:

— Sterilization techniques to be used;

— Which technique may be used on which material;

— Minimum requirements per technique to ensure the sterilization quality and how is this quality measured.

— **Incubators**

A standard on incubators for OoC applications should include minimum requirements on design and functionality. This may include:

— Providing access to retrieve biological samples;

— Optical window;

— Standard dimensions and tolerances;

— Integrated flow control facilities.

— **Microplates**

There is a need to extend the existing standards on microplate format to accommodate OoC workflows, Opportunities include:

— Orientation

— Numbering

— Plate flatness

— Plate nesting

— Labelling

— Application of Flow

— Electronic Connections

6.6 Bioprinting

Bioprinting is a technique where bio-ink (biomaterial that contains cells) is used to fabricate a tissue construct that mimics the 3D geometry and structure of native tissues. These complex physiological constructs can be incorporated / printed into OoC devices. The incorporation of cells in a controllable manner (with respect to shear stress, and desired morphology, dimensions and direction), makes this technique attractive for use in fabrication of microfluidic systems.

Examples of 3D bioprinting techniques used in fabrication of microfluidic devices are:

— Extrusion based

— Multi-material bioprinting

- Co-axial bioprinting, and
- Laser assisted bioprinting/SLA (photocurable inks)

Often the bio-ink is used in fluidic form and crosslinked using: UV radiation, or a chemical or enzymatic crosslinker. Bioprinting systems can contain one single nozzle or multiple nozzles. For OoC devices 3D printing is furthermore used in research to generate 1D, 2D and 3D printed PDMS chips that can be used to bioprint cell-laden structures in or for printing sacrificial inks to generate hollow channels within the bioprinted construct. Bioprinting is widely used in the fabrication of microfluidic chips for vasculature-on-chip, lung-on-chip, heart tissue-on-chip, liver-on-chip, kidney-on-chip, cancer-on-chip, and BBB-on-chip.

As many different approaches are taken, reproducibility is low and there is a lack of standardisation. Bioprinting fidelity is limited by printing accuracy and resolution, bioink materials, printhead size and printing speed.

Guidelines for reporting details are, at this stage, more important than standardisation of the various techniques. For example, reporting guidelines would be useful for: crosslinking, needle/nozzle production, methacrylation of bioink, bio ink viscosity, shear stress level etc. should be documented to enable reproducibility.

6.6.1 List of available standards

No standards are currently available for bioprinting.

6.6.2 Areas requiring standardisation

As this field is very much in development, opportunities for standardisation are limited. It might make sense to take the first step towards standardisation by: writing guidelines, formulating quality standards, and generating techniques to compare systems/methods of bioprinting.

- **Standardisation Requirements for printers:**
  - Dimensional Reproducibility
  - Resolution
  - Multimaterial printing (creating architectural compartments, with different cell and biomaterial types placed in discrete locations relative to each other)
  - Compatibility to substrates (dimensional)
- **Requirements for bioink:**
  - Materials available
  - Crosslinking methods
  - Compatibility to substrates (biophysical)
  - Translucency
  - Viscosity
  - Protocols / biological CAD

6.7 Recommendations

The following list of engineering topics with potential for standardisation was created based on: the research/discussions used to generate in the previous sections, extensive discussions with external experts, and the list of hardware items benefitting from standardisation as presented in in section 6.5.6. The list is presented in order of priority, where priority was determined by a survey of 170 OoC experts. Sub points (a, b, c, etc...) were generated by discussion among the focus group members when reflecting on the survey results. The following list is therefore complementary to the rest of this section on engineering, but also has some overlapping information.

1. Describing rules for sterilization quality guidelines.
    a. What sterilization techniques are currently being used by the OoC community?
    b. What standards exist for sterility or sterilization techniques in other fields (e.g. cell biology, medicine, food production)
    c. What are the criteria by which sterilization is tested?
2. Making clear definitions of OoC applications.
    a. List of OoC applications
3. Standards that would help to integrate microfluidics based OoC with the standard microplate workflow.
    a. Describe standard microplate workflows.
    b. How does microfluidics fit in these workflows.
4. Defining what material properties are relevant for OoC and should be reported by the suppliers of materials and components.
    a. What are the relevant material properties?
    b. How are these properties characterised / measured? Including batch – batch reproducibility.
    c. Are these measurements covered by ISO standards?
5. Standards for modular integration of a microfluidic system, including making microfluidic connections.
    a. Bring interested suppliers together to reach consensus.
    b. Define operational conditions for such system / requirements.
    c. Define connection methodology that fits best to the requirements.
6. Setting standards for connection of sensors and actuators to instrumentation. See point 5 a, b, and c.
7. Making guidelines for the measurement of flows and fluids, leakages etc. This topic is covered by the MFMET project, results will be published soon. See www.mfmet.eu
8. Standards that would help to integrate microfluidic based OoC devices in incubators.
    a. Define classes of environmental conditions based on temperature, humidity, gas composition and time to operate inside the incubator. Can they be linked to certain applications?
    b. Are existing methods for testing and reporting durability with respect to humidity and temperature sufficient? Or are new standards needed?
9. Setting clear requirements for OoC sensors (dead volume, flow rates, etc.) and a standard interface to enable easy and reliable integration of sensors in OoC systems, either tube based or tube-less integration. See point 5 a, b, and c.
10. Creating a set of symbols for the hardware elements used, to visualize a OoC system / experiment setup.

a. What symbols from existing standards can be used in OoC?
b. What other symbols are currently being used by the community?
c. Create a list of useful symbols.
d. Give examples how such symbols can be used to visualize an experimental setup.

11. Working on microplate limitations / missing standards
12. Setting quality standards for bioprinting
13. Development of one standard way to parameterize micro-reaction-chambers
    a. What are the distinguishing characteristics of micro-reaction-chambers?
    b. How are these characteristics measured
    c. Are there classes of comparable micro-reaction-chambers and can they be linked to certain applications?

7 Hardware parameters, experimental design and data management

7.1 Introduction

OoC provide improved physiological relevance, and thus offer great potential for application in a number of areas. However, OoC models, and therefore experiments, are complex integrated systems comprised by biological and engineering components, with inherent multiple factors to consider and control, as these could potentially confound the results and/or introduce variability. It is therefore important to identify, account for, and control these factors to ensure the conclusions drawn from OoC experiments are robust and reproducible (Cairns et al., 2023). Such factors can include fit-for-purpose hardware aspects, including flow rate and mechanical stimuli, as well as experimental aspects such as cell source and analytical techniques, but also experimental design features such as control groups and power. Furthermore, all the data generated from OoC experiments, both biological and technical data, must be carefully documented and evaluated for accurate interpretation, (computational) analysis, and reproducibility. This includes documenting details of the hardware and data collection methods in addition to the more commonly recorded parameters from in vitro studies such as cell number or cell viability. As such, the aim of this WG is to identify the need for standards for these three broad aspects of biological experiments using OoC: **hardware parameters, experimental design, and data management**. Adopting standards in these areas will ensure the generation of robust and reproducible data. Specifically, they will:

— Ensure the production of reproducible experimental data across laboratories and operators

— Enable wider adoption of MPS/OoC technology

— Help demonstrate the improved predictivity of these models over current ‘gold standards’/state-of the-art, such as animal models or other simpler cell-based *in vitro* assays.

7.2 Hardware parameters that directly impact experimental data

While the manufacture of OoC-associated hardware, and the required standards for this, are documented in Section 5, this chapter refers specifically to the requirement for standards for measuring and documenting parameters controlled by hardware that have a direct impact on the experimental results. These could be from OoC-specific hardware, or other hardware important for the OoC experiment, such as incubators. Standards for recording such parameters before, during and/or after an OoC experiment, to demonstrate the hardware processes are as specified are necessary to ensure reliability and robustness. For example, where a specific flow rate is

required for an OoC experiment, a standard procedure for measuring and recording the actual output is important; any discrepancy in the flow rate could have an impact on the experimental results, and thereby the conclusions drawn, ultimately impacting the robustness and reproducibility of the data if such discrepancies are not accounted for.

7.2.1 List of available standards

There are some existing standards of relevance to this section (Guidance Document on Good In Vitro Method Practices (GIVIMP)(OECD, 2018);ISO 13485:2016 Medical devices - Quality management systems - Requirements for regulatory purposes), which may be able to be applied, in part, or be used to guide OoC-specific standards.

7.2.2 Areas requiring standardisation

Below is a list of aspects of **Hardware Setup Processes** that would benefit from standardisation.

— **Incubator Conditions (Temperature, humidity, CO_2/other gas levels)**

It is important that conditions inside an incubator are carefully controlled to ensure optimum cell growth and viability. It is important that the temperature, CO_2 and humidity level the incubator is set to, and the actual values are the same. To verify this, the temperature, CO_2 and humidity levels of the incubator should be recorded independently, and the values recorded at defined points throughout the experiment using calibrated instruments. The type of instrument and calibration standards, as well as a SOP for the frequency and number of replicates of readings to be recorded, are required. National Institute of Standards and Technology (NIST) and/or ISO guidelines for the type of instrument and calibration procedure may exist, but an SOP for recording incubator temperature, CO_2 and humidity during an OoC experiment needs to be defined. Furthermore, an acceptable range needs to be defined, which may be cell type and/or OoC-specific.

— **OoC Hardware**

Pressure can be applied to certain OoC devices to mimic stretching e.g. breathing motion of a lung. Gas and liquid flow to the cells in an OoC device can also be controlled. Flow can also be applied to induce shear stress. As such, these parameters may not be present in all OoC experiments, but where they are, they should be independently verified to ensure the output matches the setting on the hardware.

Flow sensors (gas or liquid) are available, possibly with associated standards for operation and/or calibration, as discussed in section 6.3.4, but SOPs for using these in an OoC experiment, including the frequency and number of replicates of readings to be recorded, are required.

Oxygen saturation is an important parameter typically driven from the incubator. In complex OoC systems, oxygen saturation requirements may vary throughout the different components. SOPs are required how the oxygen saturation levels are controlled and monitored in OoC systems.

SOPs are also required for the following aspects that influence experimental results, such as leak-tightness of tubing and other characteristic quality management.

Some OoC devices incorporate mechanical stimuli, to improve the physiological relevance; the different types of mechanical stimuli applied to OOC systems include shear flow, compression, and stretch/strain. These are reviewed in (Kaarj & Yoon, 2019). SOPs to record the level of mechanical stimulus the cells are subjected to are required.

Area that needs standardisation	**What is missing/needed**
Incubator	

Temperature	NIST and/or ISO guidelines for the type of thermometer and calibration procedure may exist, but an SOP for recording incubator temperature during an experiment needs to be defined.
CO_2/other gas levels	NIST and/or ISO guidelines for the type of gas analyser and calibration procedure may exist, but an SOP for recording incubator CO2 or other gas levels during an experiment needs to be defined.
Humidity	NIST and/or ISO guidelines for the type of hygrometer and calibration procedure may exist, but an SOP for recording incubator humidity levels during an experiment needs to be defined.
OoC hardware	
Pressure	SOP for controlling and monitoring pressure in OoCs
Flow rate	SOP for controlling and recording of flow rates needs to be defined.
Mechanical stimuli	SOP for controlling and recording of mechanical stimuli, such as pressure or shear stress, needs to be defined.
Leak-tightness of tubing	SOP for verifying of leak-tightness of tubing and connections needs to be defined.
O_2 saturation	SOP for controlling and recording of oxygen saturation needs to be defined.

7.3 Experimental Design

Experimental design refers to how to set up an experiment, including technical, operational and biological aspects.

Experimental design is a critical component of conducting biological experiments as it ensures that the results obtained are accurate, precise, and reproducible (intra-/inter-laboratory). State-of-the-art experimental designs for biological experiments typically involve careful consideration of sample size, statistical power, and control groups (biological controls), as well as selection of appropriate biological materials and measurements. For instance, when designing experiments using cell-based in vitro assays, factors such as cell source, culture conditions, and passage number must be carefully monitored and documented to minimize experimental variability. Similarly, the selection of appropriate analytical techniques, such as transcriptomics or proteomics, can be critical for obtaining accurate and meaningful results. There are challenges in achieving optimal experimental design, including variability, instability and bias in biological systems, lack of standardized protocols, and ethical considerations when working with living organisms. The ARRIVE guidelines are a resource for best practice in designing and reporting animal studies (https://arriveguidelines.org/arrive-guidelines)(Percie du Sert et al., 2020). Regarding in vitro studies, the OECD published a Guidance Document on Good in vitro method practices (GIVIMP; https://www.oecd.org/env/guidance-document-on-good-in-vitro-method-practices-givimp-9789264304796-en.htm)(OECD, 2018) and recently a set of recommendations has been published for what to include when publishing in vitro studies (RIVER; https://osf.io/preprints/metaarxiv/x6aut/)(The RIVER working group, 2023). However, these are for in vitro studies generally, and do not refer specifically to OoC. Regarding OoC studies, two publications refer to experimental design, specifically for the experimental set

up (Cairns et al., 2023) or automated imaging of OoC (Peel et al., 2019), and another recent publication presents a technical framework for enabling high quality measurements in New Approach Methodologies (NAMs), of which OoC are one type (Petersen et al., 2023). However, broad guidelines and/or standards for experimental design of OoC studies are not yet available. Therefore, the aim of this section is to identify and define standards for the experimental design and execution of biological OoC experiments. Overall, the establishment of standardized protocols for experimental design and execution would enhance the reliability and translatability of OoC experiments, thereby enhancing confidence in, and adoption of, these models.

The identification and definition of standards for experimental design and execution of biological OoC experiments can have several specific use cases and applications. For instance, these standards can be used to improve the drug discovery process by enabling accurate and reproducible assessment of drug efficacy and toxicity. Additionally, OoC models can be used for disease modelling, which can aid in the development of new therapeutics and personalized medicine. Standards for experimental design can also be applied in the field of regenerative medicine, where OoC models can be used to develop and test new tissue-engineering strategies.

7.3.1 Areas requiring standardisation

All aspects of setting up an experiment require standardisation to ensure the generation of robust and reproducible data. These have been divided into the following aspects: Biological characterisation, Compound characterisation, Study design, and the current status of standardisation of these will be discussed in the following subsections.

- **Biological characterisation**

  - **Number of cells and/or cell viability:** To be able to reproduce and compare data the number of cells is of great importance. How cells are counted depends on type of cells, e.g. hepatocytes are binuclear and hence cannot be counted on all cell counting instruments (Friedrich & Gilbert, 2023). The viability of the cells also needs to be assessed, both before the start of an experiment and during the time course of the experiment to make sure the system is viable all through the incubations. Viability tests also depends on type of cells and type of experiment, cell viability after thawing is typically done with trypan blue exclusion test which cannot be used to test viability on formed organoids. For viability during experiment a soluble marker such as lactate dehydrogenase (LDH), which leaks out in the medium could be used, whereas at the end of an experiment disruptive measures such as ATP concentration could be of greater value. Viability marker is also dependant on the type of OoC which is used, e.g. if it is a flow-through or a recirculating system. It is important that SOPs are defined, and where standard procedures already exist for cell counting and viability measures exist, that these are followed, however it is important that the approach taken is chip, organ and CoU-dependent; how to count and measure viability of cells in OoC should not be standardised.

    Recommendation: Develop a list of available methods and recommendations in which settings they are applicable.

  - **Baseline characteristics of cells or organoids in OoC, cell specific functionality:** As in any in vitro experiment the details about cells or cell lines, such as identity, source, pre-characterisation etc, need to be clearly stated. This is well described in the RIVER recommendations but what would be useful is a more detailed description of how to characterise functionality of the cells/organoids and how well they represent the native cell or tissue.

Such characterisation could consist of molecular readouts (e.g. gene expression patterns) but where possible functional characterisation is also aspirational. Cell specific functionality could be e.g. albumin secretion from hepatocytes, TEER values on barrier forming cells, or beat rate on cardiac organoids. Cell-specific functionality is needed to demonstrate that cells under the control settings react as expected (Baudy et al, 2020, Lab-on-Chip). Time points for such measurements are dependent on type of experiment, if it is a short- or long-term experiment, but also depends on the type of measurement (eg if it is disruptive, if it is secreted into the medium). As such, SOPs for recording the baseline characteristics of the cells/organoids in the OoC should be established, and where standard procedures are in place for particular methods, these should be followed, however it is important that the approach taken is chip, organ and CoU-dependent; what baseline characteristics to record, and how should not be standardised.

Recommendation: develop a list of available methods for the most common cell types and recommendations in which settings they are applicable

— **Compound characterisation**

There are a number of aspects relating to compound characterisation that should be recorded to ensure data can be interpreted and analysed effectively: compound identity and purity, fractions of unbound compounds in media and non-specific binding to chip surface, stability in media over time, method of sample collection, material of collection tube and storage conditions to ensure minimal loss of compound/analyte.

An SOP for recording the compound characterisation should be established, and where standard procedures are in place for particular methods, these should be followed, however it is important that the approach taken is chip, organ and CoU-dependent; how to record these aspects should not be standardised.

Recommendation: develop an SOP for the recording of aspects of compound characterisation listed above

— **Study Design**

Below is a list of aspects of experimental design that would benefit from standardisation.

— **Appropriate positive and negative controls for each arm:** A well-designed experiment should include positive and negative controls (where possible) and the inclusion of reference item(s), which benchmark the response of the test system to the test (OECD GIVIMP), as appropriate. Considerations of what to include and report are well defined for in vivo (ARRIVE guidelines) and in vitro studies including NAMs (OECD, 2018; Petersen et al., 2023; The RIVER working group, 2023). For OoC studies, there are some publications that include guidelines on drugs/compounds to test for (i) specific applications (PK, PD, Tox, Safety, Efficacy) used e.g. in ADME-related applications (Fowler et al., 2020) or (ii) per specific organ (e.g. (Baudy et al., 2020). However, these are not available for all organs or applications, and developing a list of standard test compounds for organ- or application-specific effects would ensure consistency in the evaluation of organ models and new compounds, thereby increasing confidence in OoC.

Recommendation: develop a standard list of positive and negative controls for specific organs and applications

— **Sample size (number of experimental units):** Sample size relates to the number of experimental units in each group. Both the ARRIVE and RIVER guidelines outline clearly how to define experimental and biological units, and how these should be decided on and reported. While not directly for OoC studies, these guidelines are applicable to such studies. To ensure correct inclusion and reporting, it would be useful to have examples of the appropriate experimental unit allocation for different MPS. Publications by (Cairns et al., 2023; Peel et al., 2019) define the experimental unit in their specific OoC studies, which could be used for guidance, but more are needed. Moreover, it would also be useful to have guidance on how to account for the possibility of a low n for some MPS owing to the complexity leading to a small maximum n in any one study. Power analysis demonstrating the study is appropriately powered for the given number of samples/experimental units will be important to include.

  Recommendation: develop OoC-specific guidance on allocation of n/EU in OoC studies, including how to ensure robust experimental design when the maximum n is low.

— **Operators:** OoC studies typically require multiple operators owing to the technical complexity of the systems limiting the number of chips that can be reliably handled by one operator at any given time. Consequently, multiple operators will be required to handle the chips for a given time point, and different operators may be required over the course of a study due to practical/staffing limitations. This can introduce variability and/or bias into the experiment and therefore needs to be carefully controlled and standardised within an OoC study to ensure robust and reproducible data. Randomisation of the operators to conditions/chips needs to be carefully considered and included in the standard guidance on randomisation (see 'Randomisation' section); for example, if two operators are performing the experiment, control and treated chips should be distributed between the operators so as not to confound treatment effects with operator effects.

  Recommendation: develop standard guidance on considerations regarding the need for multiple operators in a study to ensure the study is robust. Moreover, randomisation of the operators to conditions/chips and across timepoints needs to be considered and included in the standard guidance on randomisation (see 'Randomisation' section). It is not appropriate to standardise the number of operators, since this will vary depending on a number of factors such as the chip system being used and the size of the study.

— **Randomisation:** Randomisation is a strategy to minimise potential confounders through appropriate distribution of experimental variables. The process for randomisation is well defined (ARRIVE, RIVER) but limited applications of this to OoC studies have been reported (Cairns et al., 2023, Peel et al., 2019). OoC studies tend to have more potential confounders than standard in vitro studies, such as multiple operators (OoC studies typically have multiple operators – see 'Operators' section), pump control units, and multiple chips. As such, standard guidance on how to apply randomisation to OoC studies with differing and often multiple technical variables is needed to protect against technical effects.

Recommendation: develop OoC-specific standard for randomisation across different OoC platforms accounting for multiple types of technical and biological variable

— **Sampling time points:** The ARRIVE guidelines document that it is important to describe what was measured, particularly when this can be done in different ways. This will be especially important for OoC studies, which will have different methods for accessing and sampling cells/media. Moreover, depending on the size of the OoC study, samples may need to be collected by multiple operators within a single study, thus randomisation of operator to samples/sample time points will be important and should be considered as part of the standards on randomisation outlined above. Other things important to consider would be the sample collection process (including details such as mixing, temperature, maximum time for collection, labelling procedure), minimal sample volume necessary for valid results, the timeframe for analysis and thereby storage, including tube material (to minimise compound/analyte binding). Regarding the test method for downstream sample analysis, the Technical Framework Publication (Petersen et al, 2023) calls out the need to incorporate one-time preliminary control experiments, periodic control measurements (e.g. daily, weekly, or monthly), and in-process control measurements (performed each time an assay is performed) into a method. This would be important for OoC studies, particularly when sampling is repeated over multiple timepoints. In this context, part of the guidance should consider whether samples should all be processed together at the end, or separately at each time point.

Recommendation: clear standard guidance/SOP on sampling from OoC studies, accounting for different types of chip, multiple operators and often small sample volumes. In particular, the process for collecting the sample, including tube storage material and storage conditions/times should be included.

7.3.2 Conclusion

The below table summarises the areas requiring standardisation (Table 3).

Area that needs standardisation	What is missing/needed
Positive and negative controls	A standard list of positive and negative controls for specific organs and applications
Sample size (experimental units)	Develop OoC-specific guidance on allocation of n/EU in OoC studies, including how to ensure robust experimental design when the maximum n is low
Operators	Where there are multiple operators, guidance on randomisation of the operators to conditions/chips and across timepoints needs to be considered and included in the standard guidance on randomisation (see 'Randomisation' section)
Randomisation	Randomisation across different OoC platforms accounting for multiple types of technical and biological variable

Sampling time points	Accounting for different types of chip, multiple operators and often small sample volumes. In particular, the process for collecting the sample, including tube storage material and storage conditions/times should be included

Table 3: Areas that need standardisation

7.4 Data Management

This topic applies to activities relating to data from OoC experiments including the day-to-day activities and final results. Biological data produced by OoC devices, including prolonged data collection, as well as technical data must be carefully documented and evaluated for accurate interpretation and reproducibility. This requires data management and complex analyses (e.g. computational modelling) covering aspects such as:

Experimental protocol: describing materials, e.g. pump and other hardware specifications, brand name of incubators, and **compound aspects** such as supplier, product and lot number, information on solubility, stability, binding specificity, lipophilicity, unbound fraction and non-specific binding to platform, time-concentration profiles either intracellular or extracellular in media and **other aspects** such as number of operators and details on chip/sample collection (e.g. date and time) and details on methods following a structured, transparent, accessible reporting strategy such as "STAR methods", applied by journals in the Cell Press family.

Technical/operational aspects: describing activities related to acquisition, organization and storage of raw data as well as analysis and reporting/disseminating results, providing templates for specific applications or organ-chips including formats of documents (e.g. doc(x), xls(x), ppt, pdf or any future formats).

Although standardisation on the data acquisition is of great interest, the results obtained are usually generated with equipment that are meant for biological purposes as overall and not specific for the OoCs. However, standardisation allows that the large amount of produced data can be integrated and reused by the scientific community either after publication or when exchanged with partners. The FAIR principles (Findability, Accessibility, Interoperability, and Reusability) provide a guide for scientific data management and stewardship. Concretely, this means using rich, highly structured, and interlinked metadata, stored in indexed and accessible repositories; data should be open to everybody who has the right to access, complying with GDPR and respecting IP rights and confidentiality of the data where needed. To ensure the interoperability of the data, the corresponding metadata should have multiple attributes, following relevant minimal information guidelines, to describe the content of the datasets and the context in which they were recorded, including the biological source material. The storage of data could be performed using a Laboratory Information Management System (LIMS). These systems consist of a software that allows you to effectively manage samples and associated data. This method is widely adopted in pharma and in GMP and GLP processes and should be adopted when performing OoC studies.

Computational Modelling of OoC Data: The computational modelling of data obtained from Organ-on-a-Chip experiments currently lacks standardized approaches, which is a critical gap in the field. This absence of standards affects the development, reporting, and reproducibility of computational models used to interpret OoC data and the potential translation to in-human situations.

There is a need for guidelines on developing computational models for OoC data. This includes methods for integrating biological data with physical and chemical parameters, considering the unique microenvironment of each organ chip. A standard approach would facilitate the comparison of results across different studies and enhance the predictive power of these models.

Clear guidelines are essential for reporting outcomes of computational models, such as estimated parameters and graphical visualizations. Standards should dictate the level of detail required to ensure transparency and enable other researchers to validate and build upon published findings.

The reproducibility of computational models is currently hindered by a lack of standardized coding practices and validation procedures. There is a pressing need for guidelines that cover good coding practices, model verification, and validation processes. This would ensure that models are not only reproducible but also qualified for specific applications in OoC research.

The selection of software and tools for data analysis (e.g. open-source like R) should adhere to best practices in software engineering and data science. Guidelines should recommend open-source tools where possible, to facilitate sharing and collaboration within the scientific community.

Encouraging collaborative frameworks that bring together biologists, engineers, and data scientists can foster the development of robust, standardized computational models. Such collaborations can lead to the establishment of shared repositories of models and data, further advancing the field.

Incorporating a standardized approach to computational modelling in OoC experiments is crucial. It will not only enhance the reliability and comparability of the results but also significantly contribute to the advancement of OoC technologies and their applications in drug development and disease modelling.

7.4.1 List of available standards

- STAR methods (Marcus, 2016)
- FAIR guiding principles for scientific data management and stewardship (Wilkinson et al., 2016)
- MIQE guidelines, scope is very narrow but could be a good starting point for reporting guidelines (Bustin et al., 2009)
- PRO-MaP (Leite et al., 2023)
- RIVER (Reporting In Vitro Experiments Responsibly) (The RIVER working group, 2023)
- ISO 20691:2022, Requirements for data formatting and description in the life sciences
- ISO 27001:2022 Annex A Control 8.28, This specific control in ISO 27001 emphasizes the development and implementation of secure coding processes. It includes considerations for secure coding principles during new coding projects, software reuse operations, and the use of development tools. Security testing is recommended during and after development, and there is a focus on ensuring security in the production environment.
- ISO/IEC 15408 (all parts): For more information on IT security evaluation, organizations are recommended to refer to ISO/IEC 15408.

This collection of recommendations and standards are not specific for OoCs but contain general principles that are also applicable for OoCs. A critical analysis of these principles is required to come to standardised data management and reporting for OoCs.

7.4.2 Areas requiring standardisation

- Standards that define the use of software and programming languages, e.g. open-source like R, Python
- Documentation verifying the use of FAIR
- Guidelines for using statistical software tools and tests as well as data analyses
- Reporting practices – a description of what should be included

7.4.3 Recommendations

It was found that the currently existing guidelines and standards for data management and reporting in life science experiments serve as a good starting point for OoC applications, but for specific aspects of OoC experiments standardisation is required. The table below summarizes the identified areas that require standardisation (Table 4).

Area that needs standardisation	**Identified Guidelines/Standards**	**What is missing/needed**
Experimental Protocol		The followed protocol should be completely described such that it can be reproduced.
Not applicable	PRO-MaP	The guideline "Promoting Reusable and Open Methods and Protocols"(Leite et al., 2023), proposes to stimulate the sharing of methods and protocols, that can be re-used by other scientists.
Compound- and Other Aspects		Reporting of compound- and other aspects to improve traceability and comparison of experimental results.
Not applicable	STAR	The Structured, Transparent, Accessible Reporting strategy (Marcus, 2016) gives guidelines how to publish these aspects. For internal administration, additional standardisation may be required.
Data Acquisition, Organisation and Storage		A standardised method to acquire and store data is crucial for subsequent data analysis and publication of results.
Not applicable	ISO 20691:2022	Requirements for data formatting and description in the life sciences is
Use of statistical methods and -tools		Standardisation in the use of statistical methods and -tools needs further effort.
Data analysis via computational modelling		
Not applicable	ISO 27001:2022, ISO 27002:2022, ISO/IEC 27005:2022	Currently, no specific standards or guidelines exist for the computational modelling of OoC data. There is a need for development of such standards, including guidelines on reporting modelling outcomes and ensuring

		reproducibility and qualification. ISO standards like ISO 27001:2022, ISO 27002:2022, and ISO/IEC 27005:2022, though not specific to OoC, provide a foundation in good coding practices and information security management which could be adapted for OoC computational modelling.
Reporting and Dissemination of Results		
Not applicable	FAIR principles	Data sharing principles, a general principle that also applies for OoC, it is not specific.
Not applicable	MIQE guidelines	Minimum Information for Publication of Quantitative Real-Time PCR Experiments, narrow scope but a good starting point. (Bustin et al., 2009)
Not applicable	RIVER	The general recommendations for Reporting In Vitro Experiments Responsibly should be considered and further evaluated for applicability in OoC experiments. (The RIVER working group, 2023)

Table 4: Areas that need standardisation

7.5 Conclusion

Hardware setup, experimental design and data management are essential to produce reliable, robust results from a biological system. Within the OoC area an additional layer of the design of the hardware is added and with a clear standard for the design of an OoC experiment the threshold for adapting these systems could be decreased.

So far, no unified way of reporting biological or hardware data from OoC is present. This comprises the data format and the type of data, e.g. biomarker levels on-chip or kinetics of investigated compounds or biomarkers. Additionally, no information on the used hardware is systematically stored and reported. This hinders technological adaptation in a wider community. Another aspect that hampers the wider use of OoC experiments, especially in the industry, is lack of comparability of data from different systems.

Standardisation in life sciences has many benefits, including enabling comparable research, complying with legislation, increasing patient safety, fostering innovation, and showing best practices. However, the adoption of OoC technology in the industry has been slow due to a lack of qualified assays with scientifically proven robustness, unclear applicability domains, and poor experience with the technology. To ensure that OoC models are fit for purpose, the qualification must include external aspects such as the availability of laboratory infrastructure, well-documented SOPs, and strong technical support, in addition to the characterization of the model and assay. Design specifications for OoC models must be based on the intended use or purpose. For example, the design specifications of a Lung-on-a-Chip model to study pulmonary oedema will be different from a Liver-on-a-Chip model aiming to predict drug-induced liver injury.

Presenting a framework on data reporting from OoC biological experiments would enable comparing on-chip performance across labs and operators, which would identify the best-in-class chip for a specific application. It further provides a guideline on how to setup a biological

experiment. Stored data from biological experiments and hardware in a unified database in combination with newly available machine learning and artificial intelligence algorithms may unlock unforeseen potential of these chips to impact the drug development process.

An important extension of this concept is the development of digital twins through computational modelling. Digital twins, essentially detailed and dynamic computational representations of the physical OoC models and the emulated biology, can significantly enhance the understanding and predictive power of these systems. However, the creation and use of digital twins in the OoC field face challenges due to the lack of standardized computational modelling approaches and guidelines. As such, there is a pressing need for establishing standards in this area, including the development of guidelines for reporting modelling outcomes, ensuring reproducibility and qualification of these models. Incorporating good coding practices and adhering to relevant ISO standards like ISO 27001:2022, ISO 27002:2022, and ISO/IEC 27005 could provide a foundational framework for developing these computational models. The integration of computational modelling, particularly digital twins, into this framework, is a critical step toward achieving these goals and unlocking the full potential of OoC technology.

With standards being applied, this lack of comparability could be overcome since the adoption of standards will ensure that all information is captured from all experimental aspects (biology, hardware, data, etc), else, the information about the experiment will capture only what the experimenter considers important. Following standardized guidelines will ensure that data from OoC can be reproduced and compared across labs and operators, leading to the substantial increase and build-up of relevant data in different areas (e.g. disease modelling, PK/PD modelling etc.). The increase in availability and understanding of the data from OoC experiments and the interpretation thereof would be a major advantage in presenting the data to e.g. regulatory agencies. Most importantly, a better understanding of OoC systems would be gained, enabling clinical applications and promoting the widespread use of OoC models.

Standardizing experimental design in the OoC area is essential for reliable and comparable results. The lack of comparability of data from different systems is a major challenge in the wider use of OoC experiments, especially in the industry. The application of a clear standard for experimental design can help overcome this challenge and ensure that all relevant data are captured. This will enable data from these models to be reproduced and compared across labs and operators, leading to increased understanding and implications for the clinical setting. Furthermore, the integration of computational models, particularly digital twins, into this standardisation process is crucial. These models can significantly enhance the predictive accuracy and utility of OoC systems, making them more valuable for research and clinical applications. The development and standardisation of computational modelling approaches will be a key factor in realizing the full potential of OoC technology.

8 User perspective and regulatory, legal and ethical aspects

8.1 Introduction

This chapter provides background information on the most relevant scientific applications of OoC technology, discussing potential implications within existing regulatory, legal and ethical aspects.

In section 8.2, the use of OoC devices as tools to enable precision medicine is described. OoC can be used in internal decision-making to predict drug responses in specific organs, but also to screen candidate molecules for efficacy. Interestingly, OoC are also used to provide data for drug repurposing, complementing information from clinical trials.

Considerations on the applicability of the Medical Device Regulation (MDR), In Vitro Diagnostics Regulations (IVDR) and Advanced Therapy Medicinal Products (ATMP) to OoC devices were made in section 8.3.

Section 8.4 discusses the use of OoC as non-animal tools for regulatory use across different sectors. The paragraph includes some considerations on the scientific assessment that is necessary to comply with current requirements for test methods.

This chapter ends with ethical considerations for the use of non-animal, human-based models in the EU context, with some specific considerations on OoC.

8.2 Use of OoC for medical purposes: diagnosis, treatment, drug repurposing

8.2.1 Prediction of patient-specific drug response

To date, OoC technology has mostly been developed as a means to improve the drug discovery and preclinical development processes, to provide experimental data for the development of improved in silico models, and to support the replacement, refinement, and reduction of animals used for scientific purposes. In addition to these relevant goals, OoC promises to become an important technology for understanding variability in patients' response to drugs and for enabling precision medicine in clinical use.

OoC models are increasingly populated with induced pluripotent stem cells (iPSCs)–derived cells, organoids or tissue biopsies. These models carry individual variations in genetics, physiology, and other biological factors, enabling a better understanding of the patient's disease and how they might respond to potential treatments (Peck et al., 2020). An OoC from a specific patient could be used to screen a range of drugs, drug combinations, and doses to identify which has the potential to be most effective in that patient. For example, a glioblastoma-on-chip model using patient-derived cells was shown to be predictive of patient-specific resistances for chemoradiation with temozolomide and could be used to determine drug combinations associated with more effective tumour killing (Yi et al., 2019). When multiple chips are seeded with cells from different donors representing different subpopulations or patients with a different comorbidity, OoC models might also be used to design and optimize drugs for specific subgroups, allowing patient stratification for targeted clinical trials (Ingber, 2022).

Use of OoC technology for decision-making on the treatment of an individual patient would require qualification for the specific application to meet possible regulatory requirements. This brings new challenges to the field as no clear guidelines for these models to be accepted as tools for tailor-made treatment strategies currently exist. Recently the PERMIT (Personalized Medicine Trials) Group has presented 15 recommendations to improve the robustness of preclinical methods in translational research for personalized medicine (Fosse et al., 2023). These recommendations include the development of standards to characterize new models and methods in support of their qualification for prediction of the best personalized therapy for each individual patient. This will be an important step in establishing scientific credibility and building confidence in new technologies for preclinical personalized medicine within the regulatory science community.

8.2.2 Drug repurposing

Medicines repurposing (also known as drug repurposing, drug repositioning, drug recycling and therapeutic switching) describes the process of recognising new medical indications for a medicine with an existing marketing authorisation. The sponsor/manufacturer must seek regulatory approval to broaden the approved indications or expand the treatment population on the basis of new clinical evidence gathered, as indicated in the Regulation EC/1234/2008 and related guidelines from the European Medicines Agency (European Medicines Agency, 2024c).

Although this is a very specific scenario, it represents a very practical and imminent application where OoCs could rapidly supersede conventional experimental approaches used to support medicines repurposing applications (e.g. in vivo animal studies). OoC systems have the potential

to significantly improve the standard of evidence for such regulatory submissions, whilst also enhancing the safety of human clinical trials conducted to support them.

Typically, this new evidence comprises in vivo proof-of-concept studies in relevant animal species, additional ad hoc clinical trials in patients and clinical evidence from off-label (i.e. use of a medicine for an unapproved indication or in an unapproved age group, dosage or route of administration) or compassionate use (Agency, 2024a)(i.e. medicinal products without a Marketing Authorisation that may be made available for compassionate reasons to a group of patients with a chronically or seriously debilitating disease or whose disease is considered to be life-threatening, and who cannot be treated satisfactorily by an authorized medicinal product). While there is an expectation that a substantial clinical-based evidence demonstrating the product's safety profile would already exist, additional non-clinical toxicology studies may be requested if the new treatment population is substantially different from the original one.

OoC systems could be utilised to perform proof-of-concept studies to support the proposed new clinical application(s), allowing for clinically relevant evaluations of functional activity. OoC can also be used to further characterise the response to treatment which could lead to reduction of risks associated with human clinical studies by reducing sample size required, reducing study duration, and enhancing clinical outcome assessments.

This is particularly relevant in relation to rare diseases where developers are faced with significant challenges around trial participant recruitment and retention, often leading to increased reliance on non-clinical proof-of-concept in determining the risk-benefit of the proposed treatment in such populations. Furthermore, whilst there are over 7000 rare diseases recognised by the EMA and FDA, it is estimated that less than 10% of these are actively being researched by developers due to lack of reflective animal models of disease (The Lancet Diabetes & Endocrinology, 2019). OoC will offer additional options and it is to be expected that over time drug repurposing applications for rare diseases will increasingly involve such new approaches.

The EMA and FDA have openly stated (Han, 2023) that, going forward, they will strongly support utilisation of in vitro data to expand disease indications where there is a significant lack of drug development precedent. This regulatory openness to evidentiary alternatives and novel methodologies is welcome, and OoC systems are likely to figure very prominently in this area. However, adjusted regulatory guidance and standards will be required to encourage developers to widely adopt these alternative approaches.

Real-case example *- A system composed of human induced pluripotent stem cell (iPSC)-derived motoneurons and human Schwann cells. Exposure to serum from MMN and CIDP patients led to increased autoantibody binding and activation of the classical complement cascade, a critical part of the immune system response. Additionally, patient-mediated serum exposure reduced conduction velocity and decreased action potential firing frequency in their functional model, recapitulating the clinical features observed in patients. The addition of TNT005, an antibody developed by Sanofi that inhibits the classical complement pathway, rescued neuronal function and restored spontaneous frequency and conduction velocity, which was supportive data used by Sanofi for their IND filing* (Rumsey et al., 2022)*.*

8.3 Considerations on applicability of the Medical Device Regulation (MDR), In Vitro Diagnostics Regulations (IVDR) and Advanced Therapy Medicinal Products (ATMP) to OoC devices

8.3.1 Medical Device Regulation (EU) 2017/745 (European Parliament and Council, 2017)

An OoC with a direct medical purpose, would be functionally similar to a medical device (MD, see definition in Box 1). Just as in the case of MDs, the necessity of demonstrating its safety and efficacy is required before the device is used.

BOX 1

The definition of '**medical device (MD)**, as given by the MDR 2017/745, is the following:

"'Medical device' means any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

— diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease,

— diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability,

— investigation, replacement or modification of the anatomy or of a physiological or pathological process or state,

— providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations,

and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means."

Expressly, the MDR states in article 1(6)(1) that the MDR does not apply to "transplants, tissues or cells of human origin, or their derivatives, covered by Directive 2004/23/EC, or products containing or consisting of them; however this Regulation does apply to devices manufactured utilising derivatives of tissues or cells of human origin which are non-viable or are rendered non-viable." As OoCs require viable derivatives, the OoC is exempt from the scope of the MDR. Only in case an OoC is using its diagnostic capabilities through the "examination of specimens, including blood and tissue donations, derived from the human body", a reference to the IVDR could be made (see section 8.3.3).

8.3.2 ATMP regulation (European Parliament and Council, 2007)

On the contrary, the regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products (ATMPs) could be applicable since it explicitly foresees the use of viable cells or tissues. Hence, an OoC partially meet the definition of either a 'Somatic cell therapy medicinal product' or a "Tissue engineered product', provided that the cells (or tissues) used in the fabrication of the OoC itself are considered "engineered cells" ("engineered tissues").

More precisely, the OoC itself can be considered as a "combined ATMP", owing to its multiple components (i.e. a physico-chemical and a biological component). Therefore, whereas the cells or tissues embedded in an OoC have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, the OoC itself should follow the ATMP regulation. The same applies whenever the OoC contains cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor.

However, this regulation only holds for products that are administered to the patients and thus are used inside the body. Thus, the ATMP regulation might apply to OoC once their scope is broadened to internal use in a patient.

2525 BOX 2 (European Parliament and Council, 2010)

The definition of ***'Somatic cell therapy medicinal product'***, as given by Part IV of Annex I to Directive 2001/83/EC[z] on the Community code relating to medicinal products for human use, is as follows:

'Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics:

(a) contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor;

(b) is presented as having properties for, or is used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.

For the purposes of point (a), the manipulations listed in Annex I to Regulation (EC) No 1394/2007, in particular, shall not be considered as substantial manipulations. For instance, "cell separation, concentration or purification" are not considered as substantial manipulations.

From Art 2(a) of the ATMP 1394/2007, the following definition applies:

*'**Advanced therapy medicinal product**' means any of the following medicinal products for human use:*

— a gene therapy medicinal product as defined in Part IV

of Annex I to Directive 2001/83/EC,

— a somatic cell therapy medicinal product as defined in

Part IV of Annex I to Directive 2001/83/EC,

— a tissue engineered product as defined in point (b)

From Art 2(b) of the ATMP Regulation, the following definition applies:

*"'**Tissue engineered product**' means a product that:*

— contains or consists of engineered cells or tissues, and

— is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue."

From Art 2(c) of the ATMP Regulation, the following definition applies:

*"**Cells or tissues shall be considered 'engineered'** if they fulfil at least one of the following conditions:*

— the cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved. The manipulations listed in Annex I, in particular, shall not be considered as substantial manipulations,

— the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor."

From Art 2(d) of the ATMP Regulation, the following definition applies:

*"'**Combined advanced therapy medicinal product'** means an advanced therapy medicinal product that fulfils the following conditions:*

— it must incorporate, as an integral part of the product, one or more medical devices within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of Article 1(2)(c) of Directive 90/385/EEC, and

— its cellular or tissue part must contain viable cells or tissues, or

— its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be considered as primary to that of the devices referred to."

8.3.3 IVD Regulation (EU) 2017/7468

Regarding the case of OoCs with diagnostic functions, which also fall into the category of OoCs with a direct medical purpose, it can be stated that they can be functionally similar to an IVD (in vitro diagnostic) MD, according to the definition in Box 3.

From this definition, the functional similarity of such a device to an OoC with diagnostic capabilities is evident, (e.g. for cases a, c, e and e). A companion diagnostic is an in vitro diagnostic test that supports the safe and effective use of a specific medicinal product, by identifying patients that are suitable or unsuitable for treatment. Also in this case, the functional similarity of such a device to an OoC with diagnostic capabilities is evident (e.g. for case a). A reference to its 'Annex VIII: Classification rules' may be useful as a guideline for risk class assignment of diagnostic devices, in view of a future regulatory framework for OoCs with diagnostic function.

BOX 3

The IVD Regulation defines (Article 2(1-2)):

(1) 'medical device' means 'medical device' as defined in point (1) of Article 2 of Regulation (EU) 2017/745;

(2) 'in vitro diagnostic medical device' means any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, piece of equipment, software or system, whether used alone or in combination, intended by the manufacturer to be used in vitro for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information on one or more of the following:

(a) concerning a physiological or pathological process or state;

(b) concerning congenital physical or mental impairments;

(c) concerning the predisposition to a medical condition or a disease;

(d) to determine the safety and compatibility with potential recipients;

(e) to predict treatment response or reactions;

(f) to define or monitoring therapeutic measures.

Specimen receptacles shall also be deemed to be in vitro diagnostic medical devices

The IVD Regulation also defines (Article 2(1-2)):

(7) 'companion diagnostic' means a device which is essential for the safe and effective use of a corresponding medicinal product to:

(a) identify, before and/or during treatment, patients who are most likely to benefit from the corresponding medicinal product; or

(b) identify, before and/or during treatment, patients likely to be at increased risk of serious adverse reactions as a result of treatment with the corresponding medicinal product;

Regarding the applicability of the IVDR to OoC devices, it can be argued alternatively that OoC:

- do not fall under the IVDR. An IVD MD, although being a particular type of MD, is still a medical device, as defined in the MDR. Hence, it cannot contain cells or tissues of human or animal origin, unless they are made non–viable, as implicitly stated in Annex VI of the IVDR, or.

— do fall under the scope of the IVDR. The IVDR only requires that an IVD meets the definition of medical device, which is what the literal text of the definition of IVD requires under the IVDR (cf. art. 1(1)). Furthermore, the MDR provides that it does not apply to IVDs in article 1(6)(a) MDR.

It must be noted that there is not yet any case law from the CJEU that addresses this matter. In order to dissipate any doubt about the possible applicability of the IVDR to OoCs, it is possible to ask for the opinion of the Medical Device Coordination Group (MDCG), established under Article 103 of the MDR. The MDCG plays a strategic role also for IVD devices, see IVDR, Article 3. In particular, the consultation of the MDCG is necessary: (Art. 3.1) "Upon a duly substantiated request of a Member State, the Commission shall, after consulting the Medical Device Coordination Group established under Article 103 of Regulation (EU) 2017/745, by means of implementing acts, determine whether or not a specific product, or category or group of products, falls within the definitions of 'in vitro diagnostic medical device' or 'accessory for an in vitro diagnostic medical device'."

8.4 Use of OoC as alternative tools for regulatory applications

Regulatory toxicological testing is based on internationally agreed test guidelines, covering in vivo and in vitro test methods. These guidelines are internationally issued by organisations such as OECD (OECD, 2018) and ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), 2024). Animal studies, whether for the development or production of new medicines, for physiological studies, for studying environmental effects or for the testing of chemicals or new food additives, must be carried out in compliance with EU legislation, which also includes compliance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The directive includes the principles of the Three Rs – Reduction, Replacement, Refinement – in the legal text. The term alternative includes those methods (assays, tests, methods, techniques, tools, strategies and approaches) that can:

— Obtain the required information without the use of live animals.

— Reduce the numbers of animals whilst obtaining the same level of information.

— Refine the use of live animals to cause less pain, distress or suffering, or improve the welfare of the animals.

An OoC could be considered as an alternative approach to evaluate toxicological properties for the compounds regulated in the following European regulations and directives, provided that their scientific validity is established.

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP)
Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market
Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products
Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products

Directive 2001/83/EC of the European Parliament and of the council of 6 November 2001 on the Community code relating to medicinal products for human use
Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products

The confidence in these test methods used in a regulatory context is gained through a scientific validation process, demonstrating the reliability and relevance of a particular approach, method, process or assessment for a defined purpose. Scientific validation is a prerequisite for regulatory acceptance but it is insufficient to guarantee regulatory acceptance (capability of a test method to provide answer to specific regulatory questions). Validation in multiple laboratories across regions to build a weight of evidence approach could support the efforts towards global harmonisation and regulatory acceptance.

Several test methods, such as new approach methodologies utilising OoC technology, can be combined to produce a prediction model of adverse outcomes as Integrated Approaches to Testing and Assessment (IATA) to address a toxicity or biological effect of interest. Different requirements may be applicable depending on the application of the technology and complexity of the system, for example the weight and type of evidence needed to support single organ systems versus multi-organ systems.

BOX 4

Some common toxicological endpoints are:

- Skin corrosion and irritation
- Serious eye damage/eye irritation
- Photo-induced toxicity
- Mutagenicity/genotoxicity
- Acute toxicity
- Skin sensitisation
- Repeated dose toxicity
- Carcinogenicity
- Reproductive and developmental toxicity
- Absorption, distribution, metabolism and excretion (ADME)
- Toxicokinetics (TK)
- Cardiotoxicity
- Hepatotoxicity
- Nephrotoxicity
- Neurotoxicity

— Endocrine disruption

It is possible to develop a battery of alternative tests, able to combine information from different test methods and integrating information from other sources. In these cases, a mechanistic based approached is recommended, for instance through the use of Adverse Outcome Pathways (AOPs). An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. The AOP-wiki is a useful tool for collaborative AOP building (AOP-Wiki, 2024). Two valid examples of this approach are:

— Thyroid validation study (Bartnicka J et al., 2021)

— Developmental Neurotoxicity (Blum et al., 2023)

Valid (but not validated by a validation body) methods can also be used to support regulatory decision making (e.g. pharmaceuticals) or as a decision-making tool (e.g. chemicals). Qualification is the term used in the medicinal domain to refer to the scientific assessment of the reliability and relevance for a specific context of use. An example of how qualified assays are evaluated can be found in Annex 2 of the ICH S5 (R3) guideline on reproductive toxicology (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), 2020), but many efforts are underway to bridge the regulatory needs and the OoC technological advancements. The EUROoCS Regulatory Advisory Board and the EC JRC created a catalogue of resources for developers and end-users to support validation and qualification of new technologies. The catalogue contains a curated list of relevant reading documents and a set of Frequently Asked Questions related to the main regulatory fields of interest (e.g. chemical, drug and food safety). Based on this list, a qualification framework and its practical implications were discussed by the stakeholder community (Piergiovanni et al., 2024).

Support to OoC developers that want to pursue regulatory use is available at the European Medicines Agency (EMA), in the form of scientific advice to support the qualification of innovative methods for a specific intended use in the context of research and development into pharmaceuticals (European Medicines Agency, 2024b). Moreover, EMA Innovation Task Force offers the possibility for researchers/developers to interact with regulators at a very early stage of the innovation process, to better design qualification assessment for specific contexts of use (Agency, 2024b). A similar approach is also offered by FDA, through the ISTAND programme (U.S. Food & Drug Administration (FDA), 2024).

Global harmonisation is key to the wider acceptance of the use of OoC for regulatory purposes, as varying requirements in different markets drive industry to develop large, risk-averse approaches to ensure global regulatory acceptance. However, there are no currently accepted global reporting standards that would support the wider application of OoC technology.

8.5 Ethical considerations for OoC use in the EU context

Standardized methods and technologies for the production of OoC will enable the development of these devices for preclinical, clinical and regulatory applications on a broad scale, allowing comparative studies between laboratories and applications across the research landscape. However, the wider adoption of these technologies will lead to a number of ethical considerations, relevant to the appropriate application domain, requiring standardized description or best practice to be defined. The breadth of ethical considerations that may arise in many areas of research is discussed in detail as part of the Horizon 2020 Programme self-assessment process (European Commission, 2024), however specific areas which may be relevant to OoC are summarised below.

It should be noted that the regulations, directives and guidance in place from regulatory authorities and governing bodies provide a compliance framework for research activities.

However, working in compliance does not deem a piece of research to be necessarily ethical. It is important that relevant ethical steering groups and advisory boards be in place to guide the varied ethical considerations that may emerge for the development and application of OoC technologies.

8.5.1 The use of animals and the 3Rs

OoC technology has created a promising opportunity for the replacement of animals in basic and applied research, through the provision of models that are faster, cheaper and more physiologically relevant where human tissues are used. The principles of the replacement, refinement and reduction of animals in research are embedded in the regulations that govern the use of animals in scientific procedures. Indeed, Directive 2010/63/EU specifies that wherever possible, scientifically satisfactory methods not entailing the use of live animals should be used for experimental or other scientific purposes. As the advancement in this area progresses, these technologies and their applications should be reviewed in order to identify where replacements are possible, to support the phasing out of animal procedures for research and regulatory testing (Zuang, V. et al., 2022).

Where animal tissue and organs are used for the development of in vitro methods, the principles of refinement and reduction should be applied. It should be noted that, despite different attitudes and national perceptions on the use of animals in research in EU member states, it is generally desirable to replace the use of animals to protect human and animal health as soon as it is scientifically possible to do so (Directive 2010/63/EU (European Parliament and Council, 2010)).

The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) is tasked to promote the development and use of alternatives in the area of regulatory testing and biomedical research and coordinate the validation of non-animal methods, such as those that OoC technology may offer, in collaboration with the EU Network of Validation laboratories (EU-NETVAL).

8.5.2 The use of human tissues, cells and data

The integration of human tissues and cells supports the efforts to develop more physiologically relevant models for the improvement of science. The ethical principles outlined in the Declaration of Helsinki (DoH) provide the fundamental guidance for all parties involved in medical research using human subjects, tissues and associated data. The cornerstone of these principles is the absolute requirement of an Informed Consent either from the subjects or their legal representative allowing the particular intervention and the use of personal associated data, such as any information affiliated with biological material, according to the General Data Protection Regulation GDPR Regulation (European Parliament and Council, 2016). Guidance from the European Commission for the preparation of funding applications supports the identification and management of ethics issues that may arise from research and development, including the use of human cells and tissues, and is a useful framework to assess the multi-faceted ethical considerations of research. The EU Directive 2004/23/EC further sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. Furthermore, international, national or regional regulations or requirements also apply to specific topics. ISO 15189:2022 and other clinical standards are intended to apply first and foremost for entities handling human materials procured and used for diagnostic and treatment purposes.

Specific considerations when using human tissues and cells for OoC technologies may include the agreed international Good Clinical Practice standard that applies when conducting research for clinical trials. Where human cells are genetically modified, the EC Good Practice on the assessment of GMO-related aspects (https://health.ec.europa.eu/document/download/62bc65ee-7f74-4b76-bdc3-07909ab177ee_en, 2024) should be implemented.

The applications of OoC for drug delivery/development, personalized medicine and other clinical contexts lead to further ethical considerations in terms of the use, storage and labelling of human data. Following from the DoH, the *Declaration of Taipei* enacts the ethical principles and importance of protecting the dignity, autonomy, privacy and confidentiality of research subjects regarding health databases and biobanks. As a fundamental human right, data protection must be rigorously applied by the research community to meet and be compliant with the EU's 2016 General Data Protection Regulation (GDPR). Developers are obliged to provide research subjects with what will happen to any personal data collected, and the data must be properly collected and stored. Furthermore, the reliance on computational support to manage the increasing volume, complexity and creation speed of data with minimal human intervention emphasizes the importance of the FAIR Guiding Principles for scientific data management and stewardship. These guidelines recommend how to improve the Findability, Accessibility, Interoperability and Reusability of digital assets, referring to both data (any digital object) and metadata (any information about digital objects), to support good data management. Using these guidelines to implement sound management of research data will ultimately support the advancement of discovery, innovation, knowledge integration and data reuse.

8.5.3 Commercial use of cells

The use of cells for commercial purposes is impacted by regulation over the use of human tissue, the regulation of pharmaceutical products or medical devices, and the influence of international legislation where a commercial product may be globally distributed. Importantly, the process of informed consent should include the potential uses of the tissue, particularly if there is a commercial objective or there is a possibility of a commercial outcome, which may impact the donation of tissues or cells. Furthermore, where donations are made as part of a medical diagnosis or treatment process, the commercial potential of the materials should in no way influence the application of good medical practice (Petrini, 2012). As both OoC and precision medicine technologies advance, a clear framework that can be used to govern the commercial use of cells would benefit both the developers and groups or individuals that donate tissue.

8.5.4 Ethical implications of specific OoC

The development of specific OoC systems may require particular ethical considerations. For example brain-on-chip or full body-on-chip systems raise questions regarding the potential development of consciousness or sentiency of such models. Furthermore, as the brain is the carrier of personal identity, these models may require special status to consider the wider impact of their development and maintenance. These reflections may influence the conditions by which cells are donated and could be linked to informed consent, and the implications of broad consent (e.g. for biobanking purposes).

OoCs developed for the purposes of developmental research or toxicity screening may mitigate ethical questions raised by the use of human tissues, but still raise innate ethical questions regarding the length of time, level of complexity, and level of protection that regulate such cultures. The OoC field is progressing at rapid pace and currently, without specific regulatory standards, it is important that the community determines which information is required to support informed decision-making that will ultimately protect patient autonomy (Thakar & Fenton, 2023).

8.5.5 Dual uses of OoC

Life science research is subject to the consideration of the dual-use dilemma wherein research is intended to provide a clear benefit but could be misapplied to do harm, whether through negative consequences to human health and safety, agriculture, the environment or national security (e.g. bioterrorism). The ethical implications and regulatory measures required are dependent on the identification of research that has potential to be misused (i.e. having a 'dual use' character)

(Salloch, 2018). These discussions require input from the researchers, organisations, funders, regulators and governing bodies.

8.5.6 Ethical considerations – an international and community perspective

Further discussion/consideration may be warranted for international, global research. For example, the exchange of resources, biological material and data outside of the EU (and therefore outside the reach of EU laws and standards) may require further discussion or raise specific ethical issues such as the exploitation of research participants and local resources, risks to researchers and staff and research that is prohibited in the EU. Where research involves the transfer of cells and tissues to/from non-EU countries, researchers must be compliant with the provisions outlined in Directive 2004/23/EC and consider the requirements of GDPR and data transfer to non-EU countries. The European Group on Ethics in Science and New Technologies (EGE) works to integrate ethics at an international level and act as an independent, inter-disciplinary perspective on the ethical questions posed by scientific and technological innovation, and supports the upholding of the international ethics framework.

Finally, these conversations must take place within and acknowledge the views of the broader community and public (Thakar & Fenton, 2023). The scientific community must be transparent to the public as more understanding is gained of the broader impact, benefits and risks of OoC within personalised medicine, toxicology and other applications, so that the field may continue to move forward in a meaningful, and potentially transformational, way.

9 Conclusion and future outlook

This roadmap document is the results of two years of work, 10 Focus Group meetings, numerous Working Group meetings and the active participation of around 120 experts of the CEN/CENELEC Focus Group Organ-on-Chip (FGOoC).

Next steps

Having identified needs for standardisation on Organ-on-Chip above, a next question is how to organize the work such that on the one hand European interest is guarded and the work from the FGOoC is recognized and built upon. On the other hand fragmentation should be avoided, and the focus should be on Europe's position in a global market. This requires discussion and proposals at the European level, as well as global coordination with ISO, IEC, MFMET and other relevant standards-developing initiatives. The present document provides a base for such discussion, proposals and coordination.

Future outlook

The FGOoC advises the creation of a European Technical Committee Microphysiological Systems to focus European stakeholders and interest. Furthermore, it advices this TC to put forward the work program for adoption within the ISO standardisation community, in close connection with at least ISO/TC 276 Biotechnology and ISO/TC 48 WG3 Microfluidics. By choosing to standardize at the international ISO level rather than the European CEN/CENELEC level, stakeholders recognize the widespread interest in OoC standardisation across countries and the diverse initiatives already underway. This decision acknowledges the global value chain for OoC technologies and ensures that standards are developed with input from stakeholders worldwide, fostering innovation, interoperability, and safety in this rapidly advancing field.

Through this international standardisation, stakeholders from various regions can collaborate to develop comprehensive standards that address the unique challenges and opportunities presented by OoC technologies. By leveraging international expertise and perspectives, these

standards have the potential to drive harmonization, facilitate regulatory compliance, and accelerate the translation of OoC research into impactful applications for healthcare, drug discovery, and beyond.

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Annex A Terminology List

WG1 has developed a terminology list based on a survey prior to and during the roadmap development process. Based on the results it is recommended to define the following terms in further standardisation activities.

- Actuator
- Biocompatibility
- Biological material
- Bioprinting
- Contamination
- Decellularized
- Disease-on-Chip
- Donor
- Engineered cells
- Hydrogel
- Identity verification
- Induced pluripotent stem cell (iPSC)
- Informed consent
- Interoperability
- Leakage
- Microfluidics
- Microphysiological system
- Micro-reaction-chambers
- Multipotent stem cells
- Organ-on-Chip
- Passage number
- Patient-derived primary cells
- Pluripotent stem cell (PSC)

— Primary cells

— Primary culture

— Reference compound

— Reliability

— Repeatability

— Reproducibility

— Sample

— Scaffold

— Stability

— Standard operating procedure (SOP)

— Tagging

— Translatability

— Validation

— Verification

— Viability

Annex B Identified Available Standards

This Annex lists all identified available standards.

Chapter 3 Terms and Definitions

- ASTM F3570 – 22 - Standard Terminology Relating to Microphysiological Systems
- ISO 10991:2023, Microfluidics - Vocabulary

Chapter 5 Biosciences

Cell and Tissue Sources

- Good In Vitro Method Practices (GIVIMP)
- International Society for Stem Cell Research (ISSCR) guidelines
- Guidance on Good Cell Culture Practice (GCCP)
- Guidelines for the use of cell lines in biomedical research
- ISO 20387:2018, Biotechnology - Biobanking - General requirements for biobanking
- ISO 21709:2020, Biotechnology - Biobanking - Process and quality requirements for establishment, maintenance and characterization of mammalian cell lines
- ISO 24603:2022, Biotechnology - Biobanking - Requirements for human and mouse pluripotent stem cells

Biomaterials

- ASTM F2739 – 19 Standard Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds
- ASTM F2150-19 Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products
- ASTM F2038-18 Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications Part I & II Formulations and Uncured Materials
- ASTM F2315-18 Standard Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels
- ASTM F748-16 Standard Practice for Selecting Generic Biological Test Methods for Materials and devices
- ASTM F3142-16 Standard Guide for Evaluation of in vitro Release of Biomolecules from Biomaterials Scaffolds for TEMPs

— ASTM F3354-19 Standard Guide for Evaluating Extracellular Matrix Decellularization Processes

Chapter 6 Engineering

— ISO 22916:2022, Microfluidic devices - Interoperability requirements for dimensions, connections and initial device classification

Material Specific Standards

— ASTM F2027, Standard Guide for Characterization and Testing of Raw or Starting Biomaterials for Tissue- Engineered Medical Products

— ASTM F2212, Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)

— ISO 3826 (all parts), Plastics collapsible containers for human blood and blood components

— ISO 5832 (all parts), Implants for surgery — Metallic materials

— ISO 5834 (all parts), Implants for surgery — Ultra-high-molecular-weight polyethylene

— ISO 5838 (all parts), Implants for surgery — Metallic skeletal pins and wires

— ISO 6474-1:2019, Implants for surgery — Ceramic materials — Part 1: Ceramic materials based on high purity alumina

— ISO 7153-1:2016, Surgical instruments — Materials — Part 1: Metals

Material Agnostic Standards

— ISO/TS 23565:2021 Biotechnology — Bioprocessing — General requirements and considerations for equipment systems used in the manufacturing of cells for therapeutic use

— ISO 20417:2012 Medical devices – Information to be supplied by the manufacturer

— ISO 16142-1:2016, - Medical devices — Recognized essential principles of safety and performance of medical devices — Part 1: General essential principles and additional specific essential principles for all non-IVD medical devices and guidance on the selection of standards

— ISO 7405:2018, Dentistry — Evaluation of biocompatibility of medical devices used in dentistry

— ISO 10993 (all parts), Biological evaluation of medical devices

Sensors and actuators in the Organ-on-Chip space

— **Sensors**

- ISO 14511:2019, Measurement of fluid flow in closed conduits – thermal mass flowmeter

- ISO/TS 23367-1:2022, Nanotechnologies — Performance characteristics of nanosensors for chemical and biomolecule detection

- ISO 14511:2019, Measurement of fluid flow in closed conduits – thermal mass flowmeters

- **Connection of sensors and actuators to instrumentation**

- **Measurement of flows and fluids**

  - IEC 60601-2-24:2012: Medical electrical equipment - Part 2-24: Particular requirements for the basic safety and essential performance of infusion pumps and controllers

  - AAMI TIR 101: Fluid Delivery Performance Testing For Infusion Pumps

  - ISO 4185:1980, Measurement of liquid flow in closed conduits - Weighing method

Modular integration of a microfluidic system

- ISO 22916:2022, Microfluidic devices — Interoperability requirements for dimensions, connections and initial device classification.

Hardware and Techniques from Existing Cell Culture Pipelines

- **Sterilization**

  - ISO/TS 22421:2021, Sterilization of health care products — Common requirements for sterilizers for terminal sterilization of medical devices in health care facilities

  - ISO 22441:2022, Sterilization of health care products — Common requirements for sterilizers for terminal sterilization of medical devices in health care facilities

  - ISO 11137 part 1-4, Sterilization of health care products - Radiation

  - ISO/TS 21387:2020, Sterilization of medical devices - Guidance on the requirements for the validation and routine processing of ethylene oxide sterilization processes using parametric release

  - ISO 11135 (all parts), Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices

  - ISO 11138 (all parts), Sterilization of health care products — Biological indicators

  - ISO 11140 (all parts), Sterilization of health care products — Chemical indicators

- ISO 7886 (all parts), Sterile hypodermic syringes for single use
- ISO 8536 (all parts), Infusion equipment for medical use
- ISO 8537:2016, Sterile single-use syringes, with or without needle, for insulin
- ISO 13408 (all parts), Aseptic processing of health care products
- ISO 17665-2:2006, Sterilization of health care products , Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices
- ISO 17665-2:2006, Sterilization of health care products, Part 2: Guidance on the application of ISO 17665-1
- ISO 17665-3:2006, Sterilization of health care products, Part 3: Guidance on the designation of a medical device to a product family and processing category for steam sterilization

- **Microplates**
  - ANSI SLAS 1-2004 (R2012): Footprint Dimensions
  - ANSI SLAS 2-2004 (R2012): Height Dimensions
  - ANSI SLAS 3-2004 (R2012): Bottom Outside Flange Dimensions
  - ANSI SLAS 4-2004 (R2012): Well Positions
  - ANSI SLAS 6-2012: Well Bottom Elevation
  - ANSI SLAS 4-2004 (R2012) SBS micro plate format

Chapter 7 Hardware parameters, experimental design and data management

Hardware parameters that directly impact experimental data

- ARRIVE guidelines are a resource for best practice in designing and reporting animal studies
- Guidance Document on Good In Vitro Method Practices (GIVIMP)
- ISO 13485:2016, Medical devices - Quality management systems - Requirements for regulatory purposes

Data Management

- STAR methods
- FAIR guiding principles for scientific data management and stewardship
- MIQE guidelines, scope is very narrow but could be a good starting point for reporting guidelines
- PRO-MaP

- RIVER (Reporting In Vitro Experiments Responsibly)
- ISO 20691:2022, Requirements for data formatting and description in the life sciences
- ISO 27001:2022 Annex A Control 8.28, Information security, cybersecurity and privacy protection - Information security management systems - Requirements
- ISO 27002:2022, Information security, cybersecurity and privacy protection - Information security controls
- ISO/IEC 27005:2022, Information security, cybersecurity and privacy protection - Guidance on managing information security risks
- ISO/IEC 15408 (all parts), Information security, cybersecurity and privacy protection - Evaluation criteria for IT security

Chapter 8 User perspective and regulatory, legal and ethical aspects

The use of human tissues, cells and data

- ISO 15189:2022, Medical laboratories - Requirements for quality and competence

Annex C Prioritisation for standardisation

WG 1 has set the prioritisation for standardisation chart below based on results from a survey to all WGs. This was prepared according to the protocol:

1. Each WG was asked to give input of possible items needing standardisation.
2. A list was prepared based on the items collected.
3. Each WG was asked to establish one urgency level from five possible for each prioritisation item. Five levels: 1-very important, 2-important, 3-neutral, 4-less important, 5-not important.
4. The results were collected and pondered based on the equation:

$$Pondered\ value = \sum \left(\frac{\#\ consensus}{level\ of\ importance} \right) \times \#evaluations$$

5. The priorities were organized in 10 major topics: Qualification of materials; Sterilization; Cell integrity, identity, function; Leakage; Study design; Interfaces; Fabrication related; Metrology; Symbols; and Software.
6. The pondered values were summed per topic.

The ranking presented in the table below was obtained from 256 scores on 117 items identified by the 5 WGs. 117 items were prioritized and grouped in 10 different areas of interest for OoC.

Indicates consensus about high priority/urgency for standardisation in this area							
Suggests consensus about high priority/urgency for standardisation in this area							
Suggests consensus about lower priority/urgency for standardisation in this area							
No clear indication of priority/urgency for standardisation in this area							
Item Number and Description	**1. Very important**	**2. Important**	**3. Neutral**	**4. Less important**	**5. Not important**	**Number of evaluations**	**Ponderation**
Qualification of materials							
012. Leaching of material, for instance in the case of PDMS un-crosslinked oligomers	3	1				4	13,0
011. Standards on how to measure and qualify materials	3		1			4	12,4
016. Absorption	2	2				4	10,0
015. Biocompatibility	2	1		1		4	9,3
018. What material properties are relevant for OoC users?	2	1			1	4	9,2
014. (Oxygen) permeability	1	3				4	7,0
010. Material properties and information to be supplied by the manufacturer	2	1				3	6,8
087. material of collection tube and storage conditions to ensure minimal loss of compound/analyte	1	1				2	2,5
061. Compatibility to substrates (dimensional)		2		1		3	1,7
063. Materials available		1	1	1		3	1,3
065. Compatibility to substrates (biophysical)		1	1	1		3	1,3
099. Hydrogels compatibility with OoC	1					1	1,0
100. Hydrogel Biocompatibility	1					1	1,0
109. Scaffolds compatibility with OoC (summary of the following items)	1					1	1,0
110. Scaffold Biocompatibility	1					1	1,0
112. Scaffold Biochemical properties	1					1	1,0
115. Functional coatings compatibility with OoC substrates	1					1	1,0

116. Functional coating Biocompatibility	1					1	1,0
082. Compound characterisation		2				2	1,0
083. test for compound identity and purity	1					1	1,0
062. Requirements for bioink		1				1	0,3
101. Hydrogel Mechanical properties/architecture		1				1	0,3
103. Hydrogel Degradation		1				1	0,3
111. Scaffold Mechanical properties and architecture		1				1	0,3
066. Translucency				1	1	2	0,2
Sterilization							
038. Minimum requirements per technique to ensure the sterilization quality	3			1		4	12,3
039. How is the effect of sterilization measured?	3			1		4	12,3
013. Cleanliness of the surface, for instance residues from the fabrication process	2	2				4	10,0
036. Sterilization techniques to be used	1	1		1	1	4	5,5
035. Sterilization quality	2					2	4,0
107. Hydrogel Sterilization		1				1	0,3
113. Scaffold Sterilization		1				1	0,3
Cell integrity, identity, function							
002. Cell integrity and identity	3					3	9,0
003. Cell function	3					3	9,0
004. Cell contamination	3					3	9,0
080. Biological characterisation: Number of cells and/or cell viability	1	2				3	4,5
081. Baseline characteristics of cells or organoids in OoC, cell specific functionality	1	1	1			3	4,1
005. Minimum reporting requirements for cells used in OoC systems	2					2	4,0
001. Quality controls steps during the culture and maintenance of cells	1					1	1,0
105. Hydrogel Biological properties	1					1	1,0
117. Cell type definition	1					1	1,0
Metrology							
098. Terminology	2					2	4,0
059. Resolution	1	1		1		3	3,9
041. Flow generator	1				1	2	2,1
067. Viscosity		2		1		3	1,7

020. According to what standards are these properties measured		2			1	3	1,7
022. Dead volume		1	2			3	1,4
023. Flow rates		1	2			3	1,4
043. The liquid properties		1	1		1	3	1,2
019. How are these properties characterized?		1		1	1	3	1,1
076. Shear stress	1					1	1,0
085. Stability in media over time,		2				2	1,0
070. Temperature	1					1	1,0
071. O2 saturation	1					1	1,0
050. Integrated flow control facilities			2			2	0,4
040. Measurement of flows and fluids		1				1	0,3
072. Pressure		1				1	0,3
074. Flow rate		1				1	0,3
073. Humidity			1			1	0,1
Interfaces							
024. Standard interface to enable easy and reliable integration of sensors in OoC systems, either tube based of tube less integration	2	1	1			4	9,4
026. Hardware	1	1			1	3	3,9
034. Manifold based integration: footprint of the component, position of microfluidic ports, clamping system and exclusion zone	1				2	3	3,3
048. Optical window		1	2			3	1,4
030. Standard application layer interfaces		1	1	1		3	1,3
025. Connection of sensors and actuators to instrumentation		2				2	1,0
032. Heterogenous integration: limited space for standardisation			1		2	3	0,6
031. Modular integration of a microfluidic system		1			1	2	0,6
033. Tube based integration: Tube dimensions, connection of tubing to component		1			1	2	0,6
021. Sensors			2			2	0,4
069. Hardware Setup Processes		1				1	0,3
028. Wired/wireless connectivity					1	1	0,1
Fabrication related							
037. Which technique may be used on which material	2			1	1	4	8,5

084. Fractions of unbound compounds in media and non-specific binding to chip surface	2					2	4,0
045. Integration of microfluidics and microplate workflow: TBD	1	1				2	2,5
060. Multimaterial printing (creating architectural compartments, with different cell types placed in discrete locations relative to each other)		2		1		3	1,7
058. Reproducibility of the bioprinted object is defined as the "standard deviation of the bioprinted item or channels in/for the OoC		1	1	1		3	1,3
104. Hydrogel Crosslinking method/kinetics of formation	1					1	1,0
057. Bioprinting		2				2	1,0
064. Crosslinking methods			2	1		3	0,9
049. Standard dimensions and tolerances			2			2	0,4
054. Plate flatness			2			2	0,4
055. Plate nest			2			2	0,4
Study design							
088. Study design	2	1				3	6,8
090. Sample size (number of experimental units) develop OoC-specific guidance on allocation of n/EU in OoC studies, including how to ensure robust experimental design when the maximum n is low	1	2				3	4,5
089. Appropriate positive and negative controls for each arm: develop a standard list of positive and negative controls for specific organs and applications		2		1		3	1,7
092. Randomisation: develop OoC-specific standard for randomisation across different OoC platforms accounting for multiple types of technical and biological variable		2			1	3	1,7
086. Method of sample collection		1	2			3	1,4
079. Setting up an experiment		2				2	1,0
091. Number of operators: develop standard guidance on the minimum/maximum number of operators that can be included in a study to ensure the study is robust		2				2	1,0
068. Protocols / biological CAD			2	1		3	0,9
093. Sampling time points: for different types of chip, multiple operators small sample volumes. Process for collecting the sample, including tube storage material and storage conditions/times			2			2	0,4
Leakage							
044. Leakage	3	1				4	13,0

075. Leak-tightness of tubing		1				1	0,3
Symbols							
008. What symbols are being used in OoC that are not covered by ISO standard		1	1	2		4	1,9
007. What ISO symbols can be used		2		1		3	1,7
009. How to use the symbols to visualize an OoC system of experimental setup		2		1		3	1,7
006. Symbols		2				2	1,0
Software							
097. Guidelines for using statistical software tools and tests as well as data analyses		2		1		3	1,7
095. Standards that define the use of software and programming languages, e.g. R, python			1	1	1	3	0,7
027. Software					1	1	0,1
Other							
096. Documentation verifying the use of FAIR (Findable, Accessible, Interoperable, Reusable)		2	1			3	1,8
078. Characteristic Quality Management	1					1	1,0
029. Security		2				2	1,0
094. Data Management		2				2	1,0
047. Priding access			2			2	0,4
051. Microplate limitations			2			2	0,4
053. Numbering			2			2	0,4
056. Labelling			2			2	0,4
052. Orientation			1	1		2	0,3
017. A standard specifying the information the manufacturer should supply for its product		1				1	0,3
046. Incubators				2		2	0,3
077. Characteristic			1			1	0,1
042. The microchip					1	1	0,1