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Standardisation needs for organ on chip devices†

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Organ on chip (OoC) devices represent the cutting edge of biotechnologies, combining advanced cell and tissue culture with microengineering. OoC is accelerating innovation in the life sciences and has the potential to revolutionise many fields including biomedical research, drug development and chemical risk assessment. In order to gain acceptance by end-users of OoC based methods and the data derived from them, and to establish OoC approaches as credible alternatives to animal testing, OoC devices need to go through an extensive qualification process. In this context, standardisation can play a key role in ensuring proper characterisation of individual devices, benchmarking against appropriate reference elements and aiding efficient communication among stakeholders. The development of standards for OoC will address several important issues such as basic terminology, device classification, and technical and biological performance. An analysis of technical and biological aspects related to OoC is presented here to identify standardisation areas specific for OoC, focusing on needs and opportunities. About 90 standards are already available from related fields including microtechnologies, medical devices and *in vitro* cell culture, laying the basis for future work in the OoC domain. Finally, two priority areas for OoC are identified that could be addressed with standards, namely, characterisation of small molecule absorption and measurement of microfluidic parameters.

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Introduction

According to the International Organization for Standardization (ISO), a standard is a document, established by consensus and approved by a recognized body that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context. In relation to disruptive innovation, standards can play a key role in advancing new technologies from R&D to commercial products. For organ on chip (OoC) devices, proper qualification is required to demonstrate their technological and biological relevance in order to increase their uptake and implementation by end-users, and to contribute to their acceptance in regulatory contexts of use. The development and use of standards should facilitate, for example, systematic characterisation of different devices by describing their structure and functionality, and the specification of performance requirements and test methods to verify them. In addition, such standards will provide a means for better comparison and benchmarking of OoC

devices, by ensuring a consistent use of predefined sets of parameters and measurement units. Standards dealing with proper terminology and reporting can also prove essential for effective and efficient communication and ensure a proper understanding between R&D, end-user, and stakeholder communities. Here we first review the current status of standardisation in the OoC field to understand what is already available and to identify emerging needs. Standards already existing in different but related technological domains are then presented as potential starting points for future standards development work specific to OoC. Finally, molecule adsorption and microfluidic control are described as two priority areas for OoC standardisation activities.

Current status

Various consortia have developed position papers that describe the collective vision of many stakeholders regarding standardisation needs to advance the OoC field. For example, the ORCHID project identified standardisation as a fundamental pillar for the advancement of OoC technologies at the European level.¹ The t4 (transatlantic think tank for toxicology) 2019 workshop report² summarises the view of 46 international stakeholders on the challenges for the OoC community and identifies standards as tools to support qualification and reach regulatory acceptance.

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Table 1 Standardisation in OoC – gap analysis

	Current scenario	Future needs	Priority
Definition	No uniform definition of MPS/OoC and related vocabulary	A consensus on terminology is necessary to start the qualification process	+++
Classification	Some categories can be identified (OoC focusing on barrier, parenchyma, multi organs) but there is no consensus	Identification of categories will facilitate the qualification process	+++
Functional requirements	Functional requirements are not uniformly agreed upon. Many developers perform an internal technical validation, but it is usually only partial and not fully reported	Identification of requirements and performance indicators (relevant parameters, units, measurement method, acceptability range...)	+++
Device material	There is a wide use of PDMS but also other plastics. The issue of molecule absorption is not uniformly addressed	Identify suitable test methods for molecule adsorption quantification	+++
Production process	Low TRL ^a devices are produced with soft lithography and rapid prototyping, no standardisation is usually needed	Standards for plastic materials can be used for high TRL ^a devices	++
Compatibility	Standardisation effort to create common interfaces among different OoCs and with laboratory equipment	Promote the development of standards for OoC integration	++
Sterilization and packaging	Low TRL ^a devices are sterilized with non-standard methods (UV light under biological hood or autoclave)	Standards for sterilization and packaging can be used for high TRL ^a devices	+
Quality	Some developers of commercialized OoC already perform quality control	Promote the use of GMP	+
Ancillary devices	Many standards are applicable in this field. Many products offered by major companies are already CE marked	Monitoring and updating of existing standards	+
Assays/endpoints	Relevant endpoints are organ-specific and application-specific	Standardized lists for specific context of uses have to be agreed upon and used as a basis for qualification	+++
Test compounds/drugs	Building lists of reference compounds is a widely accepted validation method in regulatory sciences	Standardized lists have to be agreed upon and used as a basis for qualification	+++
Cell source	Primary cells, iPSC and cell lines are all widely used. Standardisation in the field is rather poor	Promote the standardisation of protocols for cell culture and maintenance, sharing of best practices	++
Practical use	GLP, GIVIMP and GCCP are applicable	Disseminate and increase the use of best practices among developers	++
Other material	Some standards exist for materials used as matrixes or scaffold. Issue of molecule absorption is not uniformly addressed	Identify test methods for molecule adsorption on matrixes (measurement methods, units, ranges)	+

^a TRL = Technology Readiness Level.

Only recently, however, the OoC community started to actively involve Standards Development Organisations (SDOs) in their work and to discuss actions that could be taken together. The PSIS (Putting Science into Standards) 2021 workshop,[‡] organised by the European Commission's Joint Research Centre (JRC) and CEN-CENELEC is an important step in this direction, bringing together stakeholders from academia, industry and regulatory agencies. The Standards Coordinating Body in the USA is also targeting SDOs to steer their standards work in the OoC area. In particular, the Microphysiological System working group is coordinating

activities together with ASTM international within the Standards Advancement Project.[§]

The analysis presented here builds on these pre-normative initiatives and complements their findings. It is divided in sub-sections, each one focusing on a specific aspect of OoC technology, providing an overview of the current situation and suggesting future needs. Table 1 summarises the analysis of the current scenario, together with needs and recommendations in standardisation for OoC and a proposal on priorities.

Definition and classification

Since OoC first appeared in the scientific field, researchers and developers all over the world have been trying to find the best terminology to describe their innovations. Generally

[‡] <https://www.cencenelec.eu/news/events/Pages/EV-2021-20.aspx>

[§] <https://www.standardscoordinatingbody.org/project-organonachip-standards-landscape-assessment>



speaking, both Organ on Chip and Micro Physiological System (MPS) are currently used, often interchangeably, although for many, MPS is taken to be technically broader in scope, also including *in vitro* models such as 3D cultures, spheroids and organoids which usually lack the engineered fluidics component specific to OoC. Recently, the FDA Alternative Methods Working Group proposed some draft definitions³ but there is still no agreement on the meaning or relevance of terms such as tissue-on-chip, body-on-chip, and so on. Future work should focus on the identification of the specifics that constitute an OoC device and use this as a basis to compile a list of relevant terms.

Classification aims to group together devices that have technically similar features. This is particularly challenging for OoC since there are many different types of devices based on various technologies and design concepts, developed to represent specific aspects of biology and physiology. However, it should be possible to identify common technical characteristics that can be used as a basis to set up a classification scheme using suitable classification criteria. For instance, some devices are designed to represent the combination of multiple organs, providing two or more connected chambers where nutrients and signalling molecules are shared among different cell types. Other devices reproduce barrier functions, for example by culturing endothelial and epithelial cells of the same organ on opposite sides of a membrane. Some others focus on the co-culture of various cell types by recreating a relevant microenvironment. The use of fluid flow can also be used as a classification criterion, since many devices use a flow of continuously fresh medium, while others prefer to recirculate the medium for a certain amount of time. The use of various pumping systems also influences the performance of a device, determining the stability of the flow-rate and thus of the shear stress on the cells and medium renewal.

Technical performance of OoC devices

Functional requirements. To advance the qualification of OoC, proper performance of a device needs to be demonstrated for a particular context of use. As indicated by a survey on complex *in vitro* methods,⁴ many developers and end-users declare that they perform some sort of in-house qualification of their devices. The majority however do not follow any (generic) international qualification or validation guidelines, choosing to devise their own *ad hoc* approaches. Since such approaches are subjective by nature, they are typically limited in scope and often overlook important aspects of performance that should be characterised. Thus although some sort of qualification is being undertaken, the lack of standardisation means that even the performance of similar devices is difficult to compare, and to judge what performance specifications best match the context-of-use requirements. There is a clear need therefore to define qualification principles, processes and related performance standards for OoC. As typical in the standardisation world, this activity should be consensus-based

and performed by a cross-disciplinary expert group comprising developers and end-users from industry and the regulatory community, all of whom have a lot to gain from OoC qualification standards. A good example of a similar initiative in a related field is the MFManufacturing consortium which developed ISO-IWA 23:2016⁵ to define microfluidic dimensions and interfacing specifications for microfluidic devices. This standard also proposes a classification method based on operating pressure and temperature ranges that could be a suitable starting point also for OoC devices.

Materials. As for many instruments and consumables used in biology and biotechnology domains, the materials used for OoC need to guarantee biocompatibility and not interfere with the scientific result. The silicon based organic polymer polydimethylsiloxane, or PDMS, is the most widely used material for manufacturing microfluidic devices. In the early stage of development, where prototyping is necessary to optimise design, PDMS is very often used to produce the 'chip'. This is because it is easy to use, relatively cheap, and can reproduce micron-scale features using a soft lithography process. In more advanced development stages, OoC developers are moving towards materials that can be produced with injection moulding or 3D printing, thus bringing OoC devices closer to industrialisation. Commonly used materials at this later stage are polystyrene, polycarbonate, polymethylmethacrylate (PMMA) and cyclic olefin copolymer (COC). These materials are already widely used for consumables in biological research and medical devices, thus they have already been subject to biocompatibility testing and certification.

When selecting a primary material for an OoC device, the issue of absorption of molecules circulating in the liquid medium is a significant concern since it can compromise the functioning of the device and the accuracy of the results obtained. The absorption of small molecules by PDMS has been extensively studied through both experimental testing and mathematical modelling. However, no standards exist in terms of test methods, suitable measurement units and performance criteria (*e.g.* acceptability range). Such standards would greatly benefit the whole community by providing a sound basis for reliable characterisation and comparison of materials in different OoC setups and assays.

Production process. As with the materials, the OoC production process is also very different depending on the technology readiness level (TRL) of a device. Many devices are designed and prototyped in research laboratories which heavily rely on soft lithography of PDMS or 3D printing. As with many technological innovations, standardisation in early phases is usually not possible or even useful since it potentially constrains the R&D process. However, for devices at a higher TRL that are already mature enough to be commercialised, the production would benefit from the use of standards to guarantee such aspects as batch-to-batch reproducibility and conformity with established extrusion and moulding standards that exist for specific materials in many industrial sectors. In the assembly phase, components



such as connectors, needles, tubes and reservoirs are usually inserted as essential parts of the devices. These components are used in various fields (e.g. medical devices, industrial machinery, hydraulic systems) and are highly standardised, thus facilitating their efficient integration into OoC devices.

Compatibility. Even though most OoC devices are built using the same basic components (microfluidic channels, culture chambers, pumping system, hydraulic valves, integrated sensors for real time measurement, *etc.*), there is an evident lack of compatibility or interoperability among devices produced by different developers. For example, when considering commercial devices where pressure is used to move the fluid, there are still no common pump setups for widespread use and thus each device requires its own particular fluid control and sensor readout systems. The hDMT (human organ and disease model technology) consortium is supporting the development of the translational organ-on-chip platform (TOP), a significant effort to address this issue. The platform is designed to provide a common infrastructure for automated microfluidic chip control, which can be adapted to various OoC devices provided that they follow simple design rules. These efforts should be promoted since they can greatly boost the implementation of OoC devices also in smaller laboratories, without jeopardizing the peculiarity of each OoC device which needs to be retained to fulfil its particular purpose.

OoC devices are rarely completely standalone and thus need to be compatible with laboratory equipment. Devices are designed to be operated inside an incubator in order to provide the right conditions for air, CO₂, temperature and humidity required for maintaining cells and tissues. OoC devices that require continuous fluid flow inside the microfluidic channels need a connection with its pumping and control system. A lot of work has been done to minimise pump dimensions so that they can fit in the incubator. In addition, the number of tubes that connect a pumping system located outside the incubator with the OoC devices inside it have been minimised. Some commercial providers have implemented platforms to easily manage multiple devices in order to facilitate parallel experiments to increase flexibility and throughput.

Compatibility issues also arise in the analysis phase of an OoC method or assay. Many OoC devices provide optical access to the culture chambers through a transparent bottom but in many cases there are no standard solutions for mounting the device on microscopes or imaging platforms. Devices typically need to be physically accessed to retrieve the biological material for further analysis (e.g. immunostaining, gene-expression, *etc.*). To address these needs, OoC developers are opting to use standard dimensions and tolerances already being used for common lab ware, such as multi-well plates, glass slides, coverslips and petri dishes.

Quality assurance. Quality assurance and control standards are probably the most widely known, such as ISO 9001:2015 for quality management systems or ISO 13485:2016 which is specific for medical devices. To ensure

the quality of products and compliance with regulatory requirements, companies usually apply good manufacturing practice (GMP). GMP is composed of a comprehensive series of guidelines that provide the minimum requirements that manufacturers must meet to ensure that their products are consistently high in quality, from batch to batch, for their intended use. Some OoC companies, especially those with higher market share and visibility, are already applying GMP and using recognised quality control systems. Specifically on production, some companies declare that they apply European IQ (installation qualification), OQ (operational qualification), and PQ (performance qualification) validation protocols to their chip production, thus complying with GMP. These practices should gradually be included also in the workflow of smaller companies, to increase their credibility and strengthen their value proposition.

Packaging and sterilization. As already discussed for the materials and production process, the majority of OoC devices are still produced as prototypes in research laboratories. In this scenario, sterilization is mainly performed with laboratory equipment (mainly autoclave and UV irradiation) with no standard procedure, while packaging is mainly used to ensure sterility during transport. For high-TRL devices that are already produced under GMP, packaging and sterilization are generally included in these guidelines. Moreover, many standards were developed in the field of medical devices and these can be adapted and applied to OoC devices.

Ancillary devices. Additional equipment and materials are usually necessary to complete an OoC setup these are usually tailored to fit a specific device. Examples include pumping and control systems to deliver the correct flow rate inside the microfluidic devices, but also platforms that can be installed inside the incubators to automate operation and increase throughput. To be commercialised, this equipment needs to comply with the relevant instrumentation standards which assure safe and proper functioning. This is illustrated by the fact that the vast majority of pumps and control systems currently available in the European Union (EU) have already received the 'CE mark'.

Biological model performance

Assays and biomarkers. One frequently cited attribute of OoC devices is their ability to faithfully recapitulate specific aspects of organ function. However to assess the accuracy of biological performance, it is necessary to identify relevant biomarkers and assays. The current level of standardisation in this regard is rather poor, with most developers using their best judgement and available literature knowledge to choose the physiological parameters they believe are most representative for a certain context of use. Demonstration of OoC performance usually involves comparison with other *in vitro* cultures (usually 2D or static 3D), animal data from various species, and human data when available. Even if this case-by-case qualification related to biological performance is important to demonstrate



the validity of a single device, more complete performance assessment will only be possible through the establishment of a standard approach, at least for the more widespread applications. For example, defining standard reference values (ranges) for general parameters such as shear stress, fluid flow, liquid–cell ratio and cell–cell ratio would help the evaluation of the relevance of OoC systems and with *in vitro* to *in vivo* extrapolation (IVIVE) of data. A recent series of papers on specific organs (e.g. liver, kidney, skin)⁶ lists applicable biomarkers to evaluate normal organ functionality, as well as acceptability ranges and reference compounds to assess biological relevance. As an example, two stages of qualification were identified for a liver chip:⁷ basic functionality – where urea and albumin have to be measured, together with a baseline quantitative gene expression profiling, and complex functionality – which includes histology and requires evaluation of the activity of metabolic enzymes, transporters, and other physiologically relevant hepatic processes.

Reference chemicals. The relevance of an *in vitro* test method is often described in terms of its ‘predictive capacity’, which can be determined by testing compounds with known effects *in vivo*, and then comparing *in vitro* predictions with reality. To aid this type of evaluation of OoC performance, a list of positive and negative compounds has been published together with related biomarkers for specific contexts of use.⁶ In the case of human drug-induced liver injury for instance, liver toxicants and their mechanism of action were identified, together with less toxic comparators.⁷ These tailored lists, once agreed by the larger community of developers and end-users, will prove useful in supporting OoC qualification for various fields of application, from toxicology to biomedical research. Compiling reference lists is a challenging standardisation exercise in itself and is accepted practice in validation of alternative methods intended for regulatory use. When suitably designed, such lists not only provide a means to quantify predictive capacity, but they also help to identify scientific and technical limitations of a method and to define its applicability domain (e.g. in terms of the allowable physicochemical properties of the chemicals that can be tested). In the validation studies undertaken by the JRC’s EU reference laboratory for alternatives to animal testing (EURL ECVAM), reference chemicals lists have typically been established through expert groups and peer review and are made publically available.^{8,9} Recently, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) published a guideline on toxicity to reproduction for human pharmaceuticals.¹⁰ This includes the possibility to use qualified *in vitro* methods as a means to generate hazard data to complement or as an alternative to using conventional animal tests. The guideline sets out several qualification criteria that should be met, including the provision of test data on a prescribed set of reference compounds (positive and negative) to quantify the sensitivity and specificity of the alternative method used. Other examples of reference lists used in more of a R&D

context include the Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive Chemicals¹¹ and also the SEURAT-1 ‘gold compounds’.¹² The latter, for instance, cover a number of different organ toxicities listing compounds that affect specific biological pathways and result in well-known adverse outcomes.

Cell type and source. The choice of cells and their source is probably one of the most critical aspects of OoC assay design on which the final biological performance will heavily depend. One important consideration is the level of variability that can be tolerated, which is usually a case of finding a balance between the intrinsic biological variability of cells and the acceptable level of reproducibility of test results. Guidance documents such as Good Cell Culture Practices (GCCP,^{26–29}) and Good *In Vitro* Method Practices (GIVIMP,³⁰) are valuable references to guide users in cell selection. In particular, GIVIMP recommends the use of cells from certified providers (e.g. companies, cell banks...), who can provide proper documentation on cell source and characterisation, assuring the quality of their products. Moreover, the provider should also document the absence of contamination by external biological agents (e.g. mycoplasma, bacteria, fungi and viruses), genetic profile (identity, consistency, traceability), and stability of the declared functionality. When using the cells in an experiment, standard operating procedures (SOP) should be developed and followed which specify key steps in cell culturing and manipulation, to assure consistency, efficiency and reproducibility. Although this has been achieved to a large extent for (immortalised) cell lines, there is still much to do regarding primary cells and induced pluripotent stem cells (iPSC). Due to their natural variability and sensitive differentiation processes and states, standardisation remains challenging. One attractive prospect is to establish protocols that can be used routinely to characterise cells at different steps¹³ to ensure they remain in the desired state. One major step forward would be to increase the diffusion and uptake of method reporting standards, with particular attention on cell source, culturing and functionality assessment.

Other biological materials. OoC devices often incorporate scaffolds, membranes or a matrix to support the culturing of cells in 3D. Likewise, the cell medium flowing in the device is comprised of very many different biological materials or reagents (e.g. proteins, minerals, growth factors, cytokines, etc.) brought together in complex formulations optimised for a specific device, cell model and assay. Many of these materials are derived from animals and thus are often of an undefined chemical composition, leading to difficulties in standardisation and reproducibility.

The recent Resource Identification Initiative (RII) has built a freely accessible database that lists well-characterised biological resources and gives them unique identifiers that can be reported in research papers.¹⁴ A wide variety of biological materials is considered, such as micro-organisms and antibodies and the RII approach is gaining popularity among scientific journals. Standards covering requirements



and test methods related to scaffold and matrix materials have already been developed for tissue engineered medical products (TEMPs). These standards can be directly applied or used as a basis to develop new standards for materials specifically used in OoC. As discussed more below, a high priority for OoC is the development and use of standards to address the issue of molecule absorption by biological materials since this can influence the effective concentration of a test compound that the target cells or tissues are exposed to.

Existing standards relevant to OoC

OoC devices were developed by combining micro-process engineering and cell culture with the goal of improving the relevance of *in vitro* methods for basic and applied research. Thus OoC integrates and exploits scientific and technical knowledge from a variety of fields. Our preliminary analysis showed that about ninety published standards, originally developed in different fields, could be related to specific aspects of OoC. In particular, several existing standardised test methods and requirements refer to sterilization, pumping system safety, and materials characterization. Many standards on components widely used in design/prototyping phase (such as needles, connections, syringes) and standards on compatibility (like microplate geometry, pitch-spacing) are already available from the medical device, plastics and *in vitro* diagnostic fields. These standards are listed in the ESI† section, together with the number and title of the standard, the specific technical committee (TC) that developed it, the standard type (definition, test method, requirement, reference material, best practice) and the OoC aspect the standard relates to. The TCs that are more involved are ISO/TC 276 (Biotechnology), ASTM – F04.^{41–44} (focused on TEMPs), ISO/TC 210 (quality management and general aspects for medical devices) and CEN/TC 140 (*in vitro* diagnostic medical devices). The standards presented here cannot be immediately applied in the OoC domain, but they provide a practical starting point to identify what could be adapted for OoC and what significant OoC-specific gaps there might be. In addition, this list of relevant standards helps identify the most relevant TC that could address a particular OoC standardisation need.

General standards

Definitions. ISO 10991 – 2009¹⁵ is the first standard developed for micro-process engineering vocabulary, thus providing the basis for all the terminology that is currently used. This standard defines basic terms like ‘microfluidics’ and ‘lab-on-chip’, as well as some components that can be integrated in OoC, for example ‘micro pump’, ‘micro mixer’, and ‘micro heat exchanger’. ISO-IWA 23 – 2016⁵ defines specific dimensions to support interoperability and proposes a classification of microfluidic devices, based on operating temperature and pressure. It therefore represents another valuable starting point to further develop classification standards for OoC.

Design, prototyping and production. The SLAS Microplate Standards Advisory Committee developed a group of five standards that are largely used worldwide.^{16–20} These standards define the geometric requirements of well plates including specific pitch-distances and height and position of wells for all the well-known microtiter plates. OoC developers that want to ensure compatibility with measurement equipment and robotic instruments are recommended to adhere to these standards.

Many OoC devices are built as laboratory prototypes by university groups and research institutions. Several standard requirements and test methods related to consumables used in the healthcare sector are typically used, such as needles, tubing and connectors. Even if most current applications of OoC do not involve contact with a human subject (patient), these standards still guarantee that the components used in OoC devices meet such specifications.

For devices produced at an industry level, the batch control standard²¹ deals with the production process of any product on the market and is thus available to be used also for OoC devices. CEN/TC 102 published a series of standard requirements for sterilization for medical purposes including those using ethylene oxide and radiation, and provides chemical and biological indicators to verify the sterilization outcome. These standards can be easily adjusted to be applicable with OoC devices.

Materials. ASTM published two standards on Silicone Elastomers, Gels, and Foams Used in Medical Applications^{22,23} to address their formulation and the fabrication process. While PDMS is largely used in prototyping of OoC devices, many different plastics can be used for industrialised products. ISO standards are available for all the main plastics used with moulding and extrusion processes. Specifically, polycarbonate, PMMA, polystyrene, polyethylene, acrylonitrile-butadiene-styrene and polypropylene, are all suitable candidate materials for OoC production. The VDI (the Association of German Engineers) also published a specification standard on medical grade plastics²⁴ that can be coupled with the Standard Practice for Selecting Generic Biological Test Methods for Materials and devices²⁵ by ASTM to qualify the biocompatibility of OoC materials.

The US Pharmacopoeia (USP) identified about 10 reference materials commonly used for OoC, including collagenase, foetal bovine serum (FBS), growth factors and cytokines. The use of reference materials is generally needed to develop accurate test methods and to ensure that perform as intended. These materials can also be used during inter-laboratory comparisons to increase reproducibility.

Fluidic measurement and control. Seven standards concerning the requirements and test methods for pumping systems are currently used to grant market access for industrial use. These standards address worker health and safety during use and installation, as well as test methods to verify their correct functioning in normal and worst-case scenarios. Of note, compressors and vacuum pumps also



Table 2 GIVIMP principles

Chapter	Content
1. Roles and responsibilities	The <i>in vitro</i> method life cycle from development to the use for safety assessment purposes has a variety of key actors and the guidance identifies clearly their responsibilities, both individually and collectively
2. Quality considerations	To realise fully the potential of <i>in vitro</i> methods and allowing them to become a key tool for a new way of doing toxicology, they need to be developed and applied in a way that scientific integrity and quality is assured
3. Facilities	<i>In vitro</i> cell and tissue culture facilities should be fit for purpose and a detailed understanding of the workflow for the <i>in vitro</i> method related processes is essential. The separation of specific laboratory functions and elements that can adversely affect <i>in vitro</i> method work need to be understood
4. Apparatus, materials and reagents	Apparatus, including computerised systems, should be regularly maintained, calibrated and validated (if required). Material and reagents should be purchased from well-established sources to ensure the integrity and reliability of the <i>in vitro</i> method results
5. Test systems	With the advances in science and technology a variety of different cell and tissue culture-based test systems have been developed, but only few have been used in regulatory-approved test guideline methods due to reliability issues caused by a variety of elements described in this chapter
6. Test and reference/control items	The preparation and characterisation of test, reference and control items and their interaction with the <i>in vitro</i> environment should be well understood, to ensure the acquisition of reliable and relevant results
7. Standard operating procedures	Standard operating procedures (SOPs) and the accompanying forms, templates or worksheets should be written and prepared in a way that they will form the tools to simplify the work of the user when carrying out an <i>in vitro</i> method study
8. Performance of the method	<i>In vitro</i> method developers need to ensure that <i>in vitro</i> methods they design will produce good quality data, <i>i.e.</i> fit for purpose, thanks to a stringent assessment of the performance of the method
9. Reporting of results	Good reporting of <i>in vitro</i> methods can only be achieved when all important details are recorded in a way that allows others to reproduce the work or reconstruct fully the <i>in vitro</i> method study
10. Storage and retention of records and materials	Before collecting data from <i>in vitro</i> methods it is important to assess the format of collection, the complexity involved and requirements for traceability, storage, verification and transmission of data

have to adhere to requirements regarding their acoustic emission and mechanical vibration.

A group of seven standards developed by the American Society of Mechanical Engineering focuses on the measurement of flow rates in pipes, covering definition of key terminology, device requirements, and test methods for certain flow meters. These standards are quite general but can be applied also to flow meters for microfluidic applications. Awareness of these standards can help when assessing the flow rate in OoC devices setups.

Best practices. Even if OoC is a relatively recent technology field to emerge where the majority of activities are undertaken by developers, their use within a regulatory framework will most likely require users to follow good laboratory practice (GLP) and good cell culture practice (GCCP).^{26–29} To help the community incorporate these best practices into their processes, the Organisation for Economic Co-operation and Development (OECD) Guidance Document on Good *In Vitro* Method Practices (GIVIMP)³⁰ builds on GCCP and provides a comprehensive set of principles described in 10 chapters. These practices are applicable for a wide range of *in vitro* methods to ensure that the data generated can be used in critical decision-making (Table 2). To apply these methods for regulatory use in human safety

assessment, it is imperative that the technical quality of the results is assured in terms of reproducibility (within and between laboratories), sensitivity, specificity, and traceability. However, such practices should be applied also during research and development to expedite the translation process. Even if these principles and practices should be already known, many researchers and developers do not use them consistently thereby introducing variability in performance that can contribute to the ‘reproducibility crisis’³¹ in the life science domain. Many of the GIVIMP recommendations are directly applicable to OoC and thus should be taken up.

These efforts are crucial to standardise the test methods and thus ensure an adequate biological performance and improve reproducibility, especially for methods that are not formally validated. The OECD Guidance Document 211³² presents a harmonised approach to describing non-guideline *in vitro* test methods, which could be applied to OoC. The guidance document is addressed to developers and end-users for reporting fundamental information that a data-user would need to know such as: details of the experimental protocol; the reference chemicals used; the stage of development and validation of the method; assay acceptance criteria; data interpretation procedures; and



performance metrics including reproducibility and accuracy. A test method reporting template was also developed by the toxicological research community, setting out specific questions to be addressed and providing comments and additional notes to help the user complete the template.³³ Something similar was developed for *in vitro* methods intended for developmental neurotoxicity testing, where 'readiness criteria' were also proposed to evaluate methods with a view to their application in regulatory safety assessment.³⁴

Data reporting. Several initiatives are completed or underway by different agencies and organisations in terms of harmonising the reporting of data, *e.g.* in computational biology.³⁵ Several journals have created policies for standardising method and protocol description such as MethodX³⁶ and Cell Press Star Methods.³⁷ Another useful tool is SciRap³⁸ which provides a practical and systematic way of checking the completeness of method reporting. The EURL ECVAM test submission template indicates the information required to establish the scientific validity of a test method following a modular approach.³⁹ OECD harmonised templates (OHT) are available for reporting data to be included in chemical hazard and risk assessment processes. Among them, OHT201 was recently approved for the reporting of information on 'intermediate effects', typically of a mechanistic nature, which is therefore ideal for reporting data from *in vitro* methods.⁴⁰ A project is also underway at the OECD to develop reporting frameworks and related guidance for transcriptomics and metabolomics studies, which should also be also applicable to 'omics studies carried out using OoC devices.

TEMPs

Tissue Engineered Medical Products (*as per* ASTM terminology) or cellular therapeutic products (*as per* ISO terminology) are medical products that may achieve a therapeutic potential from cells, biomolecules, scaffolds, and other materials, and processed tissues and derivatives used in various combinations or alone.⁴¹ Standards to regulate these new products were developed between 2011 and 2019 by ASTM and ISO using a strategy that could be replicated for OoC. Firstly, standard terminology and classification of TEMPs was proposed, followed by 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. A standard guide for evaluation of *in vitro* release of biomolecules from biomaterial-scaffolds recognises the need to measure molecules released by TEMP materials,⁴² as opposed to the issue of absorption of molecules in OoC devices mentioned earlier.

Medical devices

Standards for medical devices are developed by CEN/ISO and are referred to by the European Commission in the list of harmonised standards that can be used to prove compliance

with the medical device regulation.⁴³ There are more than 200 standards included in this list; some of these can be used as a model for the OoC field. The standards related to quality management in a risk assessment framework and those on biological evaluation of medical devices are fundamental pillars of the regulatory acceptance for medical devices. Moreover, standards on the various sterilization alternatives (radiation, ethylene oxide, moist heat methods but also biological, chemical and microbiological indicators to assess reliable sterilization) and packaging requirements complement the list of relevant standards that can be used as a reference to address similar issues in the OoC field.

In vitro diagnostics

Standards on *in vitro* diagnostics are developed by CEN/ISO and are indicated by the European Commission in the list of harmonised standards that can be used to prove compliance with the EU Regulation on *in vitro* diagnostic medical devices.⁴⁴ Those of interest for the OoC field are mainly related to performance evaluation of these devices, stability-in-time of the various components (*e.g.* reagents and culture medium) and statistical aspects to be considered for acceptability of results.

Critical aspects of OoC devices that need standardisation

In addition to standardising OoC terminology and classification, there are two areas where standards could play a valuable and immediate role: small molecule absorption on OoC materials and the evaluation of fluidic shear stress. These two widely acknowledged issues in OoC applications are, however, still addressed in a very heterogeneous way, with every end-user using a different approach. The development and use of test methods and reporting standards with adequate information would greatly increase the reliability, relevance, and utility of the results obtained.

Methods to determine small molecule adsorption

As already discussed, some polymeric materials used in many OoC devices can raise doubts about their suitability, due to absorption of small molecules (*i.e.* molecular weight lower than 1 kDa) from the fluid medium. This material property can be a fundamental issue for many OoC applications, but it becomes specifically relevant in toxicity or efficacy studies, where knowing and controlling the effective concentration of a test compound acting on the cells is crucial to obtain accurate results on the biological response.⁴⁵ PDMS is the material most used for OoC devices, yet it has significant absorption issues. A number of studies were conducted to investigate this phenomenon and it is now widely known that the absorption rate depends on the chemical properties of the molecule itself. For example, hydrophobic molecules ($\log P > 1.85$) with no H-bond donor groups are the molecules most easily sequestered into PDMS.⁴⁶



Researchers and companies started to use various coatings on the inner walls of microfluidic chambers to avoid direct contact between PDMS and the small molecules present in the medium, with the goal of reducing or preventing absorption (see ESI†). Coatings are produced using various types of biological matrixes (most commonly: collagen, fibronectin, polylysine) or by growing a layer of organ-specific endothelial cells. However, small molecules can also be sequestered by coatings, bind to proteins and lipids dissolved in the medium, or can even evaporate, decreasing the amount of compound that the cells are effectively exposed to, as demonstrated for collagen I with ibuprofen.⁴⁷ Thus, while the use of a coating can solve the issues posed by PDMS, it can actually cause others.

To gain a better understanding of the problem, computational models such as the VCBA (virtual cell based assay) can be used to estimate the amount of compound that the cells are exposed to and the factors influencing it.^{48–50} These models are able to simulate the kinetics and dynamics of a chemical compound in cell-based *in vitro* assays, by integrating a transport model, a cell partitioning model, a cell growth and division model, and an effects model, together with many important characteristics of the experimental setup. In order to be adapted to OoC devices, these models will need to be modified to account for OoC specific design features. In particular, the PDMS needs to be implemented as a new material with its specific physical and chemical properties, as well as the materials used for the coatings. Moreover, current VCBA models do not include fluid flow, which is key to represent the transport of molecules in the devices. VCBA models have been built to represent typical *in vitro* assays using multi-well plates and thus they are based on a simple cylindrical geometry. This is not applicable to the majority of OoC devices, which usually employ rectangular chambers connected by microfluidic channels.

The relationship between a material and the biological entity it is in contact with is of great relevance. This certainly the case in the medical device domain, especially for permanent implants and prostheses. Two standard test methods were developed to comply with regulatory safety assessment for biocompatibility (ISO 10993⁵¹ and ASTM F748 – 16²⁵). A standard test method⁵² describing an experimental procedure to extract plastic material from a medical device, to simulate its release in a biological environment, was developed by ASTM to demonstrate compliance with regulations. A similar approach could be used to define a suitable test method that allows a correct and reproducible evaluation of the compound actually binding to the OoC system used, accounting for coatings, matrices, scaffolds and other ancillary materials involved.

Shear stress estimation

One of the major characteristics of OoC technologies is the possibility to include fluid flow, thus exposing the cell culture

to a physiologically relevant biomechanical solicitation and continuous exchange of biomolecules. This is largely possible due to the integration of microfluidic circuits that, with specific pumping systems and sometimes with the help of hydraulic valves, can modulate the flow in various chambers to achieve different fluid dynamic conditions. To evaluate the effect of the flow on the outcome, it is important to correctly understand the fluid dynamics at the microscale, how the fluid flow can be controlled with different pumps and how the device resistance can influence the flow rate.

In some OoC devices, for instance, the fluid is not flowing through empty channels, but rather through a matrix that embeds the cells in a 3D structure. In this specific situation, it is possible to estimate the hydraulic resistance with the Darcy law, which accounts for the permeability k of a porous material through which a fluid is flowing (an example from the biomedical field is cartilage):

$$R_h = \frac{L\mu}{kA}$$

where L is the length of the channel, μ is the fluid viscosity and A is the channel cross-sectional area. In these configurations, understanding what can modify the permeability is crucial. One has to pay attention to the specific formulation of the matrix, potential batch-to-batch variability in its production, and potential matrix degradation, all of which can modify the permeability over time.

The cross-section of the channel greatly influences its resistance. In particular, the relationship between fluidic resistance and channel radius (or the channel height and width for rectangular cross-section) follows a 4th order power law.⁵³ Thus, even a small variation in channel geometry will greatly influence the channel resistance and thus the fluid flow. For commercialised products, proper production control can ensure design specifications and tolerances are respected and each batch is controlled. However, this dimensional issue becomes relevant for OoC devices which are in the early phases of design and prototyping. With soft lithography, where moulds can easily have varying heights, and 3D printing, where every device is printed as a single entity or in very small batches, the geometrical features can vary from one device (or batch) to another. This uncertainty can influence the hydraulic resistance, leading to non-uniform flow rates, especially if the pumping system is not used properly (see ESI†).

Geometry is also fundamental to estimate the shear stress. For very small channels, the presence of a cell layer can significantly reduce the cross-section space, thus considerably increasing the shear stress acting on the cells.

A correct understanding and use of microfluidic systems in OoC is necessary to ensure reliability of the results, while accurate reporting of fluidic variables and protocols will ensure reproducibility. Standard methods to measure, derive and report fluidic variables are thus of crucial importance to



ensure reliability of results, clearly demonstrate the added value of OoC approaches and ultimately build end-user confidence.

Conclusions

There is a clear consensus in the OoC community on the need for standardisation to advance the field, with several initiatives now in progress worldwide. It is important to emphasise that the development and use of standards in no way impedes innovation. On the contrary, standards are an important enabler of innovation. Researchers and developers need to keep thinking ‘outside the box’ to push boundaries and drive innovation. On the contrary, standards should be considered as valuable tools that can be readily used to demonstrate the reliability and relevance of their novel devices and pave the way for their application and regulatory acceptance.

The analysis presented here builds on those efforts and has identified specific needs and priorities. Clearly, a focus on terminology and device classification are the first aspects to be tackled and in this regard, with ISO-IWA 23 (2016) and ISO 10991 (2009) providing a good basis. However, specific features and peculiarities of OoC need to be properly addressed. A second priority is the technical assessment of OoC devices, covering various aspects including materials, production processes and test methods. Some existing standards related to medical devices and IVD can be used as a starting point to develop specific standards for OoC, considering their intended use in various fields. To really boost the implementation of OoC for regulatory and biomedical sciences, it is crucial to understand their biological performance, intended as the capacity to recapitulate relevant and specific organ functions. The development of standards to facilitate the assessment of biological performance for specific contexts of use would not only advance the OoC field, but complex *in vitro* methods in general.

Even though OoC has emerged as a potentially disruptive technology in its own right, the OoC community should acknowledge the many existing standards in related fields and evaluate how best they could be exploited and expanded for the OoC domain. As proposed here, material absorption and microfluidic flow are two specific issues common across all OoC devices where a considerable amount of work has already been done and where there is a real need for standardisation to progress further. Irrespective of which priority areas are pursued first however, it is imperative that the OoC community joins forces with SDOs (e.g. CEN-CENELEC, ISO, ASTM) and with experts from related fields to devise a roadmap for bringing standards into the OoC domain.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 M. Mastrangeli, S. Millet, C. Mummery, P. Loskill, D. Braeken, W. Eberle, M. Cipriano, L. Fernandez, M. Graef, X. Gidrol, N. Picollet-D'Hahan, B. Van Meer, I. Ochoa, M. Schutte and J. Van den Eijnden-van Raaij, *Altex*, 2019, **36**, 481–492.
- 2 U. Marx, T. Akabane, T. B. Andersson, E. Baker, M. Beilmann, S. Beken, S. Brendler-Schwaab, M. Cirit, R. David, E. M. Dehne, I. Durieux, L. Ewart, S. C. Fitzpatrick, O. Frey, F. Fuchs, L. G. Griffith, G. A. Hamilton, T. Hartung, J. Hoeng, H. Hogberg, D. J. Hughes, D. E. Ingber, A. Iskandar, T. Kanamori, H. Kojima, J. Kuehn, M. Leist, B. Li, P. Loskill, D. L. Mendrick, T. Neumann, G. Pallocca, I. Rusyn, L. Smirnova, T. Steger-Hartmann, D. A. Tagle, A. Tonevitsky, S. Tsyb, M. Trapecar, B. Van de Water, J. Van den Eijnden-van Raaij, P. Vulto, K. Watanabe, A. Wolf, X. Zhou and A. Roth, *Altex*, 2020, **37**, 365–394.
- 3 Food and Drug Administration (FDA), <https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda#:~:text=Objectives of FDA's Alternative Methods Working Group Discuss,development of draft performance criteria for such assays>.
- 4 S. Batista Leite, M. Zincke Dos Reis Fernandes Cipriano, D. Carpi, S. Coecke, M. Holloway, R. Corvi, A. Worth, J. F. Viegas Barroso and M. Whelan, *Establishing the scientific validity of complex in vitro models*, EUR 30556 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-28410-9, DOI: 10.2760/376171, JRC122394.
- 5 International Organization for Standardization, *Interoperability of microfluidic devices — Guidelines for pitch spacing dimensions and initial device classification*, 2016, ISO-IWA 23, 2016.
- 6 K. Fabre, B. Berridge, W. R. Proctor, S. Ralston, Y. Will, S. W. Baran, G. Yoder and T. R. Van Vleet, *Lab Chip*, 2020, **20**, 1049–1057.
- 7 A. R. Baudy, M. A. Otieno, P. Hewitt, J. Gan, A. Roth, D. Keller, R. Sura, T. R. Van Vleet and W. R. Proctor, *Lab Chip*, 2020, **20**, 215–225.
- 8 S. Casati, P. Aeby and I. Kimber, *et al.*, *ATLA, Altern. Lab. Anim.*, 2009, **37**(3), 305–312.
- 9 D. Kirkland, P. Kasper, H. J. Martus, L. Müller, J. van Benthem, F. Madia and R. Corvi, *Mutat. Res.*, 2016, **795**, 7–30.
- 10 International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH S5 (R3) guideline on reproductive toxicology: detection of toxicity to reproduction for medicinal products including toxicity to male fertility, 2020, ICH S5 (R3).
- 11 F. Madia and R. Corvi, EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive



- Chemicals, <https://ec.europa.eu/jrc/en/scientific-tool/eurl-ecvam-genotoxicity-and-carcinogenicity-consolidated-database-ames-positive-chemicals>.
- 12 P. Jennings, M. Schwarz, B. Landesmann, S. Maggioni, M. Goumenou, D. Bower, M. O. Leonard and J. S. Wiseman, *Arch. Toxicol.*, 2014, **88**, 2099–2133.
 - 13 D. E. Ingber, *Adv. Sci.*, 2020, **7**, 2002030.
 - 14 A. Bandrowski, M. Brush, J. S. Grethe, M. A. Haendel, D. N. Kennedy, S. Hill, P. R. Hof, M. E. Martone, M. Pols, S. C. Tan, N. Washington, E. Zudilova-Seinstra and N. Vasilevsky, *Brain Behav.*, 2016, **6**, e00417.
 - 15 International Organization for Standardization (ISO), *Micro process engineering — Vocabulary*, 2009, ISO 10991, 2009.
 - 16 ANSI/SLAS, *Microplates: Footprint Dimensions*, 2004, ANSI SLAS 1–2004.
 - 17 ANSI/SLAS, *Microplates: Height Dimensions*, 2004, ANSI SLAS 2–2004.
 - 18 ANSI/SLAS, *Microplates: Bottom Outside Flange Dimensions*, 2004, ANSI SLAS 3–2004.
 - 19 ANSI/SLAS, *Microplates: Well Positions*, 2004, ANSI SLAS 4–2004.
 - 20 ANSI/SLAS, *Microplates: Well Bottom Elevation*, 2012, ANSI SLAS 6–2012.
 - 21 International Society for Automation (ISA), *Batch Control*, 2010, ANSI/ISA-88.
 - 22 ASTM International, *Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications Part I—Formulations and Uncured Materials*, 2018, ASTM F2038-18.
 - 23 ASTM International, *Standard Guide for Silicone Elastomers, Gels, and Foams Used in Medical Applications Part II—Crosslinking and Fabrication*, 2018, ASTM F2042-18.
 - 24 VDI - the Association of German Engineers, *Medical Grade Plastics*, 2017, VDI2017.
 - 25 ASTM International, *Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices*, 2016, ASTM F748 - 16.
 - 26 S. Coecke, M. Balls, G. Bowe, J. Davis, G. Gstraunthaler, T. Hartung, R. Hay, O. W. Merten, A. Price, L. Schechtman, G. Stacey and W. Stokes, *ATLA, Altern. Lab. Anim.*, 2005, **33**, 261–287.
 - 27 D. Pamies, A. Bal-Price, C. Chesné, S. Coecke, A. Dinnyes, C. Eskes, R. Grillari, G. Gstraunthaler, T. Hartung, P. Jennings, M. Leist, U. Martin, R. Passier, J. C. Schwamborn, G. N. Stacey, H. Ellinger-Ziegelbauer and M. Daneshian, *Altex*, 2018, **35**, 353–378.
 - 28 D. Pamies, A. Bal-Price, A. Simeonov, D. Tagle, D. Allen, D. Gerhold, D. Yin, F. Pistollato, T. Inutsuka, K. Sullivan, G. Stacey, H. Salem, M. Leist, M. Daneshian, M. C. Vemuri, R. McFarland, S. Coecke, S. C. Fitzpatrick, U. Lakshmipathy, A. Mack, W. B. Wang, D. Yamazaki, Y. Sekino, Y. Kanda, L. Smirnova and T. Hartung, *Altex*, 2017, **34**, 95–132.
 - 29 D. Pamies, M. Leist, S. Coecke, G. Bowe, D. Allen, G. Gstraunthaler, A. Bal-Price, F. Pistollato, R. DeVries, T. Hartung and G. Stacey, *Altex*, 2020, **37**, 490–492.
 - 30 Organisation for Economic Co-operation and Development (OECD), *Guidance Document on Good In Vitro Method Practices (GIVIMP) - Series on Testing and Assessment No. 286*, 2018.
 - 31 M. Baker, *Nature*, 2016, **533**, 452–454.
 - 32 Organisation for Economic Co-operation and Development (OECD), *Guidance document for describing non-guideline in vitro test methods - Series on Testing and Assessment No. 211*, 2014.
 - 33 A. Krebs, T. Waldmann, M. F. Wilks, B. M. A. Van Vugt-Lussenburg, B. Van der Burg, A. Terron, T. Steger-Hartmann, J. Ruegg, C. Rovida, E. Pedersen, G. Pallocca, M. Luijten, S. B. Leite, S. Kustermann, H. Kamp, J. Hoeng, P. Hewitt, M. Herzler, J. G. Hengstler, T. Heinonen, T. Hartung, B. Hardy, F. Gantner, E. Fritsche, K. Fant, J. Ezendam, T. Exner, T. Dunkern, D. R. Dietrich, S. Coecke, F. Busquet, A. Braeuning, O. Bondarenko, S. H. Bennekou, M. Beilmann and M. Leist, *Altex*, 2019, **36**, 682–699.
 - 34 A. Bal-Price, H. T. Hogberg, K. M. Crofton, M. Daneshian, R. E. FitzGerald, E. Fritsche, T. Heinonen, S. Hougaard Bennekou, S. Klima, A. H. Piersma, M. Sachana, T. J. Shafer, A. Terron, F. Monnet-Tschudi, B. Viviani, T. Waldmann, R. H. S. Westerink, M. F. Wilks, H. Witters, M. G. Zurich and M. Leist, *Altex*, 2018, **35**, 306–352.
 - 35 S. Brunak, C. Bjerre Collin, K. Eva Ó Cathaoir, M. Golebiewski, M. Kirschner, I. Kockum, H. Moser and D. Waltemath, *J. Integr. Bioinform.*, 2020, **17**(2–3), 20200006.
 - 36 Elsevier, *MethodsX*, <https://www.journals.elsevier.com/methodsx>.
 - 37 E. Marcus, *Cell*, 2016, **166**, 1059–1060.
 - 38 L. Molander, M. Ågerstrand, A. Beronius, A. Hanberg and C. Rudén, *Hum. Ecol. Risk Assess.*, 2015, **21**(3), 753–762.
 - 39 T. Hartung, S. Bremer, S. Casati, S. Coecke, R. Corvi, S. Fortaner, L. Gribaldo, M. Halder, S. Hoffmann, A. J. Roi, P. Prieto, E. Sabbioni, L. Scott, A. Worth and V. Zuang, *ATLA, Altern. Lab. Anim.*, 2004, **32**, 467–472.
 - 40 Organisation for Economic Co-operation and Development (OECD), *OHT 201 Intermediate effects - mechanistic information*, 2020.
 - 41 ASTM International, *Standard Classification for Tissue Engineered Medical Products (TEMPS)*, 2013, ASTM F2211 - 13.
 - 42 ASTM International, *Standard Guide for Evaluation of in vitro Release of Biomolecules from Biomaterials Scaffolds for TEMPS*, 2016, ASTM F3142 - 16.
 - 43 European Parliament and Council of the European Union, *Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC*, 2017.
 - 44 European Parliament and Council of the European Union, *Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU*, 2017.
 - 45 B. J. van Meer, H. de Vries, K. S. A. Firth, J. van Weerd, L. G. J. Tertoolen, H. B. J. Karperien, P. Jonkhøj, C. Denning, A. P. IJzerman and C. L. Mummery, *Biochem. Biophys. Res. Commun.*, 2017, **482**(2), 323–328.



- 46 A. W. Auner, K. M. Tasneem, D. A. Markov, L. J. McCawley and M. S. Hutson, *Lab Chip*, 2019, **19**, 864–874.
- 47 G. L. Truisci, E. D. Consiglio, C. Parmentier, C. C. Savary, G. Pomponio, F. Bois, B. Lauer, R. Jossé, P. G. Hewitt, S. O. Mueller, L. Richert, A. Guillouzo and E. Testai, *Toxicol. Lett.*, 2015, **233**, 172–186.
- 48 J. M. Z. Comenges, E. Joossens, J. V. S. Benito, A. Worth and A. Paini, *Toxicol. In Vitro*, 2017, **45**, 209–221.
- 49 R. Graepel, L. Lamon, D. Asturiol, E. Berggren, E. Joossens, A. Paini, P. Prieto, M. Whelan and A. Worth, *Toxicol. In Vitro*, 2017, **45**, 258–267.
- 50 A. Paini, M. Mennecozzi, T. Horvat, K. Gerloff, T. Palosaari, J. V. Sala Benito and A. Worth, *Toxicol. In Vitro*, 2017, **45**, 233–240.
- 51 International Organization for Standardization, Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process, 2018, ISO 10993-1, 2018.
- 52 ASTM International, Standard Practice for Extraction of Materials Used in Medical Devices, 2020, ASTM F619 - 20.
- 53 H. Bruus, *Theoretical Microfluidics*, OUP Oxford, 2008.

