Moya et al. (2018) integrated multiple ink-jet sensors in a thin and porous membrane inside a Liver-On-a-Chip device (Fig. 17 A). In tumor microtissues, instead, metabolic parameters provide information on cell growth and viability and in ref. (Misun et al., 2016) an amperometry sensor was implemented to monitor glucose and lactate levels secreted by a 3D human colon cancer microtissue.

Electrochemical impedance spectroscopy (EIS) is a related transduction technique based on changes in the electrical properties near the electrode surface. From its introduction by Giaver and Keese (Giaever and Keese, 1993), this method has been employed in lab-on-chip for facilitating several cell-based assays, including monitoring motion, attachment, growth, proliferation, spreading and differentiation of cultured cells (Arndt et al., 2004; Wegener et al., 2000; Bagnaninchi and Drummond, 2011), quantifying cell viability and heterogeneity (Han et al., 2007; Swami et al., 2021), assessing cell migration and invasive activities (Rodriguez et al., 2005; Primiceri et al., 2011; Keese et al., 2002) and evaluating the effects of biochemical compounds and cytotoxicity (Xiao and Luong, 2003; Primiceri et al., 2010; Kustermann et al., 2013; Ramis et al., 2013). Furthermore, upon electrode functionalization with suitable recognition probes, EIS sensors consent the detection of biorecognition events (Katz and Willner, 2003; Chiriacò et al., 2013, 2015, 2016; Buja et al., 2022). Recently, Sorafenib efficacy in hepatocellular carcinoma treatment was assessed on chip through EIS sensors integrated in a miniaturized platform for cell proliferation assays and drug screening (Piccinno et al., 2021). Within OOC field, in ref. (Ortega et al., 2019), Ortega et al. used EIS sensors functionalized with enzyme-linked secondary antibodies to detect interleukin (IL)-6 and tumor necrosis factor (TNF), reaching limit of detection of the order of ng/ml. Zhang et al. monitored liver and heart in a multi-OOC platform (liver and heart) achieving a two orders of magnitude lower limit of detection (LOD) (Zhang et al., 2017). Moreover, impedance spectroscopy has been exploited to measure tumor spheroid size on-chip (Schmid et al., 2016) and to study tumor spheroid viability throughout drug testing assays (Wu et al., 2018).

The transepithelial/transendothelial electrical resistance (TEER) can be very useful to monitor in real time the integrity and the functionality of cellular barriers in OOC models such as the BBB, the intestinal epithelial barrier in gut-on-a-chip or the air-liquid interface in lung-on-a-chip. In ref. (Maoz et al., 2017), Maoz and coworkers employed TEER measurements to simultaneously detect dynamic alterations of vascular permeability in heart-on-a-chip device. In the same chip, multi electrodes arrays (MEA) were integrated to measure field potentials of cardiomyocytes (Fig. 17 B). In general, the electric/electrochemical techniques described above require electrode integration in the chambers/channels housing living cells or in contact with fluids passing through the cell compartments.

Optical biosensors provide another, convenient in-situ monitoring approach (Kavand et al., 2107). Refractive index changes near the sensor surface (due for example to cellular response or analyte secretion) are measured in surface plasmon resonance (SPR) (Nguyen et al., 2015; Haes et al., 2004; Colombelli et al., 2021; Rizzato et al., 2018), optical waveguide light mode spectroscopy (OWLS), photonic crystals (PC) and resonant waveguide grating (RWG). In ref. (Li et al., 2018), SPR based on nanoholes arrays were integrated in a microfluidic platform to detect vascular endothelial growth factor. Photonic crystals and structures fabricated by colloidal lithography were used as self-reporting tools for cardiomyocytes activity or microphysiological breath (Shang et al., 2020; Zhu et al., 1002) (Fig. 17 C1–C3). Optical waveguides were fabricated in flexible PEG-based hydrogels to stimulate drug release from optogenetically engineered bacteria (Feng et al., 2020), whereas resonant waveguide grating biosensors have been used to study the role of plasma proteases on endothelial cell layers (Debreczeni et al., 2020).

Optical sensors have been also developed to investigate cell and tissue metabolism using sensitive opto-chemical tracer molecules. These sensors represent a valid alternative to electrical sensors to measure microenvironmental parameters such as pH and oxygen levels. Optical oxygen sensors exploit photoluminescence quenching produced by oxygen-sensitive dyes. Fluorophores based ruthenium- and metalloporphyrin were largely diffused due to their very high quenching constants (Kavand et al., 2107). For example, Shaegh et al. realized a bioreactor with integrated LED and photodetectors for monitoring of pH and oxygen levels (Shaegh et al., 2016). By means of a similar approach, Khalid et al. measured the absorption light variation in cell media in the presence of phenol red to monitor pH in a lung cancer-on-a-chip platform (Fig. 17 C4) (Khalid et al., 2020).

Mechanical sensors is another class which attracted attention due their potential applications in monitoring different physiological signals, mechanical (strain, pressure) or biological (for example detection of metabolic biomarkers) (Ferrari and Palma, 2020). These sensors are used in OOC to study tissue stiffness and monitor dynamic deformations typical of the striatal muscular tissues (skeletal and cardiac muscle tissues). For example, Lind et al. developed some cantilevers that contained flexible thin-film strain gauges to detect beat rate and contractile stresses of the cardiac cells (Lind et al., 2017b) (Fig. 17 D1-D2). In ref. (Sidorov et al., 2017), Sidorov et al. designed a platform to grow 3D cardiac tissue constructs (ECTCs) and to perform measurements of their mechanical and electrophysiological parameters to evaluate the ECTCs functionality in both normal and pharmacologically modified conditions, using cantilever probes.

Between mechanical sensors, surface acoustic wave are widely used since they are very sensitive toward mass loading, viscosity and conductivity variations occurring on the surface (Chiriacò et al., 2018; Rizzato et al., 2017; Rizzato et al., 2023), being the mechanical excitation strongly localized in the surface region (Maruccio et al., 2018). For example, Wang et al. used a shear horizontal-surface acoustic waves (SH-SAW) device comprising two pairs of resonators for detection and quantification of viability and growth of cells (Wang et al., 2015) (Fig. 17 D3-D4). The same authors included SAW sensors in a gravitation microfluidic platform system for monitoring the proliferation and metabolism of tumoroids (Wang et al., 2020). Liu et al. (2018) realized an integrated system of metal-enhanced fluorescence with SAW for cancer biomarker detection.

In some cases, gas sensors can provide other suitable monitoring and diagnostic tools as demonstrated by the increasing interest in their use for volatilomics and metabolomics (Milone et al., 2023; Serasanambati et al., 2019; Dummer et al., 2011; Phillips et al., 2012; Einoch Amor et al., 2019; Di Natale et al., 2003; Murdocca et al., 2021; Mougang et al., 2021). Finally, some authors developed and integrated sensors in OOC devices, equipped with miniaturized models of laboratorial instruments. For example, a 3D-printed digital fluorescence type microscope was incorporated in a lung-cancer on chip platform for the visual monitoring of the cells on the chip (Khalid et al., 2020). In ref. (Mermoud et al., 2018), Mermoud et al. presented microimpedance tomography system integrated in a flexible printed circuit board to control cell activity and membrane movements in a breathing lung-on-chip.

In summary, the integration of miniaturized sensors for in situ, automated monitoring of relevant cellular and physiological parameters represents an important frontier for further increasing the throughput and content of OOC platforms.

4.3. Materials for organs-on-chips devices

Different materials are used to develop organs-on-chips depending on the specific features required in mimicking the in vivo structural and/or biochemical environment (Table 1). In this paragraph, the advantages and disadvantages of the most popular materials explored in the design of OOC are discussed.

Glass is the oldest material employed in microfluidics. It is transparent, resistant to mechanical stress, hydrophilic, biocompatible and possess a low drug absorptivity. The major disadvantages which led to the experimentation of other materials concern its low gas permeability that does not allow long-term cell studies and the high cost of fabrication

Table 1
Materials for OOC platforms.

Categories	Materials	Advantages	Limitation	Applications
Glass		• transparency, • resistance to mechanical stress • hydrophilicity • biocompatibility • low drug absorptivity	low gas permeability,	• OOC substrate
Elastomers	PDMS	• flexible • optical transparent, • gas permeable • biocompatible, • low autofluorescent, • deformable, • ease of use, • low cost, • very useful in prototyping new devices	• hydrophobicity • incompatibility with organic solvents • tendency to adsorb protein and small analytes • leaching un-crosslinked oligomers	• OOC substrate • to mimic biological processes involving a mechanical deformation, (breathing (Huh et al., 2010b; Huh, 2015) and gut peristalsis (Kim et al., 2012)) • porous membrane to measure mechanical properties of monolayer cells (Huh et al., 2010b) • to produce microwell arrays to induce 3D cell aggregation (Lee et al., 2018b).
Thermoplastics	polystyrene (**PS**), polycarbonate (**PC**) polyurethane (**PU**)	• optically transparent, • more rigid than elastomers and • resistant to the diffusion of small molecules,	• lowly gas-permeability • inflexible • dissoluble in most organic solvents	• cell growth and adhesion (Lee et al., 2019) • Membranes (Pocock et al., 2017; Shah et al., 2016). • heart valves, pacemakers, haemodialysis membrane and artificial heart (Piccin et al., 2007) • membrane (Pourmand et al., 2018)
	polymethylmethacrylate (**PMMA**) polyethylene glycol diacrylate (**PEGDA**)	• moderate resistant to alcohol.		• OOC substrate • microfluidic valve and pump (Rogers et al., 2011, 2014)
Thermosets		• optically transparent • resistant to heat degradation and to chemical attack of most solvents	• rigidity • high cost	• negative photoresists (es. SU-8) for microchannels fabrication • high-aspect ratio and free-standing microstructures
Paper		• low cost, • lightweight • ease to use	• limited detection method • difficulty to integrate microcomponents such as valves.	• cell growth
Natural Hydrogel	**Collagens**	• biocompatible, • permeable to gas, water and solute • low-cost • similar to natural soft tissue	• weak mechanical properties • limited long term stability • batch-to-batch variability	• ECM models (Hassan et al., 2020) • cell adhesion, growth and differentiation, tumoroids (Tasoglu and Demirci, 2013; Elomaa and Yang, 2017; Fedorovich et al., 2012; Roseti et al., 2017) • artificial microvessels (Liu et al., 2015)
	Gelatin **matrigel**	• non- or low- immunogenicity • alginate and gelatin have tunable properties • collagen present motifs for cell adhesion		• cell growth • ECM models • cell adhesion, proliferation • formation of functional organoids (Hoang and Ma, 2021).
	Fibrin			• vascular network engineering • scaffold for cell encapsulation • component of ECM
	Alginate			• microfluidic channels (Choi et al., 2007)
Synthetic Hydrogel	**PEG** and its derivative **PCL**	• biocompatible, • permeable to gas, water and solute • low-cost • tunable mechanical properties and degradation rate	• absence of cell adhesion ligands on the surface	• tissue engineering • drug delivery and bone tissue scaffold

processes (Ding et al., 2020). For these reasons, the use of polymeric materials became a mainstream. Launched many years after glass chips, polymeric-based devices can today be divided into three major groups: elastomers, thermoplastics and thermosets.

Between elastomers, polyimethylsiloxane (PDMS) is the most employed for the realization of devices for life science applications (Ding et al., 2020). It is flexible, optical transparent, gas permeable and biocompatible (Ning et al., 2016). Its mechanical properties and its hydrophobicity are tunable respectively changing the ratio of PDMS base to curing agent and making surface plasma treatments (Guttenplan et al., 2021). It has a low autofluorescence and a good deformability that allows easy microfluidic connections (Piruska et al., 2005; Amani Wan Salim et al., 2013). For its ease of use and its low cost, it is very useful in prototyping new devices (Hassan et al., 2020). PDMS elasticity is also exploited to mimic biological processes involving a mechanical deformation, such as breathing (Huh et al., 2010b; Huh, 2015) and gut peristalsis (Kim et al., 2012) or to measure mechanical properties of monolayer cells (Sorba et al., 2019). Other uses of PDMS are (i) in the form of membrane for cell culture at the interface between two compartments (Huh et al., 2010b) and (ii) to produce microwell arrays to confine and induce 3D cell aggregation into spheroid, embryoid body or organoids (Lee et al., 2018b). Various fabrication methods are available for the fabrication of PDMS devices, from conventional soft lithography and micromolding techniques to new strategies such as hybrid stamp approach (Kung et al., 2015), sacrificial template methods (Cheng et al., 2016), razor-printing (Cosson et al., 2015) and 3D printing (using a PDMS resin) (Bhattacharjee et al., 2018). PDMS has some limits of applications due to its incompatibility with organic solvents (not particularly relevant for OOC applications) and its tendency to adsorb protein and small analytes (Toepke and Beebe, 2006). Some treatment with plasma, UV or coatings are performed to improve the PDMS surface (Ning et al., 2016; Khetani et al., 2020). Moreover, it can leach un-crosslinked oligomers that can cause toxicity in cells and affect their behavior, altering for example the predictions of pharmacokinetics/pharmacodynamics studies (Carter et al., 2020).

Thermoplastics are a class of polymers that can be melted and

reshaped many times through heating usually by means of thermomolding (Becker and Gärtner, 2008). These materials are mostly optically transparent, more rigid than elastomers and resistant to the diffusion of small molecules (Hassan et al., 2020), moderately resistant to alcool but dissoluble in most organic solvents (Ren et al., 2013). Unfortunately, being lowly gas-permeable, thermoplastic sealed microchannel and microchamber are not suitable for long-term cell studies (Ren et al., 2013). Moreover, their stiffness does not allow to realized diaphragm valves (Ren et al., 2013). Depending on their application thermoplastics surface can be functionalized by means of surface grafting techniques or dynamic coating (Ren et al., 2013). Thermoplastic polymers such as polystyrene (PS), polycarbonate (PC), polyurethane (PU), polymethylmethacrylate (PMMA) and polyethylene glycol diacrylate (PEGDA) are generally used in microfluidic systems (Ning et al., 2016; van Midwoud et al., 2012). PS is highly biocompatible and used for cell growth and adhesion (Lee et al., 2019). PC membranes are usually integrated among microchannels in OOC structures for modeling tissue-tissue interfaces (Pocock et al., 2017; Shah et al., 2016). PU is a high mechanical strength material, resilient and resistant to abrasion, used in the fabrication of biomedical devices such as heart valves, pacemakers, haemodialysis membrane and artificial heart (Piccin et al., 2007) but also as membrane in thermoplastic microfluidic devices (Pourmand et al., 2018). PMMA has been employed as substrate for OOC due to its stiffness, good optical transparency and low auto-fluorescence background (Chen et al., 2016b; Miller and Shuler, 2016b). PEGDA thermoplastic is used to realize different type of microfluidic valve and pump (Rogers et al., 2011, 2014). Thermoplastics are suitable for commercial production since fabrication techniques as thermomoulding can ensure high production-rate and low cost but they are not economic for prototyping (Ren et al., 2013). For fast prototyping, photocurable soft lithography compatible liquid PS prepolymer and a fast curing PMMA prepolymer have been exploited as negative photoresists and shaped by means UV or visible light exposure (Nargang et al., 2014; Kotz et al., 2018).

Differently from thermoplastics, in thermoset plastics the polymer chains cross-link into a rigid network structure that make difficult the reshaping of material. Thermosets, generally used as negative photoresists (e. g. SU-8) for microchannels fabrication, are optically transparent, resistant to heat degradation and to chemical attack of most solvents. Their mechanical strength permits the realization of high-aspect ratio and free-standing microstructures. However, their rigidity and high cost can limit their applications in biochips (Ren et al., 2013).

Paper is another material explored in microfluidics for its low cost, lightweight and ease to use. The porous structure of cellulose matrix is exploited for cell growth in 3D layout. In addition, sensor films can be integrated in paper-based devices for the monitoring of physiological microenvironment (Boyce et al., 2016; Mosadegh et al., 2015). 3D tumor cells have been growth in a paper roll device for the analysis of cellular environment and response (Young et al., 2018; Rodenhizer et al., 2016). Although the manufacturing of paper-based microfluidic device is simple, few applications have been demonstrated on paper chips (Ren et al., 2013). In these devices, the detection methods are relatively limited and it is difficult to integrate microcomponents such as valves.

Hydrogel is a hydrophilic macromolecular polymer gel constructed of a network of crosslinked polymer chains. The type and degree of crosslinking (depending on gelation and fabrication methods) affects the network properties, like swelling, elastic modulus porosity, permeability and degradability (Maitra and Shukla, 2014). Physically or chemically cross linking can provide hydrogels a three dimensional network structure and make them insoluble, allowing immobilization and release of biomolecules. The ability of hydrogels to embed a large amount of water (more than 30% w/v) make them similar to natural soft tissues, while their properties to swell under specific biological conditions allow applications in biomedical fields, such as drug delivery and tissue engineering (Maitra and Shukla, 2014). Hydrogels are also among the most used materials for mimicking the mechanics and biochemistry of native extracellular membrane (Ding et al., 2020). These polymer networks, permeable to gas, water and solute, allow cell adhesion and migration, growth, differentiation and maturation of organoids (Guttenplan et al., 2021). ECM models in hydrogel are employed to study processes such as angiogenesis (Natividad-Diaz et al., 2019), cancer metastasis (Lugo-Cintrón et al., 2020), and osteoblast migration (Movilla et al., 2018). Combining crosslinked strategies with lithography technique hydrogel scaffold are realized for 3D cell culture. Hydrogels have been likewise used to fabricate entire microfluidic devices for the study of blood vessel and to produce engineered tissue with functional vasculature (Cabodi et al., 2005). Hydrogels can be formed from natural polymers such as for example collagen, gelatin, matrigel, fibrin and alginate, or synthetic ones such as polyethylene glycol and its derivatives (PEG-DA), polycaprolactone (PCL) or synthetic/natural hybrids (Hassan et al., 2020; Liu et al., 2015). Remarkably, hydrogels are compatible with a variety of fabrication techniques, including soft lithography, 3D printing, micropatterning, electrospinning, UV curing, that enable the design of different structures (Guttenplan et al., 2021).

Collagens are the most common components of the native ECM and of connective tissues (Hassan et al., 2020). These natural hydrogels have several cell-binding sites for cell adhesion, growth and differentiation, that make them suitable for integration in platform model of heart, liver, skeletal muscle, neuronal network and tumor spheroids (Tasoglu and Demirci, 2013; Elomaa and Yang, 2017; Fedorovich et al., 2012; Roseti et al., 2017). Moreover, collagen has been employed as structural component in microfluidic devices for the development of artificial microvessels (Liu et al., 2015). Although Gelatin is similar to collagen in composition, it has a lower cost and is less antigenic. It is combined with other materials for supporting cell cultures in OOC platforms. Matrigel, a protein mixture extracted from mouse tumor cells, is used not only to model ECM in many tissue type, but also for cell adhesion, proliferation and for the formation of functional organoids (Hoang and Ma, 2021).

Within the category of natural compounds, Fibrin is an elastic protein involved in clotting, manly proposed for vascular network engineering but also used as scaffold for cell encapsulation and as artificial component of ECM. Alginate, a polysaccharide extracted from brown algae, is widely employed for several applications for its low-cost, low toxicity, easy functionalization and immediate gelation at mild condition. For example, Choi et al. (2007) realized microfluidic channels in calcium alginate for mass transfer sensing and for detection of chemical environment control surrounding encapsulated cells.

Natural hydrogels possess interesting properties for OOC applications such as high biocompatibility and biodegradability, but they have some limitations related to their relatively weak mechanical properties, limited long term stability and batch-to-batch variability. Synthetic hydrogel (and also hybrid) has been developed to provide materials with reproducible chemical and physical properties and tunable mechanical properties and degradation rate. Hydrogels based on PEG and its derivative (e.g. PEG-DA) are the most investigated synthetic hydrogel for tissue engineering. PCL found application in drug delivery and bone tissue scaffold. These synthetic biomaterials have some drawbacks related to the absence of cell adhesion ligands on the surface. To combine the advantages from both natural and synthetic biomaterials, hybrid hydrogels have been prepared. For example, as soft hydrogel is not suitable to mimic the hard and mineralized ECM of bone, fibrin incorporating hydroxyapatite (Jusoh et al., 2015) and mineralized collagen (Hao et al., 2018) are employed to study cancer metastatic into bone, bone angiogenesis and to investigate mechanical stimulation efficacy on bone formation. Finally, it is worth mentioning that “smart” responsive hydrogels able to respond to external stimuli are presently in the development phase.

4.4. Cell lines and technologies

For OOC implementation, various kinds of cells and cultures have been employed depending on the organ and the target application. In

general, simple monocultures are limited under several aspects and the use of co-cultures is crucial to reproduce organotypic microenvironments encompassing homotypic and heterotypic cell-cell interactions. Critical parameters for co-cultures include the type of cells, culture media, order in which cells are cultured, and numbers/ratio of cell types (Hodgkinson et al., 2022).

In terms of cells (Table 2), human primary cells have limited number of population doublings. Thus, immortalized cell lines are often employed to avoid senescence and improve availability, life span and reproducibility. However, while differentiating, they both show different characteristics after each passage and it is difficult to keep them in culture for long periods without changes (Geraili et al., 2018). In contrast, stem cells can differentiate into different cell types and exhibit the capacity for self-renewal. Among them induced pluripotent stem cells can be considered as the next-generation toolkit (Huang et al., 2021), in particular human-induced pluripotent stem cells (hiPSCs) which have been hailed as an effective replacement for human embryonic stem cells (hESCs). hiPSCs are represented by somatic cells, usually fibroblasts, that undergo a reprogramming process that converts already differentiated somatic cells into hiPSCs. Remarkably, the use of induced human stem cells is contributing to the development of patient-specific drugs and regenerative medicine (Geraili et al., 2018; Zakrzewski et al., 2019). Beyond cell lines, spheroids and organoids have been integrated in microfluidic platform, e.g. for tumor models with perfusable channels. Biomaterial based scaffolds and 3D cell bioprinting are also contributing in providing additional capacity for the fabrication of more advanced OOC models (Park et al., 2018).

Table 2
Cell lines for OOC platforms. [adapted from https://promocell.com/in-the-lab/human-primary-cells-and-immortal-cell-lines/]

	Human primary cells	Cell lines	HiPSC (human induced pluripotent stem cell) and ESC (embryonic stem cells)
Senescence	Can replicate over a limited period of time	Can replicate over a long period of time	Have self-renewal
Culture	more demanding: Require more skills, special media and additives (growth factors) and adjustment for each cell type	Standard culture conditions easy to work with and keep alive	Sensitive, must be handled with care, require appropriate culture media with growth factors and extracellular matrix proteins.
Identity	Maintain the characteristics of the original tissue	Questionable, misidentification possible	ESC differentiate into different cell type,
Morphology	Show healthy cell morphology	Loss of polarity, lack of key morphological features	HiPSC derive from an adult somatic cell and is induced to
Phenotype	Maintain original phenotype for a limited number of passages depending on cell type and conditions	Change in phenotypes (need to be validated), functional alteration	convert into a stem cell that will give rise to a specifically differentiated cell
Genome	Genetically stable	Altered genomic content	Stable
Relevance in vivo	high	low	High
Reproducibility of results	Lower, donor-to-donor variations need to be considered	Higher, uniform cell type	—
Cell availability	Limited	Unlimited	Unlimited (HiPSC) Limited (ESC)
Costs	High	Low	High
Ethics	Regulations for use of human tissues	Non relevant	Ethical issues relating to ESCs surmountable by HIPS

In designing an OOC, both the cell-to-liquid ratio and the surface-to-volume ratio have to be considered since they can affect the response. In this respect, it is worth noting as OOC platforms differ from the in vivo case having large media volumes if compared to the limited amount of tissues and this results in a continuous dilution of the metabolites. Furthermore, OOC also present a different surface-to-volume ratio which influences cell autocrine and paracrine signaling (Barros et al., 2021). In various MOC studies, attention has been dedicated in building the devices maintaining ratios among the different organ masses as close as possible to the in vivo case. Vascularization, barrier functions, mechanical clues (e.g. associated to flow, breath or peristalsis), inflammatory processes, gas gradients and in particular oxygen concentrations are other important aspects to be carefully taken into account.

5. Conclusions

Current drug development methodologies present severe limitations in their ability to appropriately take into account the complex in vivo physiopathology of human tissues. Both conventional 2D culture and (costly) animal models fail to provide accurate predictions on human clinical outcomes with striking discrepancies in efficacy and side effects when compared to human trials (Dhiman et al., 2019; Ren et al., 2020; Nam et al., 2015). This results in high costs and low success rate in translation to the clinic, which are responsible for the declining number of approved drugs, the increasing duration of the drug development process and a higher risk for drug withdrawal from the market at front of huge investments (Radhakrishnan et al., 2020).

To overcome these deficiencies, new platforms with enhanced predictive potential are under development. Exploiting advances in microfluidics and cell culture technologies, organs-on-chips can recapitulate pathophysiology, in vivo biophysical conditions, cell-cell/cell-matrix interactions, organ-organ crosstalk and the underlying biochemical pathways of different diseases providing accurate and versatile in vitro models. Advantages with respect to traditional methodologies have been discussed in Fig. 3, while Table 3 provides a summary of the state of the art for the OOC and MOC platforms which were discussed in detail in the previous sections.

Remarkably, recent advances have enabled to build relevant models of several human organs and diseases. Apart from drug efficacy and toxicity, enhanced in vitro models for micro-tissues vascularization and biological barriers have been optimized for investigating drug transport and barriers' permeability with increased accuracy and contribute to developing novel drugs delivery systems across relevant in-vivo barriers (e.g. exploiting nanodelivery). A recent trend consists in coupling multiple organ modules linked by vascular perfusion in order to recapitulate organ-level structures, simulate multi-organ interactions and assess drug metabolism/clearance/toxicity in a comprehensive 'body-on-a-chip' platform (Wang et al., 2018). Microfluidic technologies facilitate combinatorial approaches to assay relevant libraries of drug candidates and evaluate toxicity against multiple tissues in a more adequate and predictive way than previously possible. In addition, the integration of miniaturized sensors opens the way to further automation and number up of the assays.

These huge progresses have been facilitated by cutting-edge techniques, spanning from microfabrication processes inherited from microelectronics to soft lithographies, micromachining, rapid prototyping techniques, laser-assisted stereolithography, 3D printing and bioprinting. A further relevant building block was the emergence of protocols for the efficient directed differentiation of human induced pluripotent stem cells at high quantity and quality (Zhang et al., 2018b),

Table 3
Summary of discussed OOC and MOC platforms and their major characteristics.

ORGAN–ON–CHIP	CULTURED CELLS IN DEVICE	Main scope/aim	Key features	Diseases	Drug	Barrier
1.1 Intestine	➢ Human colorectal adenocarcinoma cells (Caco2) (Caplin et al., 2015b) ➢ Co-colture of enterobacteria and Caco2 (Caplin et al., 2015b) ➢ HiPSC derived-human intestinal-like tubules (Naumovska et al., 2020)	➢ Drug absorption and bioavailability ➢ nutrient/biomolecules exchange ➢ environmental factors ➢ gut/host-microbiota interplay and alterations of gut microbiota composition and function (dysbiosis) ➢ gut inflammation, imbalance between pro-inflammatory and anti-inflammatory signals	➢ peristaltic movements ➢ villi microstructures, ➢ gut/host-microbiota interplay ➢ gut inflammation influence	➢ intestinal dysfunction, ➢ intestinal inflammation ➢ gut severe, chronic and cancerous diseases	➢ Drug absorption and bioavailability ➢ Efficacy ➢ toxicity and side effects	➢ intestinal epithelial barrier ➢ permeability, tightness of cell junctions
1.2 Lung	➢ Human small airway epithelial cells (SAEC) (Huh et al., 2007) ➢ Human pulmonary alveolar epithelial cells (HPAEpiC) and human pulmonary microvascular endothelial cells (HPMEC) (Huh et al., 2010b) ➢ HiPSCs -derived lung cells (Zakrzewski et al., 2019) ➢ Human dermal microvascular endothelial cells (HDMEC) ➢ Human lung fibroblasts (HLF)	➢ Targeted instead than systemic administration ➢ influence of shear stress on paracellular and transcellular transport	➢ pulmonary microenvironment ➢ branching/breathing movements → cyclic strain and mechanical forces	➢ respiratory and pulmonary diseases ➢ viral and bacterial lung infections, ➢ chronic obstructive pulmonary disease (COPD) pulmonary edemas, tuberculosis and lung cancer, SARS-CoV and other respiratory virus infection injury ➢ airway repair after an injury ➢ Wound healing	➢ Drug absorption and bioavailability ➢ Efficacy drug-induced toxicity and side effects	➢ lung-blood barrier for inhaled agents ➢ alveolar-capillary barrier ➢ air-liquid interface (ALI)
1.3 Skin	➢ Human immortalized keratinocytes (HaCaT) (Ren et al., 2021) ➢ HiPSC-derived fibroblasts and keratinocytes (Geraili et al., 2018) ➢ Melanocytes, Immune cells, Neurons ➢ + integration of biopsy and off chip models	➢ Risk assessment for external agents and cosmetic products ➢ Transdermal drug delivery/ administration ➢ Drug penetration in bloodstream	➢ considerable differences vs animal skin in terms of structural and biochemical properties, lipid profile, hair density and stratum corneum thickness	➢ Dermatological studies ➢ Wound healing	➢ Transdermal drug delivery/ administration ➢ Drug penetration in bloodstream	➢ skin barrier
1.4 Vasculature	➢ Human umbilical vein endothelial cells (HUVEC) (Caplin et al., 2015b) ➢ Porcine aortic endothelial cells (PAEC) (Caplin et al., 2015b) ➢ Human embryonic stem cell (hESC)-derived pericytes (Geraili et al., 2018) ➢	➢ Intravenous administration and drug transport, ➢ recirculation at physiologically relevant perfusion rates and application of shear stress at in vivo levels ➢ vasculogenesis, sprouting angiogenesis and anastomosis ➢ blood organ barriers	➢ network of perfused microvessels ➢ Drug and nutrient transport (→increased lifespan for OOC) ➢ hemodynamic forces and vascular paarenchymal mechanotransduction vasculogenesis and angiogenesis	➢ diseases of the vascular system (i.e. atherosclerosis and deep vein thrombotic) ➢ vascularized micro organs (VMO) and micro tumors (VMT) ➢ tumor proliferation invasion, intravasation, extravasion	➢ Drug Transport, efficacy, ➢ anti-cancer and anti-angiogenic drugs	➢ blood organ barriers

(*continued on next page*)

Table 3 (*continued*)

ORGAN–ON–CHIP	CULTURED CELLS IN DEVICE	Main scope/aim	Key features	Diseases	Drug	Barrier
2.1 Blood-Brain Barrier	➢ Human brain endothelial cells (hCMEC/D3) (Caplin et al., 2015b) ➢ Primary human-derived microvascular endothelial cells (HBMVEC) (Geraili et al., 2018) ➢ HiPSC-derived neurons (Geraili et al., 2018) ➢ HiPSC-derived brain microvascular endothelial cells (BMECs) ➢ Co-colture of neurons, glial cells, endothelial cells and skeletal muscle cells	➢ mimicking in vivo physiological brain microenvironment/ barrier and relevant conditions/functions	➢ High-fidelity in mimicking in vivo physiological microenvironment	➢ neurodegenerative and brain diseases ➢	➢ Drug delivery through BBB junctions ➢ vascular network permeability	➢ functionality and disruption ➢ inflammatory disruption of BBB ➢ metabolic consequences and repair mechanisms
2.2 Tumor	➢ Patient-derived cells ➢ Tumor spheroids ➢ Variety of tumor cell lines	➢ vascularized tumor model ➢ use of (standard and liquid) biopsy & patient-derived samples, patient-specific tumor cell functions, ex vivo analysis of tumor cell dynamics and heterogeneity, ➢ microtumors arrays for testing combinatorial treatments with high throughput.	➢ 3D solid tumor tissues (tumor spheroids/ organoids/tumoroids) ➢ tumour microenvironment, extracellular matrix, cell–cell interactions, blood vasculature, and presence of nutrients, metabolites and oxygen gradients ➢ tumor angiogenesis and tumor and vasculature interactions ➢ metastatic cascade	➢ Cancer and metastatic cascade: proliferation invasion, intravasation, extravasion	➢ Drug development and screening ➢ Personalized therapies	➢ blood organ barriers
2.3.1 Heart	➢ Cardiomyocyte cells (Caplin et al., 2015b) ➢ Embryonic cardiomyoblast cell line(H9C2) (Giridharan et al., 2010) ➢ HiPSC-derived cardiomyocyte (Geraili et al., 2018)	➢ influence of mechanical cues ➢ microtissue-generated contractile forces ➢ studying cardiotoxicity and cardioprotective efficacy of cardiovascular drugs ➢ amyotrophic lateral sclerosis and neuromuscular development	➢ influence of mechanical cues ➢ microtissue-generated contractile forces ➢ cardiac/myocardial mechanics and heart muscle contractility ➢ force/strain sensors integration	➢ Cardiovascular diseases	➢ cardiovascular drugs ➢ toxicity and side effects ➢ cardiotoxicity and cardioprotective efficacy	
2.3.2 Bone	➢ Human foetal osteoblast cell line (hFOB) ➢ Co-colture osteocyte and osteoclast ➢ Osteoblast-like cell line (MG63) ➢ Chondrocytes	➢ Recreating biomechanical structures of the bone with scaffold materials and co-cultures models ➢ mechanobiology and mechanotransduction	➢ Use of scaffold materials ➢ influence of mechanical cues ➢ osteocyte-osteoclast signaling ➢ proliferation and osteogenic differentiation of foetal osteoblast cell line ➢ vascularized bone-on-chip models	➢ Bone diseases	➢ Efficacy ➢ toxicity and side effects	
2.4.1 Liver	➢ Human liver carcinoma cesll (HEpG2) and Umbilical endothelial cells (HUVEC) (Caplin et al., 2015b) ➢ HiPSC -derived hepatic progenitor cells	➢ Hepatotoxicity test of drugs and compounds ➢ Investigating drug-induced liver injury ➢Studying hepatic drug metabolism changes in humans overcoming species-specific differences in	➢ drug metabolism ➢ multi-organ interactions	➢ liver pathologies ➢ drug-induced liver injury (DILI)	➢ drug metabolism ➢ drug-induced liver toxicity	

(*continued on next page*)

Table 3 (*continued*)

ORGAN–ON–CHIP	CULTURED CELLS IN DEVICE	Main scope/aim	Key features	Diseases	Drug	Barrier
	(HPC) and mesenchimal stem cells (MCSs) and enteroendocrine cells (ECs) (Giobbe et al., 2015) ➢ HiPSC- derived hepatocytes (iHep)	➢hepatic drug metabolism				
2.4.2 Kidney	➢ Madin darby canine kidney (MDCK) (Caplin et al., 2015b) ➢ Human primary renal proximal tubule epithelial cell line (RPTEC) (Frohlich et al., 2012) ➢ Human renal epithelial cells (HREC) (Ferrell et al., 2010) ➢ hiPSC-derived human podocytes (Musah et al., 2017b) ➢ Human immortalized proximal tubular epithelial cell with organic anion transporter 1 (CiPTEC-OAT1)	➢ Nephrotoxicity test of drugs and compounds ➢ Detecting drug-induced kidney injury	➢ drug clearance ➢ multi-organ interactions	➢ kidney pathologies ➢ drug-induced kidney injury (DIKI)	➢ drug-induced nephrotoxicity studies	
2.5 Multi-organ	➢ Hepatocellular carcinoma (HepG2/C3A) and Madin-Darby canine kidney (MDCK) (Shintu et al., 2012b) ➢ Hepatic cell line (HepaRG) and human epatic stellate cells (HHStec) (Wagner et al., 2013b)	➢ miniature 'body-on-a-chip' microphysiological systems ➢ organ-organ interaction/crosstalk, ➢ physiological relations, methabolic pathways, ➢ significant biological barriers ➢ whole-body drug response with in vivo-like sequential organ-to-organ transfer of media	➢ Drug development, toxicity tests and metabolism of xenobiotics ➢Recapitulating metabolic pathways with ➢intestine models accounting for absorption and metabolism of drugs, liver for their metabolism and kidney for clearance/excretion	➢ systemic studies on orally-administered or inhaled substances or transdermal administration and subsequent drug metabolism ➢ multi-organ platforms for predicting antitumor drug response and metastasis	➢ evaluation of systemic effectiveness, accuracy and safety of drugs	➢ By integrating relevant barrier models

in order to make available the pertinent and supporting cells necessary for accurate disease and barrier models.

In conclusion, drug research is rapidly advancing with novel technologies at the disposal of researchers and companies. Today, more predictive approaches based on accurate in vitro replicas of human tissues, specific diseases and multi-organ models can respond to the pressing need for effective drug development protocols improving throughput and resource-efficiency beyond the possibilities of conventional 2D culture systems and animal testing. Furthermore, modern organ-on-chip systems offer tremendous promise for elucidating the mechanisms responsible for currently-incurable diseases. Several milestones were already achieved in this direction. Future challenges are expected to regard compromises between standardization, reproducibility and reliability in recapitulating disease microenvironment and drug response (Brancato et al., 2020) as well as to proceed toward patient-derived testing platforms for a personalized precision medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Funding

This work was funded by Italian National PON-AIM1800370-activity 2 (topic Health), by the Apulia region program "RESEARCH FOR INNOVATION" (REFIN n° 6277F79D-UNISAL036) and by FSC/PON FSE—FESR 2014–2020, project DOT1712250 code 2, XXXVI cycle, CUP F85F20000270006.