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Emerging Biosensor Trends in Organ-on-a-Chip

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Abstract Organ-on-a-chip technology is ideally suited to cultivate and analyze 2D/3D cell cultures, organoids, and other tissue analogues in vitro, because these microphysiological systems have been shown to generate architectures, structural organization, and functions that closely resemble their respective human tissues and organs. Although great efforts have been undertaken to demonstrate organotypic cell behavior, proper cell-to-cell communication, and tissue interactions in recent years, the integration of biosensing strategies into organ-on-a-chip platforms is still in its infancy. While a multitude of micro-, nano-, and biosensors are well established and could be easily adapted for organ-on-a-chip models, to date only a handful of

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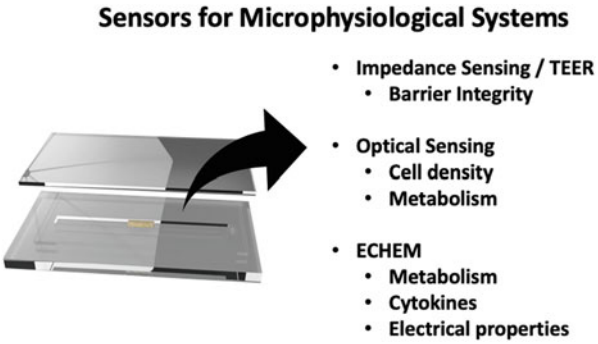
analytical approaches (aside from microscopical techniques) have been combined with organ-on-a-chip technology. This chapter aims to summarize current efforts and survey the progress that has been made in integrating analytical techniques that are being implemented for organ-, multi-organ-, and body-on-a-chip systems based on electrochemical and optical sensors.

Graphical Abstract



Keywords Biosensing, Microphysiological systems, Organs-on-a-chip, Sensor integration

1 Introduction

Two decades ago, George Whitesides and his colleagues introduced soft lithography to biomedical engineers, chemists, and biologists as a simple and fast method to develop microfluidic devices – thereby paving the way for the development of microfluidic cell-based assays made from poly-dimethylsiloxane [1]. Over the course of the last 23 years, the technology that started using simple microchannels and two-dimensional cells monolayers (so-called cell-based microfluidics) has rapidly progressed into more complex cell culture systems [2] (Fig. 1). For instance, the integration of microfluidic components – including valves, micropumps, mixers, actuators, and microsensors – has provided controlled liquid handling routines and analytical power of true lab-on-a-chip systems [3]. However, it was only in the last decade that the first microphysiological system for lung tissue (lung-on-a-chip) was developed that was capable of simulating breathing motions of the lung [1, 2]. For the first time, the scientific community witnessed a groundbreaking concept of actuating lung epithelial cell layers using flexible porous membranes to emulate a key biomechanical function of the lung. The last decade has seen a global trend toward more physiologically relevant cell cultures systems, with the aim of reengineering in vivo-like cellular microenvironments for 2D and 3D cell cultures

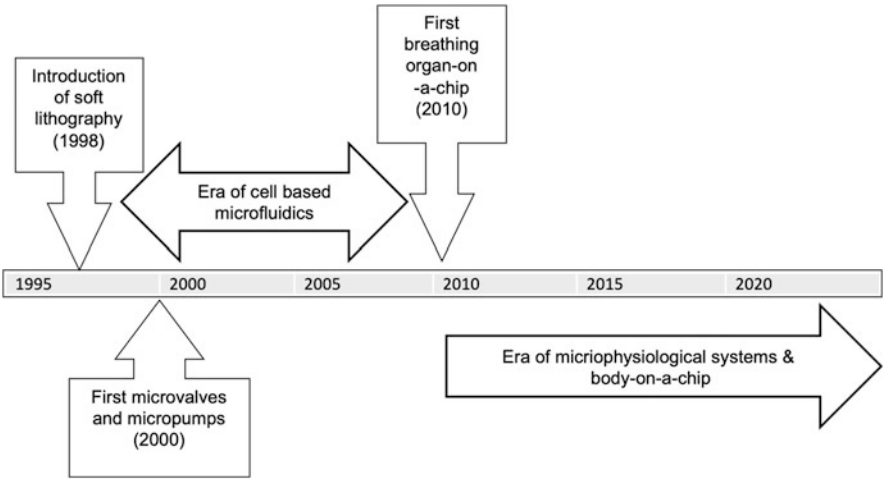


Fig. 1 Timeline for the emergence of organ-on-a-chip systems and microphysiological systems

that truly resemble actual human tissue architecture and organ function and physiology in vitro [4]. Yet notwithstanding recent biological advancements using patient-derived primary cell and iPSC-culture systems, little progress has been made to date in improving the functionality and analytic capabilities of these microphysiological systems. Even though a variety of sensors based on electrochemical, acoustic, optical, and magnetic readouts have been developed and integrated into cell-based microfluidic devices [5], only a few analytical sensing methods have actually been used in microphysiological systems so far. Among these, bipolar and tetrapolar electric cell impedance (ECIS) and resistance measurements (TEER) are mainly used to assess the integrity of human epithelial and endothelial barrier models – while multielectrode arrays (MEAs) are employed to detect electrophysiological activities of cells and electrochemical and optochemical sensors to study metabolic parameters and biomarker release such as oxygen demand, pH levels, and release of, for example, cytokines. Most organ-on-a-chip systems heavily depend on microscopy and off-chip analytical techniques, due in part to the familiarity of laboratory staff with standard techniques such as ELISA, PCR, metabolic plate-reader assays, and staining techniques for fluorescence microscopy and histology. The previous chapters by Szita highlighted how microfluidic technology can help to improve in vitro cell cultures, whereas in their chapter, Maschmeyer and Kakava elaborated on how organ-on-a-chip systems help to create a cellular microenvironment that recreates human tissue and organ responses in vitro. Accordingly, the current chapter focuses solely on the question how to make microphysiological systems (such as organs-, multi-organ-, and body-on-a-chip systems) more functional via the integration of micro- and biosensors.

2 Bipolar and Tetrapolar Electrode Approaches for Transepithelial and Endothelial Resistance (TEER) Measurements at Human In Vitro Barriers

Transepithelial/endothelial resistance (TEER) measurements as shown in Fig. 2a are considered the golden standard for analyzing barrier model integrity without the need for invasive dye leakage assays based on fluorescein or fluorescence-labelled dextran molecules. Jeong et al. integrated a TEER sensor array using etched 200 nm gold thin films within a PDMS and polycarbonate hybrid biochip to study barrier integrity of primary murine BMVECs in the absence and presence of astrocyte co-culture using a commercial EVOM2 Volt-ohm meter in combination with a multiplexer [6]. Using this setup, the authors demonstrated improved barrier integrity when using Matrigel instead of fibronectin – achieving a near doubling in resistance values at day 4. Interestingly, however, exposure to histamine resulted in loss of barrier integrity only in the presence of a monolayer culture – highlighting the protective function of astrocytes in a co-culture system. Using a similar measurement setup, Walter et al. established several tissue barrier models including intestine, blood-brain, and lung barriers within a single microfluidic device containing embedded integrated TEER electrodes fabricated either from gold or transparent indium tin oxide thin films [7]. Similar to the EVOM2, a Millicell®

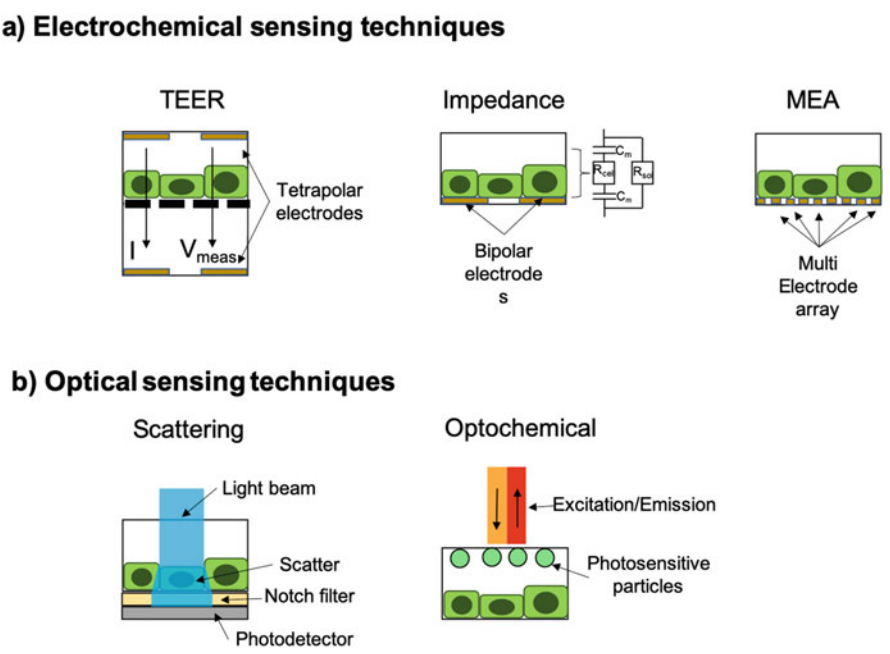


Fig. 2 Sensing principles for (**a**) electrochemical and (**b**) optical including TEER, impedance spectroscopy, MEAs, light scattering, and optochemical cell-based analysis

ERS-2 Volt-ohm meter in combination with a single integrated Ag/AgCl electrode pair was used by Ramadan et al. to establish bipolar TEER recordings for HacaT/U937 co-culture as a model for immune competent skin in the presence and absence of an air-liquid interface and toxicants [8]. A more refined measurement approach was also recently demonstrated by Henry et al., who employed a tetrapolar electrode TEER setup using a PGstat128N potentiostat to assess primary human airway epithelial cells (hAECs) over a 60-day long-term culture at the air-liquid interface (ALI) and human Caco-2 intestinal epithelial cells up to 12 days [9]. The inherent flexibility and analytical power of potentiostats enabled continuous impedance spectroscopy recordings at high temporal resolution at multiple frequencies and defined amplitudes to monitor multiple parameters such as impedance and capacitance of cell barriers. In a follow-up study, a similar TEER approach was also used by Park et al. to elucidate the influence of hypoxia on transport of drugs and antibodies at the human blood-brain barrier (BBB). Here, an increasing barrier tightness by fivefold and more was observed following exposure of iPS-derived endothelial cells to either oxygenated or hypoxic culture conditions prior seeding and differentiation into the BBB [10].

3 Bipolar and Tetrapolar Impedance Spectroscopy Approaches for Integrity Monitoring of Human In Vitro Organ and Barrier Models

A more general electrochemical approach that provides similar information to direct TEER readings is called electric cell impedance spectroscopy (ECIS; see also Fig. 2a) and involves a system wherein complex electric impedance of a cell culture system in contact with the working electrode is monitored in a time-resolved way over a broad range of frequencies ranging from a few Hz (similar frequency as TEER) to kHz, or even MHz, regimes. Depending on the applied frequency, different electrical properties of cell-based barrier models are detected – thus providing information on changes in (a) capacity of cell membranes, (b) conductivity of medium, and (c) resistive components caused by increase in tight junctions or cellular movements. In its simplest implementation, a pair of wire or thin film electrodes can be used to measure changes in impedance over time as a function of barrier integrity. By way of one illustrative example: a tetrapolar TEER measurement setup has been used to analyze on-chip μBBB integrity by inserting four platinum wires into small electrode channels and sealed with photocurable [11, 12]. Between the four platinum electrodes, a total of six different impedance values could be derived for a single chip by recording impedance values between the upper and lower electrode pair (E1 + E3/E2 + E4) for blank medium measurements or through the BBB for the remaining four electrode combinations. The results of this study showed a distinct dependence electrode pairing and obtained signal variance, suggesting the need for signal processing by averaging the four cellular

values and baseline subtracting the acellular medium recordings for an individual experiment in order to generate an average impedance curve for hCMEC/D3 cells. This approach eliminates an acellular control measurement as blank values needed for signal normalization, since control values are simultaneously recorded with the cell barriers. Moreover, Mermoud et al. have tracked of membrane deflection during the breathing motion of an actuated flexible PDMS membrane in a breathing lung-on-chip system employing coplanar impedance biosensors in a microimpedance tomography (MITO) sensing approach based on printed circuit board (PCB)-technology [13]. Furthermore, the authors showed that membrane permeabilization events (buildup and breakdown) of A549 type II alveolar epithelial cells during breathing motion can be monitored. Another bioimpedance application involves nanoparticle risk assessment at human barrier models, where, e.g., Schuller et al. [14] have developed a highly integrated placenta-on-a-chip system containing an array of embedded membrane-bound thin film impedance microsensors capable of non-invasively monitoring placental barrier integrity. Using an optimized plasma-assisted liftoff procedure, high-resolution coplanar interdigitated electrode structures with 15 μm resolution were fabricated on a porous membrane without clogging the pores [15]. Barrier integrity was continuously monitored using a cell-culture treated PET membrane that acted as a growth surface for a placental barrier of trophoblast-derived BeWo cells. Using a bipolar impedance spectroscopy approach in the absence and presence of standardized silicon dioxide (SiO_2), titanium dioxide (TiO_2), and zinc oxide (ZnO) nanoparticles, the authors demonstrated a high degree of similarity to standard tetrapolar TEER approaches. The benefits of employing membrane-based impedance biosensors include higher temporal resolution, the elimination of complex electrode pair positioning at top and bottom of the microchannel, and the removal of unwanted stray resistance originating from the porous polymer membrane (which shows high variance due to the pore fabrication process).

4 Monitoring of Organ Function Using Electrochemical Analysis Techniques: Straight Through the Heart

Electrochemical analysis techniques are well established for a variety of biological models, including neuronal and muscular in vitro models where activity of neuronal networks and beating of cardiac muscles/bodies are monitored using multielectrode arrays (MEAs; see also Fig. 2a). For instance, Oleaga et al. have presented a multi-tissue-on-a-chip platform which performed long-term analysis up to 1 month culture duration of cardiomyocyte and hepatocyte co-culture as well as a muscle and motoneuronal unit for multi-organ studies [16, 17]. Using a MEA for potentiometric measurements (electrical activity) in combination with a cantilever array, cardiac beating was evaluated after exposure with well-known drug metabolites generated by hepatic metabolism. Boudou et al. used a similar cantilever-based method for drug screening of electro-stimulated cardiac microtissues made from fibrin/collagen

3D matrices [18]. A similar approach was also used by Caluori et al. to analyze cardiac bodies derived from a patient-derived dystrophin-deficient cell line, which were reprogrammed from fibroblasts for Duchenne muscular dystrophy (DMD) studies [19]. To improve the sensitivity of electrochemical beating rate analysis, Inácio et al. applied PEDOT:PSS conductive polymer instead of metallic electrodes – which can be printed very easily using a common material inkjet printer [20]. In contrast to the pristine and tiny electrodes of MEAs, the bigger footprints of the printed MEAs enabled efficient monitor beating rates of whole embryoid bodies with enhanced signal quality. Another tetrapolar ECIS using MEAs was used by Maoz et al. to enhance analysis outcome of endothelial barrier integrity using a myocardial barrier model comprising of IPS-derived cardiomyocyte cultures and a primary human endothelium [21]. Dynamic and time-resolved alterations in the electrical activity of the cardiac model were detected following the treatment with tumor necrosis factor alpha and the cardioactive drug isoproterenol. Another modular approach was introduced by Gaio et al. using their modular "Cytostretch" platform, which combines MEA sensors with strain gauges [22]. Yet another way to measure mechanical deformation of 3D myocyte tissues using integrated copper force probes was reported by Chan et al. [23]. Additional modular modules have also incorporated porous membranes and 3D patterned microgrooves to allow for analysis of a variety of aspects important to organ and multi-organ chips including immune cell migration; measurement of electrical field potential of cardiac cells; improved cell/sarcomere orientation; and real-time monitoring of membrane stretch as a function of electrical resistance changes using a 2×2 arrayed plug and play chip. Overall, a number of reports have now demonstrated that impedance spectroscopy is not only useful to assess barrier integrity but also to monitor, for example, cardiac contraction status using high-speed impedance setup to monitor efficacy of cardioactive drugs such as verapamil and doxorubicin [24].

In addition to the abovementioned approaches, electrochemical techniques have also been used in combination with biorecognition elements (e.g., antibodies or aptamers) as ELISA-type assays to monitor on-chip secretion of cytokines. For instance, Shin et al. developed an aptamer-functionalized gold microelectrode for sensitive detection of secreted cardiac damage-associated biomarkers, which was evaluated against doxorubicin cardiotoxicity resulting in good correlation to complementary viability and beating analyses [25]. A similar sensing approach, using a more complex biological system, was presented by Zhang et al., who combined liver and cardiac organoids to detect cardiac biomarker secretion following the addition of acetaminophen (paracetamol) and doxorubicin [26]. A combination of impedimetric TEER, ELISA-like impedimetric biomarker analysis, and cardiac beating monitoring was established by Skardal et al. using a heart-liver-lung multi-organ-on-a-chip system [27]. The individual organ models printed from primary human cells were used to analyze the side effects of drugs by detecting cardiac beating using real-time microscopy, impedimetric affinity sensors to detect antibody-binding events, and transendothelial electrical resistance measurements to assess barrier integrities. The authors demonstrated that organ-organ interplay between lung tissue and a heart model can trigger severe adverse effects.

5 Monitoring Organ/Tissue Metabolism Using Electrochemical Analysis Techniques

For decades, electrochemical techniques such as amperometry and voltammetry have been extensively used to detect metabolic parameters such respiration rate, lactate levels, and glucose consumption. Sensor performance of electroanalytical techniques is constantly improving, and, with the integration of electrodes in microdevices, the rate of their application in microphysiological systems is steadily increasing. It is important to note in microphysiological cell culture systems, highly sensitive and selective analysis of metabolic parameters is a key metric for evaluating (a) an organoid's proper physiologic function, (b) the toxicity of biochemical stimuli, and (c) the onset, progression, and remission of pathophysiological processes. Yet despite the inherent advantages of employing embedded electrical biosensors, to date, the seahorse assay system remains the dominant method employed to monitor organoid cell metabolism in vitro [28]. Although widely accepted, in its current configuration (as an external inset for microtiter plate-based analysis approach), this setup is not suitable for stand-alone in situ analysis of complex cell cultures, since it cannot be integrated. To address the demands of time-resolved analysis of key physiological parameters with high spatial resolution in co-cultures and multi-organ-chip devices, a number of innovative approaches have been reported in recent years. As an example, Moya et al. presented a chronoamperometric approach to monitor oxygen consumption using inkjet printing of multiple sensors into an extremely thin, porous membrane for liver oxygen respiration monitoring using two-dimensional cultures of primary freshly isolated hepatocyte cultures [29]. Similarly, Misun et al. have measured the current density at a constant voltage of 0.65 V (e.g., amperometry) using either glucose oxidase (GOx) or lactate oxidase (LOx)-modified working electrodes to study the dynamic cellular metabolism. Using a plug and play biosensing unit that can be clicked onto a microfluidic hanging drop when sensing is initiated, lactate accumulation and glucose consumption were detected in a spheroidal HCT116 human colon carcinoma organoid model [30].

6 Monitoring of Organ Metabolism and Architecture Using Optical Sensors

Optical sensing principles as shown in Fig. 2b hold great potential for organ-on-a-chip application due to the straight forward integration and ease of use. A variety of non-invasive optochemical sensor approaches have also been developed to study cell and tissue metabolism using sensitive optochemical tracer molecules. Among these, porphyrin-based optical oxygen sensors have proven particularly useful in cell analysis applications [31]. For instance, Ungerboek et al. have implemented a ratiometric imaging system to quantify oxygen levels with high spatial resolution

in microfluidic systems [32]. Using a simple CCD camera set up, a global image of the microfluidic device was taken, and variations of local oxygen tension calculated using two different wavelengths. Additionally, Sticker et al. have demonstrated the ability to monitor hypoxic conditions in a microfluidic stroke-on-a-chip (BBB) model to emulate in vivo conditions during stroke using embedded oxygen sensor spots consisting of opto-chemical oxygen-sensitive microbeads. Importantly, the authors showed precise control over apparent oxygen levels inside a microfluidic device fabricated from oxygen-scavenging OSTEmer thiol-ene epoxies by adjusting the microfluidic flow velocities [33]. Furthermore, Zirath et al. used a similar opto-chemical sensing approach based on immobilized microbeads impregnated with porphyrin-based oxygen indicator dye to study oxygen gradients across the cultivation chambers of 2D and 3D microfluidic organ models. The authors also demonstrated the ability to tune oxygen gradients by changing flow profiles and chip materials for a three-dimensional vascular barrier model based on human adipose-derived mesenchymal stem cells in co-culture with HUVEC endothelial cells in a fibrin hydrogel scaffold [34]. An alternative optical approach to monitor morphological changes of 3D-tissue structures is predicated on using light scattering measurements to detect structural changes within on-chip 3D in vitro synovium using organic photodiodes [35]. In this approach, perpendicular 480 nm light is directed through an organoid – but only light scattered at greater than a 20° angle to the incident light beam actually passes through the notch filter and is detected by an organic photodiode. Using this simple optical setup, the authors demonstrated the ability to readily detect the onset of structural changes in the synovium that take place during inflammatory arthritic processes. Following TNF-alpha stimulation of the 3D synovial organoids, dynamic cellular matrix reorganization events were detected in a non-invasive manner with only a few days. Moreover, this setup was used as a quality control tool to assess cross-linking processes of natural hydrogels including Matrigel, collagen, and fibrin, which are known to change optical densities in the presence of uncontrolled or failed cross-linking events.

7 Conclusion

The global trend toward in vitro biological systems of increased complexity that are capable of mimicking (patho)physiology of tissue in vivo has created a renewed demand for microdevices that permit analysis of key biochemical parameters. The integration of non-invasive micro- and biosensors, therefore, presents a real opportunity to establish organs- and multi-body-on-a-chip with improved organo-typic functionality. In the last decade, a number of optical and electrochemical sensing approaches have been employed to monitor important organ functions, including barrier integrity (TEER/ECIS); metabolic parameters (e.g., porphyrin-based oxygen sensing, amperometry, and voltammetry); organ activity such as cardiac beating (e.g., cantilevers and MEAs); and as biomarker secretion (amperometry coupled with biorecognition elements). However, results obtained from a Scopus® search

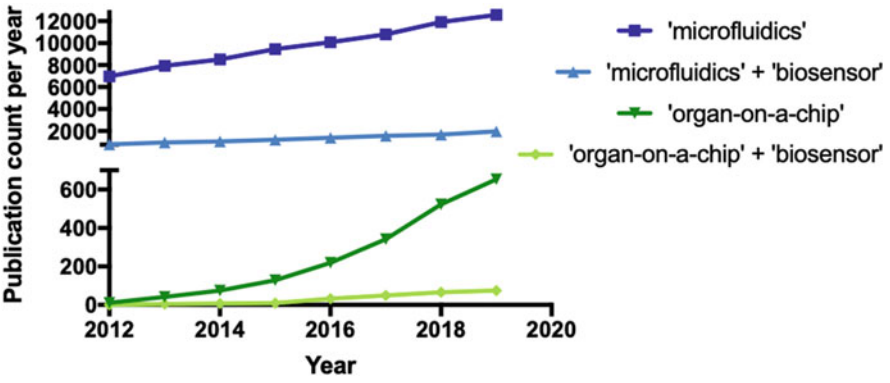


Fig. 3 Results of the Scopus research on annual publication output for the search terms "microfluidics," "microfluidics + biosensor," "organ-on-a-chip," and "organ-on-a-chip + biosensor"

seen in Fig. 3 highlight that the integration of biosensing strategies into microphysiological organ-on-a-chip systems remains in its infancy. Out of the 12,570 microfluidic papers that were published in 2019, only 13% reported embedded sensors – which is surprisingly similar to the rate of organ-on-a-chip applications (seen in 75 out of 654 papers published in 2019). These relatively low percentages underscore the need for additional technical skills and engineering expertise, costly clean room facilities, and infrastructure that all remain unfortunate predicates for sensor microfabrication. To date, improvement in microphysiological systems is mainly associated with recent progress seen in biofabrication techniques and iPSC technology to generate advanced in vitro organ models. Nevertheless, looking ahead, the authors anticipate that more and more well-established sensing techniques will be incorporated into this game-changing technology in an attempt to standardize miniaturized microphysiological systems.

References

1. Xia Y, Whitesides GM (1998) Soft lithography. Angew Chem Int Ed Engl 37:550–575
2. Charwat V et al (2014) Monitoring cellular stress responses using integrated high-frequency impedance spectroscopy and time-resolved ELISA. Analyst 139:5271–5282
3. Rothbauer M et al (2019) Monitoring transient cell-to-cell interactions in a multi-layered and multi-functional allergy-on-a-chip system. Lab Chip 19:1916–1921
4. Rothbauer M, Rosser JM, Zirath H, Ertl P (2019) Tomorrow today: organ-on-a-chip advances towards clinically relevant pharmaceutical and medical in vitro models. Curr Opin Biotechnol 55:81–86. https://doi.org/10.1016/j.copbio.2018.08.009
5. Wartmann D et al (2015) Automated, miniaturized, and integrated quality control-on-chip (QC-on-a-chip) for cell-based cancer therapy applications. Front Mater 2:60. https://doi.org/10.3389/fmats.2015.00060

6. Jeong S et al (2018) A three-dimensional arrayed microfluidic blood-brain barrier model with integrated electrical sensor array. IEEE Trans Biomed Eng 65(2):431–439. https://doi.org/10.1109/TBME.2017.2773463
7. Walter FR et al (2016) A versatile lab-on-a-chip tool for modeling biological barriers. Sensors Actuators B Chem 222:1209–1219. https://doi.org/10.1016/j.snb.2015.07.110
8. Ramadan Q, Ting FCW (2016) In vitro micro-physiological immune-competent model of the human skin. Lab Chip 16(10):1899–1908. https://doi.org/10.1039/c6lc00229c
9. Henry OYF et al (2017) Organs-on-chips with integrated electrodes for trans-epithelial electrical resistance (TEER) measurements of human epithelial barrier function. Lab Chip 17:2264–2271
10. Park TE et al (2019) Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. Nat Commun 10(1):1–12. https://doi.org/10.1038/s41467-019-10588-0
11. van der Helm MW et al (2016) Direct quantification of transendothelial electrical resistance in organs-on-chips. Biosens Bioelectron 85:924–929. https://doi.org/10.1016/j.bios.2016.06.014
12. van der Helm MW et al (2017) Fabrication and validation of an organ-on-chip system with integrated electrodes to directly quantify transendothelial electrical resistance. J Vis Exp 127: e56334. https://doi.org/10.3791/56334
13. Mermoud Y, Felder M, Stucki JD, Stucki AO, Guenat OT (2018) Microimpedance tomography system to monitor cell activity and membrane movements in a breathing lung-on-chip. Sensors Actuators B Chem 255:3647–3653. https://doi.org/10.1016/j.snb.2017.09.192
14. Schuller P et al (2020) A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells. Sensors Actuators B Chem 312:127946. https://doi.org/10.1016/j.snb.2020.127946
15. Schuller P et al (2019) Optimized plasma-assisted bi-layer photoresist fabrication protocol for high resolution microfabrication of thin-film metal electrodes on porous polymer membranes. MethodsX 6:2606–2613. https://doi.org/10.1016/j.mex.2019.10.038
16. Oleaga C et al (2018) Investigation of the effect of hepatic metabolism on off-target cardiotoxicity in a multi-organ human-on-a-chip system. Biomaterials 182:176–190
17. Oleaga C et al (2016) Multi-organ toxicity demonstration in a functional human in vitro system composed of four organs. Sci Rep 6:20030
18. Boudou T et al (2011) A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. Tissue Eng A 18:910–919
19. Caluori G et al (2019) Non-invasive electromechanical cell-based biosensors for improved investigation of 3D cardiac models. Biosens Bioelectron 124:129–135
20. Inácio PMC et al (2017) Bioelectrical signal detection using conducting polymer electrodes and the displacement current method. IEEE Sensors J 17:3961–3966
21. Maoz BM et al (2017) Organs-on-Chips with combined multi-electrode array and transepithelial electrical resistance measurement capabilities. Lab Chip 17:2294–2302
22. Gaio N et al (2018) A multiwell plate Organ-on-Chip (OOC) device for in-vitro cell culture stimulation and monitoring. In: 2018 IEEE micro electro mechanical systems (MEMS). IEEE, Belfast, pp 314–317
23. Chan V et al (2015) Fabrication and characterization of optogenetic, multi-strip cardiac muscles. Lab Chip 15:2258–2268
24. Zhang X, Wang T, Wang P, Hu N (2016) High-throughput assessment of drug cardiac safety using a high-speed impedance detection technology-based heart-on-a-chip. Micromachines 7:122
25. Shin SR et al (2016) Aptamer-based microfluidic electrochemical biosensor for monitoring cell-secreted trace cardiac biomarkers. Anal Chem 88:10019–10027
26. Zhang YS et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci 114:E2293–E2302
27. Skardal A et al (2017) Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. Sci Rep 7:8837

28. Dauth S et al (2017) Neurons derived from different brain regions are inherently different in vitro: a novel multiregional brain-on-a-chip. J Neurophysiol 117:1320–1341
29. Moya A et al (2018) Online oxygen monitoring using integrated inkjet-printed sensors in a liver-on-a-chip system. Lab Chip 18:2023–2035
30. Misun PM, Rothe J, Schmid YRF, Hierlemann A, Frey O (2016) Multi-analyte biosensor interface for real-time monitoring of 3D microtissue spheroids in hanging-drop networks. Microsyst Nanoeng 2(1):1–9. https://doi.org/10.1038/micronano.2016.22
31. Vollmer AP, Probstein RF, Gilbert R, Thorsen T (2005) Development of an integrated microfluidic platform for dynamic oxygen sensing and delivery in a flowing medium. Lab Chip 5(10):1059–1066. https://doi.org/10.1039/b508097e
32. Ungerböck B, Mistlberger G, Charwat V, Ertl P, Mayr T (2010) Oxygen imaging in microfluidic devices with optical sensors applying color cameras. Procedia Eng 5:456–459. https://doi.org/10.1016/j.proeng.2010.09.145
33. Sticker D et al (2019) Oxygen management at the microscale: a functional biochip material with long-lasting and tunable oxygen scavenging properties for cell culture applications. ACS Appl Mater Interfaces 11:9730–9739
34. Zirath H et al (2018) Every breath you take: non-invasive real-time oxygen biosensing in two- and three-dimensional microfluidic cell models. Front Physiol 9:815. https://doi.org/10.3389/fphys.2018.00815
35. Rothbauer M et al (2020) Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing. Lab Chip 20(8):1461–1471. https://doi.org/10.1039/C9LC01097A