Multiorgan-on-a-Chip: A Systemic Approach To Model and Decipher Inter-Organ Communication

Nathalie Picollet-D'hahan, Agnieszka Zuchowska, Iris Lemeunier, Séverine Le Gac

Multi-Organ-on-a-Chip: A systemic approach to model and decipher inter-organ communication

Nathalie Picollet-D'hahan [1,*], Agnieszka Zuchowska[2], Iris Lemeunier[1], Séverine Le Gac [2,*]

[1] Univ. Grenoble Alpes, INSERM, CEA IRIG BGE BIOMICS, Grenoble, France.

[2] Applied Microfluidics for Bioengineering Research (AMBER), MESA+ Institute for Nanotechnology, TechMed Center, University of Twente, 7500AE Enschede, The Netherlands.

*Corresponding authors : nathalie.picollet-dhahan@cea.fr; s.legac@utwente.nl

Social media information:

Nathalie Picollet D'hahan: ORCID 0000-0001-6307-9180; https://twitter.com/BiomicsLab; https://irig.cea.fr/drf/irig

Agnieszka Zuchowska: ORCID 0000-0003-3516-3830

Iris Lemeunier: ORCID 0000-0002-5255-5070; https://twitter.com/BiomicsLab

https://irig.cea.fr/drf/irig

Séverine Le Gac: ORCID 0000-0002-4546-6184; http://www.severinelegac.com

Keywords: Organ-on-Chip; systemic diseases; physiological barriers; cross-organ vascularization; microfluidics.

Abstract

Multi-organ-on-chip (multi-OoC) platforms have great potential to redefine the way human health research is conducted. After a brief motivation for the use of more comprehensive multi-organ models with a systemic dimension, we highlight a few scenarios where multi-organ models are advantageous. Next, we provide an overview of existing multi-OoC platforms, would those follow an integrated body-on-a-chip approach or a modular approach, with interconnected organ-specific modules. We highlight how multi-OoC models provide a unique information not accessible using single-OoC models. Finally, we discuss remaining challenges for the realization of multi-OoC platforms and their worldwide adoption. We anticipate that multi-OoC technology will metamorphose research in biology and medicine, by providing holistic and personalized models to understand and treat multi-systemic diseases.

Why go systemic?

Interactions between multiple organs are essential to ensure proper physiological functioning of the human body. While organs are physically separated *in vivo*, their communication is mediated by the blood and lymph circulation through various signals (soluble factors, exosomes, cells, etc.) to overall maintain viability and homeostasis. For instance, the journey of orally ingested substances (nutrients, chemicals, drugs, etc.) is well-orchestrated and involves different organs, through a specific sequence, each organ having a specific function: the small intestine absorbs the (digested) substances, the liver metabolizes them, before their delivery to targeted organs via the blood circulation, and the kidney excretes corresponding waste products. This complex process of **ADMET** (see Glossary), which stands for absorption/distribution/metabolism/excretion/toxicity, affects the fate, distribution, efficacy (if applicable), and possible toxicity of exogenous substances (*e.g.*, food, drugs, additives, or environmental pollutants) [1], through unwanted side-effects in secondary tissues.

In addition, many functions and processes in the body depend on regulatory pathways and feedback hormonal loops, involving organs from the endocrine system. The reproductive system, which comprises multiple tissues, relies on endocrine loops that control peripheral tissues. Similarly, Langerhans islets in the pancreas secrete insulin that promotes glucose

uptake by the liver. Altogether, this systemic and cross-organ communication is key to decipher and emulate the temporal processes involved in physiological functions.

As a direct consequence, many diseases such as sepsis, osteoarthritis, gout, infertility, and neurodegenerative diseases involve multiple organs, so systemic approaches must be pursued to accurately model them. Similarly, deciphering this cross-organ communication is essential to identify biomarkers in bodily fluids for diagnostic purposes. For instance, tumor tissues release various molecules (miRNA, ctDNA, peptides, etc.), tumor-derived extracellular vesicles (tdEVs) and circulating tumor cells (CTCs), which play a central role in cancer metastasis and are key for cancer patient management [2,3].

All these examples illustrate how essential it is to include cross-organ communication and a systemic dimension, as depicted in **Figure 1**, which is most commonly achieved by using animal models. Yet, those *in vivo* models suffer from numerous limitations: high experimental costs, limited throughput, ethical concerns, and differences in genetic background. More importantly, they exhibit large physiological differences in terms of drug effects and/or disease phenotypes compared to humans, which explains the frequent failure of clinical trials [4]. Altogether, animals do not allow dissecting inter-organ crosstalk, acquiring quantitative pharmacokinetics parameters or predicting ADMET processes, as recently highlighted [5]. Therefore, advanced *in vitro* approaches incorporating a systemic dimension and multiple organs must be developed to faithfully emulate *human* health and pathophysiology**.**

Previous efforts to study organ communication *in vitro* employed either **conditioned** medium, or co-cultures in **Transwell** platforms. However, Transwell devices use large volumes of liquids, so that communication is slow and low-concentration signaling factors are diluted, which altogether hampers cellular communication. Furthermore, culture is entirely static, which precludes emulating dynamic processes and applying controlled cell biochemical and/or physical stimulation.

Using a microfluidic format can solve some of these issues by offering sub-milliliter volumes, dynamic culture and exquisite spatio-temporal control on any physical and chemical parameters in the cell/tissue vicinity. For instance, cell-cell communication has been studied in microdevices under continuous flow, using chambers separated by porous membranes [6], pillar arrays [7], or channels [8].

Building up on these microfluidic cell cultures, **organ-on-chip** (OoC) devices aim to mimic the architecture and function of an organ by combining 3D bioengineered constructs

(*e.g.*, cell-laden hydrogels [9], differentiated epithelium [10,11], multicellular spheroids [12], organoids [13–15]), *ex vivo* tissues (*e.g.*, biopsies or explants) [16–18], re-cellularized scaffolds [19] and bioprinted constructs [20] with microfabricated structures [21], and possibly active stimulation (electrical, biochemical, or mechanical) [22–24]. For about one decade, the OoC field has been blossoming, proposing models for virtually all organs and physiological barriers in the human body [21,25,26]. These OoC platforms are revolutionizing the field of *in vitro* experimentation and holding great promises for reducing animal testing.

Still, most OoC models are based on a single cell type or tissue and lack a systemic dimension and cross-organ communication. In a major recent breakthrough, multiple organs have been modeled in one device to yield a **multi-OoC platforms** [13] (**Figure 2).** As detailed in **Box 1,** two major approaches are pursued to realize multi-OoC platforms: through coupling of single-OoC units or by integrating multiple organs in one plate (multi-OoC plates).

In this review, we will first provide an overview of existing multi-OoC platforms and discuss combinations of organs best suited for given applications. Specific areas of research will be highlighted, for which a multi-OoC approach brings superior information compared to single OoC models. Finally, we will discuss essential remaining challenges for realizing multi-OoC platforms.

Latest developments in the multi-OoC field

In the following section, we review various multi-OoC applications. For each application, discuss the set of organs considered and highlight unique information provided by this multi-OoC approach. Selected examples over the last five years are summarized in **Table 1, Key Table**.

Toxicity screening

Toxicity is closely linked to metabolization by the liver, so that multi-OoC approaches developed for toxicity purposes include a liver model, and at least one other (target) organ. For instance to examine the acute and chronic toxicity of inhaled aerosols or drugs, human liver spheroids have been combined with a 3D lung epithelium model [27] (Figure 2C). For pharmacological studies, typically an intestine model is added to the liver-target organ co-culture to mimic drug absorption [28]. Alternatively, when undesired side-effects of a therapeutic treatment are evaluated, both the target organ and the organ where side-effects are

expected, *e.g.,* the kidney (nephrotoxicity), heart (cardiotoxicity) [29] or brain (neurotoxicity) [30], are modeled in the same platform. This approach has notably been pursued to assess the impact of anti-EGFR treatment targeting lung tumor on a skin model [31]; of cyclophosphamide on heart [29]; and the deleterious effects of 2.5-hexanedione on 3D neurospheres [30]. All these studies collectively proved the importance of co-culture approaches for predicting compound safety and efficacy.

Drug metabolism

Multi-OoC platforms supporting liver-target organ communication similarly open new possibilities for testing prodrugs, which become biologically active only after their metabolization by the liver. This bioactivation process was successfully recapitulated for cyclophosphamide targeting colorectal cancer using spheroids co-cultured under flow conditions in a 96-well format [32], and in a self-adjusting modular Tetris-like microfluidic platform (TILE) [33] (Figure 2H), where it was found to effectively overcome the "apparent" resistance of metastatic OSCC tumor. The multi-OoC's efficacy was demonstrated for another prodrug, capecitabine, and its 5-fluorouracil metabolite in a liver-colorectal cancer co-culture [34] (Figure 2E). Liver bioactivation was also examined for non-cancerous drugs. Both the hepatic metabolism of vitamin D and renal bioactivation of the resulting product were mimicked in a human liver-kidney multi-OoC [35] (Figure 2A), revealing enhanced expression of vitamin D metabolizing enzymes. The same liver-colorectal cancer co-culture proved successful for the bioactivation of the nutraceutical quercetin, to eventually more potently suppress the endothelial inflammatory response [36]. Finally, using a liver-immune system co-culture, drug-induced skin sensitization was successfully predicted *in vitro*, by modeling drug metabolization by a 3D liver tissue and evaluating immune cascade activation by the resulting metabolites [37]. This last example further illustrates the importance of multi-OoC models to evaluate systemic drug effects that involve multiple processes and different organs.

Pharmacokinetics

Pharmacokinetics (PK), aiming at understanding and predicting the biological effects (therapeutic or toxic) of xenobiotics on the body, require by definition modeling various organs and their interactions. Skardal and colleagues emulated a drug response resulting from the crosstalk of heart, liver and lung [38], and combining liver and lung to a small intestine allowed evaluating the pharmacokinetics of orally administered anticancer drugs [28]. Building further upon this approach, other organs were included in this multi-OoC platform,

such as endothelium, brain, and testis[13], or liver, pancreas, gut, lung, heart, muscle, brain, skin, kidney, and endometrium [39] to very accurately study **PK/PD.** Interestingly, several studies demonstrated that quantitative drug PK parameters, as measured in patients, can be predicted using a combination of a multi-OoC platform (liver-kidney or body-on-a-chip model) and PK modeling [40–42] Pharmacokinetics often suffers from limited longevity of organ models, an issue which is overcome by connecting single OoC models, only after each organ culture has been established under optimized conditions [43].

ADMET profiling

ADMET studies typically require all necessary organs to emulate the processes of absorption (intestine), distribution (blood circulation), metabolism (liver), excretion (kidney) and toxicity (a target organ). First, a modular 2-organ platform combining 3D liver tissues and a differentiated gastrointestinal (GI) tract epithelium was proposed to emulate the absorption and metabolism of exogenous substances [44] (Figure 2F); both organ models were maintained for up to 14 days in this co-culture configuration, with enhanced CYP activities compared to liver only. Next, a quadruple co-culture platform combining skin and intestine to mimic topical and oral administrations, to liver and kidney to reproduce drug metabolization and their clearance, maintained all four organ integrity and functionality for up to 28 days [45] (Figure 2G). Each organ compartment in this platform being separately fluidically addressable, fluids could be collected at any time, which is essential to acquire precise PK parameters such as the effective drug concentration and maximum tolerable dose. A brain model was added in the same platform, all organs being built yet from **iPSCs** derived from the same healthy donor to produce an autologous system [46]. All organs were successfully cultured for 14 days using common medium without any tissue-specific growth factor, and their differentiation supported but for the kidney.

Multi-organ and metabolic diseases and reproductive medicine

Modeling multi-organ diseases suffers from the poor accessibility of some organs and the fact that different cell types are involved in metabolic homeostasis; in that context, multi-OoC approaches provide more complex disease models while giving access to key molecular mechanisms [47]. Recently, a multi-module system emulating different functions of the brain allowed dissecting the contribution of different cells to the entire organ function and revealed metabolic coupling between neurons and microvascular cells of the blood-brain barrier (BBB) [48]. Metabolites of the drug methamphetamine, produced by vascular cells, were found to directly increase the synthesis and secretion of neurotransmitters by neurons. Connecting the

liver, gut, and circulating immune cells brought new insights about the role of short-chain fatty acid (SCFAs) metabolites on liver and inflammatory gut diseases (*e.g.,* inflammatory bowel disease and ulcerative colitis) and immune response [47]. Hematopoietic dysfunction [49] or the premise of diabetes type 2 mellitus model [50] have also been modeled using multi-OoC approaches. For instance, co-cultures of human pancreatic islets and liver spheroids successfully maintained post-prandial glucose concentrations in the circulation, mimicking thereby the feedback loop to control glucose consumption and insulin secretion, while glucose levels remained elevated in both organ modules when cultured separately. In a last example, *ex vivo* tissues of all organs found in the female reproductive tract (ovary, fallopian tube, uterus, and cervix) were co-cultured with liver organoids and medium recirculation, while emulating the endocrine loop through timely hormonal stimulation, to successfully reproduce the 28-day human menstrual cycle [51].

Cancer metastasis

In cancer, cross-organ communication can lead to disease metastasis, which is the main cause of cancer mortality [52]. Metastasis, which is driven by the intravasation of CTCs and their colonization into other organs, is known to occur in preferred niches. To understand processes involved in this metastatic cascade and design new treatments, multi-organ models are highly desired, combining the tumor to potential metastatic niches. Coupling 3D colorectal cancer and liver models in a 2-organ plate, with real-time monitoring of the cancer cell migration revealed the formation of metastatic clusters in the liver [38], as well as the importance of the mechanical properties of the liver microenvironment on cancer spreading. The preference of cancer cells to home in different organs was studied in a 4-organ plate, demonstrating that breast CTCs in the perfusion were invading lung, bone and liver, but not muscle [53], which was in full agreement with animal studies. Perfusion of metastatic inhibitors stopped that invasion process, as in animal models. Spreading of lung tumor cells into different distant organs (brain, bone and liver), all equipped with a microvasculature, was similarly examined in a multi-OoC plate [54], showing the metastatic potential of cells undergoing EMT (epithelial-mesenchymal transition) to all three target organs. All these examples illustrate the potential of complex *in vitro* multi-OoC models to predict cancer metastasis and test anti-metastatic treatments.

What are the current and remaining challenges in the multi-OoC field?

In this last section, we discuss challenges we have identified for building multi-OoC platforms, some being also valid for single OoC; on the specificity and constraints of each organ environment, inter-organ coupling strategies from a biological and engineering point-of-view, and how to stimulate individual organs and measure cross-organ communication.

How simple is complex enough?

The biological question to be addressed or the physiological process to model is driving the choice to build multi-OoC models, notably in terms of type and number of organs. In some cases, a simple engineering approach is sufficient to mimic an organ function, *e.g.*, by integrating a peristaltic micro-pump to mimic the pulsatile blood flow [55]. In sharp contrast, studying the systemic toxicity of drug candidates or deciphering disease etiology requires the dynamic crosstalk between several organs [25,39,56–58], and, in turn, specialized microenvironments and interconnecting flows to offer more physiological conditions [59].

In which environment to build models?

A multi-OoC platform connects different organ models, so, a first essential question is: what are the best approach and environment to build each organ model, from both a physiological and platform point-of-view? This first element is already becoming challenging when parenchymal tissues (*e.g.*, fat, kidney, heart, adrenal glands, liver, spleen, and pancreas) are combined with physiological barriers (*e.g.*, BBB, skin, GI tract, and lung), whose modeling requires entirely both different engineering and perfusion strategies.

Physiological barriers are typically created using compartmentalized devices, different cell types being cultured on both sides of porous membranes [45]. To ensure full differentiation of the epithelial layer into a stratified structure and proper functioning, continuous perfusion is applied [60], and two independent perfusion lines with different media, are required against one only for parenchymal tissues. Barrier models are applied to evaluate drug or toxicant translocation through the blood-brain [48], the intestinal [61], the blood-alveolar barriers [62] or the skin [31], or their elimination in the kidney [63], as discussed in Section 2.

Parenchymal tissues are best modeled using 3D culture approaches, possibly mimicking the *in vivo* architecture and complexity, and combining multiple cell types. Various 3D culture strategies have been proposed. Tissue biopsies or explants emulate the full

complexity of the tissues, while being restricted in terms of supply and inter-donor variability [26,31], and they are often too large to easily be incorporated in microfluidic devices. Tri-dimensional bioengineered constructs are therefore preferred. Among those, multicellular organoids and tumoroids [2,9,13,14,16,29,34] require appropriate environments to self-organize and differentiate [15], which can be provided by adequate matrices. Natural matrices are mostly employed such as Matrigel [6,7], collagen [10,27,64], hyaluronic acid [11,13,33], and gelatin [64]. Yet, organ-specific de-cellularized matrices [65,66] more faithfully reproduce the *in vivo* environment. Alternatively, synthetic hydrogels (*e.g.*, polyacrylamide, polyethylene glycol–fibrinogen, and polylactic acid) offer more controlled, tunable and reproducible environments; support animal-free experimentation [67];[65]; and can be engineered to include molecular cues for cells to adhere, differentiate, mature and sustain proper functionality over time [67–69].

Materials

As for any microfluidic device, the choice of material(s) from which multi-OoC platforms are fabricated is critical. PDMS (polydimethylsiloxane) remains the number one material in the academic community: it is gas-permeable, optically transparent, and easy to process, and its elastomeric properties are advantageous to integrate valves and/or pumps, and to mechanically stimulate cells [70,71]. Yet, significant concerns have been raised towards PDMS, which is a porous hydrophobic material, prone to absorption of small hydrophobic molecules such as drugs and hormones, possibly releasing uncured oligomers which can interfere with the experimental outcomes [72], and incompatible with large-scale fabrication and device commercialization. Recently, non-absorbent elastomeric polymers (e.g. styrene–ethylene/butylene–styrene, polyurethane elastomers) have been developed for OoC applications [73,74]. Other promising alternatives are inert thermoplastic polymers (polymethyl methacrylate (PMMA), polycarbonate (PC), polystyrene (PS), cyclic olefin (co) polymer (COC/COP), polyetherimide (PEI), and polysulfone (PSF/PSU) [75], which are used as porous membranes in compartmentalized devices. More biomimetic solutions using soft and/or curved substrates, are currently developed to build barrier models [76]. 3D printed materials have entered the OoC field [77], with the promises to offer both faster turnover in the fabrication, and facile realization of multi-material platforms with integrated sensors [78]. Yet, 3D-printed materials are often non-transparent, which precludes *in situ* imaging, and may release toxic compounds, possibly acting as endocrine disruptors [60]. Similarly, 3D

bioprinting allows processing, in one-step, multiple materials along with different cell types to yield precisely controlled (tissue) architectures [79]. In this approach, smart materials with changeable shapes or functionalities can introduce a fourth 'temporal dimension' and stimuli-responsive structures to better mimic organ function [80,81].

Scaling

Extreme miniaturization of *in vitro* organ and OoC models, without any proper scaling, can cause significant structural reorganization and changes in organ proportions [82], which is particularly important for toxicity and drug screening assays, metabolic studies and PK/PD modeling [83,84]. Yet, scaling remains a significant challenge. Not only the size of the organ, but also the flow and shear in each organ module and the total volume of medium must scale to physiological dimensions. Disproportionately scaled multi-OoC have proven not to properly replicate organ-organ interplay [75], and to affect the residence time of medium in the recirculation, introducing a bias in the experimental outcomes [85]. Various scaling approaches (proportional, allometric and functional scaling) have been introduced in the OoC field, as discussed in a recent review [86]. Still, none of them allows emulating correctly all *in vivo* features in mini-organ models.

How to combine models prepared using different approaches?

Medium composition. To support the growth, long-term viability and function of all organs in a multi-OoC platform, proper medium must be supplied to notably fulfill each organ's requirements. Consistent with the strategy of engineering multi-tissue microenvironment, some universal blood substitute should feed all organ compartments in a multi-OoC platform. Human serum could ideally fulfill this role and maintain the physiological function of all cells types, since it nourishes *in vivo* the entire body. However, synthetic strategies in the form of "blood surrogate" have been preferred (see below) to avoid infection risks, inter-sample variability, and administrative constraints linked to donor's consents. Arguably, this human serum approach is still promising when creating patient-specific model.

Culture media contain animal-derived serum that present inter-batch variability. Chemically well-defined serum-free basal media have been developed consisting for instance of a 50:50 DMEM:Ham's F-12 mixture [40] or high-glucose medium [35], that were however able to only maintain the functionality of a few connected organs (cardiac, muscle, neuronal and liver modules) over several weeks [87]. Noteworthy, this one-medium approach is even more challenging when using more sensitive primary and/or immune cells [48].

To feed all organs in multi-OoC models, common media (MEM, DMEM or William's medium) have been supplemented with specific hormones [47,50] growth factors [31], fatty acids and lipids [47], vitamins [31] and trace elements [51] to provide organ specificity [27,30,33,45,53]. However, tedious optimization is required to include all necessary supplements while ensuring they are not detrimental for any other organ. Alternatively, common media have been prepared by mixing individual media in specific ratios [39,44,49,51,59,88], possibly using *in situ* computer assistance [39]. In this latter scenario, culture is typically initiated using organ-specific media, before inter-organ communication is established and medium composition optimized [39,89,90]. Ultimately, to identify ideal common media, biological optimizations must be combined with engineering efforts to support precise medium exchange and through integration of mixing units [90].

In a last yet more flexible approach, organs are cultured as physically separated entities in compartmentalized devices, communication occurring through porous membranes or an endothelium, when each organ is "equipped" with a blood vessel, allowing altogether media to be independently tailored [56,91,92].

Medium circulation. How OoC models are coupled typically depends on the specific purpose of the study. Medium can be perfused in one direction to study the influence of one organ on other(s) [29,35,43,49,53,54] or re-circulated to emulate reciprocal interactions [18,27,28,30,32–34,41,44,45,51,88,93–95] (**Figure 3**) and better reproduce the *in vivo* situation.

As highlighted in **Box 1**, multi-OoC models can be created (i) using capillary tubing connecting single-OoC modules [94,95] (Figure 2B), (ii) or by clicking them on a microfluidic motherboard that includes all microfluidic connections, and possibly sampling and sensing units [33,43,44], or (iii) even using a user-friendly plate approach [27,29,30,45,93] (see Figure 3), with one connecting channel acting as a vasculature-like system. In a recent modular and entirely reconfigurable approach, *Interrogator*, medium was transferred between single-OoC devices using liquid-handling robots [41]. Finally, some OoC, *e.g.*, models of physiological barriers, involve two independent perfusion lines [48,60–63], which calls for including multiple fluidic circuitries in multi-OoC platforms [45,46].

In all above-discussed scenarios, the microfluidic circuitry and perfusion parameters must be properly designed to both support and measure organ communication. Specifically, the flowrate should be optimized to ensure secreted factors reach a certain threshold

concentration to affect the next organ [96], while being measurable. Similarly, in a recirculation loop, the total volume of medium should be adjusted to prevent extensive dilution of the components of interest while ensuring enough nutrients are available and harmful components are properly removed [45,46].

Vascularization or no vascularization? *In vivo*, organs are connected through a vascular system (**Box 2**), which must be included in multi-OoC models to support inter-organ communication. Pathologies are also often characterized by alterations in the vasculature: *e.g*, malignant cancer [97], cardio-vascular diseases, diabetes, rheumatoid arthritis and central nervous system diseases [99]. Although innovative approaches for vascularization have been developed for OoC platforms (**Box 2**), in multi-OoC platforms, the "vasculature" is often merely modeled using tubing (**Figure 3**), as discussed in the previous section, without including any endothelium. The endothelium is also modeled using the well-established human umbilical vein endothelial cells (HUVECs) [48,47,54,62,33,94,99,100], which express important endothelial markers and signaling molecules associated with vascular homeostasis regulation. Yet, the vascular barrier is characterized by an organ-specific morphology (architecture), cellular composition, and function [99]. Ideally, organ-specific microvasculature endothelial cells should be used to faithfully model each organ physiology. Such organ-specific endothelial cells are not always commercially available; therefore, they must be isolated from biopsied tissues or produced through differentiation of human induced pluripotent stem cells or human mesenchymal stem cells [97].

How to stimulate models?

In vivo, organs are continuously exposed to various stimuli (mechanical, electrical, (bio)chemical, etc.), which are key for proper development, functioning, and physiology. Conversely, abnormal stimulation can trigger certain diseases (*e.g*., neurodegenerative, metabolic, and cardiovascular diseases). Mechanical stimulation plays a key role in the development, function and maintenance of articular cartilage [102] and the blood-alveolar barrier [103]. Similarly, electrical stimulation is vital for the conductive and contractile properties of the heart, while the concomitant action of electrical, mechanical and chemical stimulation is central in the homeostasis of the nerve-muscle junction. Physiological flow and associated shear ensure proper expansion of the endothelium in blood vessels and arteries.

Moreover, reproductive organs or pancreas are tightly regulated by timely hormonal exposure, as is the entire male/female physiology [64,104], which also modulates non-reproductive organs such as liver and kidney (60). Therefore, timely hormonal cues must be incorporated when designing sex-specific multi-OoC models (60). All these cues are difficult, if not impossible, to incorporate in conventional *in vitro* models, partly due to their pulsatile, chronic or periodic nature. Yet, using microfluidic technology, virtually any stimulus can be included, with accurate control on their spatiotemporal character. Examples of mechanical stimulation include shear-induced flow, surface strains [105], combination of surface strains and fluid-flow shear [106], compression [107], and combination of compressive and bulk shear forces [70]. Noteworthy, many of these modalities utilize physiological-like deformation of an elastomeric PDMS membrane. Electrical stimulation is typically produced by integrated electrodes, which have supported the differentiation of heart tissue and contraction of cardiomyocytes [29,108]. Finally, to emulate hormonal, (bio)chemical stimulation or exposure to drugs of toxicants, the perfused medium must be supplemented with those soluble stimuli.

How to measure cross-organ communication?

An associated challenge is to measure cross-organ communication. To that end, the same strategies and techniques as for single OoC devices can be applied [109], with ***in situ* measurements** (pH, oxygen levels, beating frequency, TEER measurements, and cell labeling and tracking); **online analysis** using spectroscopic techniques or classical biochemical assays; and **off-line analysis** after collection of culture medium in the circulation and/or retrieval of the cellular models from the device for further analysis, as detailed in **Table 1**.

Organ-specific phenotypes are mostly characterized *in situ* using (fluorescence) microscopy, possibly after fixation. This approach is limited to end-point measurements and only few readout parameters (*e.g.*, live/dead and TUNEL assays; and characterization of targeted functional and structural proteins). Furthermore, *in situ* imaging is highly challenging due to the three-dimensional nature of the cellular models and the use of multiple materials, which are possibly opaque. Therefore, advanced imaging techniques are employed such as light-sheet or 2-photon microscopy [110], or wavefront shaping [111,112]. Alternatively, the cellular models are extracted from the device for high-resolution confocal microcopy imaging possibly after histological sectioning or tissue clearing. Next, off-line comprehensive -omics

analysis or flow cytometry, both after dissociation of the cellular model, bring insightful information on the organ status and communication, using however sacrificed samples. Finally, off-line measurements can be conducted on intact samples or effluent medium collected manually or automatically, regularly or at given time points, to assess organ function, targeted or comprehensive metabolism, inflammation (cytokine production), viability, or drug and toxicant metabolism, using dedicated assays or MS-based analysis.

Ideally, real-time information should be continuously acquired to follow dynamic inter-organ interactions and monitor each tissue's function. To that end, on one hand, (bio)sensors or electrodes are integrated in organ-specific modules to monitor *in situ* specific culture parameters (*e.g.*, pH, oxygen), organ metabolism, the integrity of physiological barriers via trans-epithelial electrical resistance measurements, or cell beating. Of particular interest are nanoplasmonic sensors, an approach which has recently been explored for the multiplexed analysis of inflammatory cytokines in an adipose tissue on a chip model ([113]. On the other hand, on-line molecular analysis is employed using spectroscopic techniques or standard biochemical assays. A significant challenge for the latter molecular analysis scenario is to optimize the amount of sample collected to meet the sensitivity of the analytical assay without dramatically perturbing the multi-OoC microenvironment. Similarly, on-line analysis calls for minimal or no sample preparation, which can hamper the detection of low-abundant species in complex matrices. All these reasons explain why this information-rich and virtually non-invasive approach is scarcely pursued and why most analyses are still conducted off-line at the end of the experiments.

Concluding Remarks and Future Perspectives

While tremendous efforts have significantly improved the complexity, quality and robustness of OoC models, recent initiatives are now bringing this technology to the next level by generating multi-OoC platforms, to emulate entire biological processes that are in essence never limited to a single organ. Multi-OoC technology can simulate human physiology at the level of the whole organism, offering excellent accuracy and model complexity, as well as new opportunities in multiple fields, while supporting the implementation of the "3Rs" (replacement, reduction, and refinement of animal models) and the paradigm of personalized medicine. Yet, currently they remain only complementary to animal models, and there is still a long way to go before multi-OoC models are fully adopted. To achieve this Holy Grail, key

challenges remain to maintain the homeostasis of multiple organs and to incorporate all essential cues, such as hormonal stimulation, the immune system, the lymph, the microbiome, organ innervation and vascularization, which have yet been scarcely explored so far. As a next step, multimodal real-time analysis should be implemented in multi-OoC platforms through the integration of multiple sensors and coupling to on-line spectroscopic analysis (see **Outstanding Questions**).

To build patient-specific multi-OoC models, iPSCs, patient-derived organoids or *ex vivo* tissues are both equally promising. These personalized models will open new avenues to capture specific features of a person's disease, predict a patient's response to given treatment and detect possible long-term and/or side-effects of drugs, thereby supporting the concept of personalized medicine. This patient-specific approach can also capture the inherent diversity found in a population in terms of genetic and ethnic background, gender and age [25].

So far, OoC technology has undergone significant progress in an academic setting, yet many technological hurdles limit its full deployment in an industrial environment. One expected breakthrough is in the pharmaceutical industry, to notably reduce the elevated failure rate in drug development. First and above all, multi-OoC models must prove they can faithfully reproduce the *in vivo* environment, the evolution of a pathology, adverse effects of drug candidates or therapeutic outcomes. This still limited demonstration of their benefit compared to more commonly used 2D and animal models, explains why various stakeholders do not yet recognize (multi)-OoC models as reliable humanized *in vitro* models. A promising approach to validate multi-OoC models is to combine them with *in silico* modeling [40–42]. Furthermore and finally, to be more widely adopted as routine bench-tools, multi-OoC systems should be easy to use, plug-and-play, on-demand reconfigurable depending on the targeted application, highly multiplexed, and fully automated, and compatible with standard laboratory practices.

Acknowledgment

A.Z. and S. L. G. acknowledge financial support from the CHIP-ME project (Cross-organ Human In-vitro Platforms for Metastatic Environments), funded by Health Holland (project number TKI-LSH LSHM19012). N.P.D and I.L acknowledge financial support from the CEA, and the PhD program. Figures 1 and 3 and Figure I in Box 1 contain elements from SMART Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License.

BOX 1 Multi-OoC typology and applications

Multi-OoC devices can be classified into two main distinct types, a typology referring to the engineering approach pursued for their realization, namely through connection of single OoC units or using a multi-OoC plate.

First, single OoC units are connected via capillary tubing or a microfluidic motherboard to reproduce the systemic interactions between two or more organ models (**Figure IA**). This modular approach allows facile reconfiguration of the multi-OoC platform and supports the use of individual vascularized organs, using organ-specific microvasculature endothelial cells. Furthermore, the single OoC modules can first be established and matured using specific medium before they are connected with each other. In contrast, multi-OoC devices (**Figure IB**) integrate in a one-plate format all different organ models at different locations, channels in the plate acting as a vascular-like system to support inter-organ communication. This second approach is much akin the human-on-a-chip or body-on-a-chip paradigm, in which virtually all organs are modeled (**Figure IC**). Multi-OoC plates are more compact and user-friendly; they do not require manual and cumbersome connection; they limit the risks for leakage; and, in some cases, they can integrate a liquid actuation system. They are also advantageous to minimize the total recirculation volume (see *Medium circulation*). However, organ-specific vascularization is less trivial, and combining different organs modeled following various approaches (see *Organ models*) may be more challenging.

These two different multi-OoC approaches are arguably better suiting specific purposes. The former 'lego-like' approach is likely to be preferred for more fundamental research in an academic setting. Yet, they offer only low-to-moderate throughput, which is not ideal for preclinical, toxicity or drug efficacy tests. In contrast, the more integrated and turnkey plate-based platforms offer higher throughput, and are hence more appropriate for the identification of biomarkers, therapeutic targets, and for the selection and optimization of drug candidates.

Figure I: Schematic representation of the two main approaches to develop multi-OoC systems: (A) through coupling of single OoC devices, each modeling a different organ, via capillary connection or a microfluidic motherboard (B); and (C) by integrating different organ models in one plate, this approach being more in line with the body-on-a-chip philosophy.

BOX 2 Vascularization of OoC models: importance and strategies

The vascular system connects all organs in the human body and plays an essential role in each organ physiology. Specifically, blood vessels ensure proper delivery of nutrients and oxygen to all organs, removal of waste products and supports inter-organ communication through soluble and lipid-encapsulated factors in the form of exosomes and extracellular vesicles. All molecular exchanges between blood and the different organs occur through the endothelium.

Different strategies have been proposed to engineer a vascular system, this depending on the type of blood vessels to be modeled -from large structures to capillaries- and the context of the research. Endothelia in physiological barriers such as the blood-alveolar barrier [54,62,94], the gut [33,47,99] and the blood-brain-barrier [48,114], are typically created by growing endothelial cells on one side of a porous membrane (**Figure IA**). Yet, this approach does not capture the intrinsic curvature of the vasculature. In contrast, cylindrical structures in hydrogels or channels [115] incorporate this curvature. For instance, endothelial cells (ECs) have been seeded in lumens previously formed in a hydrogel matrix using (i) needles, metal rods, or fishing lines [116] which are removed after gelation; (ii) sacrificial materials such as gelatin or 3D-printed carbohydrates [117]; or (iii) the viscous finger patterning technique (**Figure IB**). In an alternative approach, perfusable capillaries have been generated through the self-assembly of ECs in a hydrogel matrix [118] (**Figure IC**), possibly under external stimulation of soluble factors (*e.g.*, VEGF) perfused or secreted by fibroblasts in another channel (**Figure ID**), to yield a complex 3D vascular architecture.

Figure I. On-chip vascularization of OoC models: **(A)** Endothelial cells (ECs) are seeded on a porous membrane to emulate a physiological barrier (in most cases, epithelial cells of the modeled barrier are grown on the other side of the membrane); **(B)** Lumen created in a hydrogel matrix before being lined with ECs to yield a cylindrical blood vessel; **(C, D**) Self-assembly of ECs in a hydrogel matrix, either spontaneously **(C)** or through exposure to external soluble factors such as VEGF (vascular endothelial growth factor) **(D).**

FIGURE CAPTIONS

Figure 1. Various multi-organ-on-a-chip combinations as a model of human physiology and pathophysiology, and for different biomedical applications. For each application (*right side*), a minimal set of organs required to build an accurate systemic model is indicated, those organs being highlighted with colored squares (*left side*).

Figure 2. Multi-Organ-on-Chip devices (A) Multi-compartment liver-kidney combination in a plate format for studying the metabolism of vitamin D3. Reprinted from [35] (B) Multi-OoC model comprising a microfluidic motherboard, an external peristaltic pump and capillary connections for *in situ* monitoring of organoid behavior using integrated sensors. Reprinted with permission from [73]; (C) Two-way communication between lung and liver models for toxicity studies, using a multi-OoC plate, incorporating liver spheroids and a differentiated lung epithelium cultured under air-liquid-interface conditions. Reprinted from [27]; (D) Tilting platform for dynamic medium perfusion in a multi-OoC plate containing spheroid models of liver and colorectal cancer. Reprinted with permission from [36]; (E) Multi-OoC plate with pressure-driven medium circulation between organs, applied here to two-organ (liver & colorectal cancer) and four-organ configurations (intestine, liver, tumor and connective tissues). Reprinted with permission from [34]; (F) Modular multi-OoC platform for the co-culture of a GI tract epithelium and 3D primary liver tissues using gravity to actuate the flow. Reprinted with permission from [44]; (G) Multi-OoC plate combining four organs (intestine, liver, skin and kidney) for ADME studies, with an on-chip peristaltic pumping module. Reprinted from [45]; (H) Self-aligning Tetris-Like (TILE) modular multi-OoC platform to study multi-organ interactions in a modular and flexible manner, with on-demand platform assembly and disassembly for analysis. Reprinted with permission from [33].

Figure 3. Various strategies for establishing communication between different organ models in a multi-OoC platform. Connected **single OoC units** and integrated **Multi-OoC plates** can reproduce one-way communication from organ A to organ B (*top row*) or two-way communication (recirculation) between organs A and B (*bottom row*). Flows are generated using an external pump or an on-chip pumping system in a Multi-OoC plate, also including bubble traps (*bottom, right*).

TABLE CAPTION

Table 1, Key Table. Overview of recently reported Multi-OoC platforms.

Abbreviations: (*GFP*)- "name of cell line" - green fluorescent protein expressing cells; (*RFP*)- "name of cell line" - red fluorescent protein transfected cells; *16HBE* - human bronchial epithelial cells; *A549* - human non small cell lung cancer cells; *AA* – amino acid; *ALI* – Air Liquid Interface; *AMSC* - airway stromal mesenchymal cells (donor derived); *APC* – Antigen-presenting cells; *BF* - bovine fetuin; *BSA* - Bovine Serum Albumin; *Caco-2* - heterogeneous human epithelial colorectal adenocarcinoma cells; *FBS* - Fetal Bovine Serum; *Fob1.19* – human osteoblast cells; *hA* – human astrocytes; *HA* – hyaluronic acid; *HA-1800* – human astrocyte cells; *HBMEC* – human brain microvascular endothelial cells*; HBVP* – human brain vascular pericytes*; HCT-116* - human colon cancer cells; *HepaRG* - human hepatic stem cells; *HEPES* - (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer; *HepG2/C3a* - human hepato-cellular carcinoma cells; *hHSteC* - human hepatic stellate cells; *HL60* - human leukemia cells; *hLSMECs* - human liver sinusoidal microvascular endothelial cells; *HM* – human microglial; *HMVEC-L*– human lung microvasculature endothelial cells; *HNC* – human neural cells; *hPCF* - human primary cardiac fibroblasts; *hRPTECs* - human renal proximal tubule epithelial cells; *HUVEC* - human umbilical vein endothelial cells; *Hw36* - human primary hepatocytes*; Kupffer cells* - stellate macrophages; *L-02* – human hepatocyte cells; *MBA-MD-231* - human breast cancer cells; *MCF-7* - human breast cancer cells; *MDCK* - human Madin-Darby Canine Kidney cells*; MEA* - measurements of neurons using the Maestro™ MEA System; *NHBE* - human Bronchial/Tracheal Epithelial cells; *NTera2/cl.D1*- pluri-potent human testicular embryonal carcinoma cells; *PEGDA* - Poly(ethylene glycol) diacrylate; *PSF* – Polysulfone; *RPTEC* – human primary renal proximal tubule epithelial cells; *RPTEC/TERT-1* – human immortalized renal proximal tubule cells; *SSC* - spermatogonial stem cells; *THP-1* - human monocyte cells; *TIG-121* - normal human diploid fibroblast cells.

GLOSSARY

ADMET: Acronym (absorption, distribution, metabolism, excretion, and toxicity) referring to the key processes that determine the safety, distribution, elimination, metabolic action, performance, pharmacological activity, and possible side-effects of a drug, or food or/and additives therein, environmental pollutants, etc.) on a living organism exposed to it.

Conditioned medium: Medium obtained from the culture of cells or tissues and that contains biologically active substances released by these cells/tissues. This medium is used to stimulate the response of other cells/tissues in terms of cell physiological function such as growth, migration, etc.

***In situ* analysis**: Characterization of a sample directly in the (OoC) device without its extraction from its location.

iPSCs: Induced pluripotent stem cells, which are pluripotent cells generated from somatic human cells (fully differentiated adult cells, *e.g*., fibroblasts) that are reprogrammed by introduction of three genes (namely, pluripotency transcription factors; Oct4, Nanog, and Sox2) or four genes with c-Myc. Of significance is their potential to create patient-specific cells, using a patient's own adult cells, which are extremely precious to generate personalized tissues for disease modeling or transplantation therapies.

Multi-organ platforms: Miniaturized microfluidic system composed of several organ/tissue models that are either built from individual chip units connected by capillary tubing or integrated into a plate.

Off-line measurement: Analysis of a sample after it has been extracted from a (OoC) device.

On-line measurement: Analysis in a continuous and real-time way, of samples eluted from a (OoC) device by using for instance tubing connecting to a measuring instrument or assay.

Organ-on-Chip: abbreviated OoC, a 3D engineered biological model implemented in a microfluidic format that mimics the structure, physiological function and biomechanics of organs.

PK/PD: Modeling integrating pharmacokinetic (PK) and pharmacodynamic (PD) mathematical aspects to describe how fast and how completely a drug is absorbed into the body, distributed through the various tissues and fluids, metabolized and eliminated from the body (via urine, feces, etc.).

Transwell insert: Semi-permeable (porous) polymeric membrane delimiting two upper and lower chambers in a microwell. These compartmentalized devices that present a basolateral and apical sides, are typically employed to model physiological barriers (*e.g.*, skin, blood-brain barrier, vessels, etc.), to study cell-cell interactions, transport or metabolic activities or to monitor cell transmigration (*e.g.*, intravasation or extravasation of cancerous cells during metastasis). Today it is used as a common name, often without its registered trademark, written “transwell” or “Transwell”.

REFERENCES

1 Cheng, F. *et al.* (2012) admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J Chem Inf Model* 52, 3099–3105

2 Rikkert, L.G. *et al.* (2020) Cancer-ID: Toward Identification of Cancer by Tumor-Derived Extracellular Vesicles in Blood. *Front Oncol* 10, 608

3 Heitzer, E. *et al.* (2019) Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nature Reviews Genetics* 20, 71–88

4 Low, L.A. *et al.* (2020) Organs-on-chips: into the next decade. *Nat Rev Drug Discov* Adv Exp Med Biol. 2020;1230:27-42

5 Ingber, D.E. Is it Time for Reviewer 3 to Request Human Organ Chip Experiments Instead of Animal Validation Studies? *Advanced Science* n/a, 2002030

6 Chung, H.H. *et al.* (2018) Use of Porous membranes in tissue barrier and co-culture models. *Lab Chip* 18, 1671–1689

7 Lembong, J. *et al.* (2018) A Fluidic Culture Platform for Spatially Patterned Cell Growth, Differentiation, and Cocultures. *Tissue Eng Part A* 24, 1715–1732

8 Zhou, Q. *et al.* (2015) Liver injury-on-a-chip: microfluidic co-cultures with integrated biosensors for monitoring liver cell signaling during injury. *Lab Chip* 15, 4467–4478

9 Patrício, S.G. *et al.* (2020) Freeform 3D printing using a continuous viscoelastic supporting matrix. *Biofabrication* 12, 035017

10 van den Broek, L.J. *et al.* (2017) Progress and Future Prospectives in Skin-on-Chip Development with Emphasis on the use of Different Cell Types and Technical Challenges. *Stem Cell Rev Rep* 13, 418–429

11 Schimek, K. *et al.* (2018) Bioengineering of a Full-Thickness Skin Equivalent in a 96-Well Insert Format for Substance Permeation Studies and Organ-On-A-Chip Applications. *Bioengineering (Basel)* 5,

12 Nashimoto, Y. *et al.* (2017) Integrating perfusable vascular networks with a three-dimensional tissue in a microfluidic device. *Integr Biol (Camb)* 9, 506–518

13 Rajan, S.A.P. *et al.* (2020) Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform. *Acta Biomater* 106, 124–135

14 Achberger, K. *et al.* (2019) Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. *Elife* 8,

15 Picollet-D'hahan, N. *et al.* (2017) Deciphering Cell Intrinsic Properties: A Key Issue for Robust Organoid Production. *Trends Biotechnol.* 35, 1035–1048

16 Schwerdtfeger, L.A. and Tobet, S.A. (2019) From organotypic culture to body-on-a-chip: A neuroendocrine perspective. *J. Neuroendocrinol.* 31, e12650

17 McLean, I.C. *et al.* (2018) Powering ex vivo tissue models in microfluidic systems. *Lab Chip* 18, 1399–1410

18 Shim, S. *et al.* (2019) Two-way communication between ex vivo tissues on a microfluidic chip: application to tumor–lymph node interaction. *Lab Chip* 19, 1013–1026

19 Wang, Y. *et al.* (2017) A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium. *Biomaterials* 128, 44–55

20 Yu, F. and Choudhury, D. (2019) Microfluidic bioprinting for organ-on-a-chip models. *Drug Discov. Today* 24, 1248–1257

21 Hinman, S.S. *et al.* (2020) Microphysiological System Design: Simplicity Is Elegance. *Curr Opin Biomed Eng* 13, 94–102

22 Kaarj, K. and Yoon, J.-Y. (2019) Methods of Delivering Mechanical Stimuli to Organ-on-a-Chip. *Micromachines (Basel)* 10,

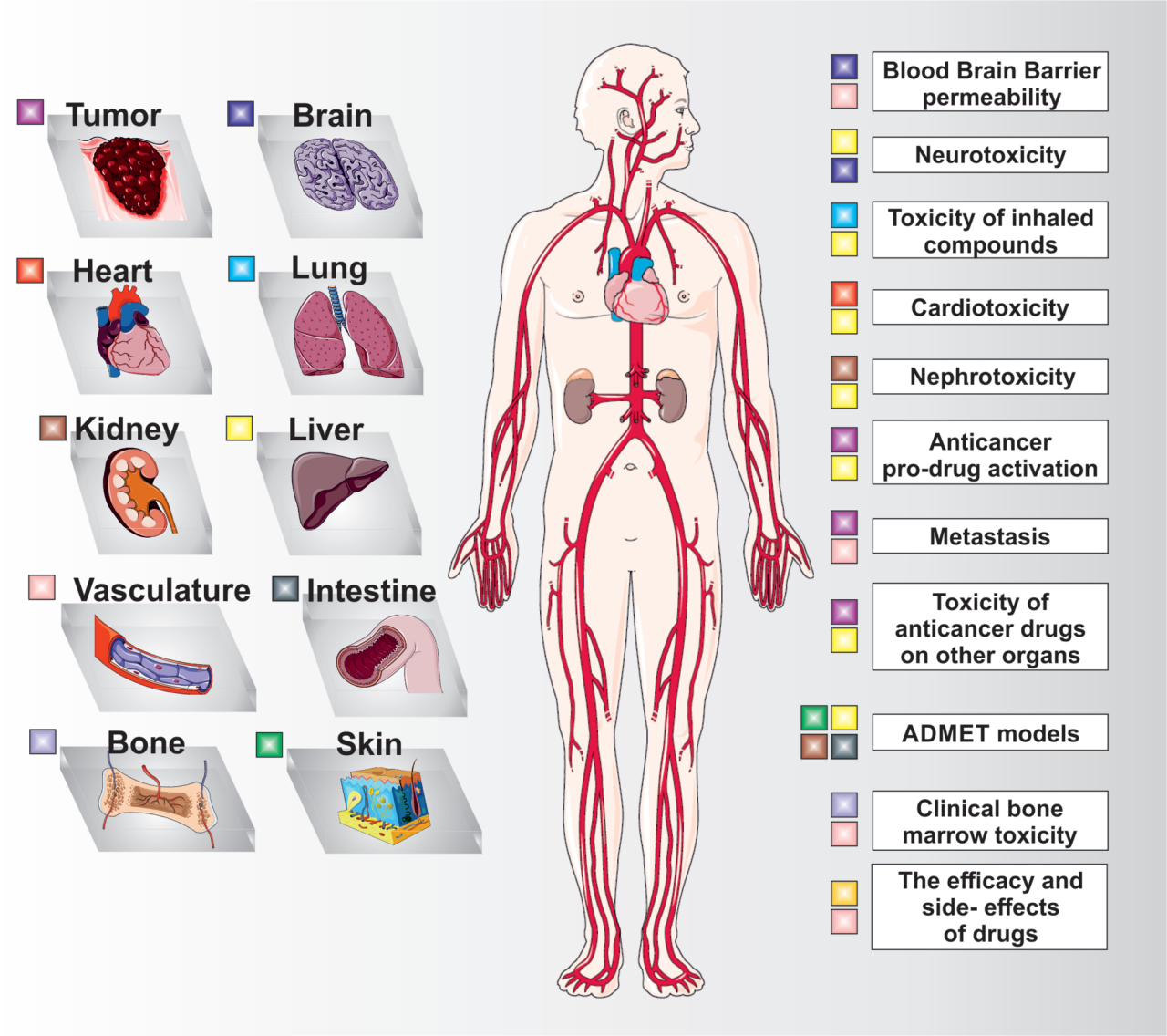
23 Gaio, N. *et al.* (2016) Cytostretch, an Organ-on-Chip Platform. *Micromachines (Basel)* 7,
24 Visone, R. *et al.* (2018) A microscale biomimetic platform for generation and electro-mechanical stimulation of 3D cardiac microtissues. *APL Bioeng* 2, 046102
25 Mastrangeli, M. *et al.* (2019) Building blocks for a European Organ-on-Chip roadmap. *ALTEX* 36, 481–492
26 Mastrangeli, M. *et al.* (2019) Organ-on-chip in development: Towards a roadmap for organs-on-chip. *ALTEX* 36, 650–668
27 Bovard, D. *et al.* (2018) A lung/liver-on-a-chip platform for acute and chronic toxicity studies. *Lab Chip* 18, 3814–3829
28 Kimura, H. *et al.* (2015) An on-chip small intestine-liver model for pharmacokinetic studies. *J Lab Autom* 20, 265–273
29 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
30 Materne, E.-M. *et al.* (2015) A multi-organ chip co-culture of neurospheres and liver equivalents for long-term substance testing. *J. Biotechnol.* 205, 36–46
31 Hübner, J. *et al.* (2018) Simultaneous evaluation of anti-EGFR-induced tumour and adverse skin effects in a microfluidic human 3D co-culture model. *Sci Rep* 8, 15010
32 Kim, J.-Y. *et al.* (2015) 96-well format-based microfluidic platform for parallel interconnection of multiple multicellular spheroids. *J Lab Autom* 20, 274–282
33 Ong, L.J.Y. *et al.* (2019) Self-aligning Tetris-Like (TILE) modular microfluidic platform for mimicking multi-organ interactions. *Lab Chip* 19, 2178–2191
34 Satoh, T. *et al.* (2017) A multi-throughput multi-organ-on-a-chip system on a plate formatted pneumatic pressure-driven medium circulation platform. *Lab Chip* 18, 115–125
35 Theobald, J. *et al.* (2019) In vitro metabolic activation of vitamin D3 by using a multi-compartment microfluidic liver-kidney organ on chip platform. *Sci Rep* 9, 4616
36 Kim, J.-Y. *et al.* (2015) 3D spherical microtissues and microfluidic technology for multi-tissue experiments and analysis. *J. Biotechnol.* 205, 24–35
37 Chong, L.H. *et al.* (2018) A liver-immune coculture array for predicting systemic drug-induced skin sensitization. *Lab Chip* 18, 3239–3250
38 Skardal, A. *et al.* (2020) Drug compound screening in single and integrated multi-organoid body-on-a-chip systems. *Biofabrication* 12, 025017
39 Edington, C.D. *et al.* (2018) Interconnected Microphysiological Systems for Quantitative Biology and Pharmacology Studies. *Sci Rep* 8, 4530
40 Herland, A. *et al.* (2020) Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. *Nat Biomed Eng* 4, 421–436
41 Novak, R. *et al.* (2020) Robotic fluidic coupling and interrogation of multiple vascularized organ chips. *Nat Biomed Eng* 4, 407–420
42 Leclerc, E. *et al.* (2016) Investigation of ifosfamide and chloroacetaldehyde renal toxicity through integration of in vitro liver-kidney microfluidic data and pharmacokinetic-system biology models. *J Appl Toxicol* 36, 330–339
43 Loskill, P. *et al.* (2015) µOrgano: A Lego®-Like Plug & Play System for Modular Multi-Organ-Chips. *PLoS ONE* 10 (10):e0139587
44 Esch, M.B. *et al.* (2016) Modular, pumpless body-on-a-chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. *Lab Chip* 16, 2719–2729
45 Maschmeyer, I. *et al.* (2015) A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab on a Chip* 15, 2688–2699
46 Ramme, A.P. *et al.* (2019) Autologous induced pluripotent stem cell-derived four-organ-chip. *Future Sci OA* 5, FSO413

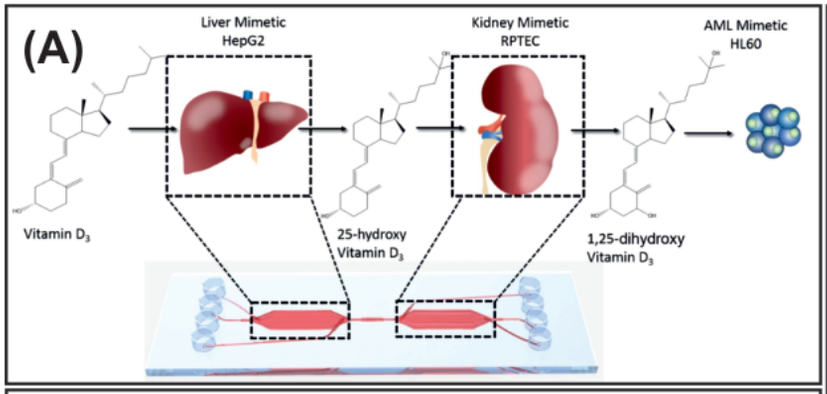
47 Trapecar, M. *et al.* (2020) Gut-Liver Physiomimetics Reveal Paradoxical Modulation of IBD-Related Inflammation by Short-Chain Fatty Acids. *Cell Syst* 10, 223-239.e9
48 Maoz, B.M. *et al.* (2018) A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat. Biotechnol.* 36, 865–874
49 Chou, D.B. *et al.* (2020) On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology. *Nat Biomed Eng* 4, 394–406
50 Bauer, S. *et al.* (2017) Functional coupling of human pancreatic islets and liver spheroids on-a-chip: Towards a novel human ex vivo type 2 diabetes model. *Sci Rep* 7, 14620
51 Xiao, S. *et al.* (2017) A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. *Nat Commun* 8, 14584
52 Siegel, R.L. *et al.* (2019) Cancer statistics, 2019. *CA Cancer J Clin* 69, 7–34
53 Kong, J. *et al.* (2016) A novel microfluidic model can mimic organ-specific metastasis of circulating tumor cells. *Oncotarget* 7, 78421–78432
54 Xu, Z. *et al.* (2016) Design and Construction of a Multi-Organ Microfluidic Chip Mimicking the in vivo Microenvironment of Lung Cancer Metastasis. *ACS Appl Mater Interfaces* 8, 25840–25847
55 Shutko, A.V. *et al.* (2017) Biocontractile microfluidic channels for peristaltic pumping. *Biomed Microdevices* 19, 72
56 Ronaldson-Bouchard, K. and Vunjak-Novakovic, G. (2018) Organs-on-a-Chip: A Fast Track for Engineered Human Tissues in Drug Development. *Cell Stem Cell* 22, 310–324
57 Tsamandouras, N. *et al.* (2017) Integrated Gut and Liver Microphysiological Systems for Quantitative In Vitro Pharmacokinetic Studies. *AAPS J* 19, 1499–1512
58 Chen, W.L.K. *et al.* (2017) Integrated gut/liver microphysiological systems elucidates inflammatory inter-tissue crosstalk. *Biotechnol. Bioeng.* 114, 2648–2659
59 Chen, H.J. *et al.* (2018) A pumpless body-on-a-chip model using a primary culture of human intestinal cells and a 3D culture of liver cells. *Lab Chip* 18, 2036–2046
60 Ferraz, M.A.M.M. *et al.* (2018) An oviduct-on-a-chip provides an enhanced in vitro environment for zygote genome reprogramming. *Nat Commun* 9, 4934
61 Kulthong, K. *et al.* (2018) Implementation of a dynamic intestinal gut-on-a-chip barrier model for transport studies of lipophilic dioxin congeners. *RSC Advances* 8, 32440–32453
62 Huh, D.D. (2015) A human breathing lung-on-a-chip. *Ann Am Thorac Soc* 12 Suppl 1, S42-44
63 Yin, L. *et al.* (2020) Efficient Drug Screening and Nephrotoxicity Assessment on Co-culture Microfluidic Kidney Chip. *Scientific Reports* 10, 6568
64 Nawroth, J.C. *et al.* (2018) Automated fabrication of photopatterned gelatin hydrogels for organ-on-chips applications. *Biofabrication* 10, 025004
65 Picollet-D'hahan, N. *et al.* (2016) A 3D Toolbox to Enhance Physiological Relevance of Human Tissue Models. *Trends Biotechnol.* 34, 757–769
66 Lu, S. *et al.* (2018) Development of a biomimetic liver tumor-on-a-chip model based on decellularized liver matrix for toxicity testing. *Lab Chip* 18, 3379–3392
67 Gjorevski, N. *et al.* (2016) Designer matrices for intestinal stem cell and organoid culture. *Nature* 539, 560–564
68 Sheehy, S.P. *et al.* (2017) Toward improved myocardial maturity in an organ-on-chip platform with immature cardiac myocytes. *Exp. Biol. Med. (Maywood)* 242, 1643–1656
69 Gjorevski, N. and Lutolf, M.P. (2017) Synthesis and characterization of well-defined hydrogel matrices and their application to intestinal stem cell and organoid culture. *Nature Protocols* 12, 2263–2274

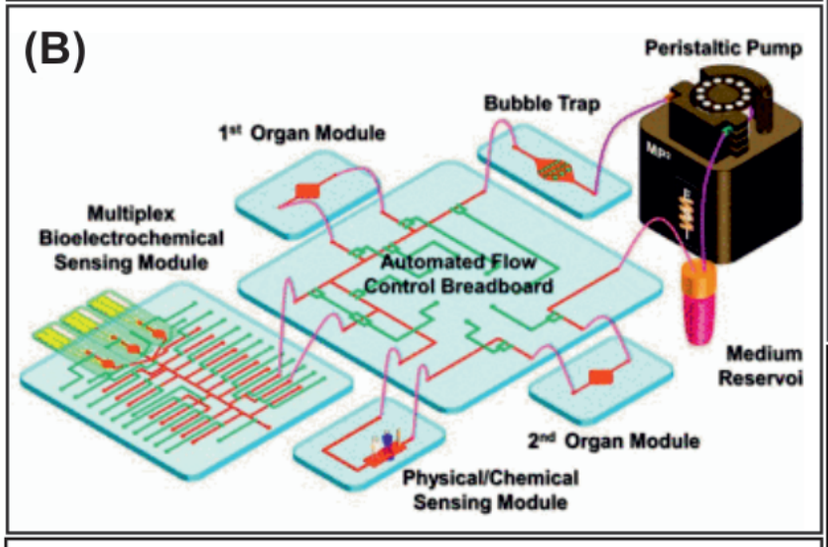
70 Paggi, C.A. *et al.* (2020) Monolithic microfluidic platform for exerting gradients of compression on cell-laden hydrogels, and application to a model of the articular cartilage. *Sensors and Actuators B: Chemical* 315, 127917
71 Delarue, M. *et al.* (2016) Self-Driven Jamming in Growing Microbial Populations. *Nat Phys* 12, 762–766
72 Pepijn Beekman, Agustin Enciso-Martinez, Sidharam P. Pujari, and Leon W.M.M. Terstappen, Han T. Zuilhof, Séverine Le Gac, Cees Otto Organosilicon uptake by biological membranes. *Microtas conference 2020, online, 4-9 Oct. 2020.*
73 Zhang, Y.S. *et al.* (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. *Proc. Natl. Acad. Sci. U.S.A.* 114, E2293–E2302
74 Lachaux, J. *et al.* (2017) Thermoplastic elastomer with advanced hydrophilization and bonding performances for rapid (30 s) and easy molding of microfluidic devices. *Lab Chip* 17, 2581–2594
75 Sung, J.H. *et al.* (2019) Recent Advances in Body-on-a-Chip Systems. *Anal. Chem.* 91, 330–351
76 Korolj, A. *et al.* (2018) Curvature facilitates podocyte culture in a biomimetic platform. *Lab Chip* 18, 3112–3128
77 Yi, H.-G. *et al.* (2017) 3D Printing of Organs-On-Chips. *Bioengineering (Basel)* 4,
78 Lind, J.U. *et al.* (2017) Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. *Nat Mater* 16, 303–308
79 Fetah, K. *et al.* (2019) The emergence of 3D bioprinting in organ-on-chip systems. *Progress in Biomedical Engineering* 1, 012001
80 Sun, H. *et al.* (2020) Combining additive manufacturing with microfluidics: an emerging method for developing novel organs-on-chips. *Current Opinion in Chemical Engineering* 28, 1–9
81 Gao, B. *et al.* (2016) 4D Bioprinting for Biomedical Applications. *Trends Biotechnol.* 34, 746–756
82 Polilov, A.A. and Makarova, A.A. (2017) The scaling and allometry of organ size associated with miniaturization in insects: A case study for Coleoptera and Hymenoptera. *Sci Rep* 7, 43095
83 Abaci, H.E. and Shuler, M.L. (2015) Human-on-a-chip design strategies and principles for physiologically based pharmocokinetics/pharmacodynamics modeling. *Integr Biol (Camb)* 7, 383–391
84 Moraes, C. *et al.* (2013) On being the right size: scaling effects in designing a human-on-a-chip. *Integr Biol (Camb)* 5, 1149–1161
85 Stokes, C.L. *et al.* (2015) Physiome-on-a-Chip: The Challenge of "Scaling" in Design, Operation, and Translation of Microphysiological Systems. *CPT Pharmacometrics Syst Pharmacol* 4, 559–562
86 Park, D. *et al.* (2020) Integrating Organs-on-Chips: Multiplexing, Scaling, Vascularization, and Innervation. *Trends Biotechnol.* 38, 99–112
87 Oleaga, C. *et al.* (2016) Multi-Organ toxicity demonstration in a functional human in vitro system composed of four organs. *Sci Rep* 6, 20030
88 Aleman, J. and Skardal, A. (2019) A multi-site metastasis-on-a-chip microphysiological system for assessing metastatic preference of cancer cells. *Biotechnol. Bioeng.* 116, 936–944
89 Hughes, D.J. *et al.* (2017) Opportunities and challenges in the wider adoption of liver and interconnected microphysiological systems. *Exp. Biol. Med. (Maywood)* 242, 1593–1604
90 Coppeta, J.R. *et al.* (2016) A portable and reconfigurable multi-organ platform for drug development with onboard microfluidic flow control. *Lab Chip* 17, 134–144

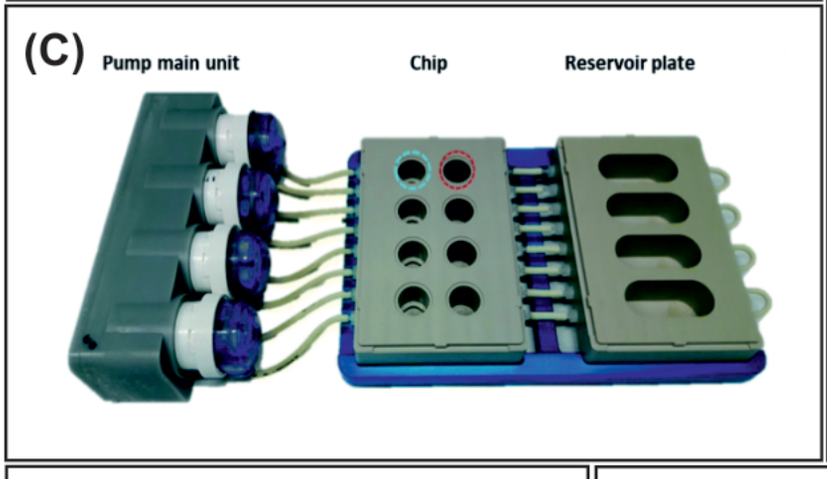
91 Boeri, L. *et al.* (2019) Advanced Organ-on-a-Chip Devices to Investigate Liver Multi-Organ Communication: Focus on Gut, Microbiota and Brain. *Bioengineering (Basel)* 6,
92 Zhao, Y. *et al.* (2019) Multi-Organs-on-Chips: Towards Long-Term Biomedical Investigations. *Molecules* 24,
93 Skardal, A. *et al.* (2016) A reductionist metastasis-on-a-chip platform for in vitro tumor progression modeling and drug screening. *Biotechnol. Bioeng.* 113, 2020–2032
94 Skardal, A. *et al.* (2017) Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep* 7, 8837
95 Zhang, Y.S. (2017) , Modular multi-organ-on-chips platform with physicochemical sensor integration. , in *2017 IEEE 60th International Midwest Symposium on Circuits and Systems (MWSCAS)*, pp. 80–83
96 Ataç, B. *et al.* (2013) Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. *Lab Chip* 13, 3555–3561
97 Roudsari, L.C. and West, J.L. (2016) Studying the influence of angiogenesis in in vitro cancer model systems. *Adv. Drug Deliv. Rev.* 97, 250–259
98 Sebastian, B. and Dittrich, P.S. (2018) Microfluidics to Mimic Blood Flow in Health and Disease. *Annu. Rev. Fluid Mech.* 50, 483–504
99 Poceviciute, R. and Ismagilov, R.F. (2019) Human-gut-microbiome on a chip. *Nat Biomed Eng* 3, 500–501
100 Chen, H. *et al.* (2016) Sulfated fucoidan FP08S2 inhibits lung cancer cell growth in vivo by disrupting angiogenesis via targeting VEGFR2/VEGF and blocking VEGFR2/Erk/VEGF signaling. *Cancer Lett.* 382, 44–52
101 Khaki, M. *et al.* (2018) Mesenchymal Stem Cells Differentiate to Endothelial Cells Using Recombinant Vascular Endothelial Growth Factor -A. *Rep Biochem Mol Biol* 6, 144–150
102 Salinas, E.Y. *et al.* (2018) A Guide for Using Mechanical Stimulation to Enhance Tissue-Engineered Articular Cartilage Properties. *Tissue Eng Part B Rev* 24, 345–358
103 Hsia, C.C.W. (2017) Comparative analysis of the mechanical signals in lung development and compensatory growth. *Cell Tissue Res.* 367, 687–705
104 Misun, P.M. *et al.* (2020) In Vitro Platform for Studying Human Insulin Release Dynamics of Single Pancreatic Islet Microtissues at High Resolution. *Adv Biosyst* 4, e1900291
105 Moreno, E.L. *et al.* (2015) Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture. *Lab Chip* 15, 2419–2428
106 Sinha, R. *et al.* (2015) A medium throughput device to study the effects of combinations of surface strains and fluid-flow shear stresses on cells. *Lab Chip* 15, 429–439
107 Occhetta, P. *et al.* (2019) Hyperphysiological compression of articular cartilage induces an osteoarthritic phenotype in a cartilage-on-a-chip model. *Nature Biomedical Engineering* 3, 545–557
108 Ribas, J. *et al.* (2016) Cardiovascular Organ-on-a-Chip Platforms for Drug Discovery and Development. *Appl In Vitro Toxicol* 2, 82–96
109 Kilic, T. *et al.* (2018) Organs-on-chip monitoring: sensors and other strategies. *Microphysiological Systems* 2,
110 Schneider, C.A. *et al.* (2019) Imaging the dynamic recruitment of monocytes to the blood-brain barrier and specific brain regions during Toxoplasma gondii infection. *Proc. Natl. Acad. Sci. U.S.A.* 116, 24796–24807
111 Albert-Smet, I. *et al.* (2019) Applications of Light-Sheet Microscopy in Microdevices. *Front. Neuroanat.* 13,
112 Thendiyammal, A. *et al.* (2020) Model-based wavefront shaping microscopy. *Opt Lett;45(18):5101-5104.*

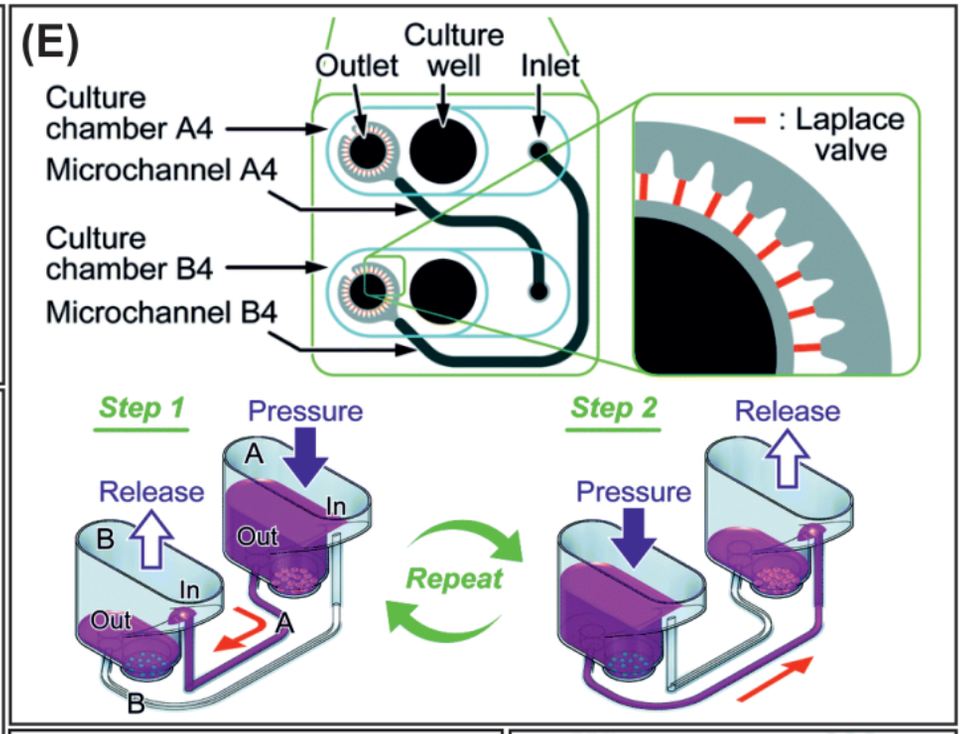
113 Zhu, J. *et al.* (2018) An integrated adipose-tissue-on-chip nanoplasmonic biosensing platform for investigating obesity-associated inflammation. *Lab Chip* 18, 3550–3560
114 Park, T.-E. *et al.* (2019) Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. *Nature Communications* 10, 2621
115 Rho, H.S. *et al.* (2020) A 3D polydimethylsiloxane microhourglass-shaped channel array made by reflowing photoresist structures for engineering a blood capillary network. *Methods* S1046-2023(19)30315-9
116 Yamada, A. *et al.* (2016) Transient microfluidic compartmentalization using actionable microfilaments for biochemical assays, cell culture and organs-on-chip. *Lab Chip* 16, 4691–4701
117 Marcu, R. *et al.* (2018) Human Organ-Specific Endothelial Cell Heterogeneity. *iScience* 4, 20–35
118 Phan, D.T.T. *et al.* (2017) A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. *Lab Chip* 17, 511–520

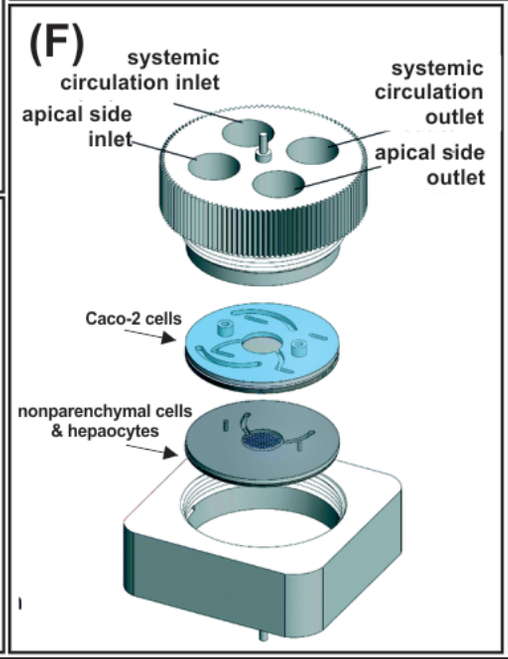

Tumor
Brain
Heart
Lung
Kidney
Liver
Vasculature
Intestine
Bone
Skin
Blood Brain Barrier permeability
Neurotoxicity
Toxicity of inhaled compounds
Cardiotoxicity
Nephrotoxicity
Anticancer pro-drug activation
Metastasis
Toxicity of anticancer drugs on other organs
ADMET models
Clinical bone marrow toxicity
The efficacy and side- effects of drugs

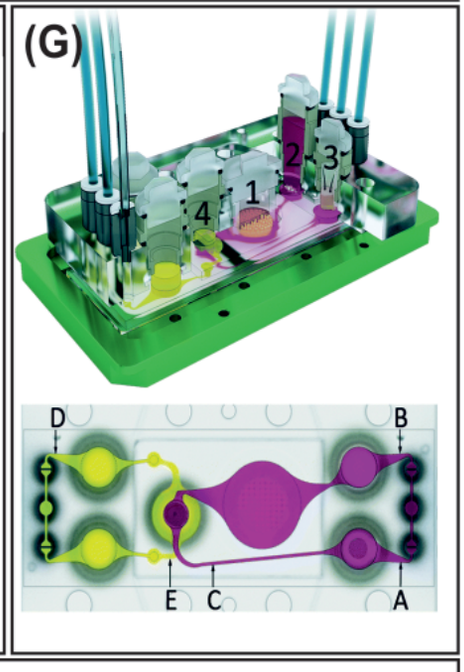

(A)
Liver Mimetic
HepG2
Kidney Mimetic
RPTEC
AML Mimetic
HL60
Vitamin D3
25-hydroxy
Vitamin D3
1,25-dihydroxy
Vitamin D3

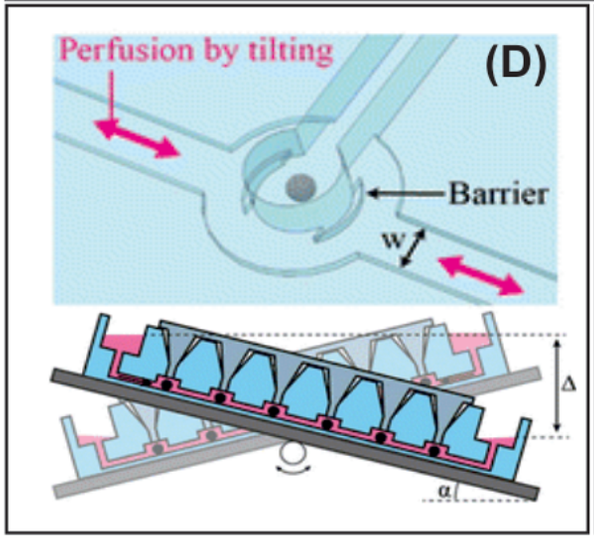

(B)
Peristaltic Pump
Bubble Trap
1st Organ Module
Multiplex
Bioelectrochemical
Sensing Module
Automated Flow
Control Breadboard
Medium
Reservoi
2nd Organ Module
Physical/Chemical
Sensing Module

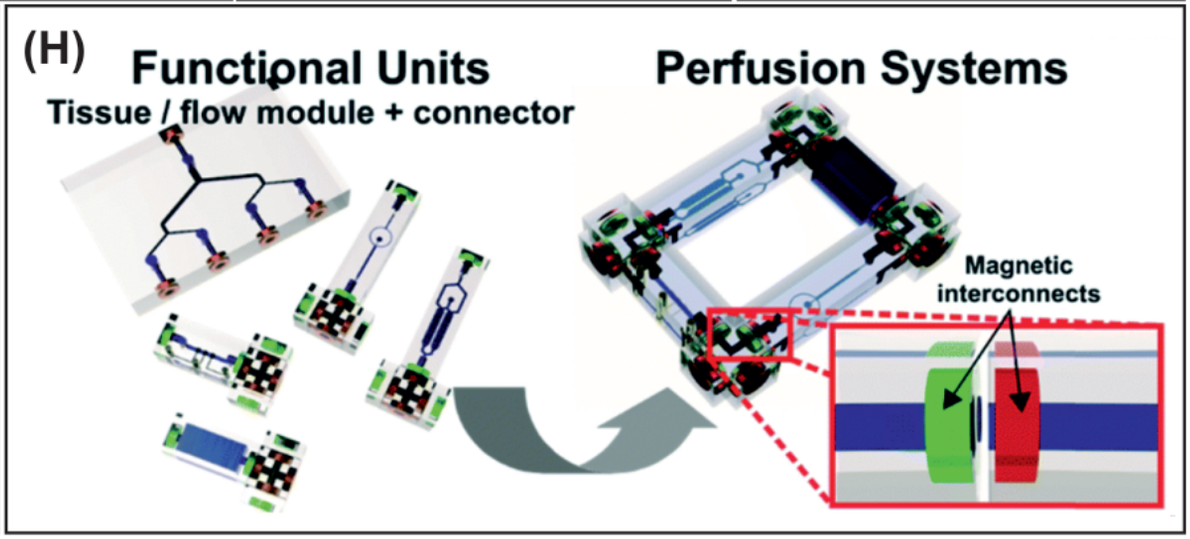

(C)
Pump main unit
Chip
Reservoir plate

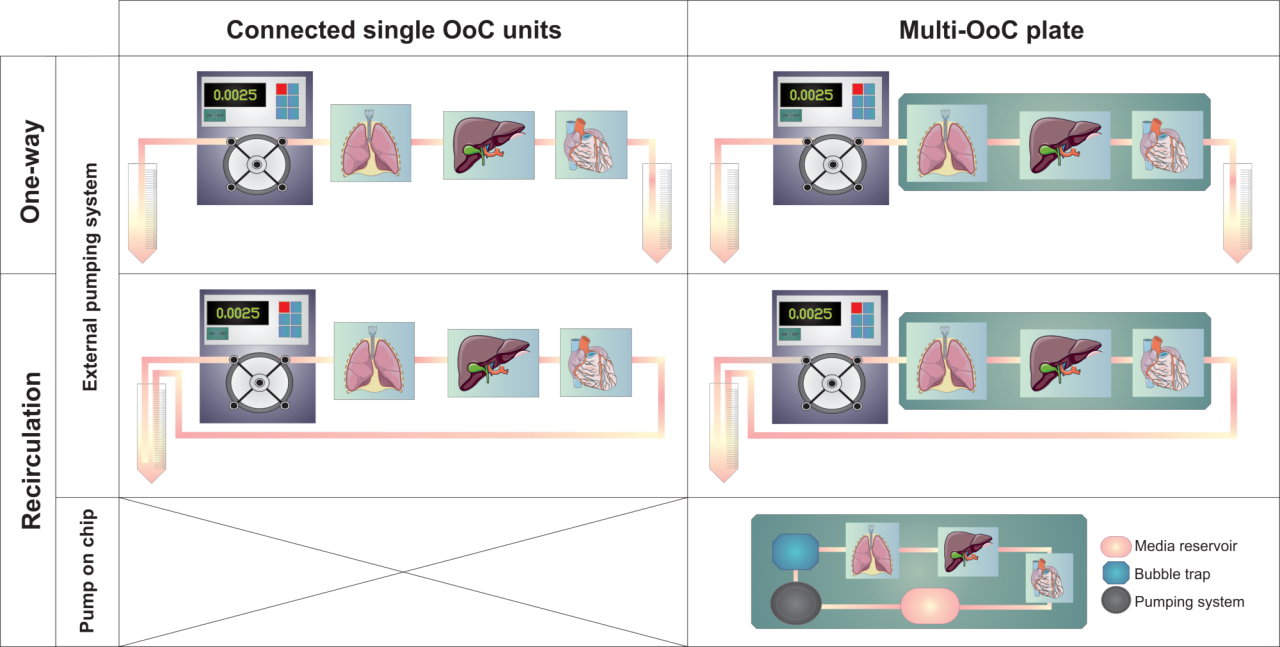

(E)
Outlet
Culture
well
Inlet
Culture
chamber A4
Microchannel A4
Culture
chamber B4
Microchannel B4
: Laplace
valve
Step 1
Pressure
Release
A
In
Out
B
Repeat
Step 2
Release
Pressure


(F)
systemic
circulation inlet
apical side
inlet
systemic
circulation
outlet
apical side
outlet
Caco-2 cells
nonparenchymal cells
& hepaocytes


(G)
1
2
3
4
D
B
E
C
A


Perfusion by tilting
(D)
Barrier
w
Δ
α


(H)
Functional Units
Tissue / flow module + connector
Perfusion Systems
Magnetic
interconnects


Connected single OoC units
Multi-OoC plate
One-way
Recirculation
External pumping system
Pump on chip
0.0025
Media reservoir
Bubble trap
Pumping system

Classification	Application(s)	Organs	Organ modeling approaches		Vascularization (strategy)	Stimulation (biochemical, hormonal, physical)	Typology of system (see BOX 1)	Material	Medium composition	(Re-)Circulation (Yes/No//Not available (N/A)	Time duration of the experiment	Analysis			Major outcome	Reference
												Mode	End-point	Assay / technique		
Toxicity screening	Evaluation of the potential toxicity of aerosols	Liver	Spheroids	HepaRG	N.A.	N.A.	Multi-OoC plate	PEEK	PneumaCult™ medium	Y	28 d	*In situ*	Characterization of the lung model	TEER and CBF (Cilia beating frequency)	Toxicity of inhaled compound reduced by liver tissue metabolism	27
												Off-line	Cell viability	ATP content (CellTiter-Glo®) LDH assay		
													Morphological changes	Immunostaining		
		Lung	ALI	Normal human bronchial epithelial cells (NHBE)									Expression levels of phase 1 metabolism-associated genes	RT-qPCR		
													CYP enzymatic activity (liver)	Dedicated assay for CYP1A1/1B1		
													Metabolic and functional activity (Glucose consumption, lactate production, and albumin synthesis)	Dedicated assays		
	Evaluation of the toxic effects of anti-cancer treatment after activation by liver	Small Intestine	Monolayers on collagen coated surface	Caco-2	N.A.	N.A.	Multi-OoC plate	PDMS PET porous membrane	DMEM (+non-Essential AA Solution)	Y	iv 3 d	*In situ*	Cell viability	Live/Dead staining	Successful replication of physiological circulation and organ ratio; new insights into the importance of small intestine and liver to evaluate the activity of anti-cancer drugs.	28
		Liver		HepG2												
		Lung Tumor		A549												
	Evaluation of neurotoxicity	Liver	Spheroids	HepaRG hHSteC	N.A.	N.A.	Multi-OoC	PDMS on Glass	HepaRG medium	Y	14 d	Off-line	Cell viability	LDH assay	Enhanced sensitivity of the	30

				(24:1 ratio)									Cell metabolic activity (Glucose consumption, and lactate production)	Dedicated assays	liver-neurosphere model than single-tissue cultures	
													Cell apoptosis and proliferation	TUNEL assay & Ki67 staining		
		Brain		NTera-2/cl.D1									Characterization of organ models (brain neuronal markers beta III-tubulin & MAP2 and pluripotent marker TRA-a-60; liver: Cyt P450 3A4, MRP-2, CK 8/18, Vimentin)	Immunostaining on tissue cryosections		
													Gene expression level (III-tubulin, Oct 4, Pol2, nestin, albumin, BSEP, CPS-1, Cyp 1A2, Cyp 2B6, Cyp 3A4, TBP)	RT-qPCR		
	"Safficacy" assay to screen EGFR inhibitors in cancer treatment	Skin	*Ex vivo* human tissue Milicell® insert		N.A.	N.A.	Multi-OoC plate	PDMS on Glass	E3 medium (+ glucose)	Y	5 d	*In situ*	ECM remodeling	Bright field imaging	Recapitulation of specific patterns observed in patients treated with Anti-EGFR therapy – such as release of inflammatory markers, and inhibition of the skin proliferative turnover	31
												Off-line	Tissue viability	LDH assay		
													Cell proliferation	MTT assay		
													Skin morphological change (Collagen IV, Vimention, E-cadherin)	Histology Immunostaining		
													Cell apoptosis and proliferation	TUNEL assay and Ki67 staining		
		Lung tumor	Spheroids	hNCI-H292									Cytokine production	V-Plex Chemokine Panel I Human kit		
													Gene expression related to apoptosis induction, inflammation, differentiation	qPCR		
	Cardiotoxicity and the impact of hepatic metabolism thereon	Liver	Monolayer on collagen-coated glass coverslips	Primary human hepatocytes Hw36	N.A.	Electrical stimulation of the cardiac muscle	Multi-OoC plate	PDMS & PMMA	HSL2 (serum free, static culture) or HSL3	N	28 d	Online	Hepatic tissue state	Urea and albumin secretion (dedicated assays); CYP enzyme activity	Impact of the presence of liver on the effect of various drugs on the heart model.	29
												In situ	Characterization of organ models (albumin)	Immunochemistry		

									(serum free, housing-based experiments)				Electrical activity of the cardiomyocytes	MEA measurements		
		Heart	Monolayer on glass coverslips	Human iPSc derived cardiomyocytes									Cardiac contractile function	Cantilever-based force measurements		
												Off-line	Cell viability and metabolism	MTT assay and AlamarBlue assay		
													Drug metabolism	LC-MS/MS		
Cancer research - Metastasis	Evaluation of the invasion potential of lung cancer cells and associated fibroblasts in distant organs (brain, bone and liver)	Lung cancer	ALI	16HBE A549 (10:1 ratio)	HUVECs + macrophages (activated THP-1 and W138 cells) in basolateral compartment	N.A.	Multi-OoC plate	PDMS	N.A	N	-	*In situ*	EMT markers in lung model and invasion in distant organs (E-cadherin, N-cadherin, Snail1, Snail2)	Immunostaining	Reproduction of cancer growth and metastasis processes. Validation using a mouse model	54
													Lung model characterization Macrophage M2 marker CD206 + fibroblast marker a-SMA + lung cancer marker CEA			
													Tight junction (E-Cadherin) in lung epithelium and endothelium			
		Brain	3D cell culture	HA-1800									Cell apoptosis	Hoechst / PI staining		
		Bone		Fob1.19									Tracking of lung tumor and stromal cells	Fluorescence microscopy (Cell Tracker dye)		
		Liver		L-02												
	Colon tumor metastasis in liver and drug screening	Colon carcinoma tumor	3D cell culture in HA/ PEGDA / gelatin hydrogel	(RFP)-HCT-116 INT-407 (1:10 ratio)	N.A.	N.A.	Multi-OoC plate	PDMS	DMEM	Y	24 d	Off-line	Characterization of organ models (ZO-1, β-catenin, MMP 9, N-cadherin, PCNA, Vinculin)	Immunostaining	Tumor cell migration influenced by the liver model mechanical properties and drug treatment	93
		Liver		HepG2								*In situ*	Tumor cell expansion and migration	Cell tracking		

	Evaluation of metastatic preferences of CTC-like breast cancer cells under chemokine stimulation	Bone Lung Muscle Liver	Transwell-like support	rBMC Rat primary murine pulmonary cells Rat primary murine muscle cells Rat primary murine hepatocytes	Vascular endothelial barrier (HUVECs grown in basolateral compartments)	Chemokine stimulation to trigger metastasis	Multi-OoC plate	PDMS on Glass	DMEM F12 (1:1 ratio) or L-15 medium	N	30 min	*In situ* Off-line	Breast tumor CTC-like cell invasion Characterization of the endothelium (ZO-1 marker) Cell viability CXCL12 secretion from lung, liver, bone and muscle models Expression of CXCR4 receptor in CTC-like breast tumor cells under chemokine stimulation	Cell tracking Immunostaining Live/Dead staining ELISA Flow cytometry analysis	CTCs showed mainly metastatic potential to lung over muscle, and to liver then bone marrow over muscle in the microfluidic model Model and result validation using a mouse model.	53
	Evaluation of metastatic preference of colorectal cancer cells	Colorectal cancer Liver Lung	"organoids" 3D cell culture in hydrogel (Heprasil, Gelin-S and Extralink, ratio 2:2:1)	(RFP)-HCT116 HepG2 A549	Endothelium mimicked as an organoid not as blood vessels	N.A.	Multi-OoC plate	PDMS on glass	DMEM-10 EGM-2 (3:1 ratio)	Y	15 d	In situ	Cell viability HCT116 cell tracking	Live/Dead staining Cell permanent labeling (RFP)	Preferential colonization of colorectal cancer cells in liver and lung, as observed in vivo.	88
	Modeling tumor–lymph node interactions	Lymph node Breast tumor	*Ex vivo* mouse tissues (slices)		Innate vascularization (*ex vivo* tissues)	N.A.	Multi-OoC plate	PDMS PC porous membrane	RPMI-1640 (+mercaptorethanol, pyruvate, non-essential AA, HEPES)	Y	1 d	Off-line	Tissue viability T-cell activity in lymph node slices (IFN-gamma)	Live/Dead staining ELISA	Successful modeling of some features of the immune-tumor interactions	18
Drug metabolism	Modeling bioactivation of nutraceuticals and anti-cancer prodrugs	Liver Oral squamous carcinoma (primary or metastatic)	Spheroids	HepaRG HN137 and OSCC	Vascular endothelial barrier (HCAEC cells)	N.A.	Multi-OoC chip	PDMS Magnets embedded	Liver-endothelium HepaRG medium EGM2 (1:1 ratio) Liver-tumor RPMI1640 HepaRG (1:1 ratio)	Y	2 d	Off-line *In situ*	Gene expression related to apoptosis Characterization of organ models and inflammation of the endothelium (Vimentin, E-cadherin) Cell viability	qPCR Immunostaining Live/Dead staining	Successful bioactivation of nutraceuticals and prodrugs Plug-and play and easily reconfigurable Multi-OoC platform	33

	Prediction of drug-induced skin sensitization using a liver-immune co-culture, and testing of three drugs known to cause cutaneous reaction	Liver	Spheroids	HepaRG	N.A	N.A	Multi-OoC plate	PDMS on Glass	Liver: HepaRG medium Immune cells: RPMI1640 (+ glutamine, HEPES, sodium pyruvate)	N.A.	2 d	Off-Line	Gene expression level (Liver: CYP1A2, CYP2A4, CBZ-E, 2-OH CBZ, 3-OH CBZ, p-HPPH, Oxipurinol; Immune cells for their activation: IL8, IL1β, CD86)	qPCR	Liver-immune co-culture system supporting organ culture and maintaining organ function. Successful triggering of APC activation response. Robust assay to assess the potential skin sensitization of systemically ingested drugs.	**37**
													Organ characterization (CD86 +)	Immunostaining		
		Immune system	Cells in suspension	U937									Analysis of drug metabolites	LC-MS/MS		
	Activation of anti-cancer pro-drug by liver models	Liver	Ex vivo rate tissue	rLiMTs	N.A.	N.A.	Multi-OoC plate	PDMS on Glass	Proprietary Liver microtissue medium	Y	8 d	Off- line	Albumin production	ELISA	Activation by the liver required for activation of the pro-drug cyclophosphamide	32
													Quantification of pro-drug and its metabolites	LC-MS/MS		
		Colorectal cancer	Spheroids	(eGFP)-HCT116								*In situ*	Tumor spheroid size	Microscopy		
													Characterization of organ models (DPPIV/CD6; actin/nuclear stain)	Immunostaining		
													Cell viability	ATP assay (CellTiter-Glo) and PI staining		
		Intestine	Monolayer on glass or Transwell	Caco-2	N.A.	N.A.	Multi-OoC plate	PDMS PC membrane Glass	Medium 670	Y	3 d	Off-line	Cell proliferation	AlamarBlue assay	Successful emulation of the processes of absorption (intestine), metabolism (by liver) and cell killing for tumor cells and connective tissues.	34
		Liver		HepaRG									Drug concentration and its metabolites	LC-MS/MS		
		Colon cancer		HCT-116								*In situ*	Cell viability	Live/dead staining		
		Connective tissue		TIG-121									Quality of the intestine epithelium	TEER		

	Evaluation of the efficacy and side-effects of drugs on multiple organs	Liver	Organoids	HA/gelatin hydrogel with liver ECM solution	Human primary hepatocytes hHSteC Kupffer cells (80:10:10 ratio)	Endothelium (HMVEC-L) grown in lung module below the Transwell membrane	N.A.	Multi-OoC chip	PDMS on glass PET porous membrane Capillary connection	α-MEM	Y	9 d	Online	Stability of Heart model	Beat rate analysis	Response to drug depending on tissue-tissue interactions; simultaneous effect of drug efficacy and side-effects on other organs	94
		Heart		Fibrin-gelatin hydrogel	Human iPSc-derived cardiomyocytes hPCF (90:10 ratio)								Off-line	Cell viability	Live/dead staining		
														Metabolic profiling	LC-MS/MS		
		Lung		Multi-layer culture on ECM-coated membranes	AMSC NHBE (layer by layer)									Secretion of inflammatory products (IL-8 & IL-1beta) by lung tissue	ELISA		
														Biomarker detection	Electrochemical impedance spectroscopy (EIS)		
	Proof-of-concept study to demonstrate the capability of a Lego Plug-and-Play system for Multi-OoC studies	Multiple heart models	Multilayer		Human iPSc derived cardiomyocytes	N.A.	N.A.	Multi-OoC chip	PDMS on glass	RPMI 1640 (+B27, Insulin)	N	3 d	In situ	Cell viability	Live/Dead assay	Successful maintenance of multiple heart tissues in one platform.	43
														Functionality of the cardiac tissues	Bright field imaging		
	Patient-specific Multi-OoC model prepared using iPSCs from the same healthy donor	Intestine	Transwell Spheroids		Human iPSC derived intestinal organoid Human iPSC derived stromal cells	N.A.	N.A.	Multi-OoC Plate	PDMS on glass PC membrane	HepaRG medium	N	14 days	Off-line	Characterization of organ models (Liver: albumin, ZO-1,4 alpha, SLC10A1, Cyt 8/18, Vimentin, Ki67; Intestinal: CdX2, Na+/K+-ATPase, Cyt 8/18, Vimentin, ZO-1, Ki67; Kidney: Cyt 8/18, vimentin, Aquaporin 1, Na+/K+-ATPase, ZO-1, Ki67; Brain: TUBB3, PAX6, nestin, TBR1, MAP2, ZO-1, Ki67)	Immunostaining	Patient-on-a-chip platform Successful maintenance of three organs in differentiated state for 14 days using one single medium; no differentiation of kidney model.	46

													Gene expression level	qPCR		
		Liver	Spheroids	Human iPSC derived hepatocytes Human iPSC derived stromal cells									Tissue differentiation	RNA sequencing		
													Metabolic activity	Glucose assay		
		Brain	Spheroids on Transwell	Human iPSC derived neuropsheres									Tissue viability	LDH assay		
		Kidney	Monolayer on porous membrane	Human iPSC derived renal cells									Cell apoptosis and proliferation	TUNEL assay & Ki67 staining		
	Reconstruction of complex metabolic interactions in a liver-kidney model, and the activation of leukemia cells by the metabolized drug	Liver	Monolayers on collagen coated surface	HepG2	N.A.	N.A.	Multi-OoC plate	COP	DMEM (high glucose no supplement)	N	24 h	Off-line	CYP mRNA expression analysis in all organ models	RT-qPCR	Successful emulation of metabolism of vitamin D3 by the liver and its bio-activation by the kidney; enhanced expression of Vit D3 metabolizing enzymes	35
													Differentiation of HL60 cells	Flow cytometry analysis		
													Analysis of Vit D3 metabolism and fate of its metabolites	LC-MS/MS		
		Kidney		RPTEC									Production of albumin by liver model	ELISA		
												In situ	Functionality of kidney model	Albumin uptake (FITC-albumin); fluorescence microscopy		
	Body-on-a-chip platform with pumpless medium perfusion	Liver	3D scaffolds	Human primary hepatocytes Non-parenchymal cells (primary human fibroblasts hHSteC, Kupffer cells, hLSMECs, vascular, biliary epithelial cells) (5:3 ratio)	Endothelial cells added to liver models	N.A.	Multi-OoC chip	3D printed Veroclear polymer PC porous membrane	DMEM # L3SNB-500 (1:1 ratio)	Y	14 d	*In situ*	GI tract epithelium function	TEER	Maintenance of tissue function in the liver-intestine model including enzymatic activity to response to toxicants	44
												Off-line	Liver cell viability	Level of aspartate aminotransferase		
		GI tract epithelium	Transwell-like support	Caco-2									Function of liver tissue	Urea and albumin synthesis		

													Liver tissue response to toxicants	Cyt P450 enzyme activity assay		
	ADME profiling with repeated dose systemic toxicity testing of drug candidates	Intestine	Reconstructed model (EpiIntestinal™)		Innate vascularization of skin (*ex vivo* tissue)	N.A.	Multi-OoC plate	PDMS on Glass PET porous membrane	Small intestine culture medium (+glucose, human serum) HepaRG medium, (+glucose, human serum) Proximal tubule cell medium (+glucose)	Y	28 d	Off-line	Cell viability	LDH assay	Reproducible homeostasis of all organs within 2- 4 days Maintenance of tissue functionality over a period of 28 days	45
		Liver	Spheroids	HepaRG hHSteC (24:1 ratio)									Cell metabolism (Glucose consumption & lactate production)	Dedicated colorimetric assays		
													Cell function (albumin synthesis)			
		Kidney	Differentiated "3D" epithelium	RPTEC/TERT-1									Gene expression level (Intestine: SGLT1/SLC5A1, Na-K-ATPase, MDR1; Kidney: SGLT2/SLC5A2, Claudin 10, TJP3/ZO-3; Liver: Albumin, BSEP/ABCB11, GSTA2, CPY3A4, UGT1A1, MRP2)	RT-qPCR		
		Skin	*Ex vivo* human biopsy Air-liquid interface culture in a Transwell										Tissue-specific markers (liver: Cyt P450 3A4; skin: CK 10 & 15; intestine: CK 19)	IHC		
													Kidney epithelium: CK 8/18 & NaK-ATPase	Immunostaining		
												In situ	Barrier function of the intestine	TEER		
	Parallel assessment of drug efficiency (3-organ model) and toxicity on multi-organoid models (6-organ models)	Liver *3 and 6 organ platform*	Organoids in HA/gelatin hydrogel	Human primary hepatocytes hHSteC Kupffer cells LECs (75:10:10:5 ratio)	Vascularized organoids and endothelium in 6-organ model (HUVEC cells in hydrogel)	N.A.	Multi-OoC plate	Adhesive film on glass	Testis organoid media EGM media (without FBS) (1:1 ratio)	Y	14 d	*In situ*	Cell and organoid viability	Live/Dead staining	Successful metabolization of the alkylating prodrug isfomamide by the liver to induce neurotoxicity.	13
		Heart *3 and 6 organ platform*		Human iPSCs derived cardiomyocyte Cardiac fibroblasts Cardiac ECs (75:20:5 ratio)												
		Lung		A549 *(3-organ model)*												

		3 and 6 organ platform		Lung fibroblasts HBEC (80:20 ratio)												
		Testes *6 organ platform*		SSC Leydig Sertoli cells (80:10:10 ratio)												
		Brain *6 organ platform*		Primary HBMEC HBVP, hA, HM, HO, HNC (30:15:15:5:15:20 ratio)												
	In vitro Multi-OoC model to provide quantitative PK/PD data	Gut	3D culture (villi) on porous membrane	Caco-2 BBe	Organ-specific microvasculature incorporated in all organs	N.A.	Multi-OoC chip	PDMS PET membrane	Common "blood substitute": DMEM F12 EGM-2 (+growth factors) Specific medium for each organ parenchymal compartment	Y	10 d	*In situ*	Barrier permeability	Translocation of fluorescent tracers	Excellent prediction of PK parameters for nicotine (oral administration) and the anti-drug cancer cisplatin (intravenous injection). Agreement with cisplatin PD data with data acquired on patients.	40
		Liver	Transwell-like support	Human primary hepatocytes primary hLSMECs										TEER		
		Kidney		primary hRPTECs								Off-line	Liver function (Albumin secretion)	ELISA		
													Cytochrome P450 CYP3A4 activity	Dedicated assay		
													Quantification of CYP2A6 and P-glycoprotein	Western-Blot BCA assay		
		Bone marrow	3D cell culture in fibrin gel (apical)	Human primary bone marrow CD34+ progenitor cells									Analysis of bone marrow cells	Flow cytometry		
													Quantification of Nicotine and its metabolites	LC-MS/MS		
	Establishment of a Multi-OoC platform with 4, 7 or 10 organ models, or physiome-on-a-chip for quantitative pharmacology study	Liver/Immune *4, 7 and 10 organ platform*	3D culture on scaffold	HPH Kupffer cells (10:1 ratio)	N.A.	N.A.	Multi-OoC plate	PSF on acrylic PU membrane	Mixed medium (N/A ratio)	Y	14 d 21 d 28 d	Off-line	Organ function Liver (albumin); endometrium (IGFBP-1); pancreas (C-peptide); muscle (myostatin)	ELISA	Maintenance of phenotypic markers for 2 weeks (*4 organ platform*) Robust operation and maintenance of phenotypic function for 3 weeks (*7 organ platform*) Maintenance of phenotypic function for 4 weeks and PK analysis	39
		Lung *4, 7 and 10 organ platform*	ALI	NHBE												
		Gut/Immune *4, 7 and 10 organ platform*	Transwell	Caco-2 or C2BBe1 HT29-MTX-E21 (9:1 ratio) Dendritic cells (basal side)												
		Endometrium *4, 7 and 10 organ platform*	Multilayer culture tHESCs in PEG hydrogel,	Ishikawa cells tHESCs												

			Ishikawa cells on hydrogel Transwell												(*10 organ platform*)	
		Brain *7 and 10 organ platform*	3D culture *(7 organ platform)*	NPCs									Brain function (N-acetylaspartate)			
			Transwell *(10 organ platform)*	Human iPSCs derived astrocytes and neurons									Pharmacokinetics of anti-inflammatory drug and its metabolites	LC-MS/MS		
		Heart *7 and 10 organ platform*	Transwell	Human iCell cardiomyocyte 2												
		Pancreas *7 and 10 organ platform*	3D culture PS scaffold *(7 organ platform)* Alginate hydrogel *(10 organ platform)*	Rat pancreatic islets								*In situ*	Cardiomyocyte beating frequency	Video microscopy		
		Kidney *10 organ platform*	Transwell	RPTEC												
		Skin *10 organ platform*	Culture on collagen matrix Air-liquid interface	Human keratinocytes									Epithelial barrier integrity (Gut, lung, skin and kidney)	TEER		
		Skeletal muscle *10 organ platform*	Transwell	Human primary skeletal muscle myoblasts												
Metabolic and multi-organ diseases & Reproductive medicine	Evaluation of the ovarian hormone control of downstream human female reproductive tract and peripheral tissues (liver)	Ovary / follicle *1, 2 and 5 organ platform*	*Ex vivo* tissues	Mouse tissues	Innate vascularization (*ex vivo* tissue)	Hormone stimulation (Continuous perfusion of prolactin during luteal phase day 0-14)	Microfluidic motherboard	-	αMEM F-12 (+BSA, BF, Insulin, Transferrin, Selenium) (1:1 ratio)	Y	28 d	Off-line	Stability of organ models Morphology analysis	Histology	Emulation of endocrine loops between organs. Murine ovarian follicles able to reproduce the 28-menstrual cycle.	51
		Fallopian tube *1, 2 and 5 organ platform*		Human tissues									Oocyte spindle morphology Chromosome alignment Tissue fonction (endometrium Ki67, CK, ER, PR; cervix, PR & Ki67)	Immunostaining		
		Uterus *1, 2 and 5 organ platform*											Stability of fallopian model (OVGP1 and alpha-tubulin)	Immunoblot analysis		

		Cervix *1, 2 and 5 organ platform*												Monitoring of fallopian epithelial cilia beating		
		Liver *1 and 5 organ platform*	Microtissues in alginate or 3D-printed on gelatin scaffolds	Human primary hepatocytes Non-parenchymal cells (Kupffer cells, hLSMECs, hHSteC)								Hormone production (E2 - oestradiol, P4 - progesterone, inhibin A, inhibin B, FSH, hCG) Chemokine (IL8, VEGF-A) Liver function (albumin)	Immunoassays (e.g., ELISA, chemiluminescent assays)		
	Establishing a model for type 2 diabetes	Pancreas (islets of Langerhans)	Human pancreatic islets of Langerhans		N.A.	High glucose stimulation	Multi-Ooc plate	PDMS on Glass	HepaRG medium (without insulin)	Y	15 d	Off-line	Measurement of pancreatic (insulin, glucagon and CK8/18, vimentin and albumin) and liver function (CK8/18, vimentin, albumin, CYP3A4)	Immunohistochemistry	Establishment of a functional coupling, with release of insulin in response to glucose stimulation and enhanced glucose uptake in presence of insulin.	50
													Insulin receptor expression level	RT-qPCR		
		Liver	Spheroids	HepaRG phHSteC (24:1 ratio)									Glucose concentration	Dedicated assay		
													Insulin production albumin expression and AKT expression (phosphorylated vs. non phosphorylated)	ELISA		
	Modeling of the gut-liver-immune axis in a Multi-OoC platform while mimicking ulcerative colitis (UC)	Liver	3D culture on polystyrene scaffold	Human primary hepatocytes Kupffer cells (10:1 ratio)	N.A.	Short chain fatty acids (SCFAs): acetate, sodium propionate, sodium butyrate	Multi-Ooc plate	PSF PET membrane	William's E medium (+cell maintenance supplement pack, IL-2, Hydrocortisone, glucose, insulin)	Y	4 d	Off-line	Liver function (albumin production)	ELISA	New insights into the link between UC, liver function and SCFAs. Impact of SCFAs on UC positive or negative depending on the activation state of the immune system.	47
													Metabolomic analysis; analysis of SCFA concentration	LC-MS/MS		
		Gut	Transwell	Colon organoids prepared from									Cytokine and chemokine analysis	Dedicated multiplexed assay		

				patient biopsy (apical side) Monocytes-derived dendritic cells and macrophages (basal side)									Influence of the organ model interaction and the SCFAs on gene expression level	RNA sequencing		
		Immune system	Cell suspension	CD4+ Treg Th17 (2:1 ratio)									Characterization of organ model (F-actin, CD14))	Immunostaining		
												In situ	Gut model integrity	TEER		