“Allgemeine und systematische Pharmakologie und Toxikologie” WS 19/20

Seminarthema: Chemische Kanzerogenese


Dr. Katharina Ernst

Institut für Pharmakologie und Toxikologie

Raum N26/427, Tel. 0731-50065528, Email: katharina.ernst@uni-ulm.de

Referat 1: Tabak-induzierte Kanzerogenese


In diesem Referat sollen die mechanistischen Grundlagen der Tabak-induzierten Kanzerogenese, die wichtigsten kanzerogenen Inhaltsstoffe des Tabak-Rauchs und die gesundheitliche Bedeutung von E-Zigaretten erläutert werden.

Referat 2: Alkohol-induzierte Kanzerogenese

Innerhalb des Referates sollen die verschiedenen Mechanismen der Alkohol-assoziierten Kanzerogenese erarbeitet werden. Ebenso soll kurz auf epidemiologische Grundlagen und genetische Faktoren eingegangen werden.

Referat 3: Humane „organs-on-chips“ in der Krebsforschung

In diesem Referat soll ein Überblick über den Stand der Technik im Bereich Organ-Chips in der Krebsforschung gegeben werden. Die in der Literatur beschriebenen Modelle zur Untersuchung verschiedener Schritte der Krebsentstehung (Tumorwachstum & Expansion, Angiogenese, Tumorzellinvasion und Metastasierung) sollen vorgestellt werden.
Preclinical development of cancer therapeutics commonly involves testing of potential anticancer agents in tumour cell cultures, but developmental therapeutic programmes do not advance without results from animal models. Animal studies often involve treating tumours implanted subcutaneously in rodents, but these models are widely accepted to be poor models of human cancer, as they lack features of the native tissue-specific microenvironment. In response, there has been a move to implant human tumour xenografts in mice at the same organ site from which the tumours were derived because these in vivo orthotopic cancer models have been shown to better mimic tumour growth and metastasis. Nevertheless, orthotopic mouse models still do not recreate the tissue–tissue interfaces, organ-level structures, fluid flows and mechanical cues that cells experience within living organs, and furthermore, it is difficult to collect samples from the different tissue microcompartments. In this Review, we outline how recent developments in microfluidic cell culture technology have led to the generation of human organs-on-chips (also known as organ chips) that are now being used to model cancer cell behaviour within human-relevant tissue and organ microenvironments in vitro. Organ chips enable experimentalists to vary local cellular, molecular, chemical and biophysical parameters in a controlled manner, both individually and in precise combinations, while analysing how they contribute to human cancer formation and progression and responses to therapy. We also discuss the challenges that must be overcome to ensure that organ chip models meet the needs of cancer researchers, drug developers and clinicians interested in personalized medicine.
layers of purified extracellular matrix (ECM) molecules (for example, collagen or fibronectin) or a 3D ECM gel composed of collagen or Matrigel with medium present on both sides. Tumour cell trans-endothelial migration assays can be performed in a similar fashion with the exception that a confluent monolayer of endothelial cells is cultured on one side of the ECM-coated surface of the membrane before initiating the experiment. 3D cancer cell spheroids also have been developed that can recapitulate cell–cell and cell–ECM interactions between tumour cells and the surrounding tumour microenvironment, and if grown large enough, spheroids may generate oxygen and nutrient gradients that result in formation of a necrotic core similar to that observed within central regions of poorly vascularized tumours. But neither transwell models nor spheroids reproduce the complexity observed in the 3D tissue architecture of living organs or incorporate mechanical forces (for example, fluid shear stress, hydrostatic pressure and tissue deformation) that can substantially influence cancer cell behaviour. Moreover, neither of these models is perfused by blood or nutrient-rich medium flowing through an endothelium-lined vasculature, which results in a lack of important tissue–tissue interactions and an inability to study recruitment of circulating immune cells, as well as physiological dosing of test therapeutic agents.

Organoid culture technology, which involves culturing either normal or cancerous epithelial stem cells isolated from patients in ECM gels to form self-organizing organotypic structures called organoids, has recently emerged as a new in vitro tool for drug discovery and personalized medicine. However, organoids are commonly closed structures, which precludes direct experimental access to the epithelial lumen, and again, they lack the tissue–tissue interfaces between epithelial tumour cells and the surrounding vasculature and stroma that are important contributors to cancer control and progression. Furthermore, none of these models retain normal mechanical cues, which cells experience within whole organs (for example, breathing motions in lung or cyclic peristaltic deformations in intestine), that also can contribute to tumour behaviour.

This Review outlines a new approach to modelling cancer based on the recent development of microfluidic organ-on-chips (also known as organ chips) that enable experimentalists to recapitulate the multicellular architecture, tissue–tissue interfaces and the physiologically relevant physical microenvironment of cancers growing within living human organs while sustaining vascular perfusion in vitro (Fig. 1). These microfluidic cancer organ chip models also enable the use of a synthetic biology approach in which important molecular, biochemical, cellular and tissue components can be varied individually and in combination in a controlled manner to gain further insight into human cancer pathophysiology in an organ-relevant context. We first review how microfluidic culture models have been used to study key steps in the cancer cascade, including angiogenesis, tumour progression, expansion, invasion and metastasis, and thereby enable in vitro analysis of how local microenvironmental cues and chemical gradients influence these processes. We then describe the development of orthotopic human primary and metastatic cancer models in which organ-specific cancer cells are grown in organ chips lined by normal human cells from the same primary organ or a distant organ site where these lesions form in vivo and how they recapitulate cancer behaviours within primary tumours and metastatic lesions observed in human patients. We also discuss how cancer organ chip models are being used to assess responses of human cancers to drug therapies, as well as how these studies have led to new insights into the mechanisms by which the cellular, biochemical and physical microenvironment cues modulate tumour sensitivity and resistance to therapy. Finally, we examine the challenges that must be overcome for organ chip technology to be more centrally integrated into cancer research programmes and drug development efforts, as well as how it may enable the study of metastatic spread in fluidically linked multi-organ chips and advance personalized medicine programmes by incorporating patient-derived cells.

Organ chip technology

Organ chips are microfluidic cell culture devices composed of optically clear plastic, glass or flexible polymers, such as polydimethylsiloxane (PDMS), that contain perfused hollow microchannels populated by living cells that recapitulate in vivo organ-level physiology and pathophysiology by recreating tissue-level and organ-level structures and functions in vitro. They are called ‘chips’ because they were initially fabricated using micromanufacturing methods adapted from computer microchip fabrication (Box 1). Microfluidic devices can be used to create ‘tissue chips’ with a single channel lined by cells from one type of tissue or more complex organ chips that combine two or more tissue types that can be interfaced directly across a porous ECM-coated membrane or separated by an ECM gel that fills one or more microchannels. The viability of the cells can be maintained over extended time periods (weeks to months) by flowing culture medium through either the endothelium-lined vascular channels, parenchymal channels or both. Furthermore, when organ chips have been supported with culture medium through endothelialized vascular channels, the culture medium can be replaced by whole blood, at least for a few hours of culture.

One of the most complex organ chips contains individual parenchymal and vascular microchannels separated by a porous ECM-coated membrane with organ-specific epithelial cells on one side and stromal cells (for example, endothelium, fibroblasts or immune cells) on the other (Box 1), thereby recreating tissue–tissue interfaces that are crucial for reconstituting organ-level structures and functions. The cells cultured on either surface of these membranes deposit their own basement membranes that span across the numerous pores that are large enough for cells to extend processes through, and form direct contacts with, the ECM of the adjacent tissue. These devices also may be flexible and contain hollow side chambers through which cyclic suction can be applied to rhythmically stretch and relax the organotypic tissue interfaces, thereby mimicking organ-relevant mechanical cues, in addition to replicating...
air–liquid interfaces (ALIs), fluid flow and associated physiologically important shear stresses.

By recapitulating the multicellular architectures, tissue–tissue interfaces, chemical gradients, mechanical cues and vascular perfusion of the body, these devices produce levels of tissue and organ functionality not possible with conventional 2D or 3D culture systems. They also enable high-resolution, real-time imaging and in vitro analysis of biochemical, genetic and metabolic activities of living human cells in a functional human tissue and organ context. Numerous organ chip microdevices have been created that reproduce structural, functional and mechanical properties of key functional units of whole human organs, including lung alveoli and bronchioles, kidney tubules, and glomeruli, small intestine, liver, bone marrow and the blood–brain barrier (BBB). Importantly, these organ chip microfluidic devices can reproduce organ-level responses to drugs, toxins, radiation, cigarette smoke, pathogens and the normal microbiome, as well as flow-dependent recruitment of circulating immune cells and organ-specific inflammatory reactions in vitro. Organ chips can also effectively mimic many types of organ-specific disease states, including Barth syndrome, pulmonary oedema and thrombosis, chronic obstructive pulmonary disease and inflammatory bowel disease, and as a result, these devices are currently being used for drug development and new therapeutic discovery.

Models of the cancer cascade

Some of the earliest applications of microfluidic cell culture technology focused on modelling specific steps in the cancer cascade, including tumour growth and expansion (Fig. 2), angiogenesis (Fig. 3), progression from early to late stage lesions involving an epithelial–mesenchymal transition (EMT), tumour cell invasion (Fig. 4) and metastasis (Fig. 5).

Tumour growth on-chip. Organ chip models have been used to identify how neighbouring normal parenchymal cells (for example, epithelial cells, endothelial cells, stromal cells and immune cells) and ECM in the local tissue microenvironment can influence the growth of various types of cancer. For example, co-culture of T47D human breast carcinoma cells with immortalized human mammary fibroblasts (HMFs) and different combinations of various ECM components in a microfluidic device revealed that HMFs promote increased growth of breast cancer cell clusters, especially in fibronectin-rich ECM compared with monolayers. This platform also facilitated the analysis of the morphology and growth of T47D cell clusters in response to broad-spectrum inhibitors of matrix metalloproteinases when cultured alone or with HMFs under different ECM conditions. Oestrogen receptor-α (ERα) expression in tumour cells is a defining feature of hormonally responsive tumours and is used in the clinic as a predictive measure of the effectiveness of hormonal therapies. As metastatic tumours exist in a microenvironment different from the primary tumour, the correlation between ERα expression and tumour cell growth stimulation is not well established. Leveraging the ability of microfluidic devices to model interactions between breast cancer cells and relevant stromal microenvironments of both primary and metastatic breast cancer sites also revealed a previously undescribed correlation between the growth of breast cancer cells and ERα protein downregulation. A more recent version of this microfluidic model that uses a heterotypic co-culture approach including up to three different cell types (breast cancer cells, stromal cells and monocytes) in combination with gene expression analysis uncovered how the different cell types interact through paracrine signalling via production of transforming growth factor-β (TGFβ) by breast cancer cells and expression of corresponding receptors by stromal cells.

In another study, the rapid growth of human non-small-cell lung cancer (NSCLC) cells was found to depend entirely on local microenvironmental factors produced by normal lung alveolar epithelial cells and lung endothelial cells that were interfaced across a porous ECM-coated membrane and cultured under an ALI within a lung alveolus chip (Fig. 4); the same cancer cells failed to grow when cultured alone in the same medium on a standard plastic culture dish. Specifically, in this lung cancer chip, conditioned medium from the healthy alveolar epithelium was shown to be sufficient to promote cancer cell proliferation even though secreted factors from the endothelial cells partially suppressed tumour growth. Interestingly, when cyclic strain was exerted on the alveolar–capillary interface of the lung alveolus chip by applying cyclic suction to hollow side chambers of the flexible device to mimic physiological breathing motions, cancer growth was inhibited by 50%. This was found to be mediated by mechanical strain-induced changes in epithelial growth factor receptor (EGFR) phosphorylation, which also resulted in increased resistance to the third-generation tyrosine kinase inhibitor rociletinib. These responses, which could not have been identified without the use of this type of organ chip technology,
Neovascularization. Angiogenesis — the outgrowth of new capillary blood vessels from existing blood vessels — is a crucial step in the cancer cascade, as the initiation of neovascularization is required for precursor lesions to progress from hyperplasia to neoplasia and hence represents an important control element restricting cancer progression and growth. Multiple microfluidic angiogenesis models have been developed that recreate capillary sprouting and vessel formation in vitro using engineered microvessels. This might explain why rampantly growing lung cancers that impinge on alveolar movement can respond to therapies, as well as why resistance to therapy is high in patients with cancer with minimal residual disease and restored lung functionality. Collectively, these studies are excellent examples of how microfluidics may be used to investigate with high resolution the complex interactions between multiple cancer-associated cell types and ECM molecules that are found in the local tissue microenvironment.

Box 1 | Microfabrication of two-channel organs-on-chips

| Step 1 | Manufacturing of organ-on-chip (organ chip) devices begins with a silicon wafer spin-coated with a layer of an ultraviolet-curable polymer, SU-8. The layer thickness will become the height of the microfluidic channel. |
| Step 2 | A photomask bearing the pattern of a feature that will become the microfluidic channel is placed over the SU-8 layer and exposed to ultraviolet light. The ultraviolet light crosslinks the polymer within the exposed part of the SU-8 layer, solidifying the feature. |
| Step 3 | The wafer is then developed by exposure to a solvent that dissolves the unpolymerized SU-8, leaving behind only solid polymer in the form of the channel feature. |
| Step 4 | Liquid polydimethylsiloxane (PDMS) polymer is then cast on top of the wafer. When the PDMS is baked in an oven, it turns into a clear solid, but flexible, material surrounding the SU-8 channel feature. |
| Step 5 | The PDMS device with a central hollow channel is then pulled off of the wafer, and a biopsy sample punch is used to create fluidic ports. |
| Step 6 | In the final assembly of the two-channel organ chip, a porous PDMS membrane is sandwiched between two PDMS sheets containing channels manufactured as shown in steps 1–5. The porous membrane is created using a similar casting method except that the SU-8 polymer is shaped into multiple tall micropillars to generate the pores in the thin PDMS membrane when it is lifted off the silicon wafer. Different cell types can be cultured on opposite sides of the same porous membrane in adjacent channels to recreate relevant tissue–tissue interfaces (for example, lung alveolar epithelial cells overlaid with air in the top channel and endothelial cells covering all walls of the lower vascular channel, as shown). Once completed and fluidic quality control has been passed (for example, no leakages), the device is ready for cells to be added. |

Cell seeding in a two-channel organ chip

- Before cell seeding, the porous PDMS membrane is coated with extracellular matrix (ECM) by first infusing ECM solutions or gels (for example, Matrigel) through both channels.
- To increase adherence of the ECM molecules to the PDMS membrane, the PDMS surface is either exposed to oxygen plasma or to chemical crosslinkers before ECM addition.
- The channels are then gently flushed with culture medium to remove residual uncoated ECM, and medium containing cells at the appropriate density is introduced into the lower and upper channels and allowed to adhere for a few hours under static conditions before tissue-specific medium at physiological flow rates is applied.
- Subsequently, the device is connected to a peristaltic pump to continuously perfuse culture medium.
- Devices may also be made with hollow side chambers to which cyclic suction may be applied to rhythmically extend and relax the adjacent channel side walls and attached porous ECM-coated membrane along with the cells.
Parallel endothelium-lined microvessels formed within microfluidic channels that were created by depositing sacrificial materials during gel formation. These materials were solubilized after the gel solidified, and endothelial cells were then plated on the internal walls of these channels and perfused with culture medium to form the microvessels. When tumour angiogenic factors were perfused through one set of these engineered microvessels, capillary sprouts could be observed to extend from neighbouring vessels, eventually linking to establish new functional capillary tubes. This model was also used to investigate the mechanisms of action of angiogenesis inhibitors, including the vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) inhibitor semaxanib and the sphingosine-1-phosphate receptor (S1PR) inhibitor fingolimod as well as angiogenesis stimulators, such as VEGF, monocyte chemotactic protein 1 (MCP1; also known as CCL2), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF)47.

In vitro models of one of the most aggressive forms of brain cancer, glioblastoma, are frequently designed to investigate brain tumour cell–endothelial cell interactions because this form of brain cancer can become vascularized to such an extent that it resembles an arteriovenous malformation in vivo. One method used to study these interactions physically separates the different cell types via micropillar partitions within a microfluidic device. For example, human umbilical vascular endothelial cells (HUVECs) and malignant human U87MG glioblastoma cells have been co-cultured on opposing sides of a central fibrin gel-filled channel within a microfluidic device53. Interestingly, HUVECs cultured in devices with the cancer cells exhibited faster initial growth upon seeding than those without cancer cells, and this was presumably caused by factors secreted by the tumour cells. The sustained outgrowth of the HUVECs was similar in devices with and without cancer cells.
Angiogenesis is the process during which endothelial cells in pre-existing vessels sprout to form new blood capillaries. Vasculogenesis occurs when dispersed, singular endothelial cells or endothelial progenitor cells coalesce to assemble into functional microvessels. Angiogenesis and vasculogenesis are induced by growing solid tumours to ensure continuous delivery of nutrients and oxygen, which are required for tumour growth and progression.

A schematic of a microfluidic device that enables analysis of sprouting capillary and new microvessel formation during angiogenesis in vitro is shown (left). This angiogenesis chip is filled with an extracellular matrix (ECM) gel (in this case, collagen type I) in which hollow microchannels have been created using sacrificial materials. Endothelial cells are cultured on the inner surface of one microchannel perfused with medium, whereas a parallel channel is perfused with medium containing angiogenic factors (for example, hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), monocyte chemotactic protein 1 (MCP1), vascular endothelial growth factor (VEGF) and sphingosine-1-phosphate (S1P)). This results in multicellular sprout-like structures extending into the intervening ECM gel from the endothelium-linked channel. These extending capillary sprouts eventually form functional connections with the angiogenic factor source channel. The functional perfusion of this entire engineered microvascular network was confirmed using time-lapse imaging of 3 μm red fluorescent beads perfused through the vessels. This device was also used to quantify inhibition of angiogenic sprouting using inhibitors of VEGF receptor 2 (VEGFR2) signalling, such as semaxanib (indicated by the schematic to the right).

A schematic of a microfluidic device for generating and studying self-organized microvascular networks during vasculogenesis in vitro is shown (left). The device contains four medium-containing reservoirs, two gel loading ports that fill three diamond-shaped cell chambers with ECM gel and two cell loading ports that are used to introduce endothelial cells (derived from endothelial colony-forming cells isolated from human cord blood) into the system. The endothelial cells migrate along the laminin-coated microfluidic channel and spontaneously form a capillary network inside the ECM gel within the diamond-shaped chamber. The functional integrity of the network was validated by perfusing the microvessels with medium containing 15 μm fluorescent particles. Parts b (left) and c are adapted with permission from REF. 47, PNAS, and REF. 52, Royal Society of Chemistry, respectively, and part d is reproduced from REF. 97, CC-BY-4.0 (https://creativecommons.org/licenses/by/4.0/).
cells; however, when co-cultured with cancer cells, the microvessels formed from endothelial cells exhibited more frequent tortuous branching, with atypical fusions between neighbouring cells, which are features similar to those observed in the tumour microvasculature in vivo.

Another microfluidic angiogenesis model used primary human clear cell renal cell carcinoma cells to naturally produce a gradient of angiogenic stimuli that induced co-cultured HUVECs to extend capillary sprouts, an angiogenic response that could be blocked pharmacologically. In a different microfluidic device, an ECM gel was placed between a channel lined by endothelium and a parallel channel containing chemotactants to analyse trans-endothelial cell migration of neutrophils in situ in response to chemical gradients. This study showed that the number of neutrophils migrating through the endothelial layer is mainly influenced by the concentration of VEGF rather than other chemokines.

Microfluidic models of vasculogenesis — the process by which multiple endothelial progenitor cells coalesce to form new blood vessels — have been created. These contain a series of multiplexed chambers, with each chamber being filled with a single ECM gel containing multiple dispersed endothelial cells derived from endothelial colony-forming cells isolated from human cord blood that spontaneously self-assemble into a network composed of functional branching capillaries. This model has been used to study how fluorescently labelled tumour cells interact with capillary networks and how each cellular component influences the behaviour of the other, including vessel permeability as well as cancer cell intravasation and extravasation, as described in more detail below.

### Cancer progression and the epithelial–mesenchymal transition

Microfluidic cancer organ chip models have been used to study steps during cancer progression that are accompanied by an EMT. Co-culture of multiple human pancreatic cancer cell lines and human HT-29 colon cancer cells with their corresponding cancer-specific fibroblasts, a human pancreatic stellate cell line and normal human CCD-18Co colonic fibroblasts revealed that these stromal cells are able to induce EMT in the cancer cells. For these studies, a microfluidic device containing multiple parallel channels was loaded with tumour spheroids in the central channel, medium was flowed through the surrounding channels, and fibroblasts were cultured in the outer channels. This setup allowed close proximity co-culture without direct cell contact to investigate the role of soluble factors. Induction of EMT occurred only when the fibroblasts were cultured in close proximity to the cancer cells, and interestingly, induction of this mesenchymal phenotype diminished the response of the cancer cells to chemotherapy paclitaxel treatment. A microfluidic 3D model with epithelial ovarian cancer NIH:OVCAR5 cells that were grown in clusters similarly revealed that the gene expression and morphology of the cancer cells shifted towards an EMT phenotype, including down-regulation of E-cadherin and upregulation of vimentin, when cultured under continuous fluid flow. Thus, the presence of a dynamic fluid stream more like that observed within tissues in vivo induced a switch to a motile and aggressive ovarian cancer phenotype that could not otherwise have been observed in traditional 2D cultures.

In addition to modelling the EMT, microfluidic devices have been implemented to investigate epithelial tumour cell–stromal cell interactions during progression from dysplasia to invasive carcinoma. A novel way to implement a side-by-side co-culture of two different cell types in two different adjacent ECM gels is to leverage the laminar flow properties of microfluidic devices that restricts two incoming fluid streams from mixing. By seeding one stream with HMFs in a liquid ECM solution and another with breast ductal carcinoma in situ (DCIS) cells (a xenograft-derived cell line, MCF-DCIS) in a different liquid ECM solution and subsequently gelating both solutions, it was possible to create a culture in which the two cell types were able to communicate chemically and physically. Using this approach, it was revealed that the transition to an invasive phenotype occurs only when fibroblasts are in direct contact with the tumour cells.

### Cancer cell migration and invasion

Understanding mechanisms involved in the metastatic spread of primary tumour cells is of great interest, as the growth of metastases substantially worsens the prognosis of patients with cancer. Metastasis is a complex, multi-step process that begins with cancer cells within the primary tumour gaining the ability to invade through the basement membrane that separates the epithelium from the underlying stroma. These invasive cancer cells must then migrate through the interstitial ECM and intravasate into a capillary blood vessel or lymphatic vessel to spread to distant sites. These metastatic cells must survive when circulating through the bloodstream or lymphatic flow and attach to the endothelium at a distant site, where they then need to extravasate from the vessel and implant in the interstitium of another organ to establish a new metastatic tumour.

The use of microfluidic culture technologies has unveiled the contributions of different cell types to tumour cell migration and invasiveness. For example, a microfluidic chip equipped with pneumatic micro-valves was used to analyse the paracrine loop between human CL1-0 lung adenocarcinoma cells and MRC-5 lung fibroblasts. TGFβ secreted by the cancer cells was shown to stimulate the fibroblasts to transform into myofibroblasts, which then produced soluble factors that fed back to increase the migration speed of the cancer cells. Similarly, when primary human macrophages were co-cultured with human MDA-MB-231 breast or PC3 prostate tumour cells or MDA-MB-435S melanoma cells in a microfluidic device, cytokines secreted by these immune cells increased cancer cell migration speed and persistence. When studies were carried out with two-chamber organ chips, one lined by prostate cancer cells and the other by either normal fibroblasts or cancer-associated fibroblasts (CAFs), the chip with the CAFs was found to increase fibronectin fibril alignment,
which was shown to provide paths for directed cancer cell migration.70.

Tumour cell invasion also has been studied on-chip by measuring invadopodia formation and ECM degradation by human lung cancer A549 cells growing within an ECM gel-filled microfluidic channel.71 In another study, metastatic human MDA-MB-231 breast cancer cells and mouse RAW 264.1 macrophages were placed within neighbouring ECM gels composed of collagen type I and Matrigel, respectively, within a microfluidic device. The macrophages invaded into the gels when the breast cancer cells were also cultured in the device but not when the cancer cells were absent.72 In a different four-channel microfluidic device, HUVECs were cultured in an outer channel, next to a channel containing collagen matrix with or without different subtypes of human monocyte-derived macrophages, adjoining another collagen channel with A549 lung adenocarcinoma cell aggregates with or without macrophages that was adjacent to a medium channel.73 This co-culture system revealed that when macrophages that were induced to form M2A macrophages were in direct contact with A549 cancer cell aggregates, the result was both an induction of an EMT (as detected by downregulation of E-cadherin) and increased cancer cell dispersion. Furthermore, this study also provided a possible mechanism for proximity-induced, contact-dependent dissemination of cancer cells and an EMT that is mediated

**Invadopodia**
Dynamic actin-rich cell membrane protrusions that can be associated with cancer cell invasion.

**M2A macrophages**
A subpopulation of alternatively activated macrophages that are associated with a T helper 2 cell immune response and are induced as a result of stimulation by the cytokines interleukin-4 (IL-4) and IL-13 or during fungal and helminth infections.
Orthotopic invasive versus dormant lung cancer organs-on-chips.

Schematics are depicted showing that cancer cells can either proliferate randomly and invade the epithelium, underlying basement membrane and endothelium or remain dormant, often depending on the tissue location of the cell of origin. Primary lung adenocarcinoma cells from a patient with non-small-cell lung cancer (NSCLC) cultured in the lung alveolus chip and small airway chip recapitulate the growth patterns observed in patients, exhibiting preferential growth in the alveoli rather than the bronchioles, and similarly, cancer cells grew more slowly or remained dormant in the small airway chip. Fluorescently labelled H1975 NSCLC cells were cultured with either primary lung alveolar epithelium or bronchiolar epithelium on the upper surface of an extracellular matrix (ECM)-coated porous membrane within a two-channel microfluidic device, whereas primary lung microvascular endothelial cells were grown on the lower surface of the same membrane to recapitulate the human alveolar–capillary interface. Air was introduced into the upper channel to create an air–liquid interface, and the entire culture was maintained by perfusing culture medium exclusively through the lower vascular channel, as occurs in vivo. Studies were carried out with or without applying cyclic suction to parallel hollow chambers within the alveolus chip to rhythmically extend and relax the lateral side walls and attached porous ECM-coated membrane along with the adherent endothelium and epithelium, which mimics physical cues associated with physiological breathing motions in the lung.

Fluorescently labelled tumour cells in these cultures were imaged live by confocal microscopy, which revealed that mechanical breathing motions in the lung alveolus chip inhibit both cancer cell growth and invasion through the pores of the ECM-coated membrane and into the vascular channel below. Imaging of implanted clusters of green fluorescent protein (GFP)-labelled NSCLC cells revealed that the cancer cell mass did not substantially expand in size when viewed at 1, 14 and 28 days after addition to the lung small airway chip. Part b is adapted with permission from REF. Elsevier.

Collectively, these studies have led to potentially important new insights into how cancer cell invasive behaviour could be controlled, for example, by targeting stromal cell interactions with cancer cells; however, without including more components of the TME (for example, other stromal cells and multiple types of immune cells), it is difficult to say whether these observations will translate to novel therapeutic approaches.

Other studies have leveraged organ chip technology to study the effects of mechanical forces on cancer cell migration and invasion. For example, normal prostate tissue-associated fibroblasts (NAFs) were cultured on a membrane in a flexible, two-channel, microfluidic device that can be stretched by applying a vacuum to hollow side chambers. Application of mechanical strain altered the structure of fibronectin, secreted by the NAFs, from a random, mesh-like structure to a more organized, linear-aligned form, which caused co-cultured human SGC61 head and neck squamous cancer cells to migrate in a more persistent manner. Interestingly, the phenotype of the stretched NAFs is similar to that displayed by CAFs, suggesting that mechanical stress associated with tumour expansion may contribute to the formation of CAFs via activation of normal fibroblasts.

The effects of mechanical forces on cancer cell invasion were also studied in the two-channel lung cancer chip described above that detected suppression of tumour cell growth when physiological breathing motions were mimicked (Fig. 6). Importantly, the presence of pores at defined positions in the ECM-coated membrane separating the epithelium and endothelium enabled direct visualization and quantification of tumour cell invasion through this tissue–tissue boundary. Interestingly, much like with the growth response, when cyclic strain was applied to the alveolar–capillary interface to replicate physiological breathing motions on-chip, cancer cell invasion through ECM and the underlying endothelium was also inhibited by 50%. Taken together, these studies emphasize the importance of varying the physical properties of the cancer microenvironment when trying to model and study cancer behaviour at the tissue and organ level using organ chips.

Metastatic cancer cell intravasation and extravasation. Different types of cancer vary in their ability to metastasize, and, thus, it is of interest to interrogate those with high invasive potential in order to understand the mechanisms that underlie this behaviour. In one study in which a microfluidic device was used to visualize interactions between invasive HT1080 fibrosarcoma cells and human microvasculature endothelial cells, it was found that treatment of the endothelium with tumour necrosis factor (TNF) resulted in more rapid and substantially increased numbers of tumour cell–endothelial cell attachment events (Fig. 5). Thus, this work suggests that changes in tumour cell–endothelial cell dynamics, which may promote cancer cell intravasation and/or extravasation during metastatic spread, can be regulated by soluble factor signals produced by tumour cells and transmitted to the endothelium.

The permeability of the endothelial barrier differs between normal and tumour vasculature, which in turn determines the ability of tumour cells to intravasate and extravasate. The increased permeability of tumour vasculature is a result of the secretion of cytokines and chemokines by cancer cells. This feature of the TME was replicated using a perfused microfluidic platform containing a vascular compartment with primary human breast tumour-associated endothelial cells that formed a vascular network separated by a porous interface provided by micropillar arrays from a tumour compartment. The permeability of the vessels (measured with small molecule fluorescent tracers) significantly increased in response to the presence of either tumour cells or tumour cell-conditioned medium. The increase in permeability of the endothelium was greater when the endothelial cells were co-cultured with metastatic MDA-MB-231 breast cancer cells than with non-metastatic MCF-7 breast cancer cells. Moreover, this response was associated with increased disruption of both tight junctions and adherens junctions by the metastatic cancer cells, which has also been observed in vivo.

Analysis of circulating tumour cell (CTC) spread in vivo has revealed that these disseminated tumour cells preferentially adhere to endothelium within organs that express high levels of the CXC-chemokine ligand 12 (CXCL12; also known as SDF1). Importantly, this phenomenon could be reproduced in vitro using a multi-layered microfluidic device containing MDA-MB-231 breast cancer cells in medium flowing on top of an endothelium-covered nanoporous polyester membrane, beneath which CXCL12 was perfused to stimulate the in vivo setting. Using a simple microfluidic system with a peristaltic pump, other researchers found...
that higher levels of shear stress consistent with those experienced in the circulation during exercise induced high rates of apoptosis of CTCs compared with the lower shear stresses present in human arteries at the resting state. Interestingly, this could be one of the benefits of regular exercise\(^8\).

Microfluidic models also have been used to identify tumour cell integrins, secreted factors and ECM components that play an essential role in organ-specific cancer cell extravasation across endothelium-lined vessels in vitro. One microfluidic device contained a central channel with an interconnected microvascular network embedded in a bone-mimicking or muscle-mimicking microenvironment\(^6\). The bone environment was produced by seeding the central channel of a three-channel device with ECM containing HUVECs, which formed a perfusable microvascular network, primary human bone marrow-derived mesenchymal stem cells (BM-MSCs) and osteoblasts; for the muscle microenvironment, the ECM gel was populated with HUVECs and C2C12 myoblasts. Medium containing metastatic breast cancer cells was flowed through the outer endothelium-lined channels of the device, and their extravasation capability was investigated. The extravasation rate was much higher in the bone microenvironment than in the one mimicking muscle, an effect that could be explained by the higher vessel permeability of the bone environment than the muscle. The addition of adenosine, a nucleoside present in the TME, decreased the ability of the breast cancer cells to extravasate into the bone matrix while blocking the adenosine A3 receptor increased cancer cell extravasation into the muscle microenvironment, indicating a role for adenosine in reducing cancer cell extravasation\(^9\).

In a similar study, extravasation was modelled by flowing MDA-MB-231 breast cancer cells through an interconnected microvascular network formed by HUVECs in the central channel of a microfluidic device. This model revealed that β1 integrin expression is required for cancer cells to be able to invade through the endothelial basement membrane\(^9\). Another vascularized microfluidic model showed that primary human monocytes are able to directly reduce MDA-MB-231 breast cancer cell extravasation in a non-contact-dependent manner but likely mediated by paracrine signalling through cytokines and chemokines. Once the monocytes transmigrate through the vessel wall and exhibit features of differentiated macrophages, they have little effect on cancer cell extravasation\(^9\). This finding is important, as it could be exploited to develop novel anti-metastatic therapeutic strategies. In addition, both cancer cell intravasation\(^7\) and extravasation\(^8\) have been analysed in a more relevant 3D context in vitro by
incorporating tumour cells into on-chip vasculogenesis models that contain ECM gels.

**Orthotopic cancer organ chips**

Given the success in creating organ chip models that mimic the structure and physiology of various human organs, these microfluidic culture devices also have been exploited to create in vitro human orthotopic cancer models. For example, in the lung alveolar chip model described above, cells from an adenocarcinoma form of human NSCLC that is known clinically to emerge in the distal region of the small airway but grows preferentially within alveoli in patients\(^8\) were implanted orthotopically within an alveolar epithelium. Under these conditions, the NSCLC cells grew rapidly\(^9\). However, when these same NSCLC cells were cultured in an orthotopic human lung small airway chip lined with a human small airway epithelium cultured under an ALI and adjoining a perfused lung microvascular endothelium, the characteristic dormant growth patterns of the adenocarcinoma were faithfully recapitulated in vitro\(^9\).

In another study, an orthotopic model was created to study the growth of the plasma cell malignancy multiple myeloma because culture and survival of these cells depend on local cues that are present in only the bone marrow microenvironment\(^3\). The perfusion-based microfluidic device was designed to incorporate a pre-made bone-like scaffold achieved by culturing osteoblasts in the microfluidic chamber for 4 days before use; this scaffold supported the subsequent seeding of bone marrow mononuclear cells (BMMCs) taken from patients with multiple myeloma. Importantly, this microenvironment was found to sustain the proliferation of multiple myeloma cells through interactions with BMMCs and osteoblasts, thus confirming that the endosteal surface is critical for multiple myeloma cell growth and maintenance\(^4\). Using another perfusion-based microfluidic device, patient-derived CD138\(^-\) mononuclear cells were co-cultured with primary CD138\(^+\) multiple myeloma cells from the same patient, and this platform was used to develop an in vitro chemosensitivity and resistance assay capable of predicting non-responders to the proteasome inhibitor bortezomib\(^5\). The tumour cell response to bortezomib segregated into clinical response clusters only when the patient-derived multiple myeloma cells were grown with the CD138\(^-\) mononuclear cells and did not correlate if the cells were cultured by themselves.

Organ chips have been used to generate orthotopic models of metastatic lesions as well, which have enabled insight into how these distant tumours respond to microenvironmental cues. Bone is a common site of metastasis for nearly every type of cancer and one that ultimately leads to substantial morbidity\(^6\). For this reason, many groups are interested in understanding how the bone microenvironment influences growth of metastatic cancers. In one study that applied organ chip technology to investigate this phenomenon, human BM-MSCs were seeded in a collagen gel solution within a microfluidic channel and allowed to differentiate into osteocytes, at which point adjacent channels were seeded with endothelial cells\(^7\). When breast cancer cells were flowed through the vascular channels, the rate of extravasation of the breast cancer cells into the engineered bone environment and the distance migrated were much greater than with the collagen gel matrix alone. Furthermore, once the cancer cells had migrated into the bone matrix, they formed viable micrometastases\(^8\). Results obtained with this organ chip cancer model also suggested that interactions between the osteocyte-derived chemokine CXCL5 and the breast cancer cell-expressed CXC-chemokine receptor 2 (CXCR2) may mediate primary breast cancer metastasis to bone\(^9\). In other similar studies, breast cancer cells were observed to self-organize into micro-colonies, and this was associated with a reduction in osteoblastic tissue thickness as well as an increase in osteoclastogenesis, reminiscent of the vicious cycle of bone degradation observed in bone metastases in vivo\(^10,11\). Moreover, using a BBB chip, other researchers have discovered that astrocytes similarly influence the ability of cancer cells to metastasize into the brain parenchyma\(^12\).

**Modelling responses to cancer therapies**

Many groups have used microfluidic culture devices to analyse how microenvironmental factors influence tumour cell responses to anticancer therapies. When A549 lung alveolar epithelial cancer cells were exposed to the anticancer drug tirapazamine (which is converted from an inactive to an active free radical form in low-oxygen conditions) under various oxygen gradients created within a microfluidic device, increased drug-induced cancer cell killing under hypoxia was detected\(^13\). This study provides an excellent example of how precise control over chemical (for example, oxygen) gradients, made possible using microfluidics, can be used to gain new insight into fundamental processes involved in cancer growth control and therapeutic targeting. A simple microfluidic device containing 3D chambers seeded with A549 lung cancer cells connected to a chamber seeded with CAFs, and fluidically linked to a linear microfabricated concentration gradient generator, also revealed that production of HGF by CAFs inhibits paclitaxel-induced apoptosis in lung cancer cells\(^14\).

Functional analysis of hundreds of patient-derived, single chronic myeloid leukaemia (CML) cells or normal haematopoietic stem cells (HSCs) using a microfluidic culture platform revealed tumour cell-specific responses to the US Food and Drug Administration-approved tyrosine kinase inhibitor dasatinib. HSCs exhibit a substantially elevated cell death rate compared with CML cells; however, dasatinib markedly reduces CML cell migratory behaviour by disrupting SRC kinase-mediated signalling pathways\(^15\). In another study, a lung cancer cell line, a mixture of lung cancer and stromal cell lines, or cells from fresh lung cancer tissues were cultured in 3D gels within a microfluidic device and exposed to different concentrations of chemotherapeutic agents generated on-chip using a microfabricated concentration gradient generator\(^16\). Using this device, the sensitivities of different anticancer drugs were assayed in parallel, and optimized doses of single drugs (either the targeted EGFR inhibitor gefitinib or the broader

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**Endosteal surface**

A layer covering the endosteum, which is a thin membranous tissue containing bone stem cells, blood vessels and connective tissue fibres that coats the inside of long bones and surrounds the bone marrow-filled medullary cavity.
A microfluidic model was used to identify drugs that are cytotoxic effects on normal epithelial cells, whereas the model, the anticancer drug paclitaxel had negligible endothelial cells. Interestingly, the cancer cells showed on breast or lung cancer cell aggregates and surrounding tested the effects of alternating electrical field therapy vasculature. Another interesting model first cultured as VEGFR2 and PDGFR, which led to regression of the vascular networks in this model because they targeted between drugs that were not effective in disrupting the for survival. This microfluidic device could distinguish on nutrient delivery through living perfused microvessels human lung fibroblasts into 3D microtumours that rely human colorectal or breast cancer cells and normal tissue interfaces and an in vivo-like microenvironment is for the evaluation of anticancer therapies.

Standard of care cytotoxic therapies, such as 5-fluorouracil, or vascular-targeting agents have been evaluated by developing organ chips that incorporate human colorectal or breast cancer cells and normal human lung fibroblasts into 3D microtumours that rely on nutrient delivery through living perfused microvessels for survival. This microfluidic device could distinguish between drugs that were not effective in disrupting the vascular networks in this model because they targeted only VEGFRs and drugs that had multiple targets such as VEGFR2 and PDGFR, which led to regression of the vasculature. Another interesting model first cultured human LS174T colon carcinoma cells as spheroids and then transferred them to microfluidic devices and exposed them continuously to flowing medium to mimic chemical gradients surrounding blood vessels in the TME of a solid tumour. This device was used to both measure the diffusion coefficient of the chemotherapeutic agent doxorubicin and quantify the accumulation of a therapeutic bacterium, Salmonella enterica subsp. enterica serovar Typhimurium, that was introduced into the model. Finally, a novel approach using microfluidics tested the effects of alternating electrical field therapy on breast or lung cancer cell aggregates and surrounding endothelial cells. Interestingly, the cancer cells showed reduced proliferation and lower metastatic potential, as measured by cell dispersion, when exposed to these fields, while the endothelial cells were not substantially affected.

Nanoparticle-based therapeutics are playing an increasing role in the development of novel anticancer therapies. An essential first step is the evaluation of safety and efficacy both in vitro and in vivo. A microfluidic co-culture device containing breast cancer 4T1 cells and endothelial cells was utilized to assess the penetration of therapeutic nanoparticles from the vascular compartment into cancerous cell clusters under physiological flow. This study revealed that, even though nanocrystals loaded with the cancer drug camptothecin penetrated the cancerous cell mass only superficially under flow, the cytotoxic effect of camptothecin could be observed throughout the cancer cell mass, suggesting that drug delivery to the exterior of a tumour cell mass is sufficient to enable diffusion throughout.

Delivering drugs and antibodies over the BBB for the treatment of brain tumours and metastases still poses a great challenge. A two-channel microfluidic device was created in which HUVECs were grown under flow conditions in an outer compartment while either rat brain astrocytes (to mimic the BBB) or metastatic HER2 (also known as ERBB2)- mouse breast cancer cells (to mimic the blood–tumour barrier) were cultured in the inner compartment under static conditions. When the monoclonal therapeutic antibody trastuzumab, which targets HER2, was perfused through the outer chamber, there was a considerable uptake of the antibody into the inner compartment under both BBB and blood–tumour barrier conditions; however, the quantities were small and likely to not be efficacious. Importantly, similar results were obtained in a mouse study carried out in parallel. Furthermore, both the in vivo and the in vitro studies showed that there was no clear correlation between the size of the metastases or metastatic cell clusters and antibody uptake across the blood–tumour barrier. This study emphasizes that there is still a great need to develop suitable preclinical models, including human-relevant BBB models, to aid in the development of more effective therapeutic monoclonal antibodies.

Microfluidic technology also has been used to develop a preclinical in vitro tool to evaluate the antitumour efficacy of T cell receptor (TCR)-redirected engineered T cells for cancer immunotherapy, which typically requires the use of animal models. In this study, human HepG2 liver carcinoma cells were cultured either dispersed or as aggregates within a collagen matrix within one channel of a microfluidic device, and engineered T cells were introduced in the adjacent channel to investigate their ability to migrate from the medium channel into the solid 3D matrix and kill the tumour cells. This microfluidic model was able to detect that lower oxygen levels (2%) led to reduced killing of dispersed cancer cells by engineered T cells compared with higher levels of oxygen (20%) and that the addition of the inflammatory cytokines interferon-γ (IFNγ) or TNF significantly increased killing of cancer cell aggregates by the engineered T cells.

Another group used an intrahepatic microfluidic model of hepatitis B virus (HBV)-related hepatocellular carcinoma to investigate the immunosuppressive potential of monocytes towards HBV-specific TCR-engineered T cells and the role of programmed cell death protein 1 (PD1)–PD1 ligand 1 (PD1L) signalling because monocytes have been reported to impede natural T cell function through modulation of PD1–PD1L signalling in the TME. The central channel of a three-channel...
microfluidic device was filled with a hydrogel containing embedded HepG2 cancer cell aggregates transduced with a construct containing the gene of the preS1 portion of the envelope protein from HBV genotype D, while the outer channels were filled with medium containing engineered T cells. This microfluidic model was able to identify a suppressing effect of monocytes via PD1–PD1L, whereas this was not the case in a 2D model. Collectively, these two studies demonstrate the potential of using microfluidic tools to predict TCR-engineered T cell efficacy in a preclinical setting.

Taken together, these findings demonstrate that microfluidic organ chip technology can be used to screen novel types of anticancer molecular, cellular and nanotechnology-based therapies, optimize treatment parameters and investigate effects of combination therapies in an in vivo-like TME. In particular, an application where organ chips could be particularly helpful in the future is in the development and tuning of new tumour-penetrating therapeutics (especially nanoparticles and nanotherapeutics), as the ability to quantify the diffusion of therapeutics into different tumour microcompartments will be essential. Given the ability to modulate fluid flow in vascularized organ chips, it may be possible in the future to explore how cancer therapy responses are potentially influenced by organ-specific differences in physiological blood flow rates.

**Pros and cons of cancer organ chips**

The key to success in developing any organ chip model is to focus on mimicking organ-level physiology or pathophysiology observed in vivo. Validation of these cancer organ chip models will require demonstration that they effectively mimic cancer behaviours and drug responses observed in vivo. Examples of cancer organ chips that do mimic cancer phenotypes and responses to therapy seen clinically in human patients already exist; however, this field is young, and this type of validation should continue to be the benchmark for success. Future researchers who seek to develop cancer organ chip models should also consider deeply which specific pathophysiological processes they desire to model before initiating their studies. The fact that microfluidic models, by definition, provide perfusion (for example, continuous, cyclic or intermittent) offers a major advantage over static models, including transwells, spheroids and organoid cultures. This is especially important in the context of studies that focus on neo-vascularization, invasion and cancer cell dissemination, as well as for maintaining cell viability and functionality over extended time periods. Inclusion of a perfused endothelium-lined vasculature also offers greater clinical relevance for studying drug delivery, as well as modelling pharmacokinetics and pharmacodynamics (PK–PD), which are greatly influenced by drug transport into and across the vascular endothelium. The ability to establish controlled chemical gradients, and ALIs to replicate organ-relevant mechanical environments, as well as fluid shear stresses and hydrostatic pressures that exist in the in vivo TME represents an additional major advantage of organ chips, as does the ability to collect samples selectively from either the parenchymal, stromal or vascular compartments for further molecular, cellular, genetic or pharmacokinetic analysis.

At the same time, however, organ chips are more difficult to use than many other 3D culture systems, and they usually have a lower throughput than can be obtained with highly multiplexed static culture chambers. Indeed, in terms of the time it takes to carry out an assay, the level of throughput, their high content screening and their physiological relevance, it is perhaps more appropriate to think of organ chips that contain multiple cell and tissue types as replacements for animal studies instead of conventional culture assays. Technical robustness is another challenge, as the small scale and complexity of microfluidic systems that experience controlled fluid flow require that many factors must interplay perfectly to achieve optimal functionality, and simple factors, such as bubble formation, can ruin an experiment. For long-term studies, there is the challenge of maintaining cell viability and functionality and structural integrity of multiple tissues and different cell types using a common media, stable ECM coatings and consistent fluid flow. In the past, it was necessary to have specialized equipment, microfabrication facilities and engineering knowledge to create and use these devices in cancer research laboratories. However, with the introduction of multiple commercial sources of mass-produced organ chips and user-friendly automated instruments for culture and fluidic control, the ease of use should considerably increase over time. In the end, it will be the ability of cancer organ chips to better mimic human cancer pathophysiology than other models that justifies the extra effort or cost involved in their use.

**Future considerations**

Human cancer organ chips have the potential to transform cancer drug development by better predicting clinical trial outcomes than current animal models. Testing anticancer drugs using organ chips could provide greater insight into molecular mechanisms of both drug action and toxicities before these candidates enter clinical trials. Validation of cancer organ chips as effective preclinical models will require studies with multiple different types of cancer drugs that demonstrate similar efficacies and toxicities in organ chips to those observed in vivo and with similar pharmacokinetics. Interestingly, many biologics, such as therapeutic monoclonal antibodies being developed by the biotechnology industry, are now so specific that they do not cross-react with related proteins in other species, and, therefore, their evaluation in preclinical animal models is extremely challenging if not impossible; therefore, human cancer organ chip models offer a potential solution to this problem. Normal organ chips also may be used in parallel studies to define toxicity mechanisms that emerge during clinical trials, which were not previously observed in preclinical animal models. This latter point was recently demonstrated in studies with a monoclonal antibody intended for the treatment of autoimmune disorders that resulted in the adverse event of lung thrombosis; this drug led to unexpected deaths in phase I clinical trials, which could be mimicked using organ chips but not by animal models.
Cancer organ chip models also offer the possibility of elevating personalized medicine to a higher level by more faithfully recapitulating patient-specific, organ-level, cancer pathophysiology and responses to therapy. However, there are still many technical challenges along the way before patient-matched, tumour-derived, organ chips can be integrated into the clinical arena. As a first step, cancer tissue xenografts from patient biopsy samples have been cultured in microfluidic devices for patient-specific drug evaluation and have shown promising results. Nevertheless, one of the main hurdles to developing patient-specific cancer organ chip models that enable the sophisticated level of analysis described above is the need to separate and isolate all the cell types (cancer cells, endothelial cells, stromal cells and immune cells) from the same patient and then to build organ chips with the appropriate cell types in the correct relative proportions and location to accurately mimic in vivo behaviours and responses. Each of these cell types requires specific isolation protocols and culture conditions to guarantee the maintenance of cell-specific functionality. Furthermore, the meaningful integration of multiple different cell types into microfluidic devices can be time consuming and requires thorough optimization. Only when this can be done correctly and rapidly, and has been validated against known patient responses, will it be possible for cancer organ chips to be used to evaluate anticancer therapies for individual patients in a robust, effective and meaningful manner.

Another potential limitation in the field is that the most common material used to fabricate organ chips is PDMS; although it has excellent optical clarity, flexibility and gas permeation properties, PDMS is also known to nonspecifically absorb small molecules, including certain drugs. Despite recent studies using PDMS-based organ chips for PK–PD studies showing that these...
Potential absorption problems can often be circumvented using experimental measurements combined with computational modelling\textsuperscript{119}, there is still a need for new materials in this field. Various thermoplastics (for example, polyurethanes, styrene ethylene butylene styrene (SEBS), cyclic olefin polymers and copolymers) are being explored\textsuperscript{109–111}, but to realize the full potential of mechanically actuable organ chips, novel materials that are both flexible and optically clear while minimally absorptive of drugs and cell nutrients will need to be developed. For example, the development of fully degradable porous membranes and their integration into organ chips could increase direct tissue–tissue interactions to establish if this influences the cancer phenotype. Recent advances in 3D bioprinting also open up possibilities of creating complex perfused tissue chips, in which cells interact without any artificial membrane\textsuperscript{112}.

Finally, given recent advances in fluidically coupling multiple human organ chips to create human body-on-chip models\textsuperscript{113–117}, we begin to consider the possibility of creating multi-organ models of metastatic spread of cancer (FIG. 6). One possibility would be to create an orthotopic primary tumour model in one organ chip and then analyse how tumour cells intravasate into the endothelium-lined vascular channel, survive in the flowing medium (or whole blood), extravasate into a second fluidically linked organ chip and establish a dormant or active metastatic lesion at this distant site. This type of model could be used to dissect the cellular and molecular mechanisms involved in all these stages of cancer metastasis. The same approach could be coupled with normal organ chips that are often targets of drug toxicities (for example, bone marrow, liver, kidney and heart) to evaluate both efficacy and specificity of novel cancer therapies, such as adaptive engineered immune cells, monoclonal antibodies, nanotherapeutics or small molecule inhibitors, in vitro in completely humanized systems to improve safety and reduce costs associated with novel treatments entering clinical trials. Given that these multi-organ chip models have already been used to model human drug PK–PD parameters in vitro\textsuperscript{105,115,118,119}, this approach also could be used to establish initial dosing schedules in humans and thereby shorten phase I clinical trial times\textsuperscript{120}. This possibility is supported by a recent study that showed that drug combinations could be screened using microfluidic devices containing cells from patient biopsy samples of solid tumours for less than US$150 per patient\textsuperscript{121}. However, many challenges remain, including overcoming culture medium incompatibility problems between multiple organ chips, devising experimental designs that ensure optimal organ-specific differentiation protocols over different time frames in parallel, developing instrumentation that can fluidically link and sustain the viability of multiple different types of organ chips, developing physiologically relevant linking protocols that replicate fluid flow between different organs in the human body and creating approaches for on-chip imaging and non-invasive sample collection and analysis over extended time periods.

**Conclusion**

One of the great strengths of organ chips is that they provide a way to carry out synthetic biology at the cell, tissue and organ levels. Using this technology, one can start simple and study the effects of a single microenvironmental component or drug on cancer cells, cultured either alone or in various combinations with normal epithelial cells, fibroblasts (or CAFs), endothelial cells, immune cells, numerous mechanical cues and even microbiota, while visualizing cellular responses in real time at high resolution. It is also possible to carry out multi-omics analysis and to use primary cells or induced pluripotent stem cells isolated from patients to build these chips. All these features provide an unprecedented ability to identify key molecular, chemical, cellular and biophysical contributors to human cancer formation and progression under controlled conditions in vitro. Use of this synthetic biology approach with orthotopic organ chips has enabled the discovery of novel mechanisms of cancer progression and led to new insights into the control of tumour growth and invasion, which could not have been discovered using traditional in vitro systems. The integration of human organ chips into drug development pipelines as well as clinical care holds great potential if we can overcome the near-term challenges relating to simplifying their fabrication and use and increasing their robustness. The recent commercialization of these technologies by multiple companies should enable many academic, industrial, government and clinical groups to explore the value of this approach in their own laboratories\textsuperscript{106}.

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This study describes the development of a human lung microphysiological system that can be used to evaluate the efficacy and toxicity of an anticancer drug.


This study describes the generation of an early-stage breast cancer organ-on-a-chip that can be used to study cancer cell extravasation.


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