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Microfluidic technologies for *ex vivo* tissue biopsies: A review

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ABSTRACT

Traditional *in vitro* models and animal models often lack the physiological complexity or the accuracy to obtain predictive responses that are clinically translatable to humans. With the advent of microphysiological systems over recent years, new models that are able to mimic human biology more closely have been developed. The culture of whole tissue samples within microfluidic devices promises to bridge preclinical and clinical research, and has the potential to be applied in personalised medicine, environmental sciences or the food industry. However, many challenges must be addressed in terms of tissue maintenance *ex vivo* or methods for analysing samples, particularly in real-time. In this review, we explore the microfluidic strategies that have been reported for the culture of tissue biopsies *ex vivo* and the different techniques that have been explored in order to expand their life span, control the microenvironment and interrogate the samples. Current challenges facing the field are also discussed.

1. Introduction

There is a growing acceptance of microfluidic models that aim to recapitulate human physiology outside the body amongst different stakeholders. These platforms hold the promise to speed up discovery science and drug development, and reduce the costs and difficulties involved in studying such a complex system as the human body. The drug discovery and development process is costly, lengthy, and inefficient (Wong et al., 2019; Wouters et al., 2020). It takes an average of 12–13 years from the discovery and isolation of a compound to its release onto the market, at an estimated cost of €1926 million (DiMasi et al., 2016); with only one or two out of 10,000 substances that start the process successful (European Federation of Pharmaceutical Industries, 2019). This is generally due to the high attrition rates in late development stages, even though half of all the funding allocated by companies for Research and Development is invested in them (European Federation of Pharmaceutical Industries, 2019). The failure of potential therapies at this stage is mainly due to efficacy and safety issues (Kola and Landis, 2004) and methods for screening compounds in the preclinical phases fail to predict the drug effects observed in humans (Seyhan, 2019). Thus, there is a need to improve these models, to increase their predicting power, translatability into the clinical stage and reduce the attrition rates.

1.1. Traditional research models

Model systems that aim to mimic the physiological environment enable researchers to measure responses that would not normally be accessible. *In vivo* models using animals aim to reproduce physiological systems with high accuracy, but they assume that the animal is a good predictor of the human response. In contrast, 2D *in vitro* models enable the use of human cells (normal or disease-derived) and are able to predict a response upon variation of an experimental parameter; however, they are limited by their lack of physiological complexity. Research models are greatly dependent on their context of use and are generally relevant for the specific question they are trying to answer.

Animal models are widely used for research and play a pivotal role in the understanding of pathological and non-pathological processes. They

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provide complex dynamic environments that include factors such as circulation, hormones and mediators to allow for a complete study of the interactions of different organs and the test substances. Furthermore, animals, such as rodents, can be genetically modified to reproduce certain human diseases, thus partially recapitulating the human condition. Animal models have been instrumental in the development and testing of vaccines against many pathogens leading to the eradication of polio and saving many millions of lives from infection with other pathogens (Garattini and Grignaschi, 2017). Although they are more holistic than many *in vitro* models, animals do not always capture the human physiology and pathophysiology needed to represent the clinical observation. Species differences in physiology, metabolism and signalling pathways can lead to responses not relevant to humans, both in terms of false positives and false negatives. This can result in costly failures at clinical trials (Astashkina et al., 2012). This is the case for asthma, where murine models are commonly used, yet there are important differences between human and murine lungs in terms of development and structure and mice do not develop asthma symptoms spontaneously or long-lasting bronchoconstriction. Animals are typically from a single genetic background and are exposed to a single environmental allergen which is unlike the human disease where multiple gene-environment interactions contribute. Following sensitisation and challenge, animal models can replicate some aspects of asthma such as allergic airway inflammation and epithelial hypertrophy. However, the drugs that were developed to target particular immune pathways failed to reach the clinic due to lack of efficacy in humans (Blume and Davies, 2013). Additionally, parametric analysis is significantly more challenging because all the bodily processes occur simultaneously, and it is difficult to identify and isolate the effect of a substance on its target. It is also important to recognise that there are pressing ethical concerns regarding the use of animals with efforts to replace and reduce their use as research models, where scientifically justified.

Traditional *in vitro* cell culture models commonly use dissociated cells cultured in planar, monolayer monocultures, in homogeneous environments with periodic media changes at discreet time points. They have provided invaluable insight into biological, physiological and pathological processes (Astashkina et al., 2012). They enable the investigation of specific cellular processes in great detail, providing a deeper understanding of disease pathogenesis and the investigation of compound effects in an isolated manner. One of the major advantages of cell-based studies is their scalability, with a wide throughput range, from a few screenings to several thousand, useful for both academic and pharmaceutical research. This enables the evaluation of a large number of compounds in a fast, reproducible and cost-effective manner. However, these *in vitro* models greatly depend on their context of use, and the methods to create them may not always replicate the tissue architecture. This represents the ultimate simplification of the *in vivo* situation and although results can be informative, they can also be misleading or irrelevant to human physiology (Wikswo, 2014). These models are crucial for initial early research but are limited due to poor predictivity so that more complicated models are required to complement and validate the data before translation to clinical trials.

Recently more complex models have been developed using primary or stem cells, co-cultures of multiple cell types or adding a third dimension to the cultures to reproduce more closely the *in vivo* morphologies (stratified barrier models or organoids) (Benam et al., 2015; Shamir and Ewald, 2014). The most promising of these complex *in vitro* models are microphysiological systems (MPS). These are defined as platforms that include several of the following features: a multi-cellular environment within biopolymer or tissue-derived matrix, a 3D structure, mechanical cues such as stretch or flow, primary or stem cell-derived cells, and/or immune system components (Fabre et al., 2020). These platforms can be used to study interactions and pathological mechanisms, model disease states, help in the discovery, optimisation and selection of potential drug candidates, predict toxicity, dosage, adverse events, efficacy and safety, and also to develop monitorable biomarkers and for personalised medicine (Ekert et al., 2020). They can even be interconnected to study inter-organ interactions to evaluate drug disposition and ADME-Tox profiles (Absorption, Distribution, Metabolism, Excretion and Toxicity). MPS have the potential to overcome many of the current challenges of traditional research models, providing high-throughput testing with a high degree of fidelity. These new models can increase our understanding of human physiology and pathophysiology, resulting in an improvement in the success rates of drug development, reducing the overall costs, and making healthcare more affordable and accessible to everyone. Furthermore, they can help physicians in the selection of better treatments and individually focused therapies, opening new avenues for personalised medicine. The most common MPS platform is the organ-on-chip, a field that has grown significantly over the last decade (Cavero et al., 2019; Kimura et al. 2018; Wu et al., 2020; Zhang and Radisic, 2017). This review focuses on one aspect of MPS systems - emerging methods and techniques for long-term culture of whole tissue biopsies in microfluidic devices otherwise known as tissue-on-chip.

2. Tissue-on-chip technologies

Organ-on-chip is a fit-for-purpose MPS model containing living, engineered organ substructures in a controlled environment. These functional units recapitulate one or more aspects of an organ's dynamics, physiology, functionality and responses *in vivo* with real-time monitoring (Marx et al., 2020; Mastrangeli et al., 2019). However, prediction of these responses remains limited, as the cells exist within an artificial architecture, without a native Extracellular Matrix (ECM) and its unique architecture, nor the cell type diversity found *in vivo*. Culturing tissue slices and biopsies provides a further step in the complexity of organ-on-chip models, as they preserve the original architecture, morphology, ECM and cell heterogeneity. However, the culture of tissue explants has proven difficult and the life span of the biopsy samples is generally very limited. This is due to many reasons, predominantly the lack of vascularisation caused by the extraction of the tissue from the body; nutrient and oxygen supply and waste removal that occurs only through diffusion. Thus, the inner layers do not receive enough nutrients, reducing the viability of the sample. It is known that culturing tissue samples with continuous perfusion improves circulation in and around the tissue biopsies, improving mass transfer into the tissues, and increasing the viability of the samples compared to static (no-flow) models (Fig. 1) (Fisher et al., 1995; Schumacher et al., 2007). Microfluidic technology provides an ideal means of perfusing tissue samples, increasing the supply of nutrients and oxygen, along with continuous removal of waste. Thus, placing a tissue biopsy in a microfluidic chip (or tissue-on-chip) offers the possibility of extending sample viability and lifetime, with a tight control of the microenvironment, delivering chemicals and treatments with high spatiotemporal resolution, and a means of probing, analysing and monitoring chemical and biochemical responses. An example of this is shown in Fig. 1b where liver slices are grown in conventional static culture and with continuous perfusion. The improvement in culture conditions extends viability for dynamic compared to static culture, seen through the reduction in protein marker expression after 24 h for no-flow conditions.

2.1. Oxygen and nutrient delivery

The culture of tissue slices gained scientific recognition after the early work of Henry McIlwain. In 1957 his group developed a conical glass chamber in which a piece of brain tissue sat at the interface between air and saline solution providing a means of electrical recording and stimulation (Li and McIlwain, 1957). Resting membrane potentials were recorded for the first time from cortical slices *in vitro*. Since then, the standard for brain slice culture was set by Haas et al., in 1979. A tissue slice sits on a nylon mesh where a medium flows by capillarity from the inlet into the slice and out of the device (Haas et al., 1979). This

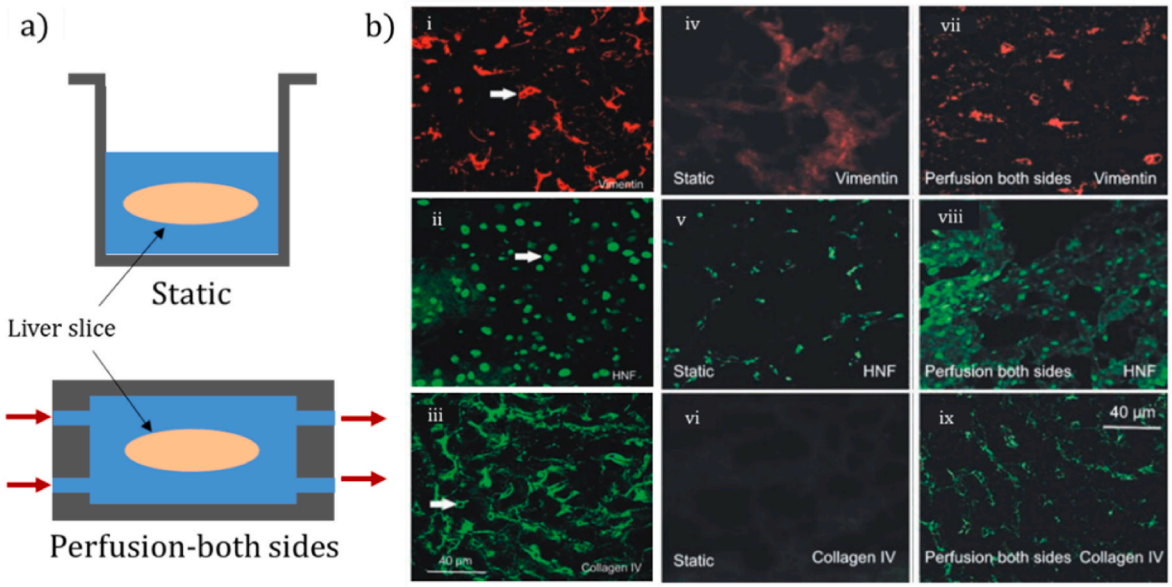

Fig. 1. *a) Comparison of static and dynamic culture (sample continuously perfused). b) Images i), ii) and iii) correspond to freshly isolated liver slices. iv), v) and vi) are liver slices after 24 h with no-flow and vii), viii) and ix) with continuous perfusion (after 24 h). The reduction in protein expression in static cultures indicates the loss of function, changes in phenotype and degradation of ECM, compared to dynamic cultures. Figure b adapted from* (Schumacher et al., 2007) *(with permission of Mary Ann Liebert Ltd).*

work was probably the first example of continuous perfusion, long before the advent of microfluidic technology. The first integration of a brain slice into a microfluidic device was reported by Passeraub et al., where the nylon mesh of the Haas chamber was substituted for an array of microposts (400 µm high) on top of which the tissue sat with cell medium flowing between the posts. Brain tissue slices could be kept viable for 5 h, enough time to record spontaneous activity (Passeraub et al., 2003). However, the short-term viability restricted the number and type of experiments that could be performed, and long-term conditions could not be investigated. Building on this work, Blake et al. developed a device in which a slice was held between two sets of microposts (top and bottom), allowing perfusion from both surfaces simultaneously (Fig. 2a) (Blake et al., 2007). The device subjects different regions of the slice to various environments. However, the tissue only remained viable for 3 h, which may have been due to the thickness of the slice (530–700 µm), which is critical when nutrient and oxygen supply is controlled solely by diffusion. Other approaches for continuous perfusion of submerged tissue samples (Fig. 2b and c) include a system where the tissue sits bathed in media that is continuously refreshed by perfusion and withdrawal of the fluid using microchannels (Bakmand et al., 2015; Cheah et al., 2010; Hattersley et al., 2008; Queval et al., 2010).

To improve the viability of *ex vivo* tissue slices and increase their lifespan, new methodologies have been developed such as precision-cut tissue slices (PCTS). PCTS can be obtained as thin as a few tens of micrometres but to preserve tissue architecture, while ensuring adequate diffusion of nutrients and oxygen, thicknesses of 200–300 µm are more commonly used. de Graaf et al. established a standardised protocol for the culture of liver and intestinal slices *in vitro* (de Graaf et al., 2010), and in 2010 they described a microfluidic system for the maintenance of liver slices to study metabolic activity over extended periods. The device consisted of a droplet-shaped chamber with an inlet at the bottom and an outlet at the top, with the flow directed perpendicular to the surface of the slice. The slices were held in the chamber with polycarbonate filters that also distributed the fluid flow velocity more uniformly (Fig. 2d). The behaviour of slices cultured under continuous perfusion was comparable to standard cultures in static well plates (van Midwoud et al., 2010a). To extend the lifespan of the slices, they were embedded in a hydrogel to avoid disaggregation and attachment to the polycarbonate filters, reducing damage to the tissue. In this manner, the viability of the slices was maintained for 72 h, and even though the metabolic rate decreased over time, it was less pronounced than in static cultures (van Midwoud et al., 2011). Although an improvement, it still could not be used to observe the long-term effects of treatments.

To extend the life of thicker (>300 µm) tissue slices, different perfusion methods have been proposed. Choi et al. developed an intra-perfusion system, in which 400 µm thick brain tissue slices were pinned to an array of microneedles that served as microjets, infusing media directly into the tissue rather than around it (Fig. 2e). They observed that after 4 h, the viability of the static model was almost non-existent while in the perfused sample it was largely intact (Choi et al., 2007). Using the same approach, Khong et al. maintained the viability of liver tissue slices of up to 1 mm thick for 3 days (Khong et al., 2007). The difference in lifespan could be due to the viability assessment methods, the length of the experiments or the sensitivity of the different organs to disruptions and *ex vivo* culture. The slices maintained their metabolic activity with albumin and urea production at values significantly higher than for traditional static cultures. Even though the technique was promising, enlarged interstitial spaces were observed after 3 days of culture, indicating tissue dissociation following injection of media through the needles. Rambani et al. proposed a different strategy using what they called “interstitial perfusion”. The tissue was placed on top of a gold grid in a chamber the exact size of the slice, and fluid was infused from the bottom, forcing it through the tissue and into the top chamber, where the medium was collected (Fig. 2f). 700 µm-thick brain slices could be kept viable for up to 5 days (Rambani et al., 2009).

Some tissues in the body constitute physiological barriers in which each side is exposed to a different environment, for example, the skin, the airways or the intestine. This poses particular challenges when replicating a multiplicity of environments. In the case of the intestine, punch biopsies have been inserted into a chamber the exact size of the biopsy so that each side of the intestine is perfused separately, with different media irrigating the serosal and luminal sides (Fig. 3a) (Dawson et al., 2016; Eslami Amirabadi et al., 2022; Richardson et al., 2020). An interphase system similar to a Transwell™ integrated within microfluidic channels has also been used for continuous perfusion (Fig. 3b)(Baydoun et al., 2020). Yissachar et al., cultured whole gut

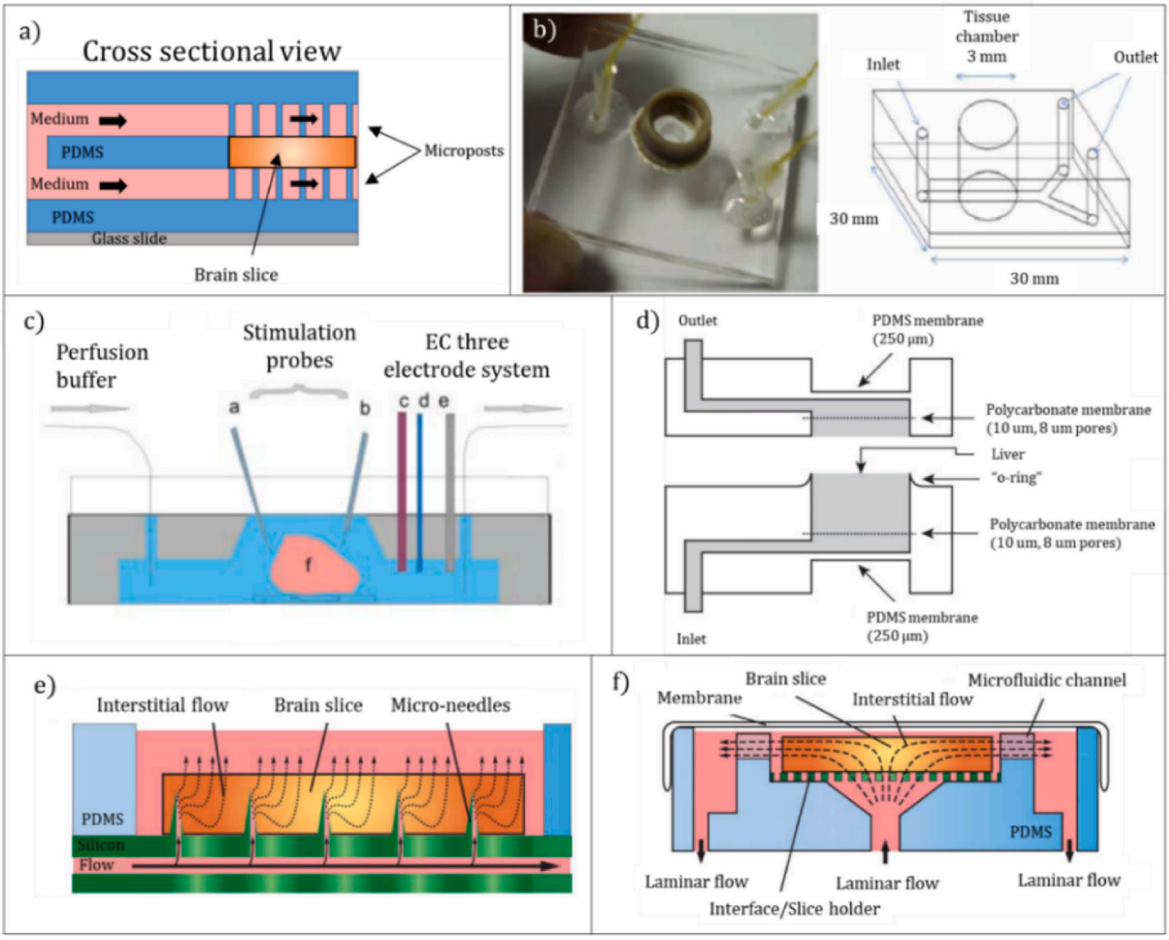

Fig. 2. *Microfluidic devices for continuous perfusion of whole tissue samples. a) This device holds a slice between microposts and the media flows continuously around the top and bottom surfaces. The devices in b) and c) have an inlet and an outlet that provide and remove the media to and from the chamber in which the sample sits* (Hattersley et al., 2008), (Cheah et al., 2010). *d) Perfusion design in which the tissue slices are placed perpendicular to the flow direction. This technique enhances nutrient and oxygen delivery to inner cells* (van Midwoud et al., 2010a). *e) Intra-perfusion device, where the tissue is pinned into the port needles and the media is delivered directly inside the sample, instead of around it. f) Interstitial perfusion device. The media is forced to flow through the slice, flowing from the bottom into a chamber the exact size of the sample slide and exiting from the top* (Huang et al., 2012). *Figures b, c, e, and f adapted from* (Cheah et al., 2010; Hattersley et al., 2008; Huang et al. 2012) *with permission of the Royal Society of Chemistry. Figure d adapted from* (van Midwoud et al., 2010a) *with permission from John Wiley and Sons.*

segments by separately perfusing the sample internally and externally by sewing the excised piece to input and output ports (Fig. 3c) (Yissachar et al., 2017). Other approaches to extend the viability of tissue slices include reducing the temperature to reduce metabolic activity and using UV light to sterilise the medium before perfusion to reduce the presence of bacteria and slow bacterial growth (Buskila et al., 2015). Other more creative solutions include air-propelled fluid circulation (Dondzillo et al., 2015) or maintaining the slice in a perfused droplet (Liu et al., 2016). However, none of the devices reported to date has had a major impact on the field. Tissue-on-chip technology is still emerging and most of these devices are still in the developmental stage, not ready for scalability and commercialisation. Furthermore, every design uses different tissues or tissue regions, from different species and with different thicknesses and sizes. Each assesses the viability with different protocols making it very difficult to directly compare performances and outcomes.

2.2. Control of the microenvironment

The ability to deliver compounds spatiotemporally and thus control the microenvironment can add value to MPS platforms. In the device described above (Fig. 2a), Blake et al., exploited the laminar flow characteristics of their microfluidic device to expose different regions of a brain slice to different microenvironmental conditions, such as Na^+-free or ice-cold solutions, in order to block rhythmic activity in selected regions (Blake et al., 2007) (Fig. 4a). Similar devices have been presented in (Ahrar et al., 2013; Blake et al., 2010; Thomas et al., 2013). In these platforms, relatively large regions are exposed to a specific condition. Others have proposed methods to create gradients across tissue samples using an H-channel (they only used coloured dyes but with the potential to use other molecules) (Tokuoka et al., 2021). However, in some cases, the delivery of compounds is required in much more localised regions. To achieve this, Mohammed et al., presented a system for controlled and localised delivery of factors to the brain slices that could be used as an "add-on" to commercial electrophysiology chambers, expanding their capabilities (Fig. 4b). The design consisted of four microchannels running underneath an "off-the-shelf" perfusion chamber, with ports to deliver fluid from the microchannels into the chamber. This device allowed delivery of factors such as dopamine, while still maintaining viability and allowing electrophysiological measurements. The device provided a new way of controlling the microenvironment and delivering compounds to specific regions of the brain (Caicedo et al., 2010; Mohammed et al., 2008; Ross et al., 2017). Tang et al., developed a similar system but with additional suction ports that held the tissue slice in place without the need for mechanical forces enabling better control of the jets through the ports and improving the spatial resolution

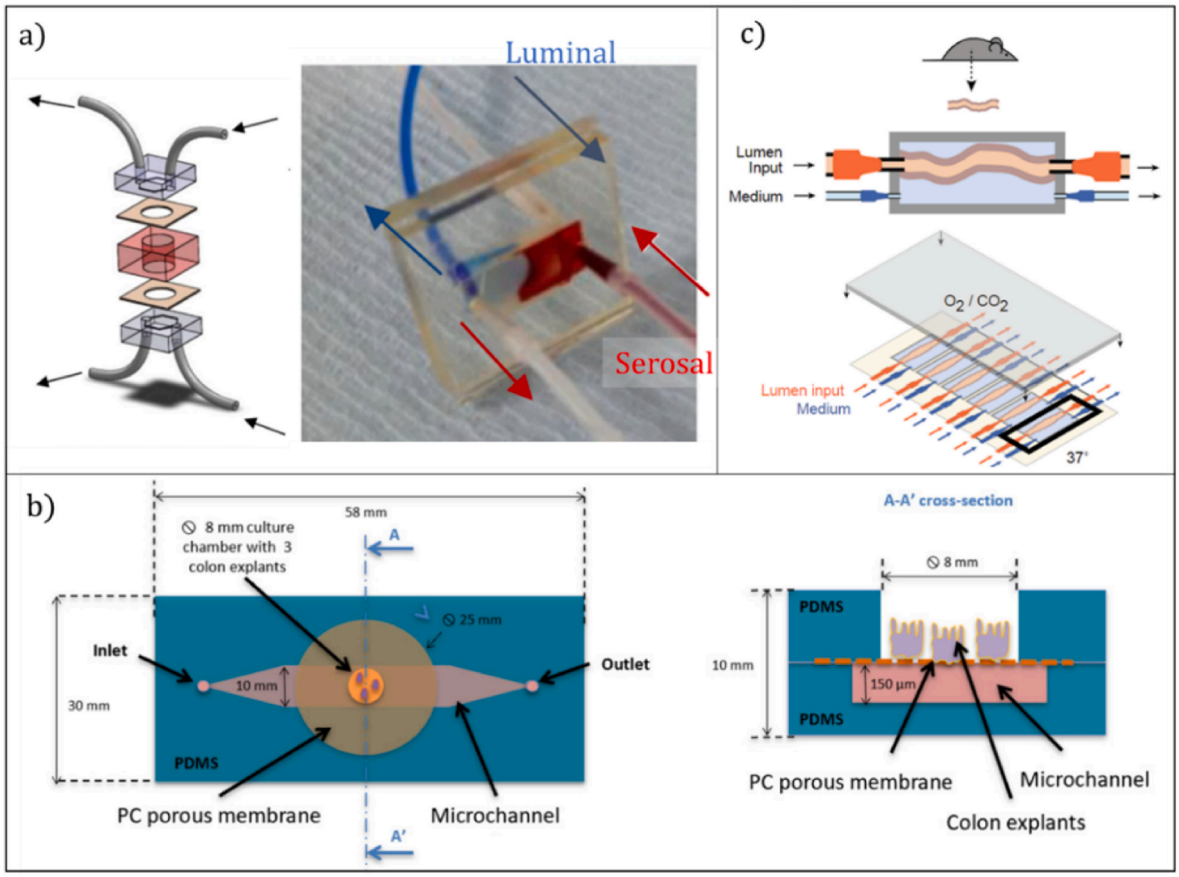

Fig. 3. *Microfluidic devices for the culture of tissue explants of physiological barriers, providing specific environments to each side. a) Intestinal biopsy punch subjected to dual flow. The luminal (blue) and serosal (red) sides are continuously perfused with different media* (Dawson et al., 2016). *b) culture of colon biopsies at an air-liquid interface, with continuous perfusion to the bottom of the sample, maintaining the stratified architecture of the epithelium* (Baydoun et al., 2020). *c) Culture of whole intestinal segments. The lumen is threaded and fixed to connection ports that introduce molecules or microbes into the lumen, and the sample is submerged in media that is continuously refreshed* (Yissachar et al., 2017). *Figure a) adapted from* (Dawson et al., 2016) *with permission from AIP Publishing. Figure c) adapted from* (Yissachar et al., 2017) *with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)*

of drug delivery (Dodson et al., 2015; Tang et al., 2011). In these devices, the tissues are placed manually and exact alignment with the fluidic ports with the regions of interest is difficult and time-consuming, therefore the experimental reproducibility is poor (Fig. 4b). To circumvent this and increase throughput, Catterton et al., created mobile ports beneath the tissue to supply the components to the desired locations (Catterton et al., 2021). A different approach was described by Chang et al., where they placed a brain slice on a removable membrane on top of an array of parallel open channels. The permeable membrane allowed simultaneous perfusion of the slices and exposure to multiple substances in localised regions (Fig. 4c). The compounds could be easily and rapidly changed, and the membrane rotated so that orthogonal exposures could be achieved, enabling several treatments and combinations to be tested on the same piece of tissue (Chang et al., 2014; Horowitz et al., 2020a; Rodriguez et al., 2020).

Van Midwoud et al., sequentially connected two chambers containing an intestinal and a liver slice that directed metabolites produced in the intestine into the liver for further metabolism (van Midwoud et al., 2010b). This replicated the homeostasis of bile acid, with potential for exploration of ADME-Tox studies. Recirculating media through multiple tissue chambers or devices provides homogenous and continuous exposure of the tissue to various factors within the media. The EVATAR system (Fig. 4d) developed by Xiao et al., replicated a human 28-day menstrual cycle hormone profile by interconnecting up to five different tissues from the female reproductive tract (Xiao et al., 2017). TissUse HUMIMIC Chip model combined reconstructed small intestine, liver spheroids, kidney proximal tubules and a skin biopsy with two separated recirculating flows (blood surrogate and excretory). They maintained homeostasis and functionality of the four organs for over 28 days, showing the potential of this platform for the generation of ADME profiles (Maschmeyer et al., 2015).

MPS technologies provide ways of controlling the microenvironment including temperature (Buskila et al., 2015) and dissolved gas concentrations (Mauleon et al., 2012). Oxygen is one of the most important factors for the survival of tissues, and tissue explants in microfluidic devices are usually cultured with standard incubator oxygen concentrations (atmospheric N_2 and O_2 and 5% CO_2). However, this level of oxygen is not physiologically relevant in most *in vivo* tissues where the partial pressure of oxygen is much lower and varies from tissue to tissue (Carreau et al., 2011). High oxygen concentrations during culture could lead to oxidative stress and other deleterious effects (Jagannathan et al., 2016) which is removed from physiological conditions. Although microfluidic technology offers the possibility for fine spatial control of oxygen and the generation of gradients, and there have been many reports of using disaggregated cells (Chiang et al.; Peng et al., 2013; Rexius-Hall et al., 2017), to the best of our knowledge, only one group has integrated oxygen control with tissue explants in microfluidic devices (Mauleon et al., 2012). To achieve this, they designed a microfluidic add-on consisting of a channel and a gas-permeable membrane on top of which sat a perfusion chamber. By supplying gas with the desired oxygen concentration to the microfluidic channel, the gas diffused through the membrane and brain slices were cultured at specific oxygen tensions. It was also possible to subject different parts of the slice to different concentrations. This is an important line of investigation and

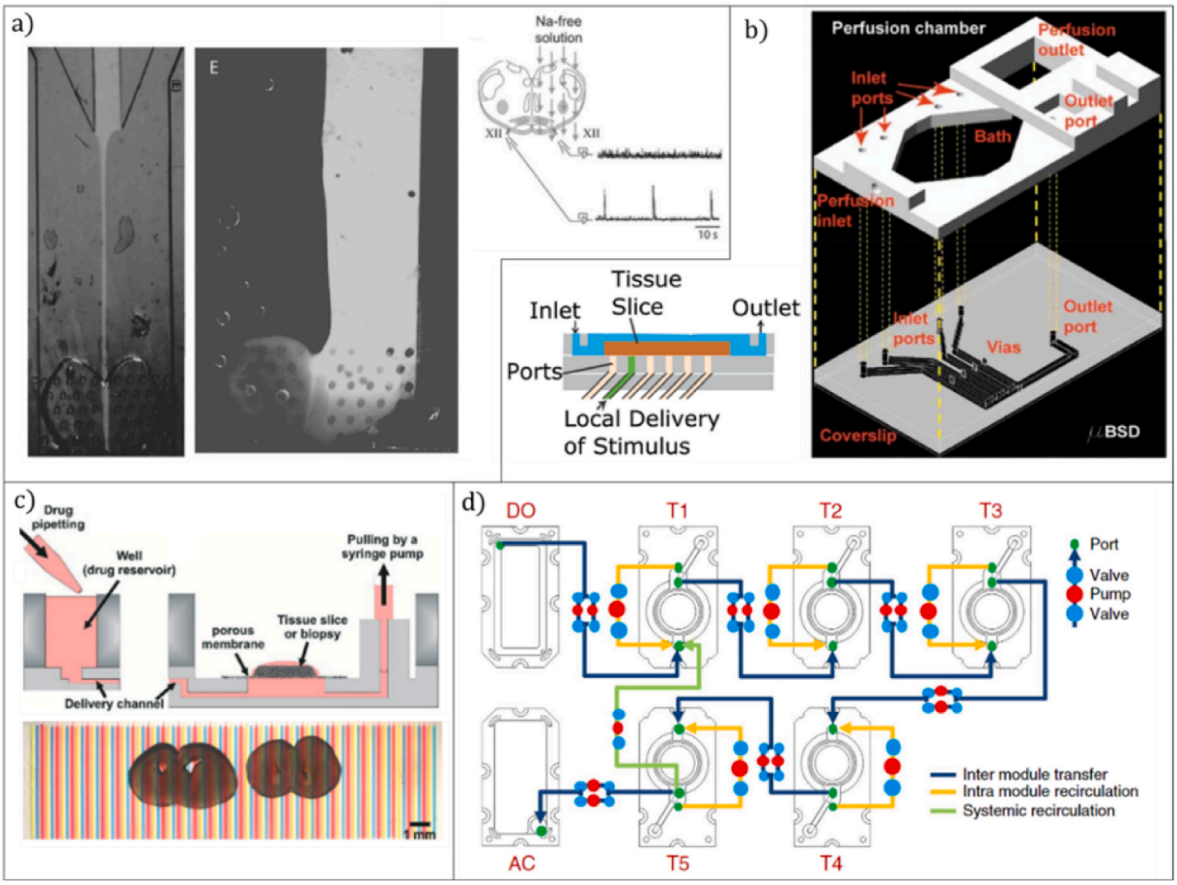

Fig. 4. *Mechanisms for the manipulation and control of the microenvironment during tissue slice cultures. The design in a) takes advantage of the properties of laminar flow to perfuse the same sample with two different media* (Blake et al., 2007)*, while b) and c) used techniques that allow a higher spatial delivery control through the use of ports* (Mohammed et al., 2008) (Ross et al., 2017) *and open channels* (Chang et al., 2014) *respectively. d) is a diagram of the EVATAR system, connecting up to 5 chambers containing different tissues interconnected to allow recirculation and homogeneous mixing of secreted factors* (Xiao et al., 2017)*. Figures a, b and c adapted from* (Blake et al., 2007; Chang et al., 2014; Mohammed et al., 2008; Ross et al., 2017) *with permission from The Royal Society of Chemistry. Figure d adapted from* (Xiao et al., 2017) *with permission from Springer Nature.*

could provide important insight into the optimal conditions for tissue culture *ex vivo* or on the effect of hypoxia on tumours, for example.

2.3. Tissue interrogation

Microfluidic devices for analysing tissue explants should include methods for optical analysis, sampling of molecules, and chemical or electrical stimulation and recording. This means that access to the sample is an important factor in the design process. For example, electrophysiological measurements for neurological investigations are usually performed by inserting electrodes into a brain slice. Blake et al., modified their system (described in Fig. 2a) to allow access to the top and side for insertion of electrophysiological probes (Fig. 5a). This design allowed measurements from the top and from the edges of the brain slice using multi-electrode probes. Multi-site measurements were simultaneously obtained with continuous perfusion of the sample (Blake et al., 2010). These open chambers are of great advantage since they can be used with instruments and probes that already exist in most laboratories. Non-invasive techniques of recording from electroactive cells are generally via microelectrode arrays (MEA) (Berdichevsky et al., 2009) which support the tissue, provide electrical contact to the bottom surface and allow perfusion with medium. However, MEAs can only record activity from neural cells from the surface in contact with the electrodes, which is often compromised due to poor perfusion or injury suffered during the slicing process. MEAs also do not give any information on the spread of signals throughout the entire thickness of the slice. To increase the viability of the bottom surface of a slice, Killian et al., integrated a perforated MEA (pMEA) into the interstitial flow chamber developed by Rambani et al., (Fig. 2f), to replace the gold grid sustaining the brain slice (Fig. 5b). This allowed recording of spontaneous and evoked electrical activity and stimulation of the slice. The device provided oxygen and nutrients to the bottom surface (Killian et al., 2016), improving tissue viability by up to 5 days. Other modifications of the MEAs include three-dimensional features in the form of tip-shaped protrusions to record from deeper tissue (Heuschkel et al., 2002), or integrating the recording electrodes with microfluidic ports that are inserted into the sample (Rajaraman et al., 2007). Scott et al., presented a method that combined multi-site electrical recording with multi-site localised chemical stimulation. Their device was based on the same fluid port concept presented previously (Fig. 4b), using ports for stimulation (chemical and electrical) and recording (Scott et al., 2013). Optical methods have also been used to record brain activity, using fast voltage-sensitive dye and laser photostimulation. Ahrar et al., presented a device to culture brain slices while generating two distinct chemical environments: with or without the presence of caged glutamate (an excitatory neurotransmitter) or Ca^{2+} (a key signalling cation in synaptic activity). The sample was laser photostimulated resulting in evoked neuronal activity at the point of exposure, measured with voltage-sensitive dyes. This tool enabled visualisation of neural dynamics over a larger surface of the brain, not restricted to the site where the electrode is inserted, and also fine spatial control of the microenvironment and signal initiation and propagation (Fig. 5c) (Ahrar et al.,

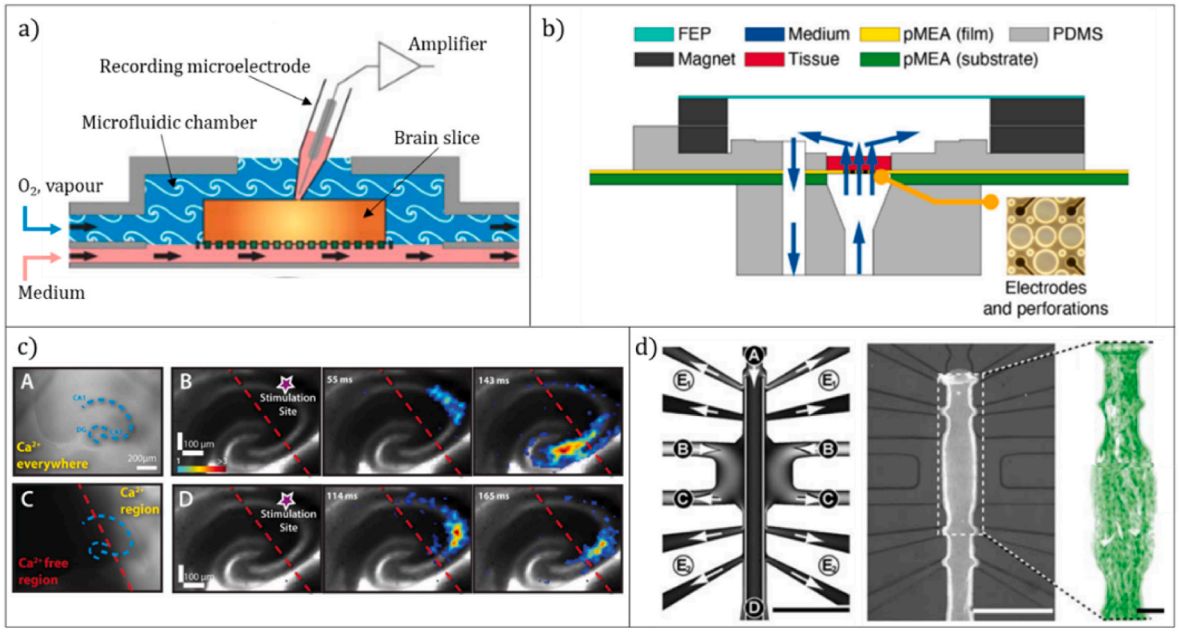

Fig. 5. *Examples of methods for interrogation of tissue samples. a) Electrophysiological measurements using microelectrodes for recording electrical brain activity* (Huang et al., 2012). *Non-invasive techniques have also been developed, such as microelectrode arrays* (Killian et al., 2016) (b) *or optical methods based on fast voltage-sensitive dyes (c)* (Ahrar et al., 2013). *d) The artery-on-chip platform in which the artery segment is fixed and the inner and outer walls are subjected to different conditions* (Günther et al., 2010). *Figures a, c and d adapted from* (Ahrar et al., 2013; Günther et al., 2010; Huang et al. 2012) *with permission from The Royal Society of Chemistry.*

2013). Although promising, the method has limitations due to fluorescence photobleaching or disturbance of the signal due to the fluid flow.

For assessment of viability or functionality of tissue, the most widely used technique is the analysis of effluents. Continuous perfusion means that any secretions can be continuously collected. This provides a way of continuous monitoring markers of cell death or cell metabolism. Another important benefit of using microfluidics is the reduction in sample volumes, which minimises dilution of the metabolites or target compounds, so that their presence can be detected and quantitatively measured. van Midwoud et al., observed that the production of metabolites was similar in static well plates to continuously perfused chips, but because of the reduction of volume in the chip, the concentration in the latter case was 4.3 times higher (van Midwoud et al., 2010a). This evidences the potential of microfluidic miniaturisation to increase the physiological relevance of the models when cytokines and other factors are used to elicit responses in neighbouring tissues.

Very simple devices have been developed by Hattersley et al., to maintain tissue biopsies. The devices consist of a chamber with one inlet and two outlets, in which a liver tissue biopsy of approximately 4 mm^3 is continuously perfused. The tissue could be maintained and monitored for 70h, with cell effluent collected periodically to measure lactate dehydrogenase (LDH) release to assess cell damage, and albumin and urea to evaluate hepatocyte function. Cell viability was assessed using fluorescent markers (Calcein AM and Propidium Iodide) (Hattersley et al., 2008). They used this system to study the toxicity induced in the liver by ethanol and observed a concentration-dependent response to increasing concentrations of ethanol in tissue viability via a cell proliferation assay (WST-1) and tissue functionality measuring albumin and urea secretions (Hattersley et al., 2011). They also explored the potential for personalised medicine, using the device as a tool to assess and guide therapeutical treatment decisions exposing head and neck squamous cell carcinoma (HNSCC) samples to chemotherapeutic drugs for 7 days (Hattersley et al., 2012), using LDH release as the marker for tumour cell death. The longer lifespan of these samples could be due to the reduction in sample volume (3 mm^3) or because tumour cells viability is not affected by a necrotic core. The effect of radiotherapies was also examined with this platform by irradiating the samples with a photon beam while being cultured in the microfluidic device (Carr et al., 2014; Cheah et al., 2017). Although they did not observe an increase in LDH release after treatment, their results showed an increased apoptotic rate and a concentration-dependent reduction in Ki-67 expression (proliferation marker) following irradiation (Cheah et al., 2017). A similar perfusion system was used to stimulate cardiac tissue with changes in reactive oxygen species (ROS) monitored electrochemically (Cheah et al., 2010). More recently, a newer version of this system has been described with a user-friendly "plug-and-play" interface for maintenance of precision-cut slices of HNSCC. The devices allowed irradiation treatments and off-chip analysis combining radiotherapy with chemotherapeutics (Kennedy et al., 2019; Riley et al., 2019). In their latest work (Kennedy et al., 2019; Olubajo et al., 2020; Riley et al., 2019), they used human samples, demonstrating the applicability of these platforms for personalised medicine and translation of tissue-on-chip methods into the clinic.

MPS provide a platform for investigations that are too difficult or cumbersome with traditional methods. For example, Günther et al. presented an "artery-on-chip" for routine assessment of artery structure and function. The traditional methods used for these studies are complicated, laborious and require skilled personnel as well as being non-scalable. Their microfluidic system trapped a whole artery segment and subjected it to varying conditions on the outer walls (e.g. stepwise increases in drugs such as phenylephrine or acetylcholine), and even to different conditions on either side of the arterial wall (Günther et al., 2010; Yasotharan et al., 2015) (Fig. 5d). They observed virtually identical vasoconstriction in mouse mesenteric arteries following phenylephrine stimulation compared to results using conventional pressure myography and highly localised responses to challenges that do not spread to the non-stimulated sides, in agreement with previous findings. This system caused less damage to the tissue, and reduced the technical demand for preparation, increasing the ease of use and scalability, which should allow for higher throughput screenings.

Diseases are complex and very difficult to model. Using primary disease tissue would give more accurate and predictive responses and could help researchers gain more insight into the healthy and diseased states. A tissue-on-chip system can also have a significant impact on

disease modelling. Some skin conditions are particularly difficult to recreate and using platforms like the aforementioned TissUse could allow disease tissue biopsies to be studied. Dawson et al., extracted tissue from inflammatory bowel disease (IBD) patients and cultured them on-chip to study differences in the intestinal wall permeability between healthy and diseased samples (Dawson et al., 2016). They showed that the system preserves inflammation throughout the experiment. This potential has also been explored in cancer research by trapping tumour biopsies obtained directly from patients in tissue-on-chip systems. These samples maintained the *in vivo* cell heterogeneity inherent in tumours and allowed different treatment strategies to be used to assess chemosensitivity (Astolfi et al., 2016; Holton et al., 2017; Horowitz et al., 2021; Mulholland et al., 2018). For example, Astolfi et al. observed variability in the response to the same treatment for samples from different individuals but also for samples from the same patient (Astolfi et al., 2016), a trend also observed by Cheah et al. (2017). This could be due to the high heterogeneity of cancer tumours inter and intra-patients, and highlights the importance of personalised medicine, as different patients respond differently to the same treatments and universal treatments are not always the most effective.

3. Applications, challenges and future work

Tissue-on-chip systems could have significant future impact as research tools with important applications in the drug discovery and development process, screening compounds, testing toxicities and efficacy, or used as models for ADME-Tox profiling. They are also important for fundamental biomedical research, helping to elucidate tissue function and mechanisms, as well as disease modelling, increasing our understanding of pathological processes. The use of primary tissue and living tissues means that these devices could also be utilised for precision and personalised medicine, and even for clinical trials on-chip, helping bridge the gap between preclinical and clinical research (Low and Tagle, 2017). However, no organ-on-chip or tissue-on-chip to date has been fully incorporated into the drug discovery pipeline or established as a complete validated model for biomedical research and drug development. Tissue-on-chip systems need to overcome hurdles before becoming widely accepted and utilised as validated and clinically relevant research tools. MPS technologies are generally challenging to fabricate and use by non-experts, material biocompatibility of the chip is important, and experimental issues such as the presence of bubbles or damage to the tissues during handling remain.

The models used to mimic organ or tissue function have to recapitulate the *in vivo* physiological environment as closely as possible, but increasing the level of complexity of the model also makes the system more difficult to use. Thus, it is important to identify the minimal functional unit that has an acceptable level of complexity for the context of use, and focus on replicating that only. This raises questions on which physiological cues or other native components should be included, and whether the systems are designed to address a very specific question (only "fit-for-purpose"), or more broadly to mimic the whole organ function and finally the "body-on-chip" system. Once a piece of tissue is isolated, it requires external intervention to provide adequate biomechanical cues, soluble factors, metabolic cues, etc. Which of these are necessary to sustain the minimal functional unit needs to be established, along with how these can be incorporated, or even replaced with computational models (Cyr et al., 2017). Some of the factors needed to sustain tissue functions originate in other organs, thus connecting two or more organ units could help overcome this problem and improve prediction. Another major hurdle is the culture media. Most cell cultures use customised media specifically designed to enhance the survival and functionality of a particular cell type. If different cell types use different customised media, which media is most appropriate when multiple organ units/tissues are cultured together?

Not only does the purpose of the model need to be carefully considered, but also the methods in which the tissue will be analysed and assessed. Traditional methods are, in general, not suited to analyse whole living tissue, as they have been developed for examination of cell supernatant, body fluids, homogenised cells or fixed tissue slices, lacking temporal resolution (Belanger et al., 2020). Microfluidic devices that maintain the viability of tissue provide a useful tool for the study and analysis of intact living samples and methods for interrogating living tissue within microfluidic devices have been summarised in this review, and also in Reference (Horowitz et al., 2020b). However, there are still several challenges. Even though most methods replicate physiological conditions during interrogation, damage to the tissue can occur when inserting probes, or after exposure to light (for imaging), or cytotoxic reagents that modify cell activity. Most reagents have been optimised for traditional *in vitro* cultures, but given the nature of tissue samples, other factors have to be taken into account such as hindered transport due to the presence of obstacles (higher cell density, organelles, fibres …) or off-target reactivity of compounds due to the presence of the tissue matrix and diluted/secreted factors (Belanger et al., 2020).

One of the main drawbacks specific to tissue-on-chip is the biological low throughput, particularly given the difficulty in sourcing human samples, which limits the number of replicate experiments. Miniaturised systems are of particular advantage when working with scarce tissue samples, providing multiplexing and scalability. Microfluidic technologies reduce the sample volume, reagent consumption and increase the response times.

Tissue-on-chip can be used as research tools to gain insight into (patho)physiological processes, but can also be applied in the pharmaceutical field for target identification and validation, efficacy testing and physiological response prediction. Thus, not only do they need to recapitulate the *in vivo* physiology of the tissue, but also provide a means to deliver and distribute the drugs of different modalities into the tissue in a physiologically-relevant manner. There is a wealth of ADME-Tox data available on thousands of compounds that have already been thoroughly studied. There is detailed information on metabolism, persistence, efficacy, dosing, expected and adverse effects and other outcomes. Using this database, the feasibility and reliability of tissue-on-chip could be evaluated and established by mimicking these known effects.

If tissue-on-chip systems are to be used for personalised medicine, efforts need to be directed towards the personalisation of the systems. This will not only include using a patient's primary tissues or body samples, but also identifying specific biomarkers or biomarker levels to recapitulate the patient's phenotypes, using biomedical imaging and biometric data to mimic geometries, parameters or lifestyle and environmental data (van den Berg et al., 2019).

There is also a need to establish analytical performance standards in order to evaluate the qualification of these platforms for their context of use, such as reliability and reproducibility and their features, such as throughput, inter- and intra-laboratory reproducibility, integration and compatibility with other existing laboratory processes. For example, the Emulate lung-on-a-chip has already demonstrated a well-defined domain of validity by recapitulating human pulmonary oedema and has shown clinical translatability that is human- (Huh et al., 2010) or species-specific (Kennedy et al., 2019). Another important bottleneck against wider adoption by end-users is the lack of qualification of these models for regulatory acceptance. MPS face obstacles in productisation and commercialisation, especially when comparing their costs to traditional 2D models in the larger scales. Full acceptance by the scientific community will occur when MPS are used routinely with user-friendly features, extended shelf life and at a cost-effective price. The field is moving rapidly but to accelerate uptake the scientific community will have to collaborate with the industry and regulatory agencies for these systems to reveal their full potential and benefits to society.

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References

Ahrar, S., Nguyen, T.V., Shi, Y., Ikrar, T., Xu, X., Hui, E.E., 2013. Optical stimulation and imaging of functional brain circuitry in a segmented laminar flow chamber. Lab Chip 13 (4), 536–541.
Astashkina, A., Mann, B., Grainger, D.W., 2012. A critical evaluation of in vitro cell culture models for high-throughput drug screening and toxicity. Pharmacol. Ther. 134 (1), 82–106.
Astolfi, M., et al., 2016. Micro-dissected tumor tissues on chip: an ex vivo method for drug testing and personalized therapy. Lab Chip 16 (2), 312–325.
Bakmand, T., et al., 2015. Fluidic system for long-term in vitro culturing and monitoring of organotypic brain slices. Biomed. Microdevices 17 (4), 1–7.
Baydoun, et al., Jan. 2020. An interphase microfluidic culture system for the study of ex vivo intestinal tissue. Micromachines 11 (2), 150.
Belanger, M.C., Anbaei, P., Dunn, A.F., Kinman, A.W.L., Pompano, R.R., Dec. 2020. Spatially resolved analytical Chemistry in intact, living tissues. Anal. Chem. 92 (23), 15255–15262.
Benam, K.H., et al., Jan. 2015. Engineered in vitro disease models. Annu. Rev. Pathol. 10 (1), 195–262.
Berdichevsky, Y., Sabolek, H., Levine, J.B., Staley, K.J., Yarmush, M.L., Mar. 2009. Microfluidics and multielectrode array-compatible organotypic slice culture method. J. Neurosci. Methods 178 (1), 59–64.
Blake, A.J., Pearce, T.M., Rao, N.S., Johnson, S.M., Williams, J.C., 2007. Multilayer PDMS microfluidic chamber for controlling brain slice microenvironment. Lab Chip 7 (7), 842–849.
Blake, A.J., et al., May 2010. A microfluidic brain slice perfusion chamber for multisite recording using penetrating electrodes. J. Neurosci. Methods 189 (1), 5–13.
Blume, C., Davies, D.E., 2013. In vitro and ex vivo models of human asthma. Eur. J. Pharm. Biopharm. 84 (2), 394–400.
Buskila, Y., Breen, P.P., Tapson, J., van Schaik, A., Barton, M., Morley, J.W., May 2015. Extending the viability of acute brain slices. Sci. Rep. 4 (1), 5309.
Caicedo, H.H., Hernandez, M., Fall, C.P., Eddington, D.T., 2010. Multiphysics simulation of a microfluidic perfusion chamber for brain slice physiology. Biomed. Microdevices 12 (5), 761–767.
Carr, S.D., Green, V.L., Stafford, N.D., Greenman, J., 2014. Analysis of radiation-induced cell death in head and neck squamous cell carcinoma and rat liver maintained in microfluidic devices. Otolaryngol. Head Neck Surg. (United States) 150 (1), 73–80.
Carreau, A., El Hafny-Rahbi, B., Matejuk, A., Grillon, C., Kieda, C., Jun. 2011. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. J. Cell Mol. Med. 15 (6), 1239–1253.
Catterton, M.A., Ball, A.G., Pompano, R.R., 2021. Rapid fabrication by digital light processing 3d printing of a slipchip with movable ports for local delivery to ex vivo organ cultures. Micromachines 12 (8).
Cavero, I., Guillon, J.M., Holzgrefe, H.H., 2019. Human organotypic bioconstructs from organ-on-chip devices for human-predictive biological insights on drug candidates. Expet Opin. Drug Saf. 18 (8), 651–677.
Chang, T.C., Mikheev, A.M., Huynh, W., Monnat, R.J., Rostomily, R.C., Folch, A., 2014. Parallel microfluidic chemosensitivity testing on individual slice cultures. Lab Chip 14 (23), 4540–4551.
Cheah, L.-T., et al., 2010. Microfluidic perfusion system for maintaining viable heart tissue with real-time electrochemical monitoring of reactive oxygen species. Lab Chip 10 (20), 2720.
Cheah, R., Srivastava, R., Stafford, N.D., Beavis, A.W., Green, V.L., Greenman, J., 2017. Measuring the response of human head and neck squamous cell carcinoma to irradiation in a microfluidic model allowing customized therapy. Int. J. Oncol. 51 (4), 1227–1238.
Chiang, H.-J., Yeh, S.-L., Peng, C.-C., Liao, W.-H., Tung, Y.-C., Feb. 2017. Polydimethylsiloxane-polycarbonate microfluidic devices for cell migration studies under perpendicular chemical and oxygen gradients. JoVE 120, 1–8.
Choi, Y., McClain, M.A., LaPlaca, M.C., Frazier, A.B., Allen, M.G., Jan. 2007. Three dimensional MEMS microfluidic perfusion system for thick brain slice cultures. Biomed. Microdevices 9 (1), 7–13.
Cyr, K.J., Avaldi, O.M., Wikswo, J.P., 2017. Circadian hormone control in a human-on-a-chip: in vitro biology's ignored component? Exp. Biol. Med. 242 (17), 1714–1731.
Dawson, A., et al., Nov. 2016. A microfluidic chip based model for the study of full thickness human intestinal tissue using dual flow. Biomicrofluidics 10 (6), 064101.
de Graaf, I.A.M., et al., 2010. Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. Nat. Protoc. 5 (9), 1540–1551.
DiMasi, J.A., Grabowski, H.G., Hansen, R.W., 2016. Innovation in the pharmaceutical industry: new estimates of R&D costs. J. Health Econ. 47, 20–33.
Dodson, K.H., Echevarria, F.D., Li, D., Sappington, R.M., Edd, J.F., 2015. Retina-on-a-chip: a microfluidic platform for point access signaling studies. Biomed. Microdevices 17 (6), 1–10.
Dondzillo, A., Quinn, K.D., Cruickshank-Quinn, C.I., Reisdorph, N., Lei, T.C., Klug, A., Sep. 2015. A recording chamber for small volume slice electrophysiology. J. Neurophysiol. 114 (3), 2053–2064.
Ekert, J.E., et al., Dec. 2020. Recommended guidelines for developing, qualifying, and implementing complex in vitro models (CIVMs) for drug discovery. SLAS Discov 25 (10), 1174–1190.
Eslami Amirabadi, H., et al., 2022. Intestinal explant barrier chip: long-term intestinal absorption screening in a novel microphysiological system using tissue explants. Lab Chip 22 (2), 326–342.
European Federation of Pharmaceutical Industries and Associations, 2019. The Pharmaceutical Industry in Figures.
Fabre, K., et al., 2020. Introduction to a manuscript series on the characterization and use of microphysiological systems (MPS) in pharmaceutical safety and ADME applications. Lab Chip 20 (6), 1049–1057.
Fisher, R.L., et al., Jan. 1995. Dynamic organ culture is superior to multiwell plate culture for maintaining precision-cut tissue slices: optimization of tissue slice culture, Part 1. Toxicol. Methods 5 (2), 99–113.
Günther, A., et al., 2010. A microfluidic platform for probing small artery structure and function. Lab Chip 10 (18), 2341.
Garattini, S., Grignaschi, G., 2017. Animal testing is still the best way to find new treatments for patients. Eur. J. Intern. Med. 39, 32–35.
Haas, H.L., Schaerer, B., Vosmansky, M., 1979. A simple perfusion chamber for the study of nervous tissue slices in vitro. J. Neurosci. Methods 1 (4), 323–325.
Hattersley, S.M., Dyer, C.E., Greenman, J., Haswell, S.J., 2008. Development of a microfluidic device for the maintenance and interrogation of viable tissue biopsies. Lab Chip 8 (11), 1842.
Hattersley, S.M., Greenman, J., Haswell, S.J., 2011. Study of ethanol induced toxicity in liver explants using microfluidic devices. Biomed. Microdevices 13 (6), 1005–1014.
Hattersley, S.M., Sylvester, D.C., Dyer, C.E., Stafford, N.D., Haswell, S.J., Greenman, J., 2012. A microfluidic system for testing the responses of head and neck squamous cell carcinoma tissue biopsies to treatment with chemotherapy drugs. Ann. Biomed. Eng. 40 (6), 1277–1288.
Heuschkel, M.O., Fejtl, M., Raggenbass, M., Bertrand, D., Renaud, P., Mar. 2002. A three-dimensional multi-electrode array for multi-site stimulation and recording in acute brain slices. J. Neurosci. Methods 114 (2), 135–148.
Holton, A.B., Sinatra, F.L., Kreahling, J., Conway, A.J., Landis, D.A., Altiok, S., 2017. Microfluidic biopsy trapping device for the real-time monitoring of tumor microenvironment. PLoS One 12 (1), 1–21.
Horowitz, L.F., et al., 2020a. Multiplexed drug testing of tumor slices using a microfluidic platform. npj Precis. Oncol. 4 (1).
Horowitz, L.F., Rodriguez, A.D., Ray, T., Folch, A., Dec. 2020. Microfluidics for interrogating live intact tissues. Microsystems Nanoeng 6 (1), 69.
Horowitz, L.F., et al., 2021. Microdissected 'cuboids' for microfluidic drug testing of intact tissues. Lab Chip 21 (1), 122–142.
Huang, Y., Williams, J.C., Johnson, S.M., Dec. 2012. Brain slice on a chip: opportunities and challenges of applying microfluidic technology to intact tissues. Lab Chip 12 (12).
Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., Ingber, D.E., Jun. 2010. Reconstituting organ-level lung functions on a chip. Science (80-.) 328 (5986), 1662–1668.
Jagannathan, L., Cuddapah, S., Costa, M., Apr. 2016. Oxidative stress under ambient and physiological oxygen tension in tissue culture. Curr. Pharmacol. Rep. 2 (2), 64–72.
Kennedy, R., et al., 2019. A patient tumour-on-a-chip system for personalised investigation of radiotherapy based treatment regimens. Sci. Rep. 9 (1), 1–10.
Khong, Y.M., et al., Sep. 2007. Novel intra-tissue perfusion system for culturing thick liver tissue. Tissue Eng. 13 (9), 2345–2356.
Killian, N.J., Vernekar, V.N., Potter, S.M., Vukasinovic, J., Mar. 2016. A device for long-term perfusion, imaging, and electrical interfacing of brain tissue in vitro. MAR Front. Neurosci. 10, 1–14.
Kimura, H., Sakai, Y., Fujii, T., Feb. 2018. Organ/body-on-a-chip based on microfluidic technology for drug discovery. Drug Metabol. Pharmacokinet. 33 (1), 43–48.
Kola, I., Landis, J., Aug. 2004. Can the pharmaceutical industry reduce attrition rates? Nat. Rev. Drug Discov. 3 (8), 711–716.
Li, C.-L., McIlwain, H., Dec. 1957. Maintenance of resting membrane potentials in slices of mammalian cerebral cortex and other tissues in vitro. J. Physiol. 139 (2), 178–190.
Liu, J., Pan, L., Cheng, X., Berdichevsky, Y., Jun. 2016. Perfused drop microfluidic device for brain slice culture-based drug discovery. Biomed. Microdevices 18 (3), 46.
Low, L.A., Tagle, D.A., 2017. Tissue chips – innovative tools for drug development and disease modeling. Lab Chip 17 (18), 3026–3036.
Marx, U., et al., 2020. Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. ALTEX 37 (3), 365–394.
Maschmeyer, I., et al., Dec. 2015. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 15 (12), 2688–2699.
Mastrangeli, M., et al., 2019. Organ-on-Chip in development. ORCHID Final Rep. 36 (4), 650–668.
Mauleon, G., Fall, C.P., Eddington, D.T., Aug. 2012. Precise spatial and temporal control of oxygen within in vitro brain slices via microfluidic gas channels. PLoS One 7 (8), e43309.
Mohammed, J.S., Caicedo, H.H., Fall, C.P., Eddington, D.T., 2008. Microfluidic add-on for standard electrophysiology chambers. Lab Chip 8 (7), 1048.
Mulholland, T., et al., 2018. Drug screening of biopsy-derived spheroids using a self-generated microfluidic concentration gradient. Sci. Rep. 8 (1), 1–12.

Olubajo, F., Achawal, S., Greenman, J., 2020. Development of a microfluidic culture paradigm for ex vivo maintenance of human glioblastoma tissue: a new glioblastoma model? Transl. Oncol. 13 (1), 1–10.
Passeraub, P.A., Almeida, A.C., Thakor, N.V., 2003. Design, microfabrication and analysis of a microfluidic chamber for the perfusion of brain tissue slices. Biomed. Microdevices 5 (2), 147–155.
Peng, C.-C., Liao, W.-H., Chen, Y.-H., Wu, C.-Y., Tung, Y.-C., 2013. A microfluidic cell culture array with various oxygen tensions. Lab Chip 13 (16), 3239.
Queval, A., et al., 2010. Chamber and microfluidic probe for microperfusion of organotypic brain slices. Lab Chip 10 (3), 326–334.
Rajaraman, S., et al., Jan. 2007. Microfabrication technologies for a coupled three-dimensional microelectrode, microfluidic array. J. Micromech. Microeng. 17 (1), 163–171.
Rambani, K., Vukasinovic, J., Glezer, A., Potter, S.M., Jun. 2009. Culturing thick brain slices: an interstitial 3D microperfusion system for enhanced viability. J. Neurosci. Methods 180 (2), 243–254.
Rexius-Hall, M.L., Rehman, J., Eddington, D.T., 2017. A microfluidic oxygen gradient demonstrates differential activation of the hypoxia-regulated transcription factors HIF-1α and HIF-2α. Integr. Biol. (United Kingdom) 9 (9), 742–750.
Richardson, A., Schwerdtfeger, L.A., Eaton, D., McLean, I., Henry, C.S., Tobet, S.A., 2020. A microfluidic organotypic device for culture of mammalian intestines: ex vivo. Anal. Methods 12 (3), 297–303.
Riley, A., et al., Dec. 2019. A novel microfluidic device capable of maintaining functional thyroid carcinoma specimens ex vivo provides a new drug screening platform. BMC Cancer 19 (1), 259.
Rodriguez, A.D., et al., 2020. A Microfluidic Platform for Functional Testing of Cancer Drugs on Intact Tumor Slices. Lab Chip.
Ross, A.E., Belanger, M.C., Woodroof, J.F., Pompano, R.R., 2017. Spatially resolved microfluidic stimulation of lymphoid tissue ex vivo. Analyst 142 (4), 649–659.
Schumacher, K., Khong, Y.-M., Chang, S., Ni, J., Sun, W., Yu, H., Jan. 2007. Perfusion culture improves the maintenance of cultured liver tissue slices. Tissue Eng. 13 (1), 197–205.
Scott, A., Weir, K., Easton, C., Huynh, W., Moody, W.J., Folch, A., 2013. A microfluidic microelectrode array for simultaneous electrophysiology, chemical stimulation, and imaging of brain slices. Lab Chip 13 (4), 527–535.
Seyhan, A.A., 2019. Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. Transl. Med. Commun. 4 (1), 1–19.
Shamir, E.R., Ewald, A.J., 2014. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nat. Rev. Mol. Cell Biol. 15 (10), 647–664.
Tang, Y.T., Kim, J., López-Valdés, H.E., Brennan, K.C., Ju, Y.S., 2011. Development and characterization of a microfluidic chamber incorporating fluid ports with active suction for localized chemical stimulation of brain slices. Lab Chip 11 (13), 2247–2254.
Thomas, M.G., Covington, J.A., Wall, M.J., 2013. A chamber for the perfusion of in vitro tissue with multiple solutions. J. Neurophysiol. 110 (1), 269–277.
Tokuoka, Y., Kondo, K., Nakaigawa, N., Ishida, T., 2021. Development of a microfluidic device to form a long chemical gradient in a tissue from both ends with an analysis of its appearance and content. Micromachines 12 (12).
van den Berg, A., Mummery, C.L., Passier, R., van der Meer, A.D., 2019. Personalised organs-on-chips: functional testing for precision medicine. Lab Chip 19 (2), 198–205.
van Midwoud, P.M., Groothuis, G.M.M., Merema, M.T., Verpoorte, E., Jan. 2010. Microfluidic biochip for the perifusion of precision-cut rat liver slices for metabolism and toxicology studies. Biotechnol. Bioeng. 105 (1), 184–194.
van Midwoud, P.M., Merema, M.T., Verpoorte, E., Groothuis, G.M.M., 2010b. A microfluidic approach for in vitro assessment of interorgan interactions in drug metabolism using intestinal and liver slices. Lab Chip 10 (20), 2778.
van Midwoud, P.M., Merema, M.T., Verweij, N., Groothuis, G.M.M., Verpoorte, E., Jun. 2011. Hydrogel embedding of precision-cut liver slices in a microfluidic device improves drug metabolic activity. Biotechnol. Bioeng. 108 (6), 1404–1412.
Wikswo, J.P., Sep. 2014. The relevance and potential roles of microphysiological systems in biology and medicine. Exp. Biol. Med. 239 (9), 1061–1072.
Wong, C.H., Siah, K.W., Lo, A.W., 2019. Estimation of clinical trial success rates and related parameters. Biostatistics 20 (2), 273–286.
Wouters, O.J., McKee, M., Luyten, J., Mar. 2020. Estimated research and development investment needed to bring a new medicine to market, 2009-2018. JAMA 323 (9), 844.
Wu, Q., et al., 2020. Organ-on-a-chip: recent breakthroughs and future prospects. Biomed. Eng. Online 19 (1), 1–19.
Xiao, S., et al., Apr. 2017. A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Nat. Commun. 8 (1), 14584.
Yasotharan, S., Pinto, S., Sled, J.G., Bolz, S.S., Günther, A., 2015. Artery-on-a-chip platform for automated, multimodal assessment of cerebral blood vessel structure and function. Lab Chip 15 (12), 2660–2669.
Yissachar, N., et al., 2017. An intestinal organ culture system uncovers a role for the nervous system in microbe-immune crosstalk. e12 Cell 168 (6), 1135–1148.
Zhang, B., Radisic, M., 2017. Organ-on-a-chip devices advance to market. Lab Chip 17 (14), 2395–2420.