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Highlights

- Microfluidic technologies are designed to manipulate fluids on the scale of femtoliters to microlitres and hence can be used to manipulate single cells, to perform flow chemistry with enhanced experimental control and to analyse assays in high-throughput.
- A droplet-based microfluidic multi-organ-on-a-chip device that mimics the female reproductive system can be used to investigate drug behaviour *in vitro*.
- The power of droplet microfluidics has been leveraged to perform assays for combinatorial drug screening on patient-derived tumour samples to create personalised drug therapies.
- Microfluidic technologies have been used to create a new type of *in vitro* pharmacokinetic compartment model using artificial cells.
- The lack of both a clear regulatory pathway and of academic-industry collaborations are stifling the use of these technologies for drug discovery and development.

There is a point in the evolution of every new technology when questions need to be asked regarding its usefulness and impact. Although microfluidic technologies have drastically decreased the scales at which laboratory processes can be performed and have enabled scientific advances that would have otherwise not been possible, it is time to consider whether these technologies are more disruptive than enabling. Here, my aims are to introduce researchers in the broad fields of drug discovery and development to the advantages and disadvantages of microfluidic technologies, to highlight current work showing how microfluidic technologies can be used at different stages in the drug discovery and development process, to discuss how we can transfer academic breakthroughs in the field of microfluidic technologies to industrial environments, and to examine whether microfluidic technologies have the potential to cause a fundamental paradigm shift in the way that drug discovery and development occurs.

"Tiny" scale research using microfluidic technologies

Researchers in traditional chemistry laboratories manipulate glassware to mix reagents to perform reactions on scales ranging from a few millilitres to several hundred. Researchers in traditional biochemistry laboratories work on smaller scales, from microliters to millilitres, and use adjustable pipettes to dispense fluids into 96-well plates to perform assays. In an analogous manner, microfluidic researchers build devices with features such as pipes (or **channels**, see Glossary), valves or electrodes that allow us to manipulate fluids on much smaller scales, from femtoliters [1] to microlitres [2]. There is no agreed-upon definition for what constitutes a **microfluidic technology**. For the purposes of this article, microfluidic technologies are defined as devices designed to manipulate tiny volumes of fluids with high accuracy and reproducibility. Even the quantification of "tiny" is debated by microfluidic researchers, though we have shown that most of the advantages associated with fluid flow on microfluidic scales occur when the most significant

(**characteristic**) dimension of the device is below 500 μm [3]. Advantages that are relevant to the fields of drug discovery and development include the use of drastically less reagents with an associated reduction in cost per reaction or assay point [4], enhanced experimental control that enables the manipulation of, for example, single cells [5], and the ability to perform reactions or analyses at rates of thousands per second [6]. In this Opinion piece, I discuss how these technologies enable scientific advances that are not otherwise possible, specifically for drug discovery and development.

The advantages and disadvantages of microfluidic technologies for pharmaceutical research

Although there are many different types of microfluidic technologies, they can be divided into two main categories based on how fluid manipulation occurs on the device. In **continuous flow** devices, fluids are manipulated on the microfluidic platform as an entity, as for example when using flow chemistry for the commercial synthesis of drugs [7,8]. Continuous flow devices can be visualised as the miniaturisation of a chemical manufacturing plant, where pipes move reagents from one location to another to enable a chemical synthesis to occur. Hence, flow chemistry is more straightforward to commercialise, though challenges remain before they can reach their full potential [3]. In segmented (non-continuous) flow microfluidic devices, flow is discretised. The most common example is **droplet** microfluidics [9], where (usually) aqueous droplets are made in an oil phase at high-throughput and provide the ability to do batch processes. Each droplet is considered to be an individual reaction chamber. In other words, we can manipulate the content of each droplet individually, and analyse it independently. However, a significant complication when contemplating the transfer of droplet-based microfluidic technologies from academic laboratories to industrial settings is the need to stabilise water-in-oil droplets using a **surfactant**, especially when they need to be stored for longer than the milliseconds they usually spend on a microfluidic

device. Surfactants are one of the least studied areas in the field [10], and even though their effect on on-chip assays needs to be carefully quantified [11], it is often ignored by academic researchers because it is too complex to correct.

Microfluidic technologies can be made from a wide variety of materials. The choice of material affects many factors such as cost per unit [12], the surface chemistry of the device and hence the surface-dependant assays that can be performed on it [13], and the minimum size of the device features and hence reagent volumes [14]. The most commonly used material for device fabrication in academia, **polydimethylsiloxane (PDMS)**, is easy and cheap to use but ill-suited to commercial applications where mass production and reproducibility of the device itself are fundamental considerations for viability of a new venture [15]. Crucially, drug absorption into PDMS is a well-known phenomenon [16]. Translation of an assay or reaction from PDMS platforms to those made of more commercially attractive materials, such as injection-moulded polymers [17] or thermoplastics for hot embossing [18], is non-trivial, and hence the reliance of most academic research on PDMS microfluidic devices limits commercial application. Although this highlights a disconnect between academic research and industry requirements, in recent years more attention has been paid to translation to commercial applications [19–22].

An important part in understanding microfluidic technologies is the intricate relationship between device design and scientific application. Device design includes the fluid manipulation strategy (continuous or segmented) and device material, but also the on-chip features that allow the required actions to occur on the microfluidic device. Since fluid manipulation takes place in features the size of a human hair, actions as simple as mixing two reagents together require complex architectures [23]. Typical microfluidic technologies consist of a device the size of a postage stamp that is connected to the “lab around the chip”, which usually includes a method to insert fluids into

the device, a microscope and a high-speed camera for visualisation. However, the simplest microfluidic technologies, which are generally used for flow chemistry, consist of pieces of tubing joined together with unions or T-pieces originally made for high-performance liquid chromatography (HPLC) instruments. When some or all of the components that form the lab around the chip, such as fluid pumps [24] and detection instruments [25], are integrated into the device itself, these technologies are called a "lab-on-a-chip". The correlation between commercial uptake of a new technology and the minimisation of its disruptiveness to established processes encourages the academic community to develop components that allow processes such as on-chip sensing [26] and sample purification [27] to be integrated into the device, in addition to the core fluid manipulation geometries. However, it is worth remembering that the integration of the "lab around the chip" into microfluidic devices is not always common practice in academic laboratories.

The design of microfluidic technologies for use in the pharmaceutical sciences should start by considering the end user, and hence balance the competing factors that relate to materials science, fluid flow on the microscale, manufacturing limitations, ease of use and integration into industrial processes, in addition to cost. Some recent examples that connect design features with a specific scientific application include continuous flow chemistry performed in simple fluoropolymer tubing for medicinal chemistry [28], intricate chambers accessed through automated valves in PDMS devices that enable the multiplexed screening of biomarkers in high-throughput [29], and droplet-based microfluidic technologies that allow drug-loaded nanoparticles to be produced with high reproducibility for the delivery of cytotoxic drugs and their prolonged release *in vitro* [30]. However, most academic laboratories do not have the capacity to both develop microfluidic technologies tools to perform cutting edge new science, and to also incorporate non-standard

instrumentation and manufacturing processes into their research workflow simply to streamline the uptake of microfluidic technologies in the pharmaceutical industry. Hence the disconnect between research innovation and commercial application starts early on in the design phase of microfluidic devices.

How microfluidic technologies enhance the traditional drug discovery and development process

Discovering and developing new drugs is a long and arduous process, taking on average 10-15 years and costing around 2.6 billion US dollars [31]. The current paradigm for the development of new drugs follows a set of established steps, which can be broadly described as: identification of a biological target, an iterative design process to create libraries of molecules which are then screened against this target *in vitro* to establish a smaller number of promising lead candidates, followed by pre-clinical *in vivo* studies of the lead candidates in animals, and finally three phases of clinical trials in humans [32]. Table 1 shows examples of companies that use microfluidic technologies to enhance and revolutionise target and lead identification, and pre-clinical studies. The most crowded space is the organ-on-a-chip sphere, whereas companies focusing on target or lead identification are using a broad range of innovative strategies. In this section, I discuss three salient recent examples where microfluidic technologies have provided significant advantages in some of these stages of drug discovery and development. These examples highlight the different types of microfluidic technologies available, demonstrate a significant advantage over the traditional non-microfluidic methods currently used and to show innovations from academic laboratories that have the potential to impact the way the pharmaceutical industry develops new drugs.

Microfluidic platforms for the creation of organs-on-a-chip

The aim of *in vitro* testing is to start to understand the biological behaviour of a drug candidate without the complexity associated with animal models. Limitations of cell-based *in vitro* tests include a lack of physiological relevance to the *in vivo* environment in which cells grow [33,34]. A microfluidic organ-on-a-chip model can mitigate some of these limitations, such as the two dimensionality of cell cultures and the lack of physiological flow conditions, and allow the study of drug behaviour in terms of absorption, distribution, metabolism, and excretion (ADME) in the body by linking different artificial organs together on one chip [35]. Organ-on-a-chip platforms are the most common microfluidic technology used for drug discovery and development (Table 1). Microfluidic platforms to build organs-on-a-chip tend to be composed of wells where the organs are grown, linked by channels that allow perfusion between them [36]. More complex body-on-chip platforms, where multiple organs are connected via cell-lined channels that model the circulatory system, further address many of the limitations of current pharmacokinetic and pharmacodynamic modelling, such as the interdependence of organs in the human body [37,38]. To achieve their full potential, these platforms will require the inclusion of on-chip biosensors [39], and the implementation of novel mathematical models for interpretation and correlation with *in vivo* drug behaviour [40,41]. Recent reviews discuss both the advances and the history of the field within the context of commercial application [42–44]. A salient example of microfluidic multi-organs-on-a-chip shows the culture of four female reproductive tissues (ovary, fallopian tube, uterus and cervix) together with the liver [45], which is the most important organ in terms of drug metabolism (Figure 1). Since the authors were also able to model hormonal signalling between these organs, platforms such as these could be used to mitigate the lack of knowledge regarding hormonal effects on drug behaviour *in vivo*, and the fact that it is extremely hard from

158 an ethical perspective to test drugs on pregnant people. Looking forward, although organs-on-a-
159 chip represent the most mature of the microfluidic technologies used for drug discovery and
160 development, it is clear that uptake is far from universal in the pharmaceutical industry even though
161 there is a clear need for *in vitro* models that provide a full prediction of drug behaviour in humans
162 [46]. Microfluidic organs-on-chip are poised to become a main contributor in the process of drug
163 discovery [47–49] once a clear regulatory pathway has been codified (see Box 1).

164 *Microfluidic technologies for personalised medicine*

165 One of the key advantages of droplet microfluidic technologies is their inherent ability to enable
166 high-throughput assays. Recent reviews provide more information about the multitude of available
167 droplet control and formation strategies useful for high-throughput screening [50] and how they
168 have been used in drug screening assays [51]. An elegant example of the power of droplet
169 microfluidic technologies is seen in work using patient-derived pancreatic tumour biopsies to
170 screen for personalised drug combination therapies [52] (Figure 1). The PDMS microfluidic
171 platform uses valves to automate the creation of a library of droplets containing different
172 combinations of sample plus drugs. Patient tumour samples were initially separated into single
173 cells and then encapsulated in droplets, together with the drugs to be tested. Each assay point
174 (droplet) contained around 100 cells (10-100 times less that non-microfluidic assays), which means
175 that they are able to screen a large number of conditions from a small patient sample. In fact, each
176 patient sample was screened using 56 different conditions, assays were reproduced 20 times, and
177 the authors were able to identify specific drug combinations for each patient, which they validated
178 using mouse xenograft models. From a commercialisation perspective, assays took only 48 hours
179 to perform and cost 150 US dollars per patient. Although the use of PDMS does limit
180 commercialisation of this platform, the fact that the authors designed the droplet system to not

need a surfactant would simplify the transfer to a commercial product. There are several biotechnology companies that leverage the high-throughput capabilities of microfluidic technologies for target and lead identification (see Table 1). The integration of these technologies into the discovery process is more straightforward since they provide information which can then be verified using mechanisms that are approved as part of the regulatory pathway. However, the use of high-throughput microfluidic technologies to replace current toxicity or pre-clinical studies suffers from the same lack of regulatory clarity as organs-on-a-chip.

Microfluidic platforms for the creation of artificial cells and tissues

In the early stages of drug discovery, the aim is to determine the chemical structure of a molecule that interacts beneficially with a chosen biological target. Drug screening is complicated because of the sheer numbers of molecules that have to be tested against a target to identify a lead compound. In addition, the future *in vivo* behaviour of the lead compound needs to be predicted to ensure that it can reach the biological target when tested in humans. In the same way that *in vitro* systems are used as predictors of more complex *in vivo* environments, the inherent complexity of cells means that simpler *in vitro* models based on artificial cells or tissues can be used to understand how drugs enter and interact with cells. The hypothesis is that biomimetic tests that can be used early on the discovery process would help select lead candidates with a greater degree of confidence regarding their future *in vivo* behaviour. We have developed a microfluidic device to build bespoke artificial cells from the bottom up for use as a new type of **pharmacokinetic compartment model** for intestinal epithelial permeability [53] (Figure 1). In this work we used a droplet-based PDMS device to model the path that a drug proxy takes from the intestine, into an enterocyte (cells that line the intestine) and then into the blood stream. We were able to model the pH and electrolyte environment present in each of these compartments and hence quantify how

the molecules move between them by calculating the **apparent permeability coefficient** (P_{app}), half-life and flux. Interestingly, our new *in vitro* model predicted a P_{app} value that was three times closer to the P_{app} of cells than the current state-of-the-art technology, parallel artificial membrane permeability assays (PAMPA). We are currently working on modelling different disease states, on building an artificial cell-based platform that allows us to test drugs against a variety of membrane proteins and on building artificial tissues, such as the blood-brain barrier. These technologies represent the most early-stage and blue-sky of those discussed here. The key question to be answered is whether these types of artificial cells and tissues enable *in vitro* to *in vivo* extrapolation of drug behaviour.

Concluding remarks and future perspectives

If we use this journal as a measure of trends in the fields of drug discovery and development, it is interesting to see that only 40 articles in the online archives include the word "microfluidic", and that the majority of these have been published in the last 5 years. Equally, as discussed here, there is a significant body of work showing how microfluidic technologies enhance the current drug discovery and development process. Others have discussed the technological challenges that remain to be mastered for microfluidic technologies to reach their full potential [54] and, more specifically, the current status of organs-on-chips, as the most mature microfluidic technology in the pharmaceutical industry [46,55]. Here I focus on more fundamental philosophical changes that are limiting the application of these technologies. An outstanding question (see Outstanding Questions) that remains to be answered is: why are microfluidic technologies not having more significant impact in drug discovery and development? The main problem is the failure in

communication between end users in the pharmaceutical industry or pharmaceutical researchers, and the engineering-based academic researchers that develop the most cutting-edge microfluidic technologies. This means that most academic research is currently unfit for translation to industry and hence impactful academic innovations are overlooked by the pharmaceutical industry. Academic researchers need to question some of the prevalent methodologies in the field, most importantly whether it is even worth developing this type of microfluidic technologies using PDMS as the device material. If the aim of the technology is to access new science, perhaps the answer is yes. Controversially, if the aim of the technology is to incentivise change in the drug discovery and development process, I would argue the answer is no. However, academia is not designed to provide fully validated technologies, rather we are incentivised to innovate. Therefore, the burden of technology validation needs to be split between industry and academia through formal collaborations that start as soon as new ideas are conceived. These formal collaborations would also enable clear regulatory pathways for these technologies to be developed as early as necessary, rather than becoming a roadblock as is currently the case for organ-on-a-chip technologies.

I have shown how microfluidic technologies can be used to predict cell-based drug behaviour using simple models based on artificial cells, and how they can be used to build more complex models based on organs-on-a-chip to simulate how interactions between organs affect drug behaviour *in vivo*. I have also shown how microfluidic technologies can be used to develop personalised therapies in high-throughput and have shown examples where commercial microfluidic technologies are currently used for drug discovery and development (see Table 1). It is clear that microfluidic technologies have the potential to become new cutting-edge tools in many parts of the drug discovery and development process. Given the breadth of new scientific discoveries that

249 microfluidic technologies can enable, they have clearly not yet reached their full potential.
250 Specifically, do they have the potential the precipitate a paradigm shift in the pharmaceutical
251 sciences? This question will be answered in the affirmative only if microfluidic technologies cause
252 a fundamental change in the way we design, develop and approve drugs. Challenging this
253 paradigm is controversial and requires a transformation in the way we think about the drug
254 discovery process. For example, although current *in vivo* testing processes have enabled great
255 progress in the treatment of disease in the last century, questions remain regarding whether animal
256 studies can accurately predict drug behaviour in the human body and around 30% of drug
257 candidates fail due to inaccuracies in ADME understanding and species translation [56,57]. Hence,
258 microfluidic technologies will cause a paradigm shift in the pharmaceutical sciences when they
259 are able to predict *in vivo* behaviour better than animal models, and when these new technologies
260 are commonly used as part of the drug approval process. This remains to be fully proven for
261 organs-on-a-chip, though the evidence is mounting.

262 Learning from the past we see that paradigm-shifting technologies are disruptive, but that when
263 they become commonplace they enable great leaps in our knowledge and abilities. Looking to the
264 future, we might one day be able to use microfluidic technologies in hospitals to develop
265 personalised therapies *in situ*, use personalised human-on-a-chip models that predict *in vivo*
266 behaviour instead of animal testing, and use microfluidic wearable sensors to quantify drug
267 response during clinical trials. The choice is ours as to whether we allow this change to take place.

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Glossary

Apparent permeability coefficient (P_{app}): number that quantifies how well a drug is taken up into cells *in vitro* and correlates to *in vivo* drug absorption.

Channel: jargon used in the field of microfluidics to describe a pipe-like feature in a microfluidic device. Channels tend to evoke an image of an open structure, whereas closed pipe-like structures are generally used in microfluidic devices.

Characteristic dimension: engineering term that describes the size of the feature on a microfluidic device that has the most impact on fluid flow. The characteristic dimension is used in calculations that allow the quantification of fluid flow.

Continuous flow: type of flow in a microfluidic device where the fluid is not discontinuous, as it is in droplet-based systems.

Droplets: microfluidic jargon for each individual component in an emulsion. Droplet-based microfluidic technologies are used to create (usually aqueous) femto- to microlitre sized droplets in a continuous non-miscible (usually oil) phase.

Microfluidic technologies: devices or chips made from a variety of different materials that allow the manipulation of fluids on the scale of femto- to microlitres.

Pharmacokinetic compartment model: a (usually) mathematical model that simplifies the concentration and rate of distribution of a drug in all human tissues into averaged compartments.

Polydimethylsiloxane (PDMS): a transparent, flexible polymer used to make microfluidic devices in academia because it is relatively straightforward to prototype, cheap and can be used to make small features with high fidelity.

Surfactant: also called a stabiliser or emulsifier, a surfactant is a molecule used to stabilise droplet systems. Surfactants usually have a water-loving (hydrophilic) moiety and an oil-loving (hydrophobic or lipophilic) moiety and hence sit at and stabilise the water-oil interface in droplets.

TEXT BOX: The regulatory pathway for microfluidic technologies

Organs-on-a-chip are the most mature microfluidic technology for use in drug discovery and development, and can hence be used as a case study for the adoption of microfluidic technologies in the pharmaceutical industry. There is clear interest in these technologies from the pharmaceutical industry and a plethora of emerging companies are commercialising a wide variety of different types of both organs-on-a-chip and of disease models based on organs-on-a-chip (see Table 1). Detailed recent reviews summarise the current commercialisation status of organ-on-a-chip technologies, and the research requirements for broad adoption in the pharmaceutical industry [46,58]. However, there are two main problems that limit the broader adoption of organs-on-a-chip. Firstly, the vast majority of these technologies have only been validated in laboratories, and

311 secondly there are no standard validation methods or specific regulatory guidelines for these
312 technologies. Right now, these represent a “foe” to the widespread adoption of all types of
313 microfluidic technologies by the pharmaceutical industry. Therefore, perhaps the most effective
314 single mechanism to propel microfluidic technologies to a mainstay in the discovery and
315 development of new drugs is to codify a regulatory pathway specifically for organs-on-a-chip. This
316 will showcase how the adoption process can occur and will provide a roadmap that enables the
317 introduction of other types of microfluidic technologies into the drug discovery and development
318 pipeline. Key learning objectives will include how to minimise the disruption these technologies
319 cause, and how to integrate them with current industrial processes. This will also lay the
320 groundwork for collaboration and discourse between the developers of these technologies and the
321 end users. Biotechnology start-up companies are much more likely to succeed when they benefit
322 from strong interactions with the pharmaceutical industry [59], and a similar effect will most likely
323 be found for emerging companies based on microfluidic technologies. It is also worth considering
324 that although microfluidic technologies are used as a tool in a wide range of fields, it is rare to find
325 as much innovation and enthusiasm both in academic laboratories and in emerging companies as
326 is found in the research and development of organ-on-a-chip technologies. Hence, to become a
327 “friend” to the integration of microfluidic technologies in the drug discovery and development
328 process, regulatory pathways and standardisation mechanisms must be developed in tandem
329 between academic researchers, start-up companies and the pharmaceutical industry to minimise
330 the risk of stifling innovation in this field.

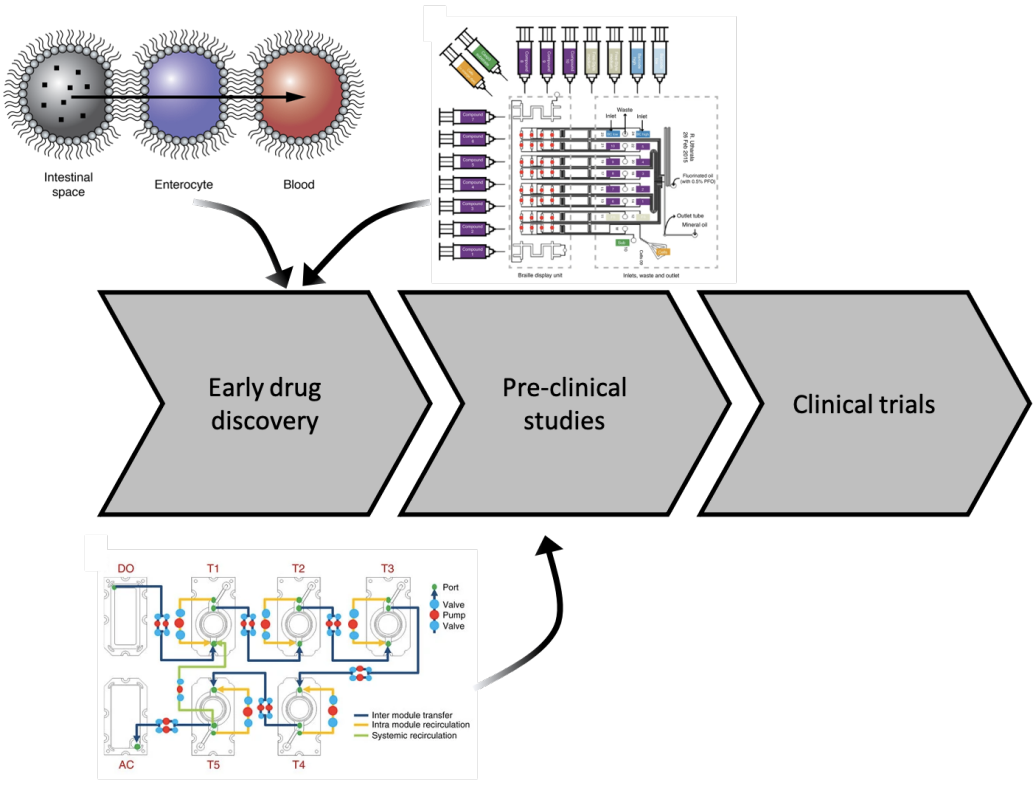

Figure 1. Microfluidic technologies for drug discovery and development. This diagram shows where the three example microfluidic technologies fit within the regulatory pathway for drug discovery and development. The top left image shows the artificial cells that can be used to form a new type of *in vitro* pharmacokinetic compartment model using droplets to determine drug behaviour early in the discovery process. Reproduced from reference [53] with permission from The Royal Society of Chemistry. The top right image shows the design of the microfluidic device for combinatorial drug screening on patient-derived samples using droplets. Adapted from reference [52] through the Creative Commons Attribution 4.0 International License. The bottom image shows the design of the microfluidic device to form a multi-organ-on-a-chip model using five wells that hold the five tissues that represent the ovary, fallopian tube, uterus, cervix and liver. Adapted from reference [45] through the Creative Commons CC BY license.

	Company	Technology highlights
Target and lead identification	Abcellera	Screening of human antibodies to find lead drug candidates using single-cell analysis
	Correlia biosystems	Fast multiplexed protein biomarker immunoassays for target identification, collaborations with four pharmaceutical companies
	Creoptix sensors	Label-free quantification of binding kinetics and affinity for target identification
	Glauconix	Tissue models for screening and target identification of ocular drugs, can also be used for pre-clinical safety and toxicity studies
	InVivo Biosystems	Phenotype-based drug discovery using non-mammalian organisms for compound efficacy and mechanism of action studies
	Tara Biosystems	Cardiac tissue that mimics heartbeats to test drug efficacy and safety
Pre-clinical studies	CN Bio	Organ-on-a-chip with multiple types of organs, compatible with existing lab equipment, FDA recognised
	Dynamic42	Organ-on-a-chip with multiple types of organs, live cell imaging, gas model for inhaled drugs and intestinal model with villus-like 3D structures
	Emulate	Organ-on-a-chip with multiple organs and disease models that can recreate tissue interfaces and the effect of mechanical forces on cells such as breathing
	Hesperos	Organ-on-a-chip with multiple organs and a pump-free, customisable technology
	Mimetas	Organ-on-a-chip with multiple types of organs and disease models, up to 96 tissues models per chip, used by Roche for drug screening
	TissUse	Human-on-a-chip, can use any tissue source, compatible with existing lab equipment, design can be customised

Table 1. Examples of commercial microfluidic technologies for drug discovery and development.

Outstanding questions

- Why are microfluidic technologies not having significant impact in the fields of drug discovery and development?
- Is there a material that has the advantages of PDMS and is easy to use in academic laboratories, while also providing a more straightforward route to commercialisation?
- What is the most efficient way to develop microfluidic technologies for use in the pharmaceutical industries that marries the expertise of academic laboratories and the requirements of end users?
- How can microfluidic technologies be integrated into current drug discovery and development processes, both in academia and in industry?
- Can microfluidic technologies also help during the clinical trial and approval stages of drug development?
- Can microfluidic organ-on-a-chip technologies remove the need for *in vivo* studies?
- Do microfluidic technologies have the potential the precipitate a paradigm shift in the pharmaceutical sciences?

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