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Review paper

30 years of microfluidics

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ABSTRACT

Microfluidics provides a great opportunity to create devices capable of outperforming classical techniques in biomedical and chemical research. In this review, the origins of this emerging field in the microelectronics industry are detailed. We also appraise how factors such as government funding influenced the development of new materials and fabrication techniques. Current applications of microfluidics are also examined and we highlight areas where work should be focussed in the future to ensure that the technology realises its full potential.

1. Introduction

1.1. Overview

Microfluidics has often been heralded as a game changer in life sciences research and industry [1]. However, despite a great deal of work over the last few decades, it has not been the harbinger for scientific advancement that it was initially predicted to be and is now more commonly referred to as a discipline in “adolescence” [2]. Microfluidics, that is systems with a width/height scale between 100 nm and 100 μm [3], is a field that has seen a great deal of research in recent times with many devices now capable of outperforming their classical ancestors as well as the development of new devices that have allowed for novel functionality and the study of phenomena that were elusive to macroscale devices. In this review, we look back at the developments that have had the greatest impact on microfluidics with many of the key advances summarised in Fig. 1. To begin with, we explain the physics of fluids on the microscale to understand the effects that dominate the behaviour of liquids and mixtures. These effects explain many of the advantages of microfluidics such as faster reaction times and simple kinematics. We then look at the origins of microfluidics in the microelectronics industry and look at how this informed the manufacture of early devices before new technologies such as replica moulding, embossing, and injection moulding were developed and adapted to better suit the needs of the growing field. Manufacture is also dictated by the choice of material. We again look at this from a historical perspective and discuss how material selection for.

microfluidic devices changed as new fabrication technologies became available and the requirements of microfluidic devices (such as optical transparency) meant that materials such as silicon were superseded by glass and plastics. Finally, we look at the most recent developments in the field and discuss directions for future research to ensure that microfluidics reaches its full potential.

1.2. Physics of microfluidics

To understand the full benefit of these miniaturized systems, it is important to first understand the physics of fluids on this scale and how this affects their behaviour. Firstly, the ratio of inertial forces to viscous forces in a fluidic system is described by the dimensionless Reynolds number (*Re*) which is given in Eq. 1 [4]:

$$Re = \frac{\rho \nu L}{\mu} \tag{1}$$

Here, ρ is the density of the fluid, ν is the velocity, L is the characteristic linear dimension of the system and μ is the dynamic viscosity. From this equation, it is apparent that as the characteristic dimensions of the system are reduced, the Reynold's number is also reduced. As Reynolds number falls below 2000, the system enters what is known as the laminar flow regime which carries several differences over turbulent flow ($Re > 4000$). Firstly, laminar flow is highly predictable meaning mathematical modelling of these systems is less intensive. Additionally, molecular transport in the laminar regime differs from the turbulent as there is no convective mixing, only diffusion, which again leads to highly predictable kinetics. In microfluidic systems, *Re* is almost always in the laminar flow regime. In addition to the Reynolds number, the Péclet Number (Eq. 2) [5] also gives information on the mass transport of a fluid.

$$Pe = \frac{\nu L}{D} \tag{2}$$

Here D is the coefficient of diffusion, and *Pe* describes the ratio of advective to diffusive transport of molecules in a fluid. From Eq. 2, reducing the dimensions of a system leads to a reduction in the Peclet

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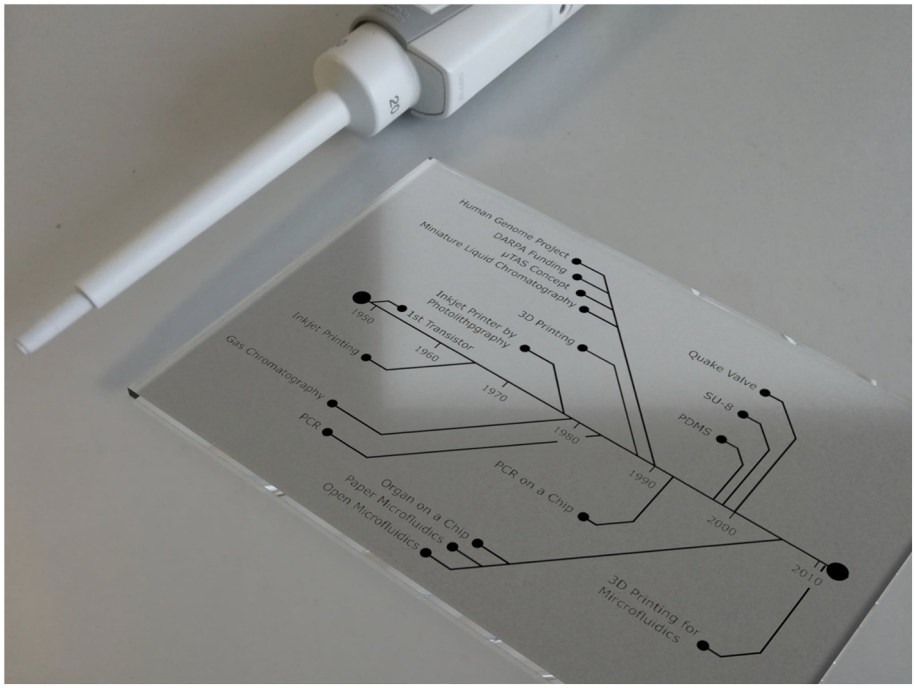

Fig. 1. Timeline highlighting the main advances in the field of microfluidics starting with the invention of the transistor leading up to the rise in 3D Printed devices.

number. As with the Reynolds Number, this means that the kinetics of a system are more predictable. Secondly, the behaviour of a fluid's surface differs between the macro- and the microscale. Surface tension describes a fluids affinity to modify its surface to air interface to reduce its free energy. Interfacial tension describes the same phenomena but in two immiscible fluids, for example, oil in water. This phenomenon has been utilised to great effect in the field of droplet microfluidics [6] which will be discussed in section 5.1. On the microscale, these forces dominate with respect to gravity (the dominant force on the macroscale) and can be used as a method to drive fluids without the need for pumps. Thirdly, capillary forces also begin to dominate gravitational forces as the characteristic dimensions are reduced. Capillary forces describe the force on a fluid that allows it to travel through a porous material or narrow capillary. Again, at the microscale, this dominates over gravity and lead to the development of many analytical devices such as blood glucose meters and cheap pregnancy tests as well as the development of paper analytical devices (PADs) [7], also discussed in more detail in further sections. Finally, reaction times in microfluidic systems are much quicker than conventional devices. This is due to the smaller dimensions of the systems leading to a shorter diffusion time for any given molecule. An approximation for diffusion time is shown in Eq. 3 [5]:

$$t \approx \frac{x^2}{2D} \tag{3}$$

Where x is the distance travelled by one molecule of solute along one axis after time, t, and D is the diffusion coefficient of the solute. From the above equation, it is apparent that:

$$t \propto x^2 \tag{4}$$

Therefore, it becomes clear that as the characteristic dimensions of a system are decreased, the time taken for molecules to diffuse across said system are reduced, thus leading to faster reaction times in microfluidic devices. This becomes increasingly important when larger molecules with a lower coefficient of diffusion, such as DNA, are considered.

With the above phenomena in mind, researchers have been able to leverage these affects in devices that can perform tasks with great value to biological and chemical studies. Additionally, due to their reduced size, microfluidic systems also consume less reagents than conventional fluidic platforms making them an ideal tool when the cost of chemicals is an issue. For example, blood glucose meters only require a small drop of blood and can give a readout of blood glucose concentration in seconds allowing for patients to monitor their condition and comply to their treatment plans from their own homes. However, despite a great deal of work and possibilities, microfluidics has not caught on in the way they were initially predicted to. The promise of “lab-on-a-chip” style devices has been realised in a “chip-in-a-lab” fashion and a lack of standardisation has meant that microfluidics has, in large, remained an academic research tool. Furthermore, the disconnect between the end users and the manufacturers of these devices has meant there has been a great deal of solutionism in the design of these systems. That is, unnecessarily intricate and complicated devices have been designed and manufactured by engineers despite the device having little or no “real world” applications [2,8]. In this review, we look back at the origins and highlight the major developments in the field that have brought microfluidics to where it is today.

2. Birth of the field

Although many of the advancements in microfluidics took place towards the end of the 20th century, its origins begin in the same manner as microelectronics. Driven by a need to improve the reliability of the mechanical relay systems used in telephone lines, William Shockley, Walter Brattain and John Bardeen of the Bell Telephone Laboratories invented the transistor in 1947 [9]. Building on this work, Jay Andrus patented the technique of photoengraving, which had been used previously to create patterns for printed circuit boards, to create much finer details that allowed for semiconductor devices such as those demonstrated by Shockley, Brattain and Bardeen, to be fabricated in silicon [10]. This is the process of photolithography (shown in Fig. 2) which would later become the standard in microelectronic manufacturing. This work was furthered by Jack Kilby at Texas Instruments (patented in 1964) which details how many discrete components such as transistors, resistors and capacitors, can be manufactured in an individual silicon crystal to form an oscillator circuit [11]. This demonstration of the first ever integrated circuit (IC) brought about a revolution in microelectronics and ushered in the “silicon age” as

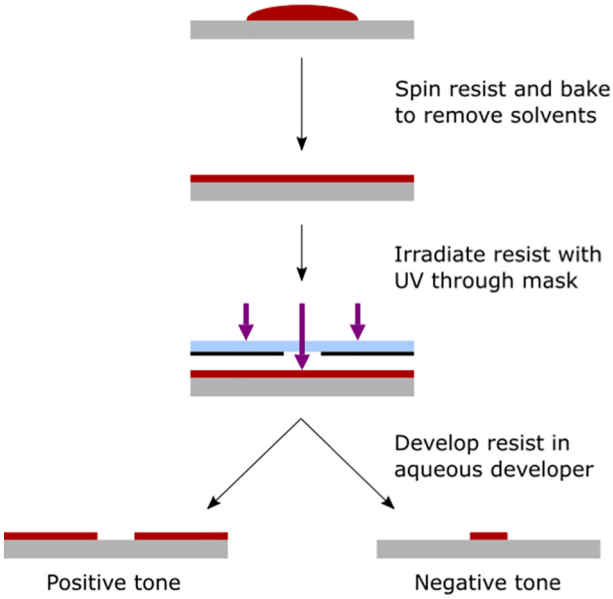

Fig. 2. A typical photolithography process. Process consists of spinning a photoresist to a desired thickness on the substrate before heating to remove any solvents. Resist is then irradiated with UV light through a photomask before a post exposure bake (if required) accelerates the curing of the resist. For positive tone resits, the areas not exposed to the radiation are removed during the development while the opposite is the case for negative tone.

companies sought to create smaller, more reliable consumer electronics. The impact of this was so significant that Kilby was awarded a Nobel Prize in 2000.

As the Silicon Valley revolution was getting underway, another new technology emerged that would have a great influence on the microfluidics industry in the future: Inkjet printing. Although first realised in a working device by Richard Sweet in 1965 [12], the mechanics behind the printer was first described by Walter Rayleigh in 1879 [13]. Rayleigh explained how a falling, continuous stream of fluid breaks up into discrete droplets to minimise their surface area (with respect to a column of the same volume), and thus surface energy. Sweet utilised this phenomena by forcing ink through a small, vibrating nozzle with a 35 µm diameter. As the ink exited the nozzle, discrete droplets were formed which were then charged by an input electrode. The droplets then fell through a uniform electric field which deflected the drops depending on their charge before they hit the paper. As the paper is moved underneath the jet, a trace of the charge of each drop with respect to time is obtained which Sweet demonstrated as an oscillograph capable of moderate frequency recording but with increased convenience and cost over optical based methods. In the design and manufacture of this oscillograph, Sweet demonstrated what can be regarded as the first microfluidic device. Further work on this technology by Bassous et al. at IBM in 1977 showed that through photolithography (a process which had become the standard for silicon manufacturing), an array of inkjet nozzles could be fabricated in an individual silicon wafer [14]. While this not only helped commercialise inkjet printers and make them more affordable and reliable for consumers, this process also illustrated that silicon could be used as a material for the mass manufacture of microfluidic devices.

As time progressed and a wider range of sensors and transducers as well as more refined photolithographic and etching techniques for silicon were developed, researchers began to turn their attention to using these techniques to solve problem outwith electronics. Molecular analysis was the first field to receive this concentration as it became obvious that the benefits of minimising the fluidic systems that were the current standard could lead to much more robust equipment.

With the phenomena associated with microfluidics in mind, work conducted at Stanford University and published in 1979 details the design and manufacture of a microscale gas chromatography system [15]. In this seminal publication, Terry et al. describe how their device, manufactured through a combination of photolithography and etching steps, consists of an injection valve and a 1.5 m long capillary coil. A thermal conductivity sensor, also manufactured with the techniques developed in the I.C. industry, was manufactured in a batch process and attached to the end of the capillary to serve as a detector. This detector, when coupled with the capillary to separate the gases in the system, could provide highly sensitive and specific analysis of the composition of the injected gas mixture. Furthermore, the paper also details how reducing the cross-sectional area of the capillary led to an increase in performance of the device – in line with the theory described in section 1.2. Additionally, the valve chosen had a dead volume of ~4 nl and was capable of injecting volumes of as small as 1 nl into the capillary showing how reducing the dimensions of a device lead to lower reagent consumption. Even though this device could be manufactured on a single 5 cm silicon wafer (seen in Fig. 3), it still had comparable performance to the much bulkier techniques that were the standard at the time. This device is now widely regarded as the first “Lab on a Chip” or “micro-Total Analysis System” although these terms had not been coined at this point. Also, it is widely regarded that this paper heralded the true birth of microfluidics as a field of its own. Indeed, even today, the gas chromatography system still holds up to the definitions of a micro-Total Analysis System even though these criteria had not been put in place at this point.

3. ‘80s: early research in microfluidics

3.1. LIGA process

LIGA (Lithographie, Galvanoformung, Abformung; German for Lithography, Electroplating, Moulding) is a process that adds an electroplating step after photolithography to create moulds that can be used to produce many replicas of the original master. After photolithography, a seed layer of nickel-vanadium (NiV) is deposited onto the master by sputter coating and then a thicker, support layer is deposited with electroplating. This technique has been utilised for the production of masters for injection moulding biomimetic surfaces [16]. Other

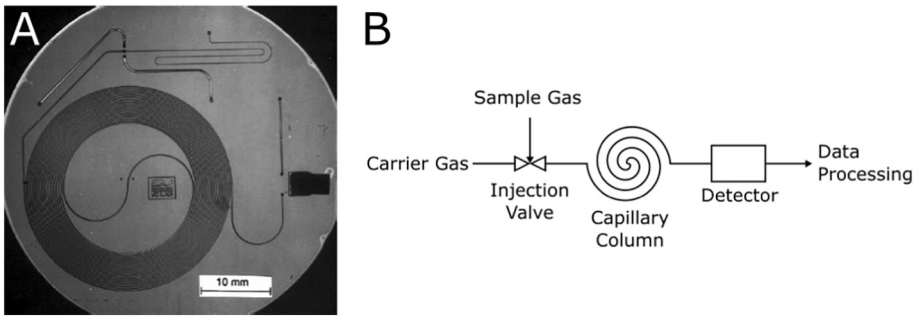

Fig. 3. A – Photograph of Gas Chromatography System described by Terry et al. Device consists of a 1.5 m long, spiral capillary column with input (top right) and exhaust (right) for gas sample. Flow within the device is controlled with a valve (top left) before the capillary coil and the detector can be seen on the right of the device. B – Schematic showing all the fluid handling components housed on the one silicon wafer. Reprinted from reference [15].

variations of this technique allow for the construction of three-dimensional coils with integrated cores as well as the manufacture of rotor elements [17]. DEEMO (dry etch, electroplating, moulding) exists as a further extension to the LIGA techniques and has been demonstrated as a viable method for the production of stamps and moulds for embossing [18]. However, as a method for producing metal parts, its uptake has been hampered by its reliance on expert operators and clean room facilities despite the high resolution and low feature size that can be achieved.

3.2. Valves

After Terry et al. published their work [15], it became apparent that microfluidics had the potential to allow researchers to develop new, more powerful tools for molecular analysis. With this in mind, they set about to create tools that would allow for the manipulation of fluids on the micro-scale, that is the pumps and valves that could control and manipulate fluid flow in such a way that robust devices could be created. The first type of valves that were made took the forms of diaphragms (similar to the one in the gas chromatography system described by Terry et al.). In a bid to make these valves more reliable, as they often required large pressures to actuate the moving parts, Huff et al. developed a valve that was balanced by the pressure of the fluid thus allowing the diaphragm to be manipulated by much smaller actuation forces [19]. Due to the increased effect of electrostatic forces at the microscale, the forces required to manipulate moving parts are too large meaning that the magnetic actuators (motors and solenoids) that were in use on the macroscale, were not up to the task. To further combat this, Jerman developed a diaphragm based valve that opened through the electrical stimulation of bimetallic contacts [20]. This valve was able to operate under the flow and pressure ranges that were useful for MEMS applications however, complex manufacturing meant that these kinds of flow regulators never really caught on in within the microfluidics community.

Similar to diaphragm valves, researchers also used simple cantilever structures to manufacture check valve to manipulate flow in MEMS devices. One such example of such a valve is the batch fabricated, non-reverse valve described by Tiren et al. [21]. This was a two-piece device with one piece harbouring the cantilever structure and another containing the inlet and exhaust while also sealing the chamber. As with the diaphragm valves, this design had the advantages of fast operation as well as small dead volumes. Due to the two-piece nature and thus complex manufacture of this valve, Ohnstein created a version that could be manufactured in one piece of silicon, thus simplifying the manufacturing process [22].

3.3. Pumps

Building on the valves created for silicon MEMS, researchers also wanted to miniaturise the pumps so that these too could be incorporated onto a single device. Examples of such devices come from Smits, who designed peristaltic pumps with the aim of delivering minute quantities of insulin to diabetic patients. His pump, consisting of piezoelectrically manipulated diaphragms and check valves to inhibit back flow could deliver a fluid with a rate of 100 µl/min [23]. Van Lintel also created peristaltic pumps and included a fail-safe so that there was no backflow when the pump was turned off [24]. The above gives a brief overview on some of the early work conducted in valves and pumps of MEMS in silicon substrates however, more comprehensive reviews on this subject can be found elsewhere [25].

Despite a great deal of work being done on the manufacture and operation of these valves and pumps, this technology never really found its places in the microfluidics community for a variety of reasons. Namely, the design and manufacture of such devices required a great deal of expertise that was not had by those in the field of molecular analysis meaning that the design and manufacture of these devices was out of reach for many of the end users of this technology. Additionally, these valves and pumps were all based in silicon as this material can be processed by thin films such as photoresists (surface machining) and etched (bulk machining) in processes that were already highly developed and understood to the point where three dimensional structures could be created [26]. Additionally, due to its use in the microelectronics industry, batch fabrication protocols exist for silicon devices allowing for an economy of scale. Furthermore, silicon is both thermally and chemically stable – attractive properties when microfluidic devices often require heaters and must be robust enough to carry out sensitive operations. Due to the crystalline nature of silicon, anisotropic etching can also be achieved depending on the orientation of the lattice structure – a property not present in many other materials and means that channels with predictable sidewall geometries can be created through wet etching. However, as microfluidics turned towards the life sciences, it became apparent that silicon may not be the ideal material.

3.4. 3D printing

The ‘80s also saw the invention of another technology that would have a huge impact on the microfluidics industry. Developed by Charles Hull in 1986, stereolithography (SLA) describes the process in which three dimensional objects are created through the stacking of two dimensional laminae [27]. In this method, computer software breaks down a three-dimensional model into a sequence of two dimensional layers which are then projected in series onto a build platform submerged in UV curable resin. After each layer has had time to solidify, the build platform is moved upwards and the next layer is cured. This process is repeated until the full object has been manufactured. This invention meant that researchers could create short production runs of bespoke parts without the requirement for expensive and specialist equipment and tooling. Although developed in the ‘80s, SLA would not become a commonplace fabrication technique until much later. The impact of this technology on microfluidics will be discussed in section 5.5.

4. ‘90s: microfluidics finds its feet

4.1. µ-TAS concept

With the trend towards these microscale fluidic devices, Manz et al. proposed that it would be possible to create total analysis systems (TAS) [28], that is, a system that could carry out all of the functions required for analysis: sampling, transport of the sample, any sample preparation steps including chemical reactions and separations, as well as detection. Furthermore, these functions should be carried out automatically. If the device in question had characteristic dimensions on the microscale, they would be termed “miniaturized Total Analysis System” (µTAS). In this influential publication [28], Manz et al. laid out how the physical advantages of fluid mechanics on the microscale would lead to faster, more efficient analysis. Additionally, it was hypothesised that many channels could be fabricated into a small area allowing for simultaneous analysis of multiple samples. This paper showed what could be possible with microfluidic devices and paved the way for many for the advances and technologies that are routine in labs today.

4.2. DARPA

With the birth of the µTAS concept, many other areas of science began to take an interest in microfluidic technologies and began to see what this emerging technology had to offer. One of these sectors was defense. As the Cold War was coming to an end, there was a perceived increased military and terrorist threat from biological and chemical weapons and with this in mind, the Defense Advanced Projects Research Agency (DARPA) funded a lot of microfluidics research in a bid to create portable, field deployable devices capable of detecting

these weapons [2]. Importantly, this focused funding lead to an increased drive to develop functional microfluidic devices.

4.3. The human genome project

Another major motivator towards microfluidics emerged towards the beginning of the '90s – the Human Genome Project (HGP). Launched in 1990, the project was set up with the aim of mapping the entire human genome within 15 years and was publicly funded by the National Institute of Health (NIH) and the US Department of Energy to the tune of $3 billion [29]. As the project began, however, it became clear that current DNA sequencing technologies would not be up to such a mammoth task.

As microfluidics turned towards biological detection, researchers began to discover the many disadvantages associated with the use of silicon. Firstly, silicon is expensive. With one of the main tenets of microfluidics being that devices should be cheaper than the alternatives, the cost of silicon lessens its attractiveness as a viable material for the mass production of microfluidics. Secondly, silicon is brittle which means the devices are often delicate so consideration must be taken when parts are to be transported. Thirdly, silicon is opaque to light in the visible and ultra violet (UV) spectrum – an important factor when many sensors, such as those for DNA analysis, use light as a detection method. Finally, the protocols to bond silicon to other silicon substrates or materials requires considerable expertise and facilities [8]. Bonding is a very important aspect when microfluidic devices are considered as the majority of chips are manufactured as sandwich structures. To move away from silicon, researchers first turned their attention to glass. Glass has similar advantageous properties to silicon – that is it can be processed by thin films in much the same way as those techniques developed in the microelectronics industry. Glass also came with the advantage of being optically transparent, which allowed for light based detection methods to be incorporated into devices, thus allowing for a new generation of optic based microfluidic biosensors.

In the decades preceding the '90s, DNA sequencing relied on slab gel electrophoresis [30]. As the preparation of these gels was laborious and time consuming and the act of carrying out the separations difficult to automate, slab gel electrophoresis was adequate for small scale research applications but would have significantly hindered the HGP. With this in mind, researchers began to apply the principles of microfluidics towards creating a more robust method of sequencing DNA. In 1990, Swerdlow and Gesteland showed that a 75 μm diameter silica capillary filled with electrophoresis gel could be used in place of a slab gel [31]. What is more, when they compared this device to slab gel electrophoresis, they found that their capillary electrophoresis (CE) was 3× faster and had a 2.4× better resolution. These advantages were due to the fact that the thermal properties of the capillaries meant that they were less susceptible to joule heating when exposed to high electric fields. This meant that up to 50× higher electric fields could be applied to CE devices compared to their slab counterparts [30] so shorter separation distances could be used which in turn contributed to the faster operation. Due to the small size of these capillaries, it was not long before photolithography techniques were used to fabricate these channels. By 1994, both Woolley and Mathies, and Effenhauser et al. had manufactured CE arrays in planar glass substrates with the function of separating DNA fragments based on their size – the first and vital step in the sequencing of DNA [32,33]. Steps taken towards on-chip DNA sequencing were taken also by Woolley et al. who demonstrated that the sequencing of fragments with ~150 base pairs was achievable with 97% accuracy [34]. Continuing on from this work, Schmalzing et al. created a theoretical framework to help optimise the design of DNA sequencing experiments [35]. Using this, they were able to sequence fragments with 400 base pairs.

Finally, Woolley et al., and Simpson et al. demonstrated that multiple CE channels could be fabricated onto one glass chip [32,36,37]. These multiplex devices meant that DNA sequencing was faster and simpler to perform than ever before. Another key advantage of these microfluidic platforms was the economic use of reagents. All of the methods described above were capable of separating DNA from sample volumes as small as a few nano-litres.

However, during this period, it was not just DNA sequencing that benefitted from microfluidics. Polymerase chain reaction (PCR) suffered from the same drawbacks as slab gel electrophoresis which motivated Northrup et al. to develop the first chip based PCR thermocycler [38]. This device meant that it was now possible for researchers to incorporate sample preparation as well as detection and analysis into a single microfluidic device in line with the requirements set out by Manz et al. for μTAS. Furthermore, these advances meant that the HGP was completed on time in 2003 as well as contributing greatly to our understanding of microfluidics.

4.4. PDMS

As described above, glass was used as an alternative to silicon for many microfluidic applications. However, glass and the plastic poly (methyl methacrylate) (PMMA) (which was also used to make devices) were materials that were available throughout microfluidics research and hence are not considered as advances within the field. Due to this, they are not examined in detail here, although how material selection changed as microfluidics progresses is discussed in section 4.7. Furthermore, glass was superseded as the standard microfluidics material by cheaper alternatives that would allow for a simpler approach to the manufacture of microfluidics while still allowing for the incorporation of valves and pumps. Towards the late 20th century, the elastomeric material poly(dimethylsiloxane) (PDMS), pioneered by George Whitesides and his group at Harvard, quickly became the most popular material for the manufacture of microfluidic devices [39]. Compared to glass and silicon, the fabrication of devices in PDMS is simple and does not require expensive clean room facilities. Firstly, a master structure is prepared (through silicon micromachining or otherwise). Next, the PDMS base and curing agent are mixed together before the solution is poured over the mould. The low surface energy of the PDMS means that it readily flows into small features and release from the mould is simple. This in turn means that features with sub 0.1 μm dimensions can be cast with ease [40]. Another main advantage of PDMS is how it can be bonded to itself or to other materials. PDMS channels can be sealed through a variety of methods. In the simplest method, tape can be used to seal channels [41] but it is more commonplace for devices to be sealed against a glass slide or with a further layer of PDMS. When placed in conformal contact with another substrate, the elastomeric nature of PDMS means that is forms a seal capable of withstanding moderate fluid pressures [39]. Additionally, PDMS can be irreversibly bonded to other materials through the plasma treatment of the two interfaces if a high-pressure seal is required [42]. Another method of bonding is to have one side with a saturation of the base in contact with a side with a saturation of the curing agent [42]. When heated, an irreversible bond between the two sides is formed without the need for an adhesive that could otherwise clog channels. These simple processes meant that prototyping of microfluidics devices became quick and cheap hence its uptake in the microfluidic community.

Concomitant to the advantages described above, another benefit of PDMS was its soft, elastic nature [43]. This lead to Stephen Quake's group at Stanford university to develop the Quake valve which would become the most commonly used valve in the field of microfluidics [42]. Driven by the μTAS concept that every microfluidics device should be able to carry out all the necessary steps required for analysis, Quake sought to recreate the valves and pumps that had been manufactured for the silicon MEMS industry in the now popular PDMS. The valve he created was constructed of a multilayer PDMS structure although 3D printed valves have been demonstrated (Fig. 4C) [44]. One layer housed the channels for fluid flow while a control line running

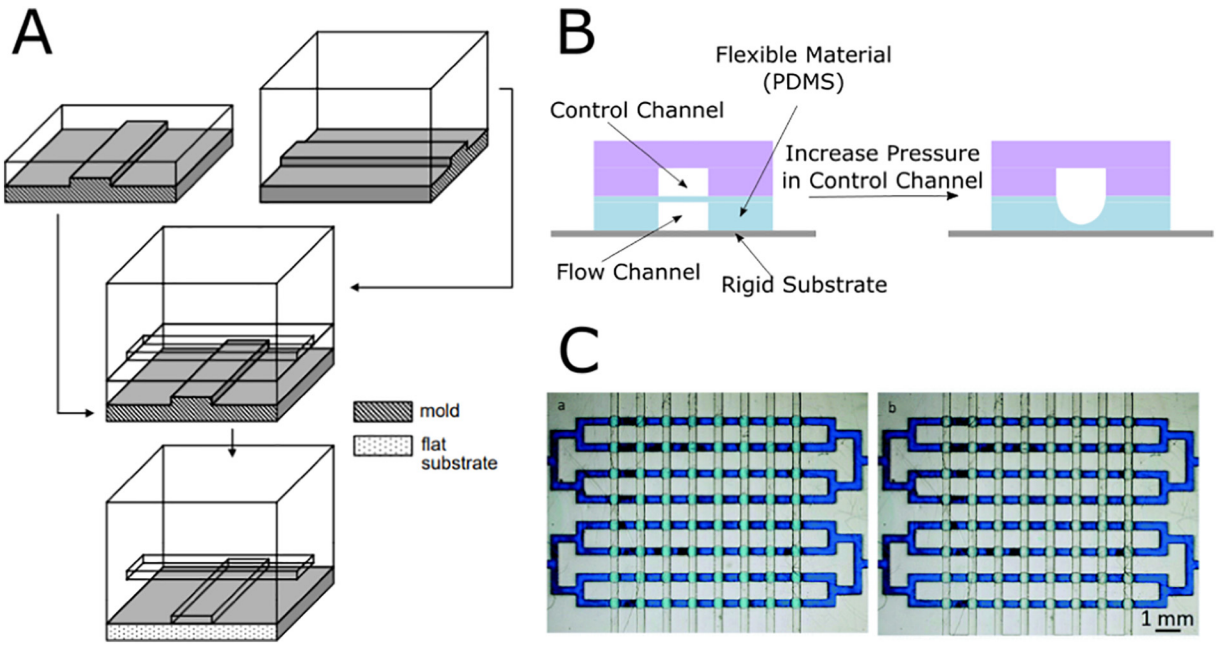

Fig. 4. A – Process of manufacture of the Quake valve. Channel is moulded in the part on the left with the pneumatic control line in the part on the right. The control line is then placed on top of the channel. During operation, an increase in pressure in the control line deforms the channel to such an extent that flow in the microfluidic channel is blocked. Reprinted from reference [43]. B shows the operation of the valve. When the valve is open, fluid can flow in the lower channel. When pressure in the control channel is increased, the flow channel is deformed and obstructs the flow. C_a – device with an array of quake valves (open) manufactured through stereolithography and closed in C_b. Reprinted from reference [44].

perpendicular to that channel was fabricated in another. When the pressure in the control line was increased, the control line bulges and deforms the channel to such an extent that the flow in the channel can be completely stymied. A schematic of this can be seen in Fig. 4. These valves also have a low dead volume and fast operation that is congruous to the requirements of μTAS. As with silicon valves, these PDMS valves can be activated in sequence to produce peristaltic pumps that come with the ease of fabrication inherent to PDMS. Examples of applications that have utilised PDMS devices include biochemical assays [45], genomics [46], chemical reactions [47], and biological detection [48]. Many of these applications were made possible by PDMS's permeability to gases making PDMS is an ideal material for use in live cell studies [49].

Not content with simply using PDMS as a substrate for microfluidics, Whitesides also pioneered the material's use as a fabrication tool. This set of techniques, termed “Soft Lithography”, due to the soft, elastomeric nature of PDMS, is discussed briefly with respect to microfluidics however more complete reviews can be found elsewhere [50–52].

4.5. Advances in micro manufacturing techniques

4.5.1. Replica moulding

Perhaps the simplest of the polymer moulding practises, replica moulding involves casting a polymer against a stamp or a mould [53]. Fig. 5 shows the replica moulding process and a device replicated from a silicon master [54]. When PDMS is used as the mould material, the mould can be deformed around curved or contoured substrates to pattern areas that are elusive to photolithography [55]. Moreover, replica moulding can be used to replicate nano-scale features and the use of elastomeric stamps means that release from the mould is not an issue when compared to rigid materials [56]. The main drawback of replica moulding to produce microfluidic parts is that it is currently not an automated process, thus it is not high throughput enough for industrial scale manufacture. Although injection moulding provides a means of replica moulding with a much higher degree of throughput, this technology will be discussed in greater detail section 4.5.5 due to the wealth of literature surrounding this topic.

4.5.2. Embossing/nano imprint lithography

Embossing, or imprint lithography, is a technique that involves the patterning of a material – usually a polymer – against a mould or a stamp with a relief pattern. This can be executed in number of ways. Most commonly, the substrate is brought to 40–50 °C above its glass transition temperature (T_g) before the stamp is then brought into contact with the substrate and both parts are cooled and the polymer returns to below T_g. The moulded part is then released from the stamp. Additionally, imprint lithography can be achieved using a pre-polymer which can then be cured to give a solid pattern. In industry, compact discs (CDs) are an example of how this technique has been used in the mass production of consumer goods [57] – although many of these processes have now been switched to injection moulding. Additionally, in research settings embossing has been used to manufacture gratings capable of coupling light into waveguides [58], as well as reliably reproducing features as small as 25 nm [59] which can then be used as mask for etching or as a sacrificial layer for the lift off of metals [60]. Stamps for imprint lithography have been manufactured with a number of techniques in a number of materials, such as PMMA [61] and quartz [62]. Furthermore, companies have developed commercial systems for the creation of embossing stamps and the imprinting of substrates [63]. Embossing is also advantageous as a replication method as it requires little flow of the polymer so there are low thermal stresses in the final part [64]. While this process can be automated, the time taken to heat and cool the substrate and tool means that the cycle time is too long for high throughput fabrication.

4.5.3. SU-8

The development of the negative resist, SU-8, by IBM, lead to the design and realisation of many MEMS and lab-on-a-chip style devices that were elusive with the thin films developed for the microelectronics industry.

First described in 1998, SU-8 allowed researchers to create

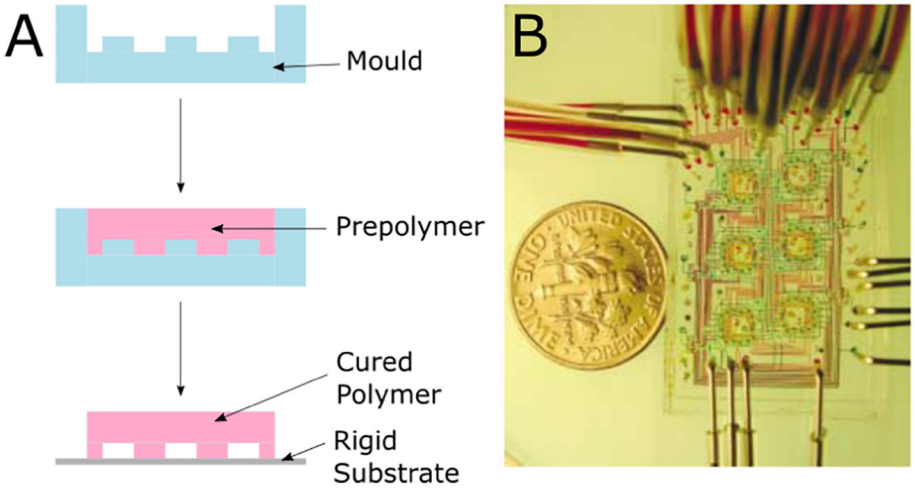

Fig. 5. A – Schematic of replica moulding process. A PDMS master is prepared before a prepolymer is poured on top. Once the prepolymer as flowed into all areas of the master, the polymer is cured and the PDMS master is removed giving a replicated part. B – Microfluidic chemostat manufactured through replica moulding. Coloured dyes used to visualise the channels and coin for scale. Reprinted from reference [54].

structures with high aspect ratio in much thicker resist layers than had been observed before (up to 1200 µm) [65]. Alongside the ability to form these thick layers, the mechanical, thermal and chemical properties that allow it to be used for the manufacture of nickel moulds for injection moulding [65], the direct manufacture of micromechanical parts such as gears [66], and thermal flow sensors manufactured directly into the photo-plastic which highlighted its suitability for microfluidics [67]. SU-8 is processed in much the same manner as other photoresists (Fig. 2) however the pre-bake and exposure times must be lengthened to compensate for the thicker layer. Additionally, a post-exposure bake is added to accelerate the cross linking of the polymer in the regions exposed to the UV radiation. SU-8 has also been used to create multi-layer structures that can be used to construct complex three-dimensional shapes. Mata et al. described a process in which 10 µm diameter posts or holes are patterned onto or around larger features with only one development step [26]. This procedure also allowed for the creation of overhanging structures that were previously only achievable with complicated, multi-step protocols involving bonding, etching and electroplating [68]. The multilayer SU-8 technique was further utilised porous scaffolds for tissue engineering while having a surface that would promote the differentiation and proliferation of cell lines. Indeed, the advent of this photoresist lead to the fabrication of microfluidics devices manufactured entirely in SU-8. Sato et al. manufactured a purely SU-8 fluidic channel with built in 3D microstructures that could generate droplets of fluid [69].

4.5.4. *Rapid prototyping*

Despite microelectronics' and microfluidics' reliance on photolithography, the process suffers from one main setback: the use of chromium on quartz masks to pattern the UV light onto the desired regions of resist. Not only are these masks expensive (~$400 per mask), they are also time consuming and require considerable expertise to manufacture – a barrier to the uptake of this technique in biology and chemistry. In order to combat this issue, Qin et al. printed patterns onto acetate films that could be used in place of the quartz masks [70]. Here, a standard laser imaging system is used to pattern black ink onto the films in the areas that are not to be exposed. This technique allows for the rapid production of photolithography masks at a fraction of the cost (~$1 per square inch) and time (~2 h from design to manufacture) of their quartz counterparts. Additionally, the mechanical flexibility of these masks means that non-planar surfaces can be manufactured. While these masks are not as durable and stable as the chromium masks, they have proved adequate for the rapid prototyping of microfluidic devices where nano-scale resolution is not an issue. If the masks are printed with a high resolution, 2400 dpi printer, dot sizes of 10 µm can be achieved which highlight their suitability for most microfluidics applications.

4.5.5. *Micro injection moulding*

While the above replicative manufacturing techniques can be utilised to provide a high throughput means of manufacture, none compare in terms of automation and throughput to injection moulding. First described in 1872, injection moulding is a process that involves the injection of a molten plastic into a cavity that allows for the manufacture of many identical parts. This technology was then rapidly expanded during World War II where the need for mass produced, affordable parts was increased. This culminated with the development of the first screw driven injection moulder which allowed for a greater control of the injection of the plastic and hence more precision and reproducibility in the parts. Towards the latter half of the 20th century, injection moulding has become an extremely efficient way to manufacture parts on an industrial scale with the market for injection moulding plastics predicted to reach a value of $162.1 billion by the year 2020 [71]. Currently, injection moulding is most commonly associated with the manufacture of CDs and Blu-ray discs where feature sizes as small as 140 nm can be achieved, although smaller features have been achieved in research settings [72]. Injection moulding has one main advantages over embossing as a replication method: the heating of the polymer melt and the cooling of the part are kept separate. This means that there is no time associated with melting the plastic for every replication so the cycle time is reduced dramatically.

In commercial injection moulding machines, the overall process is the same – molten plastic is injected into a mould where it is then solidified and the part can be released. In brief, a plastic is heated to above its melt temperature where the screw not only moves the molten material towards the mould cavity, but also mixes and homogenises the plastic melt. The material that is injected into the mould is known as the "shot" which typically consists of enough material to fill the mould once shrinkage during cooling has been accounted for plus a small quantity of material to allow for the transmission of pressure from the driving screw into the mould and stop the screw from bottoming out. The plastic then cools in the mould, with the material in the gate being the first to solidify. This means that no more material can enter the cavity so the screw retracts and prepares the shot for the next part. Once the plastic in the mould has been cooled to such an extent that it is dimensionally stable, the part is ejected and the process can begin again. This entire process can be run without supervision as it is completely automated hence why it is such an attractive technique for the high throughput production of parts from the previously mentioned CDs and Blu-ray discs to much larger parts such as bodywork for automobiles.

With regards to the moulding of microstructures, perhaps the most

important factor to consider with injection moulding is the tooling. For the manufacture of planar devices and patterns, parts are often moulded against inlays (sometimes referred to as “shims”) which are held in place inside the tool. These inlays are planar structures which contain relief patterns of the final configuration desired on the part. The tool must be designed to fit these inlays and hold them in place throughout the moulding process. Traditionally, these inserts have been manufactured by CNC milling of metals and this can be adapted to produce features on the microscale [73–75]. However, as CNC milling suffers from shortcomings such as high surface roughness and a large feature size limit, researchers have also shown a variety of means of fabricating these shims that can act as a bridge between the high-resolution manufacturing of photo and x-ray lithography and the high throughput technique of injection moulding. Examples of the materials and methods that have been used are LIGA [16], etched quartz [76], etched silicon [77,78], polytetrafluoroethylene (PTFE) backed nickel [79], SU-8 on nickel [80], UV curable polyurethane resins [81], bulk metallic glasses [82], and SU-8 on polyamide sheets [83].

Although most of the current research as consisted of manufacturing nanoscale features with injection moulding, there are some examples of microfluidic devices that have been created. Hansen et al. created injection moulded microfluidic chips [80] while Kim et al. described an injection moulded chip capable of determining blood type [84].

As previously mentioned, CDs are commonly manufactured through injection moulding and this technology has also been utilised for the fabrication of microfluidic devices. In a CD format, fluids can be manipulated within the device through centrifugal forces which can be controlled through varying the spin speed of the chip. The presence of this centrifugal force thus eliminates the need for pumps, mixers, and complex valves, and also reduces the risk of channels becoming clogged by bubbles or molecules [85]. In addition to these functions, work conducted by Marc Madou and his group have shown that functions such as PCR [86] and cell lysis [87] can also be incorporated into these microfluidic devices highlighting the flexibility of this technology. Furthermore, these devices lend themselves to high throughput manufacture techniques as demonstrated by Morelli et al. who described an injection moulded device based on a CD to screen bacteria [88].

4.5.6. Thermoplastic bonding

As devices fabricated by replica moulding, embossing, and injection moulding are planar structures, channels still need to be sealed before they can be used as microfluidic devices. One such method of sealing is gluing a cover onto the device [89]. Although relatively simple, this method has not been widely adopted as channels are prone to clogging [90]. A more common technique is thermal fusion bonding. Thermal fusion bonding involves heating the part and cover to above T_g as they are brought into contact and can be applied to a wide range of polymers [91]. However, as large areas of the device and the cover have to be heated, this technique suffers from deformation of the channel structures [90]. Ultrasonic welding is another technique that can be used to bond thermoplastic parts together [92]. Here, energy directors (or weld seams) are fabricated into the device or the cover. The parts are then aligned and brought into contact before a welding horn delivers pressure and ultrasonic vibrations to the assembly. This acts to heat the weld seams through friction which then melt to form the bond with the cover. As the energy is focused onto the weld seams, there is much less deformation of the channels when compared to thermal fusion bonding while still providing a strong fusion of the parts.

4.6. Surface treatments

With the above described advances in manufacturing techniques, there became an increased reliance on polymers within the microfluidics community. While these polymers had many desirable properties as mentioned previously, polymer interfaces tend to have poor chemical resistance and wettability – properties that are fundamental to the operation of a microfluidic device. To combat this, researchers developed a host of different techniques that can alter the surfaces of materials and imbibe them with new properties. A summary of these processes is described below with a more detailed review found elsewhere [93].

4.6.1. Plasma processing

Although commonplace as a method of cleaning substrates in microfabrication, oxygen plasma can be used to alter the surface of silicon as well as polymers which can be done in both a chemical and topographic manner. From a chemical perspective, plasma has been demonstrated to enhance the formation of a surface bound siloxane networks that can be functionalised with proteins. Gandhiraman et al. detail how oxygen plasma activates cycloolefin polymer before chemical vapour deposition of an amine provides a means of producing functionalised surfaces [94]. This method was also well suited to polymers as it provides a means of producing a hydroxylated surface without the requirement of high temperatures. With regards to topography, Evangelos Gogolidis and his group detail how by controlling the materials and conditions inside the reaction chamber, O_2 plasma could be used to provide varying degrees of nano-roughness on PMMA [95,96]. This tuneable process has applications in creating super hydrophobic surfaces through this nano-texture that could be utilised to manufacture self-cleaning materials as well as control over the optical properties of a material.

Plasma is also exploited in deep reactive ion etching protocols however, instead of oxygen, a plasma of reactive gases such as fluorocarbons and chlorine is used to etch through a substrate – typically in a pattern defined by a lithography process. As the plasma can be directed by electrodes, this is done anisotropically.

A further application of plasma processing is in the deposition of polymer films. Although traditionally performed by spin coating, plasma deposition allows for the creation of a pin-hole free film on top of a non-planar surface. Pedersen et al. describe a method whereby hexane monomer is polymerised and deposited onto a substrate to provide a coating that can be used as a resist in electron beam lithography [97]. Again, plasma deposition gives a low temperature method that is suitable for polymer processing.

4.6.2. Other surface treatments

Alongside plasma processing, there exists a wide variety of techniques that can be utilised to alter the surface properties of materials. These include the deposition of a sol-gel onto the walls of a microfluidic device to increase its chemical resistance [93] as well as irradiation of the surface of a polymer with UV light which leads to the formation of acidic groups on the surface which can be functionalised with proteins. Schütte et al. applied this technique to pattern extracellular matrix protein collagen type I to create areas that would promote adhesion and proliferation of cells within a microchamber [98]. It was found that the stability of these acidic groups was high enough to withstand additional fabrication steps before the collagen was introduced allowing for the sealing of the channels.

4.7. Material selection

As the above technologies became available to the microfluidics community, researchers had to consider how these new manufacturing methods would inform their choice of material and *vice-versa*. This section aims to summarise the materials and their available fabrication procedures and describe the benefits and shortcomings of one material over another. As stated above, materials such as glass and silicon come with the advantage of well understood manufacturing protocols and, as is especially the case with glass, superior properties with regards to microfluidics applications. That is, chemical resistance and compatibility with optical detection methods. On the other hand, glass and silicon require complex and labour-intensive fabrication steps.

After its development, PDMS became, and remains, the most common material for device fabrication due to its ease of manufacture and relative low cost. However, the cost of PDMS is often offset by the requirement on a master produced through advanced manufacture methods which, when considering PDMS is used for short production runs, can be a significant factor.

Towards the turn of the century, there was increased interest in polymers for microfluidics piqued by the development of the micro-manufacturing methods detailed above. PMMA was used as an initial candidate for microfluidics due to its rigidity, optical transparency, suitability for high throughput fabrication methods, and its compatibility with many existing biomolecular techniques [99,100]. As with glass before it, researchers developed devices in PMMA capable of a variety of functions such as electrophoresis [101], and DNA sequencing [102]. Alongside PMMA, materials such as polycarbonate, polystyrene, and cyclic olefin copolymers (COC) are also now considered when designing a microfluidic device – especially for embossed of injection moulded parts.

5. 21st century: microfluidics grows up

With the development of these new technologies, the turn of the century brought about a huge increase in microfluidics research which lead to the generation of many new microfluidic platforms with a wide range of functionalities. A small handful of these technologies are described in brief in this section. More detailed reviews on the different fields of microfluidics and their capabilities can be found elsewhere (droplet microfluidics [6], paper analytical devices [103], open microfluidics [104], and organ-on a chip [105]).

5.1. Droplet microfluidics

First described in the ‘90s, droplet microfluidics (sometimes referred to as “digital microfluidics” due to its discretised nature) involves the encapsulation of a reaction in the discrete compartments of an emulsion [106]. Typically, this involves encapsulating reagents in the aqueous phase of a water in oil emulsion, and with the development of a microfluidic platform that could rapidly produce vast quantities of uniform droplets, this technique became a means of achieving high-throughput bio-chemical analysis [107]. This means of producing the droplets is like the aforementioned method of droplet production of inkjet printing (Rayleigh criteria) but with the inclusion of a continuous oil phase that separates the droplet through viscous drag once it has reached a critical size (Fig. 6a) (which can be controlled by altering the geometry of the nozzle). The main advantage of droplet microfluidics is its highly multiplex capabilities. As reactions can be localised into compartments with a volume of a few nanolitres, it is conceivable that thousands of reactions can occur independently of each other at the same time. This concept has led to the development of this technique for high throughput PCR [108], enzyme screening (shown in Fig. 6) [109], and single cell antibody screening [110]. Further applications of this technique include the controlled manufacture of gold nanoparticles [111], which is achievable as the reagents present inside the droplet can be easily tailored to fit any need.

5.2. Paper analytical devices

In a bid to lower the cost associated with the development and production of microfluidic devices, paper has been used as a substitute for microfluidic chips. The use of paper comes with a few main advantages. Firstly, paper provides a good base for microfluidics as fluid is transported though the device by capillary action rendering the need for pumps that require power supplies obsolete. This technique has been used in the past to produce highly commercially successful devices such as lateral flow assays for home pregnancy tests, paper based pH test strips and colorimetric glucose sensors [112]. However, it was not until 2007 when researchers in the Whitesides Group showed how functionalised chromatography paper could be used to perform quick, multiplex analysis of a solution that paper analytical devices (PADs) came to the forefront of the microfluidics community [7].

This initial device can be seen in Fig. 7A. Although the initial manufacture of these devices relied on photolithography, the most standard method of fabricating these devices is now based on inkjet printing [113]. This method of manufacture involves printing a hydrophobic wax onto filter paper to create well-defined and easily programmable channels to control fluid flow. This can be done with an inkjet printer that has been altered to print wax as opposed to ink. After the printing, the paper and wax are then heated on a hot plate or in an oven to melt the wax and allow it to flow from the surface, into the fibres of the paper to create a hydrophobic barrier through the entire thickness of the sheet. The use of paper over silicon and polymer substrates also came with a significant cost reduction. With paper, it was realistic to manufacture devices with a cost as low as $0.10 per device without the need of a great deal of expertise or specialist equipment [103,114]. Furthermore, the disposal of spent PADs is simple as no sharps bins are required, devices can simply be incinerated, lowering the risks associated with the handling of infectious materials. With the above advantages in mind, many PADs have been created with a wide range of applications. Examples include a test for malaria where the extraction of DNA from whole blood is done on the device (Fig. 7B) [115] and a similar test for identifying bovine infectious reproductive diseases [116] as well PADs for tuberculosis diagnosis [117] as the realisation of many existing techniques on paper such as enzyme linked immunosorbent assays (ELISA) [118] and complex designs consisting of many layers [119] (Fig. 7C).

5.3. Open microfluidics

Another emerging field in microfluidics, is open microfluidics. The most common configuration for this to take is in the form of the microfluidic probe (MFP) developed by David Juncker et al. in 2005 [120]. The MFP simultaneously injects and aspirates a processing liquid which creates a jet of fluid between its apertures that does not mix convectively with the surrounding medium. This is termed hydro-dynamically confined flow and allows for reagents to be localised to a small area of a sample without the requirement of closed channels that are susceptible to clogging, can introduce bubbles and have a high hydrodynamic resistance which can lower the sensitivity of a device [121]. This also allows the MFP to carry out chemistry in a liquid environment with a high degree of spatial resolution allowing for cell studies to be done *in situ*. The design and working principle of an MFP can be seen in Fig. 8A. Furthermore, the MFP setup can be incorporated into a standard optical microscope to allow for real time monitoring of the process. Currently open microfluidics is an area attracting much interest and examples of this technology include single cell analysis (Fig. 8B) [122], single cell pharmacology [123], immuno-histochemistry [124], and bio-patterning [125] thus highlighting its flexibility as a tool in the life sciences.

5.4. Organ-on-a-chip

Perhaps the most rapidly expanding and keenly researched area of microfluidics at the moment is organ-on-a-chip. These systems are microfluidic cell culture devices that contain living cells arranged to simulate organ tissue. There are many potential benefits of such a device over traditional two-dimensional cell cultures. Namely, 2D cultures do not accurately represent the organisation of cells in the body in terms of spatial organisation so cannot be expected to accurately model cell behaviour. Additionally, the more recent technique of three dimensional cultures lack the mechanical cues that are important to cell response as well as the harvest of biological material for analysis can prove difficult. Organ-on-a-chip systems on the other hand, aim to

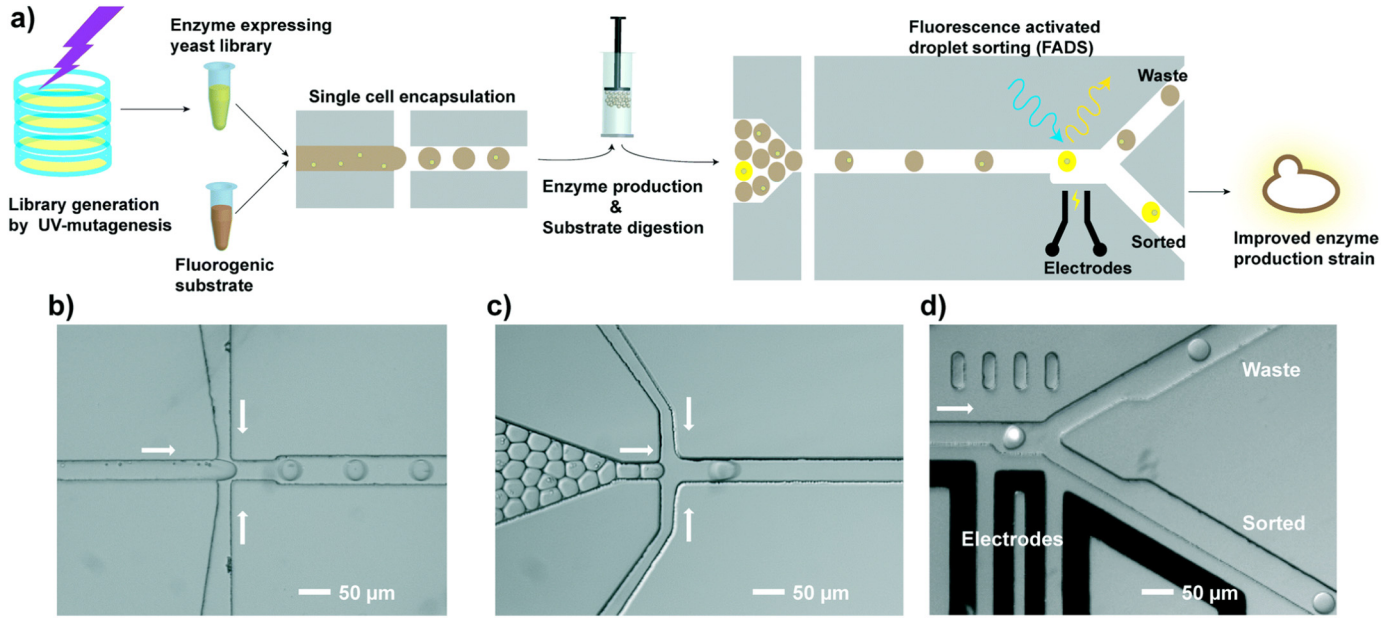

Fig. 6. A. Workflow of entire yeast screening process. First Yeast cells are mutated with UV light before they are encapsulated into droplets with a fluorogenic enzyme substrate (B). The droplets are then incubated where some cells produce an enzyme that digests the substrate and increases the fluorescence of the droplet. Droplets are then introduced to a sorter device (C) where a laser excites the fluorescent molecules and if this fluorescence is above a threshold level, the electrodes as switched on and the cells with improved enzyme production are diverted to a separate output (C) while droplets containing low enzyme producing cells flow to a waste channel. Arrows in B, C and D indicate fluid flow direction. Reprinted from reference [109].

contain many of the features that define the majority of organs. That is, porous membranes that separate cell type and constant perfusions of media – the constant flow of fluid also provides a shear force analogous to the forces exerted on cell by blood flow thus providing a more complete model of organ behaviour. Early research focussed on looking at single perfused chambers with one cell type however, as the knowledge progressed, more complicated systems consisting of multiple cell types were developed to recreate the interfaces between cells and tissues seen *in vivo*. The ability to couple microfluidics and microfabrication with cell culture also comes with a plethora of other advantages. The physics of microfluidics mean that researchers can have more control over the flow of fluid in devices. The laminar flow in microchannel has been used to create concentration gradients in chemicals to monitor cell migration amongst other behaviours. Moreover, the microfabrication methods used to manufacture these chips mean they are compatible with sensors which would allow for better monitoring of the cultures as well as allowing for a greater degree of control over the cells. The manufacturing methods also means that it is possible to create devices that provide a cyclical mechanical strain analogous to that seen in blood vessels or in the lungs. Furthermore, electrodes can be incorporated into the devices to supply electrical fields which have been used in studies of brain tissue [126]. In addition, it is conceivable that it would be possible to create organ-on-a-chip systems from the cells of a patient which would allow for the development and testing of personalised medicine. Furthermore, organ on a chip devise could be connected in series to produce human-on-a-chip systems that could be used to monitor organ-organ interactions. Steps towards this goal have been taken by Viravaidya and Shuler who demonstrated a device with separate chambers for liver, lung, fat, and “other tissue” cells in a bid to better understand the bioaccumulation of molecules between organs [127]. Other examples of organ-on-a-chip systems include chips for muscle [128], bone [129], blood vessels [130], lung (Fig. 9A) [131], gut [132] (Fig. 9B) and heart [133] however that is just a narrow selection of what has been achieved so far [105]. Currently, organ-on-a-chip style devices cannot completely replicate the true function of more complex organ systems thus highlighting the need for more work to be done. Current pitfalls of organ-on-a-chip research include the use of PDMS which, as mentioned previously, has the potential to adsorb small molecules that could have an effect on the culture. Additionally, the current microfabrication techniques require a vast amount of engineering knowledge and facilities that put this technology out of reach for a lot of researchers. However, with all the potential benefits of organ-on-a-chip, it is imaginable that these devices could replace animal assays in the not too distant future and thus lower the cost and time requirements of drug trials. Rogal, Probst, and Loskill also hypothesise that single organ chips could be coupled together to create more complex and flexible organ system models [134]. Huh, Hamilton and Ingber also hypothesise human-on-a-chip systems with this concept illustrated in Fig. 9C [135].

5.5. 3D Printing makes an impact

Despite its invention in the ‘80s, 3D printing didn't really take off within the field of microfluidics until the late ‘00s. Perhaps the most commercially successful 3D printing technology is fused deposition modelling. Here, thermoplastics are heated to above its glass transition temperature (T_g) and extruded through a nozzle onto a stage in a predetermined pattern. After each layer is completed, the stage is moved down and the next layer is deposited on top. This is the method by which many commercially available 3D printers operate. With respect to research settings, this technique has been demonstrated as a viable option to manufacture interdigitated lithium ion battery housings that can easily be integrated into MEMS while being robust enough to withstand the stresses caused by the expanding solutions during charging and discharging [136].

An emerging application of 3D printing however, is the rapid and cheap production of custom labware to either replace existing equipment or to perform entirely new protocols. Leroy Cronin and his group have described 3D printed “reactionware” vessels in which all reagents, catalysts and analysis hardware are printed and contained within a sealed environment meaning that handling steps – and the errors that they introduce – are minimised [137]. Concomitantly, it was discovered that the geometry of the reaction chamber had a significant effect on the products of the reaction. This has also lead to the production of

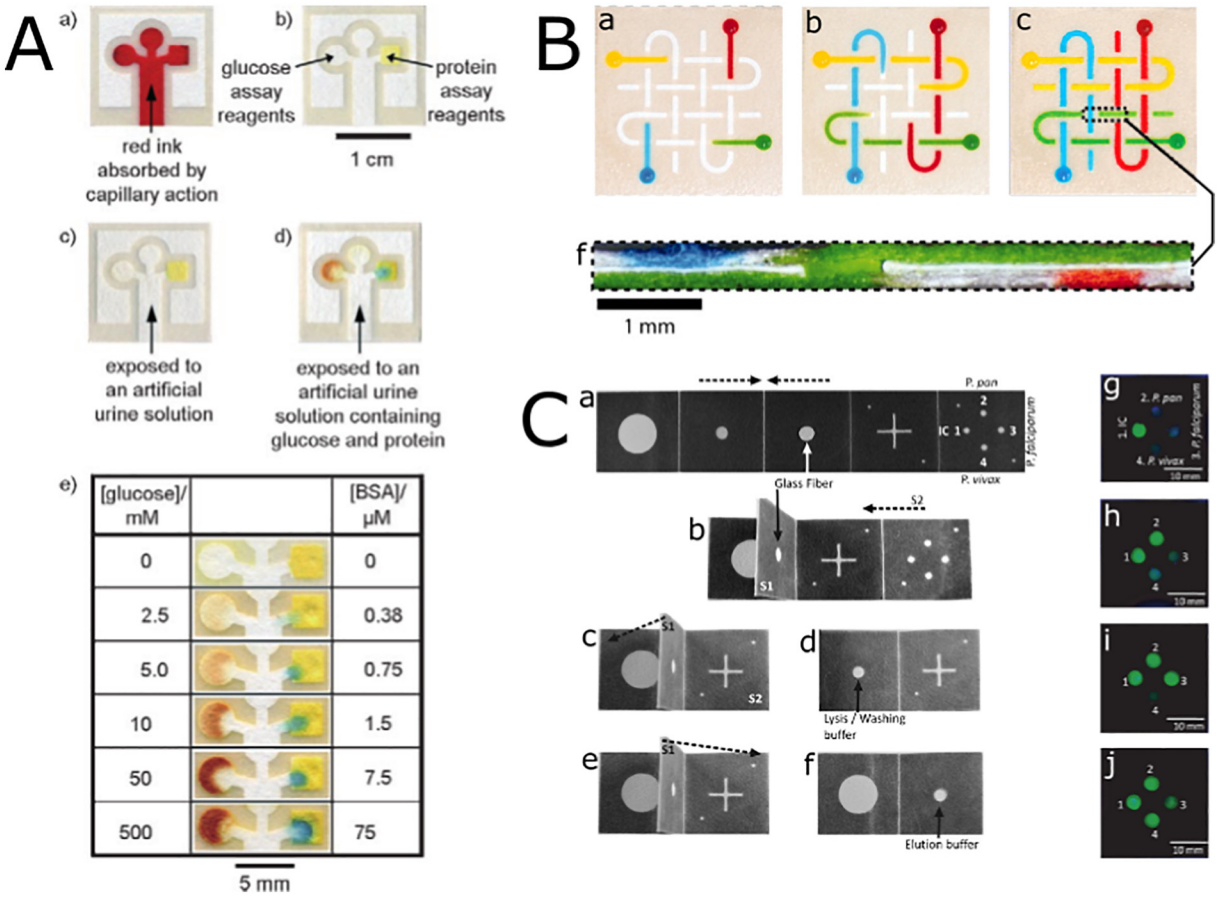

Fig. 7. Paper analytical device. A_a – Red ink is absorbed by the paper and does not penetrate the wax barrier. A_b – shows the complete device with reagents for colorimetric detection of protein and glucose. A_c – shows the device after the exposure to an artificial urine solution while A_d was exposed to an artificial urine solution containing glucose and protein respectively. Colour change in A_d. indicates the presence of glucose and protein in the solution. A_e – shows the difference in colour intensity using different concentrations of glucose and protein. Reprinted from reference [7]. B shows the 3D PAD also developed by Whitesides et al. B_a, B_b, and B_c show the device at three subsequent time points while B_d shows a cross section of the device depicted in B_c. Here the two layers of the device can be seen as well as the vias between them. Reprinted from reference [119]. C – 3D origami device described by Xu et al. C_a shows the device in its unfolded state. The device is the folded as shown in C_b and C_c and a sample is added along with along with a lysis/washing buffer. The device is ten folded as shown in in C_e before an elution buffer is added (C_f). C_g shows a test with the internal control, while positive tests for Plasmodium Pan (C_g), plasmodium pan and *Plasmodium falciparum* (C_i), and plasmodium pan, *Plasmodium falciparum*, and *Plasmodium vivax* (C_j) are also illustrated. Reprinted from reference [115]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chambers for reductive amination and alkylation reactions, large polyoxometalate synthesis and the production of gold nanoparticles [138] as well as printed devices that allow for multi-step reactions to occur in a completely sealed environment which are controlled by changing the orientation of the device [139]. Comina et al. also demonstrated 3D printing as a tool for the rapid prototyping of lab on a chip devices capable of the fluorescent and colorimetric detection of H_2O_2 and glucose [140]. Furthermore, Spivey et al. used a laser based stereolithographic system to create a microfluidic system that could capture yeast cells and remove any progeny to provide insight into the aging process of these cells [141].

Despite the advantages associated with the ability to manufacture complex geometries in a matter of hours, many affordable commercial 3D printers fall short of the resolution required for the manufacture of microfluidics. Having said that, many researchers and hobbyists have been able to assemble homemade 3D printers for a fraction of the cost of the commercial options. Homemade SLA printers are typically based on a digital projector that can project the sliced images in series onto a movable build platform submerged in photo-curable resin. With these projector-based printers, the resolution is a trade off with part size – that is, larger parts can be made with a large dot size from the printer, and small parts can be realised with really fine detail. These projects have been aided by the development of many open source software packages to run these systems on [142]. With this in mind, 3D printing offers a platform on which designs for open source designs could be downloaded and printed on open source printers which highlights the flexibility of the technique and adds to its attractiveness within the microfluidics industry.

5.6. *Two-photon polymerisation lithography*

Like 3D printing, two-photon polymerisation lithography (2PPL) involves the curing of a photosensitive resin to create three-dimensional structures. Where 2PPL differs from 3D printing however, is that an infra-red laser is focussed in 3D space inside of a photopolymerisable resin and through the mechanism of two-photon absorption, only the point at which the laser is in focus is cured giving a higher resolution than single photon techniques. The laser is then scanned through the resin in a pre-defined path to create a given three-dimensional structure. First described by Maruo et al. in 1997 [143], 2PPL has been utilised in recent years for the manufacture of complex photonic devices [144], microfluidic devices incorporating microstructures [145], devices for monitoring the mechanotransduction of cells under shear stress [146], and devices consisting of both optical and microfluidic elements [147]. With this technique, resolutions as low as 120 nm have been achieved [148].

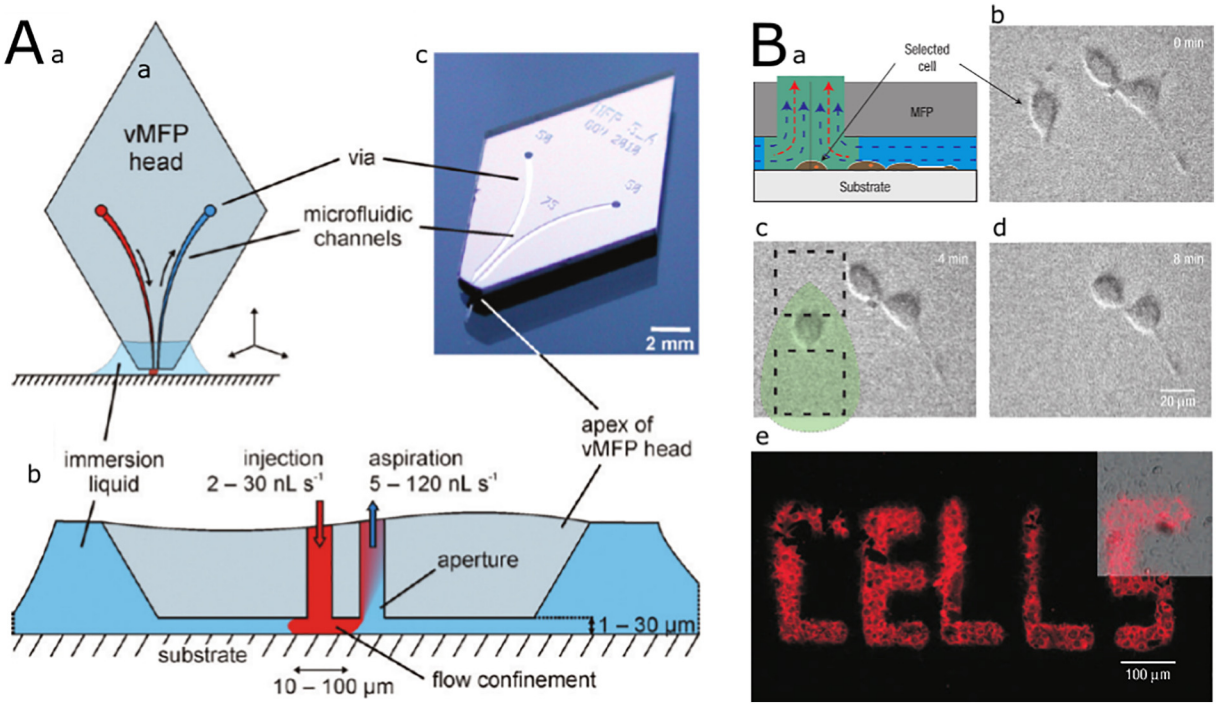

Fig. 8. Schematic (A_a) and photograph (A_c) of a microfluidic probe head showing fluid inspiration and aspiration vias and channels and the apex that sits above the substrate of interest. A_b shows a schematic of the probe with typical flow rates and distances required to produce the flow confinement region in the immersion liquid. Reprinted from reference [119]. B_a illustrates how an MFP can be used to remove a single cell from a culture while B_b, B_c, and B_d show a before, during, and after image of this process respectively. B_e shows cells that have been selectively stained using an MFP in a defined pattern with in inset showing an overlay of the phase contrast and fluorescent images. Reprinted from reference [120].

6. Conclusion: where is the field now?

Over the past 30 years, microfluidics has evolved rapidly from its roots in the microelectronics industry through the conception of μTAS devices into the diverse field it is today. The adaption of existing technologies and the development of new techniques has led to the creation of many devices that have allowed researchers to analyse systems in a much more rapid, efficient and automated manner than before. Additionally, devices have been created that have allowed for the observation of phenomena that were elusive to past technologies. Despite the plethora of new devices that have come to fruition over the last few decades, microfluidics has not yet reached its full potential in terms of its impact within chemistry and the life sciences. This could be down to a number of reasons.

Firstly, in the past, microfluidics researchers did not consider the end users of the devices they were creating. This led to the manufacture of many novel devices that failed to make an impact in the field. Nowadays, there is an increasing trend from engineers to work more closely with biologists and chemists throughout the design process of devices to ensure their usefulness as research tools and to help engineers find the right problems to solve. This type of collaboration should continue to be encouraged and extended not just to the design and functionality of microfluidic devices, but to the fabrication protocols as well. This will allow engineers to create manufacturing process that can be carried out by researchers with little expertise in manufacturing and no access to specialist facilities. Work has already been done on techniques such as 3D printing which has allowed for devices to be manufactured by those without a high level of expertise in fabrication and similar techniques that take micromanufacture out of the clean room (such as digital mask-less photolithography systems) should be developed. Additionally, the digital nature of these techniques will allow researchers to share and download device designs for free and edit them to suit their own needs. Further education of biologists and chemists into microfabrication techniques will also allow these researchers from different fields to design will enable this design and manufacture their own tailor-made devices. This will lead to a wider adoption of microfluidics as a standard experimental tool and not just a technique for those with access to expensive clean room facilities. By collaborating with those from different research backgrounds, the impact of microfluidics will not be judged on how small or fast a device is, but on a device's ability to facilitate ground-breaking and impactful research.

Secondly, microfluidics remains as an academic as opposed for a commercially successful tool. Although there has been some work conducted into the mass production of devices, a lack of standards around how a device integrates and communicates with external analysis equipment means that devices that have been designed to work in one lab, may not work in another. Only once a consensus has been reached on how to connect chips to the external world, will microfluidics become a serious industry. Furthermore, the manufacture techniques associated with prototyping do not always translate into mass production. PDMS casting from a micromachined master is currently the most common method of prototyping a device however, this technique cannot be scaled up to manufacture a large number of parts. This oversight in how devices are manufactured has meant that most microfluidics start-ups fail as although their prototypes work well, they struggle to adapt their fabrication protocols for large scale manufacture. To combat this, work must be done on creating robust manufacturing protocols that are both cheap and quick enough for efficient prototyping while also providing a simple route to automated industrial scale fabrication. However, in order to necessitate this research, there must first be sufficient demand for microfluidic devices outwith engineering communities. This will only be created by addressing the point that collaborations with other fields of research should be fostered. Only with a need for devices, will companies be able exploit this market and provide both standard and custom microfluidics to expedite novel research.

The last 30 years of microfluidics has taken the field from transistors

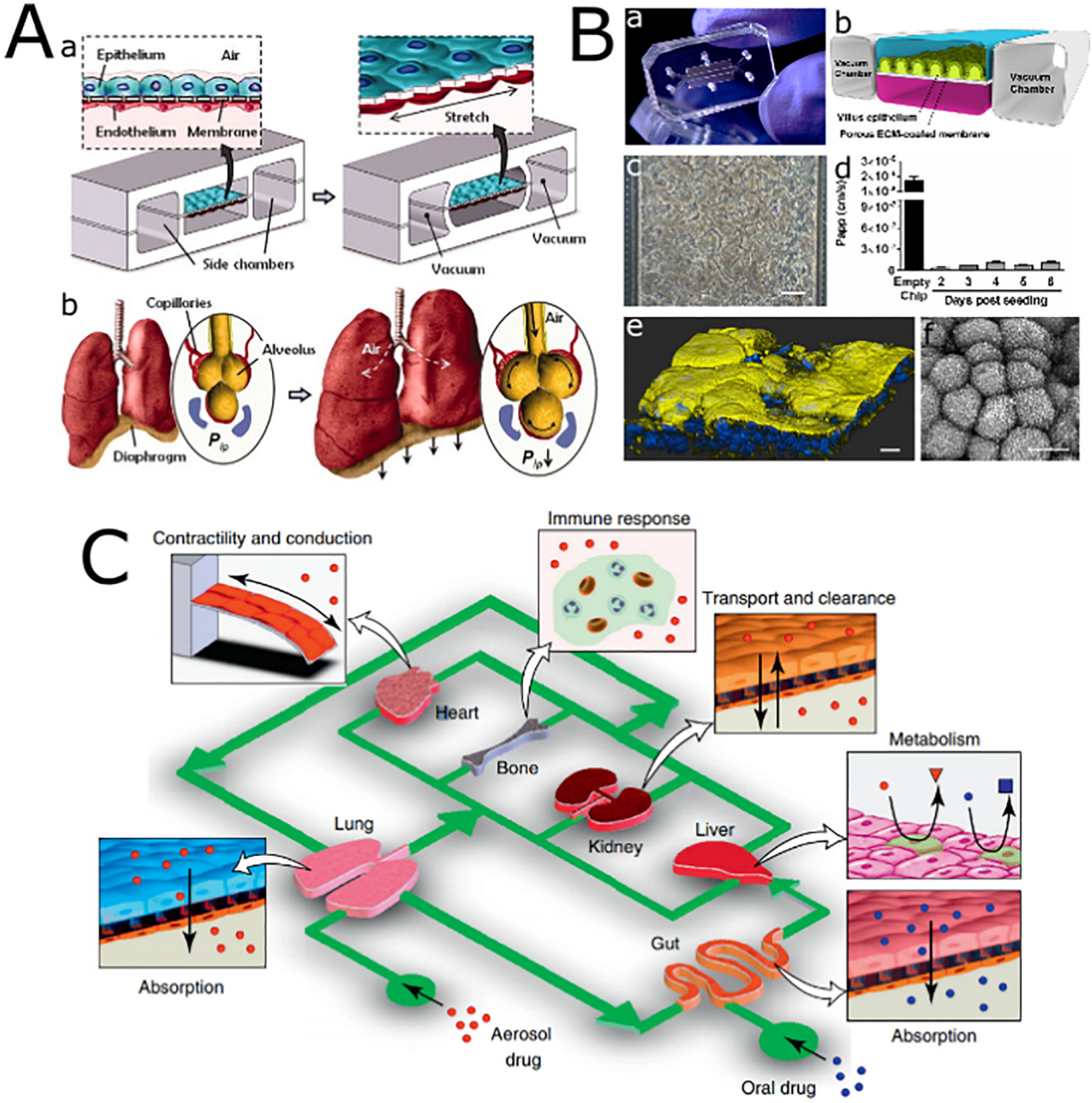

Fig. 9. A – Device to mimic lung function on a chip. A_a. PDMS microchannels are manufactured either side of an extracellular matrix coated PDMS membrane (covered in epithelial and endothelial cells) to mimic the alveolar-capillary barrier. Mechanical stresses are applied by decreasing the pressure in the two side channels to mimic the deformation of the lung during the breathing cycle. This physical deformation of the living lung is shown in A_b. B – Gut-on-a-chip device. Photograph (B_a) and schematic (B_b) of the gut-on-a-chip. B_c shows a phase contrast image of human intestinal epithelial cells cultured on the device while (B_d) shows how the permeability of a device changes when cells are seeded onto the membrane. A confocal immunofluorescence micrograph of the epithelium formed inside the device is shown in B_e. stained for nuclei (blue) and villin (yellow). Microvilli on the surface of the cells can be seen in the scanning electron micrograph (B_f). Reprinted from reference [132]. C – Human-on-a-chip concept. Chips representing individual organs are interconnected to better mimic the human body. It I hypothesised that these systems of devices could be used to gain a better understanding of the effects of a drug without the need for expensive *in vivo* studies. Reprinted from reference [135]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to tissue and if the current hurdles associated with device designs and manufacture can be overcome, the next 30 years look set to bring a whole host of new technologies and impactful research.

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