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Review

Recent advances on open fluidic systems for biomedical applications: A review

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ABSTRACT

Microfluidics has become an important tool to engineer microenvironments with high precision, comprising devices and methods for controlling and manipulating fluids at the submillimeter scale. A specific branch of microfluidics comprises open fluidic systems, which is mainly characterized by displaying a higher air/liquid interface when compared with traditional closed-channel setups. The use of open channel systems has enabled the design of singular architectures in devices that are simple to fabricate and to clean. Enhanced functionality and accessibility for liquid handling are additional advantages inputted to technologies based on open fluidics. While benchmarked against closed fluidics approaches, the use of directly accessible channels decreases the risk of clogging and bubble-driven flow perturbation. In this review, we discuss the advantages of open fluidics systems when compared to their closed fluidics counterparts. Platforms are analyzed in two separated groups based on different confinement principles: wall-based physical confinement and wettability-contrast confinement. The physical confinement group comprises both open and traditional microfluidics; examples based on open channels with rectangular and triangular cross-section, suspended microfluidics, and the use of narrow edge of a solid surface for fluid confinement are addressed. The second group covers (super)hydrophilic/(super) hydrophobic patterned surfaces, and examples based on polymer-, textile- and paper-based microfluidic devices are explored. The technologies described in this review are critically discussed concerning devices' performance and versatility, manufacturing techniques and fluid transport/manipulation methods. A gather-up of recent biomedical applications of open fluidics devices is also presented.

1. Introduction

The precise control of volume and manipulation of fluids is of the utmost importance for several scientific fields, including chemistry, analytical biochemistry, biotechnology, engineering or cell biology [1–3]. Microfluidics has become an important tool to engineer environments with precise control of the studied conditions [3,4]. In general, microfluidics alludes to devices and methods for controlling and manipulating fluids at submillimeter scale [2,4]. This technology has been presented as an attractive candidate to replace traditional experimental approaches, especially in the biomedical field [5].

The most popular and widespread approach for manufacturing microfluidic devices consists in the use of “soft lithography” of poly-dimethylsiloxane (PDMS), and has largely contributed for the technological development of this field [3,5]. The use of these materials allows the easy molding of structures with micrometric resolution by simply using a casting mold [6]. Additionally, PDMS presents valuable characteristics such as low cost, optical transparency, elasticity, permeability to gases, ease of use, and high fidelity reproducibility of patterns [5,7]. This combination allowed both the miniaturization and the parallelization of processes in compact devices, saving reagents and, consequently, costs [3,8]. Owing to the ease fabrication and flexibility of these devices, several kinds of functional microfluidic elements were described in the literature, namely sensors, mixers, separators, dispensers, pumps, valves, *etc.* [1,6,7] Furthermore, different techniques for both fluids and particles manipulation in microfluidics have been developed, using electrical, magnetic, optical, capillary and mechanical forces [1,2].

However, for biomedical research, some concerns about the use of PDMS have been raised. It was found that the leaching of non-cross-linked oligomers from PDMS may be toxic for cells [9,10]. Moreover, due to its hydrophobic properties and permeability, the absorption of

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hydrophobic small molecules by PDMS was shown [11,12]. Additionally, the permeability to water vapor can result in rapid evaporation, which may negatively impact mainly static no-flow experiments [10,13]. Nevertheless, drawbacks associated to PDMS can be mitigated, demanding additional device preparation [7]. Recently, alternative materials to PDMS were explored, namely thermoplastics (polystyrene – PS, cyclic olefin copolymer – COC, polymethyl methacrylate – PMMA, and polycarbonate – PC), paper, wax and textiles [5].

Associated to the microfluidic technology, new concepts that include lab-on-a-chip and organ-on-a-chip have arisen. Lab-on-a-chip concept postulates the full integration of several microfluidic components and procedures in a single chip with the goal of miniaturizing chemical and biological processes [1,14], while organ-on-a-chip refers to complex micro-engineered systems aiming to mimic physiological key features of specific human organs, human tissues and their interactions [15,16]. Besides novel applications, device-design associated concepts also arose from the traditional microfluidics field, which include open microfluidics. Here, we review the technological advances on this topic, namely devices' characteristics, manufacturing techniques and recent applications on the biomedical field. In this review, for a matter of accuracy, we use the expression “open fluidics”, once we also included devices at the millimeter scale.

2. Open fluidics

To understand the improvement this new concept brings to microfluidics, it is important to know the main differences and the associated advantages and disadvantages of the open fluidics technique. Open fluidic systems are characterized by, at least, one area of the device open to air, which does not happen in fully closed microfluidics systems. These open systems comprise, as brief examples, droplets on surfaces or open fluidic channels [17,18]. Besides guaranteeing the core functionality of the traditional microfluidics related to the detection and manipulation of very small volumes, it offers additional unique advantages that ensure their enhanced performance, such as simplicity of fabrication, facility to clean, and accessibility for liquid handling [19–21]. These systems can also overcome typical issues of traditional microfluidics, such as the risk of channel clogging, bulky apparatus and the occurrence of flow perturbation due to bubbles [20,22]. Open fluidic platforms can assume different geometries, which are here organized in two groups: (i) physical confinement and (ii) wettability contrast-based confinement. In the first group, the fluid is confined and manipulated using channels with rectangular or triangular shape (cross section) without one side; channels formed by opposing two vertical walls; or using the narrow edge of a solid surface. The wettability-contrast confinement comprises (super)hydrophilic areas patterned on (super)hydrophobic surfaces, using flat solid surfaces. In the wettability contrast-driven technologies, we also included paper-based microfluidics and systems in which fluid flows are driven by hydrophilic textile fibers on a supporting (super)hydrophobic surface.

2.1. Physical confinement

Platforms that combine both traditional and open microfluidics have taken advantage of the synergistic effect of the combination of both technologies in a single device [23–28]. This combination brought benefits to the systems since, for example, while the open section increases the accessibility and gas bubbles elimination, the closed section minimized the impact of evaporation of the fluid [24,27]. These closed-open platforms consisted in parallel microchannels of PDMS where the ceiling was partially removed in the middle [24] or in the end of the channels – Fig. 1A and B [23]. An open access microfluidic platform, where the parallel channels culminated in an open reservoir instead a tubing system, was also reported allowing direct manipulation of the fluid in the system with a micropipette [27]. In the channel sections without ceiling, the fluid flow remained confined to the open-microchannels due to the high surface tension of the liquid-air interface and to the hydrophobicity of the top surface of the walls [23,24]. On this topic of closed-open microfluidics, Keenan et al. reported the construction of a microfluidic gradient generator [25]. The system was able to produce soluble gradients by injecting picoliter amounts of fluid, from a closed microchannel system, into an open reservoir. Further on, we present the application of this gradient generator in neutrophil desensitization studies [26].

Regarding fully open microfluidic systems based on physical fluid confinement, the most common geometry of the channels is a rectangular cross-section with one open side [29–37]. Several reports studying the wetting phenomenon and the fluid transport dynamics on this open rectangular cross-section microchannels can be found for different materials, namely silicon [29,30,33], SU-8 [31], quartz [32], and PDMS [37]. It was found that a large variety of wetting morphologies can be observed [29,30]. Generally, the wetting behavior was dependent on both the ratio between width and depth of the channel [29,31,32,37] and the wetting properties of the underlying material [29,33]. Similarly, various wetting morphologies were also observed in open triangular cross-section channels, being dependent of the wedge angle of the channel and the liquid contact angle with the substrate [38]. Berthier et al. presented the open triangular cross-section such as an interesting solution for capillary actuation of whole blood in the point-of-care systems domain [39]. These studies aimed to determine the appropriate parameters to accomplish spontaneous capillary flows, avoiding the use of costly actuation systems [20,32,39,40].

Another promising type of open channels is channels devoid of floor and ceiling. In those systems, named “suspended microfluidics”, the liquid is supported by two opposite vertical walls – Fig. 1C [19,21,41]. These systems showed the ability to generate spontaneous capillary flows in a precise, simple and robust way. The suspended channels were easily constructed using PDMS through soft lithography [19] and thermoplastics such as COC and PMMA using milling [21,41]. Based on this technology, Casavant et al. created arrays of suspended microdots for the study of cell growth and cell invasion toward a source chemoattractant [19]. The potential of these suspended microfluidic devices was highlighted for high-throughput multiplexed screening applications with cells in 3D matrices.

Davey and Neild reported an innovative open fluidic channel that consisted in a straight open channel defined by a narrow strip of a solid surface – Fig. 1D [17]. They used the edge of a glass slide to confine the fluid, achieving a similar effect to the liquid confinement provided by a (super)hydrophilic pattern on a (super)hydrophobic flat surface – topic developed ahead [17]. The flow on the channel was induced by a syringe pump in push–pull mode, similarly to the systems commonly used in traditional microfluidics. It was observed that the use of hydrophobic needles on the input and hydrophilic needles on the output increased the stability of the system [17,42]. Additionally, it was possible to produce a stable fluid flow with a flow rate of 500 $\mu L \cdot min^{-1}$ in channels of 1 mm width and 30 mm length [17]. The channel length showed to be important for the stability of the system, since channel dewetting occurred for a length of 40 mm [42]. Using the same strategy for liquid confinement, Tan and Neild created a Y-junction open fluidic channel – Fig. 1E [43]. A system with two branches with 1 mm width merging into a main channel with also 1 mm width was constructed by 3D printing. This system was used to study the mixing of two fluids and, for high flow rates ($> 300\ \mu L \cdot min^{-1}$), the fluid mixing occurred faster than expected for molecular diffusion [43]. However, taking advantage from the air/fluid interface exposure, a rapid mixing of the fluids could be induced by simply blowing an air jet horizontally to the Y-junction. The developed device was proposed as an efficient mixer able to integrate complex systems, combining open and closed microfluidics [43].

2.2. Wettability-contrast confinement

Flat surfaces can be used as platforms to engineer open fluidic

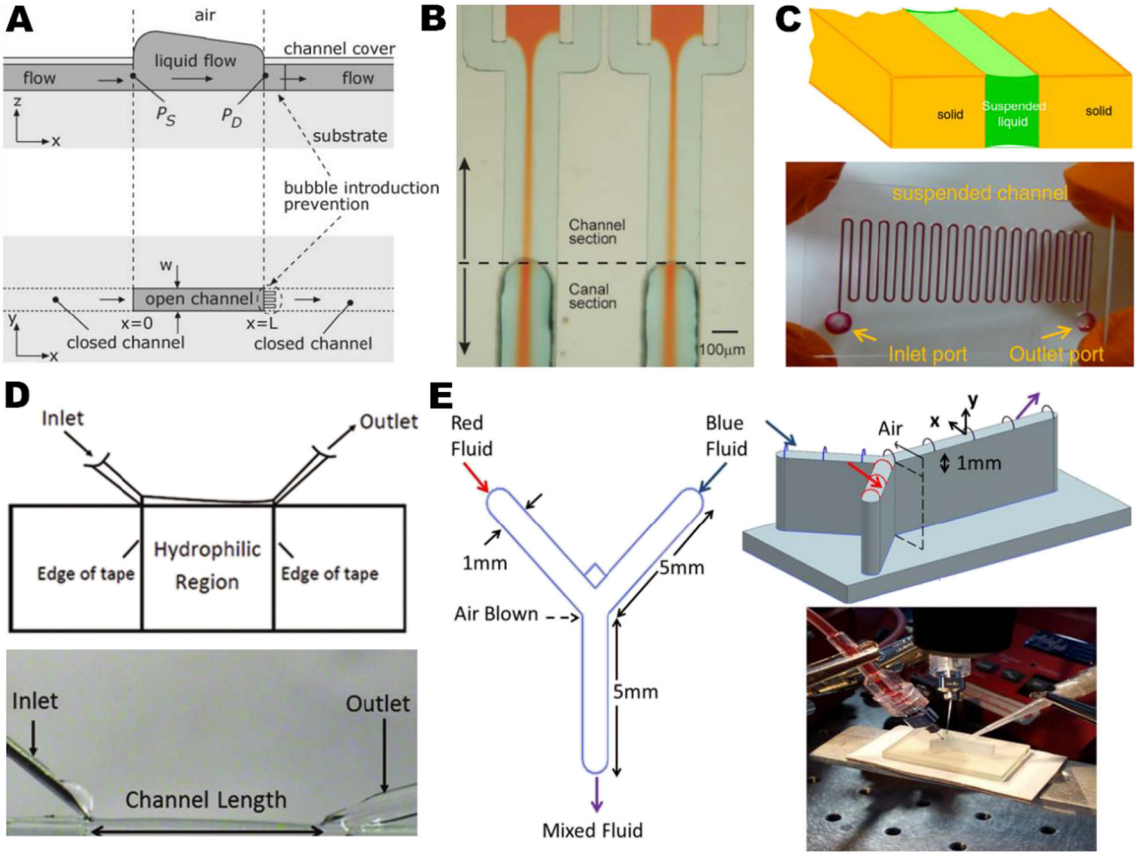

Fig. 1. (A) Cross-sectional lengthways and upper view of closed-open-closed channel. Adapted from Ref. [24] with permission of the Royal Society of Chemistry. (B) Open fluidic device combining both traditional and open microfluidics, named as channel section and canal section in the image, respectively. Adapted from Ref. [23] with permission of the Royal Society of Chemistry. (C) Suspended flow between two vertical and parallel walls and an example of a suspended channel in a PMMA plate. Adapted from Ref. [21] with permission of Springer. (D) Schematic representation of a straight open channel defined by a narrow strip of a solid surface. The flow was produced by the pressure's difference between inlet and outlet in the open fluidic channel. Flow was confined to the exposed hydrophilic region and by the thickness of the glass slide. Adapted from Ref. [42] with permission of AIP Publishing. (E) Schematic representation of the Y-junction open microchannel manufactured with two 1 mm wide branches merging into a 1 mm main channel. Fluid was infused and mixed where the branches met and then the flow was extracted at the end of the main channel. Air was blown horizontally to the Y-junction channel to help in cases where passive mixing cannot be achieved. Adapted from Ref. [43] with permission of AIP Publishing.

systems, where fluid confinement is achieved through chemical barriers capable of acting like virtual walls [44]. These non-physical walls consist in chemical patterns created on the top of a substrate, originating (super)hydrophilic areas bounded by the (super)hydrophobic substrate. Similarly to the effect of the edge of a solid surface, a fluid on a flat surface can be retained due to the large contact angle hysteresis at the (super)hydrophilic/(super)hydrophobic boundary.

By definition, hydrophilic surfaces present a water contact angle (WCA) lower than 90°, while hydrophobic surfaces present higher WCA values. Concerning to extreme water repellency phenomena, superhydrophilic surfaces are completely wettable by water, presenting a WCA lower than 5°, and high surface energy. Superhydrophobic surfaces are characterized for totally repelling water, and present static WCA > 150°, with low surface energy [45,46]. These achievement of extreme wettability phenomena are only possible by combining both surface chemistry (surface free energy) and roughness. Increasing the roughness of a substrate with high surface energy typically increases the apparent hydrophilicity of the surface, whereas increasing the roughness of a substrate with low surface energy usually increases its apparent hydrophobicity [47,48]. Additionally, air trapping that may occur on surface roughness is also essential to reach superhydrophobicity [47].

Several methods for the production of superhydrophobic surfaces have been systematically compiled in different reviews [46,48–53]. Examples include superhydrophobic surfaces produced by covalent layer-by-layer assembly of amine-reactive polymers [54,55]; UV-initiated radical polymerization of a hydrophobic monomer [56–58]; deposition of self-assembled monolayers of hydrophobic molecules in micro/nanostructured surfaces [59–62]; polymer precipitation through a phase separation method in a smooth surface [63–65]; drop-casting onto a sandpaper using a fluoroacrylic copolymer solution [66]; and vapor deposition of fluorosilane molecules in micro/nanostructured surfaces or by immersion within the fluorosilane solution [67–71].

The fabrication of surfaces with (super)hydrophobic/(super)hydrophilic patterning may rely on different principles [45,72,73]. Some examples of strategies used to achieve these patterns include (i) the protection of the wettable areas, which should remain untreated to showcase wettable properties, using an adhesive mask or an inkjet-printed sacrificial layer prior to the hydrophobization process [71,74–78]; (ii) the use of photomasks, stencil masks or sacrificial protective coatings to promote the selective exposure of the (super) hydrophobic surface to treatment targeted at the wettability increase, such as UV [79,80] or UV/ozone irradiation [81,82], or plasma treatment [67,83]; (iii) direct writing of the desired pattern by laser on the (super)hydrophobic surface [84,85]; using printing techniques, through the deposition of molecules that go through oxidative self-polymerization or lipid solutions onto (super)hydrophobic surfaces originating the desired (super)hydrophilic patterns [56,69].

A strict control of surfaces' wettability contrast has enabled holding fluids on a static perspective [58,67,79,86–89], as well as directing

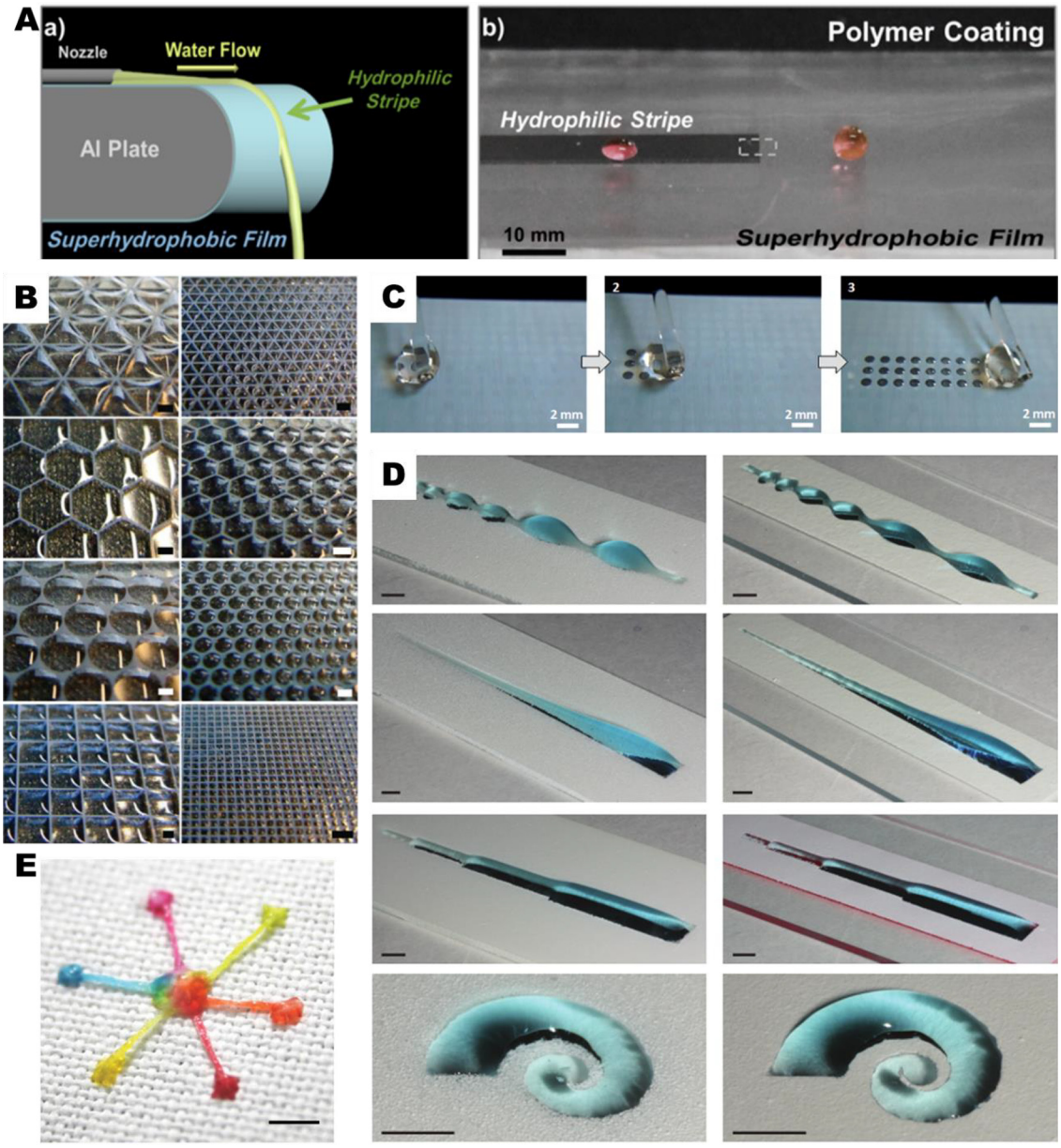

Fig. 2. (A) Schematic representation of a nozzle mounted on the top surface of an aluminum plate with a superhydrophobic/(super)hydrophilic stripe patterning. The water pumped though the nozzle was able to travel along the (super)hydrophilic stripe until separation from the solid edge at the (super)hydrophilic/superhydrophobic dividing line. Adapted with permission from Ref. [92]. Copyright 2015 American Chemical Society. (B) Microarrays of water droplets with different geometries formed on superhydrophilic/superhydrophobic patterned surfaces. Scale bars: 1 mm. Adapted from Ref. [87] with permission of the Royal Society of Chemistry. (C) Representation of the discontinuous dewetting method used for the formation of arrays of microdroplets. Adapted from Ref. [58] with permission of John Wiley & Sons. (D) Droplets formed by pipetting fluid onto sine wave, wedge, staircase, and spiral hydrophilic areas previously created by patterning. Scale bars: 3 mm. Adapted from Ref. [88] with permission of John Wiley & Sons. (E) A multi-inlet–single-outlet design on a textile platform using a hydrophilic cotton yarn sewn into the platform. Scale bars: 5 mm. Adapted from Ref. [106] with permission of the Royal Society of Chemistry.

liquid flows on flat surfaces [59,64,65,90–92]. By studying water condensation on hydrophilic stripes bounded by a hydrophobic substrate, it was observed that water assumed a highly defined cylindrical shape [86]. For small volumes, these water stripes were characterized for their stability and homogeneity. However, for an apparent contact angle on the substrate higher than 90°, the fluid stripes become unstable and the formation of a single bulge per stripe was observed. The increase of the water volume led to the coalescence of the bulges in neighboring channels [83,86,93]. Working below these critical volumes in order to avoid instability, gradients of different materials could be produced in an easy and fast way by capillary flow in a fluid stripe, promising for diagnosis, cell studies and drug screening applications [67,74,75]. Similar path designs and also non-straight paths based on both hydrophobic and superhydrophobic delimitations have been used as channels to drive continuous fluid streams [64,65,91]. Examples of the use of superhydrophobic delimited devices include a study by Dong et al., which showed how to precisely control the separation of a liquid flow from a solid edge, by simultaneously regulating the position of wettability boundary and the flow inertia – Fig. 2A [92]. In detail, the separation was achieved in different positions by moving the (super) hydrophilic/superhydrophobic dividing line at the solid edge. Interestingly, it was suggested that this strategy can be very useful for firefighting or irrigation applications [92]. Besides the stripe shape, a plethora of geometric shapes including squares, circles, triangles or hexagons can be patterned on surfaces – Fig. 2B. These platforms revealed high potential for versatile droplet-array production targeting

high-throughput assessments [80,81,89]. High-density of arrays of single droplets can be produced and assessed independently, due to the extreme wettability contrast that ensures the physical separation between very close droplets [58,87]. Additionally, arrays of thousands of droplets can be produced in a single step by rolling a droplet across the patterned surface or dipping this surface into an aqueous solution [58,77,87]. Owing to the high contrast in the wettability created on substrate, the water can be naturally removed from the superhydrophobic delimitations, while filling superhydrophilic spots, in a phenomenon named discontinuous dewetting – Fig. 2C [58,87]. Remarkably, the patterning of customized shapes onto superhydrophobic surfaces has enabled Hancock et al. to create tailored 3D droplets at the macro- and microscales – Fig. 2D [88]. This technology was suggested for the patterning of microparticles and cells, with spatially controlled gradients (*i.e.* surface concentration), such as sine waves, linear and spiral gradients [88].

Several materials have been suggested for the generation of wettability contrast-based fluidic devices. Paper-based microfluidics is a well-established field that fits the open fluidics category, with particular relevance in the wettability-contrast confinement group. Its design principles and applications, similar to other wettability contrast-based methodologies, will not be explored here; several review papers focused on this specific branch of open fluidics can be found in literature [94–100]. A particularity of the majority of paper-based platforms is related to the absorption of the fluids by the wettable components of the device. Paper's fibrous and porous structure enables the storage and delivery of controlled volumes of reagents, filtration of desired particles or contaminated samples and free diffusion of gases, avoiding air bubble formation [95] or permitting the delivery of necessary gases for cell culture. This structural property allows the development of microfluidic devices for analytical testing, as paper has the capacity of selectively filter particles that may be in a fluid [99]. Nanoparticle-conjugated immunoassays and fluorophore-based sensors have been used in paper-based microfluidics devices to detect cancer antigens, antibodies, bacteria and proteins of interest, as in electrochemical sensing to determine the concentration of targeted ions [99]. Moreover, three dimensional interconnected structures have been developed by stacking layers of this patterned paper, resulting in a multifunctional system capable of selecting different analytes through a complex network of channels [97]. This versatility permits its application in human healthcare, veterinary medicine, environmental monitoring and food safety [94].

Open fluidics devices based on textiles also allow completely flat architectures with (super)hydrophilic patterns printed on a (super)hydrophobic textile background. This technology has been presented in setups highly similar to paper-based microfluidics, or in configurations that use hydrophilic yarns on the top of a non-wettable supports [96,101–106]. For example, Xing et al. reported a microfluidic platform able to drive liquid flows using a hydrophilic cotton yarn sewn into a superhydrophobic textile platform – Fig. 2E [106]. With this device, easily controllable continuous flows were achieved by the combination of surface tension-induced Laplace pressure and capillarity presented in the fibrous structure. Similarly, Yildirim et al. engineered fiber surfaces to produce microfluidic devices using superhydrophilic polymeric fibers [107]. However, the liquid spread spontaneously on the exterior of the fibers, contrary to textile hydrophilic fibers. These fiber surfaces showed suitable for the construction of mechanically robust and flexible, lightweight and inexpensive microfluidic devices [107].

3. Fluid manipulation and transportation in open fluidics

Fluid transport and manipulation in open fluidics systems may be performed either by spontaneous/passive or energy input-dependent/active methods.

Systems based on the action of capillarity are the most widespread devices based on passive fluid transportation. Those rely on a spontaneous liquid flow that determines the physical and geometrical conditions of the fluid, whereas actively manipulated systems depend on the external provision of energy to the system, often accomplished by the use of components as pumps, valves or voltage [21,108]. Passive approaches for fluids' control have risen great interest, since they allow fully automated operations with low-cost and portable devices, facilitating the fluid effective flow in microscale systems [21]. However, passively actuated micro-fluidic devices are unable to provide high level of fluid control [108]. In contrast, active fluid actuation requires energy sources and bulky apparatus, but it can be very useful to control the fluid behavior, as it enables a large range of contact angles in hydrophobized substrates [30]. Yang et al. studied the dynamics of capillary-driven liquid flow in grooved channels with two different geometries: rectangular and curved cross-section [32]. The hydrophilic channels were created in a hydrophobic substrate and water/glycerol mixtures presenting different surface tension and viscosity were tested. It was found that the flow velocity of the tested fluids increased with decreasing the channel width, and this behavior was independent from cross-section geometry [32]. On the other hand, Feng and Rothstein proved the possibility of building open microchannels capable of restricting fluid movement in a single direction [108]. The unidirectional spontaneous flow was achieved by decorating the interior of linear channels with an array of angled fin-like-structures in both side walls – Fig. 3A. This behavior was explained by the direction-dependent Laplace pressure induced by these structures on the channel, allowing the capillary spreading of the fluid only in the predefined direction [108]. Regarding open fluidic devices based on wettability-contrast confinement, Ghosh et al. presented a wettability patterning method to produce open microfluidic paths that were able to induce on-chip liquid movement, by overcoming viscous and gravity forces – Fig. 3B [79]. Wedge-shaped patterns were used to produce superhydrophilic paths embedded on a superhydrophobic background. Driving capillary forces were responsible for the liquid movement and increased linearly with the wedge angle along of the paths [79]. Complex liquid manipulations such as liquid metering, merging, dispensing, and droplet splitting were achieved by patterning complex designs using the wedge-shaped patterns as the basic building blocks. For example, using a planar superhydrophobic surface with superhydrophilic wedge paths arranged radially and dispensing droplets on the central spot, the droplets were rapidly and equally split amongst the different paths [79].

Regarding active liquid manipulation, there are some methods used in closed microchannels that can also be applied to open fluidics, namely electrowetting [28,30,38] and pressure driven flow [17,42,43,65,92]. Using electrowetting actuation, the advancing and receding of liquid stripes in both rectangular and triangular (cross section) grooved microchannels were actively controlled – Fig. 3C [30,38]. Owing to the electrowetting effect, the apparent contact angle of liquid could be reversibly tuned and consequently the liquid movement induced. These movements were controlled by adjusting the frequency and amplitude of the applied voltage. This liquid activity showed to be capillarity driven and dependent of apparent contact angle, liquid viscosity and groove geometry [30,38]. Using a platform combining both closed and open microfluidics, Wang and Jones have showed that water droplets can also be manipulated by electrowetting actuation [28].

Similarly to the traditional microfluidics, the pressure driven flows in open fluidic systems are usually induced by mechanical pumps [17,42,43,65,92]. However, Vourdas et al. presented an innovative toll for actuation and valving in open fluidics using pressure – Fig. 3D [36]. By applying pressure at the rear face (backpressure) of porous and hydrophobic fluidic walls, that initially were sticky, the walls became slippery. Thus, controlling the backpressure, the gas pockets at the liquid-solid interface that influence wall stickiness were controlled. Consequently, the manipulation of liquid volumes became possible in an open channel with rectangular cross-section, but not applicable to other cross-section geometries [36].

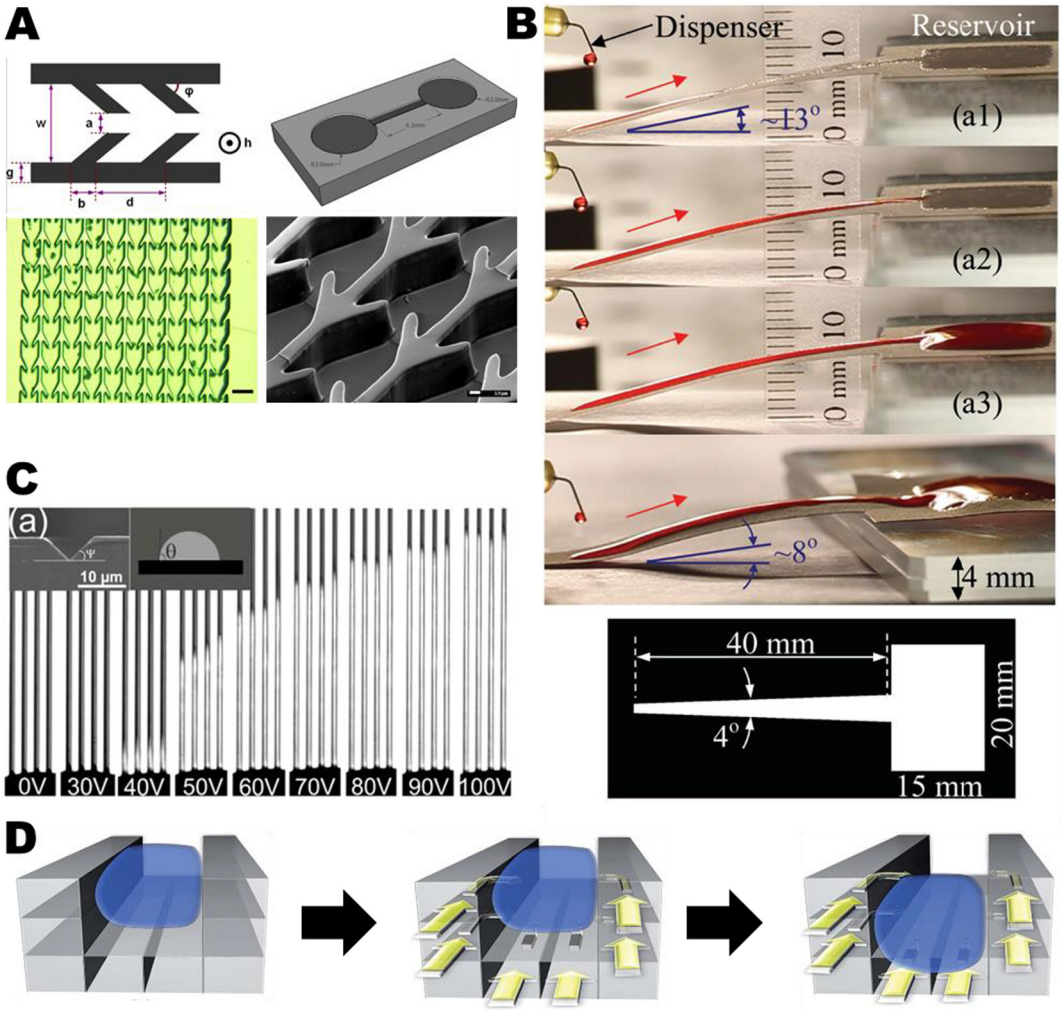

Fig. 3. (A) Schematic illustrations and images of an open channel that allowed only unidirectional spontaneous flow by decorating the interior of the channel with an array of angled fin-like-structures in both side walls. Adapted from Ref. [108] with permission from Elsevier. (B) Transport of liquid up along an inclined superhydrophilic wedge-shaped path. Water was able to move up along to an elevation of 9 mm, as shown by the sequence of images a1-a2-a3. Design of the photomasking template used for path patterning. Adapted from Ref. [79] with permission of the Royal Society of Chemistry. (C) Image of liquid stripes in triangular grooves during electrowetting. The corresponding applied voltage is given at the bottom of each stripe. Adapted with permission from Ref. [38]. Copyright 2015 American Chemical Society. (D) Schematic illustration of the valve architecture and operation. The channel with porous and hydrophobic walls accommodated the liquid and then a gas flow was applied at the adjacent channels. The backpressure increases and consequently the gas pockets at the liquid-solid interface, inducing the wall slippery and inciting the liquid movement. Adapted from Ref. [36] with permission of the Royal Society of Chemistry.

4. Open fluidic platforms for biomedical applications

The high functionality and flexibility typical of open fluidics have been translated to the development of several applications using these platforms. In this section, we report the enabling open fluidics technologies that have impacted the biomedical field. Moreover, we perform a systematic and critical analysis of the key factors used so far for the development of application-specific setups (Table 1).

4.1. Metabolite extraction

Open fluidic platforms have been reported for metabolite extraction applications using different architectures. Barkal et al. created an open platform that allowed microbial culture and the subsequent solvent extraction of the metabolites in a single device [109]. Teardrop-shaped open microfluidic channels were micromilled in polystyrene using a CNC micromilling machine. On the larger area of the channel, a grooved circular well was used for cell culture, and the opposite end of the channel was used to dispense the extraction solvent using a micropipette. This design enabled the organic solvent flow to be directed over the aqueous culture area by spontaneous capillarity (analogous to wedge-shaped paths presented by Ghosh et al.) [79], originating a stable biphasic interface. Additionally, the efficiency of processes was enhanced once the devices allowed the production of arrays and were compatible with the use of a multichannel pipette. Taking advantage from the features of these teardrop-shaped open channels, their applicability for screening analysis of biological samples such as blood, saliva, mucus, and extracellular matrix components was suggested [109].

Based on suspended microfluidics, Casavant et al. created an alternative metabolite extraction platform using a multilayer biphasic system to recover metabolites from cell culture [19]. As the capillarity flow method can also generate flow in open systems with immiscible liquid interfaces, the ability of an immiscible solvent to flow in a suspended microfluidic system over an aqueous liquid was shown. A two level microsystem was developed to promote the contact of extraction solvents with cell culture medium from cells in culture, and extract cell-secreted molecules [19].

Table 1
Key factors driving the design of open fluidic devices.

	Method	Classification	Substrate	Architecture	Driving force	Comments	Ref.
Metabolite extraction	Suspended microfluidics	Physical	–	Multilayer biphasic system	Passive: capillarity		[19]
	Micromilling	Physical	Polystyrene	Tear-shaped channels with biphasic organic/aqueous flow	Active: micropipetting	The method enabled the formation of arrays	[109]
Hydrogel preparation	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Glass; superhydrophobicity conferred by commercially available spray	Several geometries, including circles, spiral shapes and stripes	Active: micropipetting	Amenability to produce gradients; Compatibility with cell encapsulation	[88]
	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Polymeric film on a glass substrate; chemical grafting of fluorosilane and vinyl groups on patterned regions	Several geometries with sharp precision: squares, circles, heart-shaped patterns	Active: micropipetting and discontinuous wetting	Easy filling of arrays by discontinuous wetting. Amenability to sustain hydrogels in the patterned surface, or to remove them as free-standing structures after processing by adapting the crosslinking method (sandwich method); compatibility with cell encapsulation and preparation of magnetic structures	[110]
	Micromold wells	Physical	PDMS	Cubic microwells	Active: pipetting	Compatibility with cell encapsulation in the produced hydrogels	[111]
Gradients generation	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Glass slide coated with a commercially available hydrophobic spray; adhesive mask to maintain the hydrophilic patterned area untreated	Hydrophilic stripe on a hydrophobic glass slide.	Passive: surface tension and diffusion Active: micropipetting of a second solution	Possible generation of both soluble and microparticle gradients; gradient biomaterials production by crosslinking gradients of prepolymer solutions Compatible with 3D cell cultures.	[75]
	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Glass slide chemically modified to create the hydrophobic/hydrophilic pattern	Platform with hydrophilic spots	Active: pipetting		[89]
	Molded open rectangular channel	Physical	Vinyl	Rectangular grooved open channel where hydrogels with different gradients could be manufactured in a layer by layer methodology.	Active: pipetting	Multi-gradient hydrogel generation able to create relevant microenvironments for cellular studies.	[34]
	Molded microchannels placed on a glass	Physical	PDMS and glass slide	Parallel microchannels culminating in an open reservoir	Passive: diffusion	Shear forces on cells avoided, preventing its damage; cell migration and cell sensing studies; due to its design, stable gradients were generated.	[27]
	Molded tubing and reservoir	Physical	Silicone and PDMS	Open reservoir for cell culture with connected microjet arrays with inlets associated	Active: Injection	Precise amounts of the fluid injected; cell migration and morphology examined	[26]
Cell culture	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Superhydrophilic/superhydrophobic patterned surfaces for cell culture	Microarray chip composed by nanostructured multilayered films of parallel cell-containing microreservoirs.	Active: micropipetting	Cell-cell communication studies in ultrahigh-density cell microarrays with different cell types; *in situ* examination of morphological, physicochemical and biological properties of the multilayered films in the microarray chip	[78,115]
Hanging drop	Patterning of hydrophobic/ superhydrophobic regions	Wettability contrast-based	Superhydrophilic/superhydrophobic patterned surfaces	Easily handled platform with hydrophilic spots	Active: micropipetting	Reduced volume for cell growth required; direct accessibility for cell culture medium replacement or drugs/molecules addition; suitability and robustness for drug screening analysis	[77]
	Milling	Physical	Polystyrene	Asymmetric two-well platform: a larger well for cell culture and an interconnected smaller well for liquid manipulation	Active: Micropipetting Passive: From the smaller well to the larger well	Lower shear stress for cells during fluid exchange; long-term cell culture with minimal disturbance; compatible with co-cultures	[18]
	Microwells	Physical	PDMS	Circular areas for hanging droplets interconnected by channels for liquid circulation	Active: pipetting	Combination of the platform with a FACS device allows an automatic and precise single cell load into specific culturing compartments	[22]
Perfusion systems	Micromilling	Physical	Polycarbonate		Active: syringe pump	Possible incompatibility with three-dimensional cell culture	[120]

(*continued on next page*)

Table 1 (*continued*)

Method	Classification	Substrate	Architecture	Driving force	Comments	Ref.
Patterning of hydrophobic/superhydrophobic regions	Wettability contrast-based	Polystyrene	Two-level bonded polycarbonate structure where a coverslip with sliced tissues is inserted Rectangular stripes	Active: syringe pumps	Easy on-chip microscopic assessment; amenability to produce arrays; possible incompatibility with three-dimensional cell culture	[121]

4.2. Hydrogel preparation

As previously referred in Section 2, 3D droplets with fine tuning of their shape and size at the macro- and microscales could be obtained through the customization of hydrophilic patterns on hydrophobic substrates [88]. This technology enabled the fabrication of hydrogels with controlled 3D topography at the macro- and microscale. Those were synthesized by the UV-induced photocrosslinking of droplets of prepolymer solution, which were pre-shaped in the wettable regions of the superhydrophobic platforms. The formed hydrogels retained the 3D shape imposed to the prepolymer droplets with high fidelity [88]. In 2016, the rapid production of alginate hydrogels with defined sized and shapes was reported using superhydrophobic-hydrophilic micropatterns [110]. The size and shape of the particles were defined by specific patterns on the flat surfaces, likewise to the tailored 3D hydrogels. The droplet formation on micropatterns was performed through discontinuous dewetting (described before), decreasing the time required for the microarray platform preparation. Arrays of adhered hydrogels were obtained by performing the sandwiching step with the carrier of alginate droplets over the carrier of calcium chloride droplets. On the other hand, using the carrier of calcium chloride droplets on top position, freestanding hydrogel particles were produced [110]. Additionally, these hydrogel particles showed potential for tissue engineering applications, once they were able to encapsulate live cells and present magnetic properties, by incorporating magnetic beads.

Methods based on the physically constrained patterns (wall-based devices) have also been reported for the preparation of hydrogels. Patel et al. presented a simple approach to micro-manufacture arrays of bioadhesive hydrogels using a sandwiching method to achieve the ionic gelation of a prepolymer solution [111]. A gelatin-based solution was poured onto a PDMS microwell mold and, using a hydrophobic glass slide as carrier, a hanging droplet of silicate nanoparticle solution was formed. Finally, by precise alignment between PDMS platform and glass slide carrier and sandwiching both, the diffusion between two solutions occurred and the microgels formed, may containing encapsulated cells [111].

4.3. Gradients generation

Methods for generating gradients of chemicals, materials, biological molecules or cells have shown high importance in biotechnology, materials science and cell biology [112]. Several applications have been reported, namely targeting diagnostics, material screening and fundamental biological studies that include mimicking cellular and tissue microenvironments [74,89,112,113]. Open fluidic devices revealed to be very useful to generate soluble and microparticle concentration gradients, gradient hydrogels or molecular gradient for chemotaxis studies.

Simple gradient generation methods based on (super)hydrophilic/(super)hydrophobic patterned surfaces have been engineered. Hancock et al. developed a gradient technique employing an inexpensive hydrophilic/hydrophobic-patterned platform and passive mechanisms (surface tension and diffusion) – Fig. 4A [74]. This platform was created by coating a glass slide using a commercially available hydrophobic spray and protecting the hydrophilic patterned area - that should remain untreated and hydrophilic - with an adhesive mask. After removing the rectangular-shaped mask, a fluid stripe confined to the hydrophilic area was produced. In one end of this stripe, a droplet of a second solution was dispensed using a micropipette. On this step, a concentration gradient of the second fluid into the first fluid was generated by diffusion – Fig. 4A. The developed method allowed to generate both soluble and microparticle gradients [74]. Additionally, using this bench-top technique, gradient biomaterials were produced by crosslinking gradients of prepolymer solutions. As proof-of-concept, concentration gradients of encapsulated cells and with a 3D spatial distribution in the biomaterials were produced [75]. Other example of

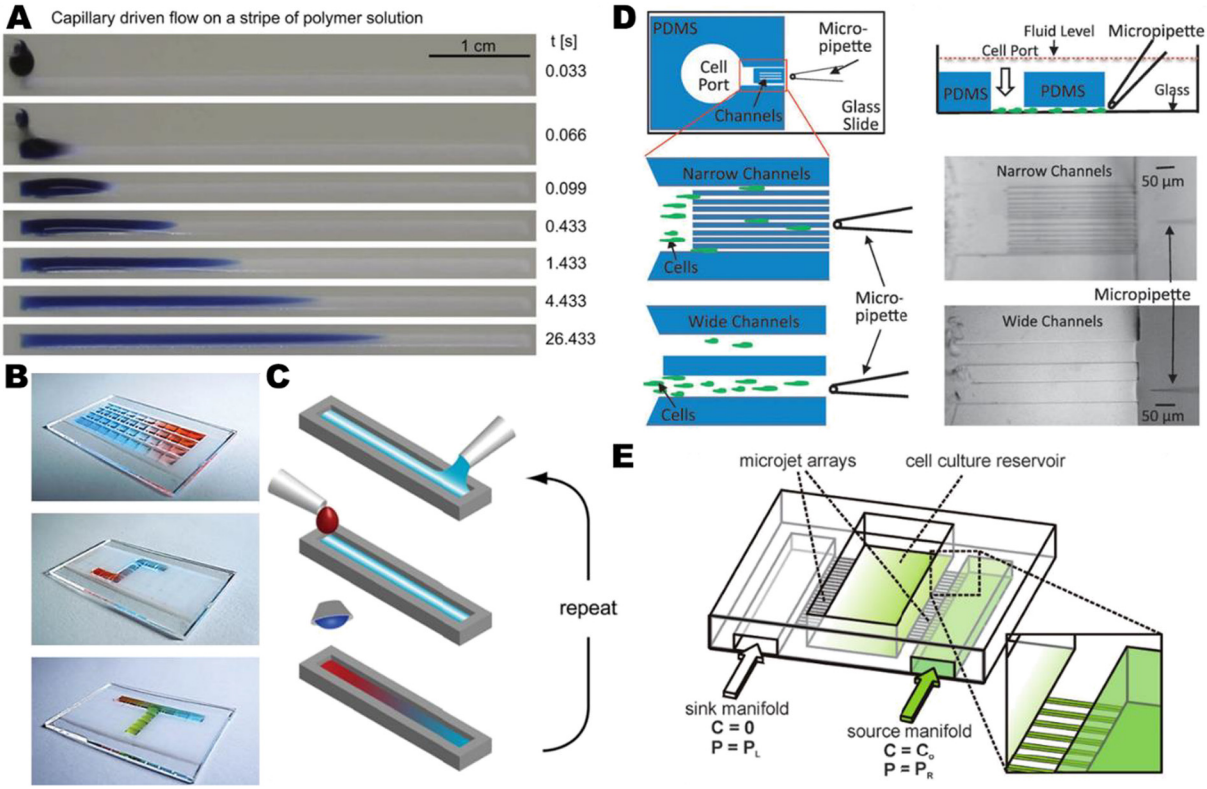

Fig. 4. (A) Images of the droplet coalescence with a fluid stripe and gradient generation of the second fluid into the first fluid over time. Adapted from Ref. [75] with permission from Elsevier. (B) Concentration gradients in liquid channels with different geometries produced using an array of hydrophilic spots. Adapted from Ref. [89] with permission of John Wiley & Sons. (C) Protocol for production of multi-gradient hydrogels through a layer by layer methodology, using a rectangular (cross section) grooved open channel. Channel was prewet and a droplet of a second fluid was added, generating a gradient. The solutions were left to achieve the desired uniformity and this gradient precursor solutions were photocrosslinked. The process was repeated several times until obtain the multi-gradient and layered hydrogel. Adapted from Ref. [34] with permission of the Royal Society of Chemistry. (D) The open microfluidic device for studying gradient sensing and cell migration. Owing to the direct access to the open reservoir where the parallel microchannels culminated, stable gradients were generated by passive diffusion using a micropipette. Then, the cellular response of cells seeded on the cell port in the opposite end of the microchannels was monitored. Adapted from Ref. [27] with permission of the Royal Society of Chemistry. (E) 3D schematic of the device showing the open architecture of the cell culture/gradient chamber, where soluble gradients were generated by injecting picoliter amounts of fluid, from a closed microchannel system, into an open reservoir. Adapted from Ref. [26] with permission of the Royal Society of Chemistry.

an open fluidic platform based on wettability-contrast confinement and able to generate gradients was developed by Efremov et al. [89]. In detail, an array platform of hydrophilic spots was created and operated in one of two modes of use: arrays of droplets with one droplet per hydrophilic spot; or liquid paths formed by neighboring droplets that were merged over the hydrophobic boundaries using a pipette tip. By injecting a solution on these liquid paths, concentration gradients of chemicals or cells were generated – Fig. 4B [89].

Other interesting concept enabled by open fluidics strategies is the generation of multi-gradient hydrogels [34]. Through the use of a rectangular (cross section) grooved open channel, hydrogels with multiple gradients could be manufactured in a layer by layer logic – Fig. 4C. Each layer could present a different gradient of particles, soluble factors, materials properties or polymer concentrations. It was proposed that the multiple gradients in biomaterials can create relevant microenvironments for cellular studies by, for example, mimicking simple systems for studying co-cultures [34].

Using an open access microfluidic device, briefly described before, concentration gradients were produced for the study of cell migration during chemotaxis [27]. Taking advantage from the direct access to the open reservoir where the parallel microchannels culminated, stable gradients were generated by passive diffusion using a micropipette – Fig. 4D. After dispensing the chemoattractant molecules in the reservoir, the gradient equilibration in the channels occurred fast due to the small dimensions of channels. Afterward, the cellular response of cells seeded on the opposite end of the microchannels was monitored. As gradients were generated by a passive method, shear forces usually present in traditional microfluidic devices were avoided. This feature was important to prevent cell damage and confounding cellular response originated by shear forces [27].

By combining closed and open microfluidics, other microfluidic gradient generator was also engineered [26]. The gradient was created by injecting the chemoattractant in precise amounts into an open reservoir, in which human neutrophils were previously seeded and allowed to settle and attach – Fig. 4E. Parameters such as cell migration and morphology were quantitatively examined. Besides the study of human neutrophils, the developed gradient generator could have potential application for assays with neurons, immune cells or embryonic stem cells [26].

4.4. Cell culture on chip

One of the major advantages that open fluidics brings to cell culture is the direct accessibility, allowing for example single-cell manipulation and probing using a micropipette [23]. Open fluidic platforms have given an important contribution on the development of array production using materials, molecules and cells for high-throughput screening application, namely using platforms based on the wettability-contrast confinement [58,78,81,85,114]. The usage of superhydrophilic/superhydrophobic patterned surfaces enabled the development of simplistic

methods for the production of ultrahigh-density cell microarrays [114]. The ability to control the arrangement and geometry of surface patterning allowed to create patterns of several different cell types on the same substrate to study cell-cell communication [115]. The method was based on a parallel formation of several cell-containing microreservoirs on the cell seeding/adhesion step that were confined by the wettability contrast between hydrophilic regions and superhydrophobic boundaries. Then, with the several cell types adhered to the platform, the platform was submerged in cell culture medium, and cell response to the neighboring cells monitored [115].

On a single platform, several different conditions can be tested simultaneously and separately in a tiny space, which allows saving time, materials and costs. Thus, different combinations of nanostructured multilayered films were produced using layer by layer methodology in a single chip for fast high-throughput screening. *In situ* examination of the morphological, physicochemical, and biological properties of the multilayered films was performed on the developed microarray chip [78]. The opposite can also be done: first, to perform cell seeding into the hydrophilic spots and then add the reagents in study to the cell droplet array. The reagent addition can be done one by one using a micropipette or simultaneously using the sandwiching method described before [58,85]. The carrier of the reagents in study, such as drugs or transfection mixtures, can be prepared using a noncontact ultralow volume dispenser with the equivalent geometry to the geometry of cell carrier [58].

These new tools, virtually accessible in any laboratory, showed high potential to be used in fields such as regenerative medicine/tissue engineering, diagnosis, cellular biology and drug discovery.

4.5. Hanging-drop systems

Owing to their great potential for cell therapy, drug discovery and tissue engineering applications, 3D spheroids have raised interest in the biomedical field. Thus, the development of new tools for cell spheroid production has gained momentum in the last years. Some reported platforms for the production of these 3D cellular structures are based on open fluidics, with main emphasis on the hydrophilic/superhydrophobic patterned surfaces technology [77,116–119]. In general, droplets with cells in suspension are placed on the hydrophilic spots – Fig. 5A. Then, the platforms are immediately turned upside-down, taking advantage of the high adhesiveness between droplets and spots for maintaining the droplets on the platform surface. Due to the gravity effect, the cells in suspension on the droplets aggregate to create cell spheroids. The spheroid size and morphology have been precisely controlled by adjusting the droplet size and cellular density [116,118,119]. Additionally, these same hanging droplet platforms allowed to produce arrays of cell spheroids for high-throughput drug screening tests [77,117,119]. This category of platforms presented several advantages over the conventional hanging droplet methods, namely the use of reduced volume for cell growth, direct accessibility that allowed easy cell culture medium change and the addition of drugs or other molecules, and both suitability and robustness for combinatorial high-throughput drug screening analysis [77,117].

Alternatively, other open fluidic platforms for hanging droplet cell culture were developed based on the suspended microfluidics concept. One example is a two-well hanging droplet platform, consisting in a larger well for cell culture and an interconnected smaller well for liquid manipulation using a pipette – Fig. 5B [18]. The device was produced using PS by CNC milling. As the liquid manipulation was performed in an adjacent well to cell culture well, lower shear stress was experienced by cells during fluid exchange. This asymmetric two-well droplet system opened the possibility for performing long-term culture with minimal disturbance to the cell culture conditions, using shear-sensitive or non-adherent cells. In order to demonstrate the platform versatility, co-culture experiments were also performed, testing both direct and indirect co-culture conditions [18].

Other example of a suspended microfluidic system for hanging droplet cell culture was presented by Birchler et al. [22]. This system consisted in a PDMS platform with circular areas for hanging droplets and interconnected thought channels for liquid circulation – Fig. 5C. Combining the developed open fluidic platform with a fluorescence-activated cell sorting (FACS) device, specific single cells could be directly loaded into defined culturing compartments of the platform in an automatic and precise way. Through this combination, cells were directly sorted into a ready-to-use platform without unnecessary manipulation [22].

4.6. Perfusion system for cell culture

Applying the open fluidic concept, a fluidic system was created to improve long-term *in vitro* culturing and monitoring of organotypic brain slices [120]. The platform was built using polycarbonate by micromilling and was constituted of two levels. The bottom level contained fluidic structures with channels and a circular chamber aligned with the culturing area, where the culture medium was perfused. Medium perfusion was actively controlled through a syringe pump connected to the channels, allowing a constant supply of nutrients and waste removal from the system. The upper level consisted in a hole to accommodate a porous membrane insert in the culturing area, where the slices of tissue were placed. Due to the continuous perfusion of cell culture medium, the brain slices were cultured for longer periods with reduced handling of the tissues during culturing, and the *in vivo*-like environment could be better mimicked [120].

On a different study, the focus was the influence of the shear stress forces caused by a fluid flow in contact with cells [121]. The culture medium stream was confined based on the wettability-contrast of a platform composed of hydrophilic paths set on a planar superhydrophobic platform. Osteogenic differentiation of C2C12 myoblast cells under flow perfusion dynamic conditions combined with biochemical modulation using bone morphogenic protein-2 (BMP-2) was studied, and cellular morphology and osteogenic commitment was assessed on-chip using standard microscopy techniques [121].

5. Summary and future outlook

This review approached the emerging topic of open fluidics. A thorough analysis of associated manufacturing techniques, devices' characteristics, as well as recent advances on biomedical applications were provided. A contextualization of traditional microfluidics technical features and commonly used materials for the fabrication of fluidics devices was performed to establish a comparison with recent and most innovative technologies presented throughout the review. Open fluidics was explored based on principle driving the design and fluid confinement on the devices: physical (wall-based) or wettability-based. Innovative systems were described, such as liquid flow confinement and its control by a narrow strip of a solid surface and the suspended microfluidic concept, a promising technology in the open fluidics topic. Regarding the wettability-contrast confinement, basic concepts related to surface wettability were added, followed by the introduction of some platforms such as (super)hydrophobic substrates with (super)hydrophilic patterns, textile-based and paper-based microfluidics. Hydrodynamic conditions to reach stable fluid flows and precise fluid manipulation on these patterned platforms were discussed. Methods for fluid transport and manipulation in open fluid devices were also addressed. Those included passive methods - such as capillary-driven liquid flow and unidirectional spontaneous flow, as well as active methods that comprise electrowetting actuation and pressure driven flow. Finally, valuable applications of these platforms in the biomedical field were reviewed, namely metabolite extraction, hydrogel production, gradient generation, microarray systems, hanging-drop systems and perfusion systems for cell culture. A critical analysis of the key factors used in the design of biomedical devices was

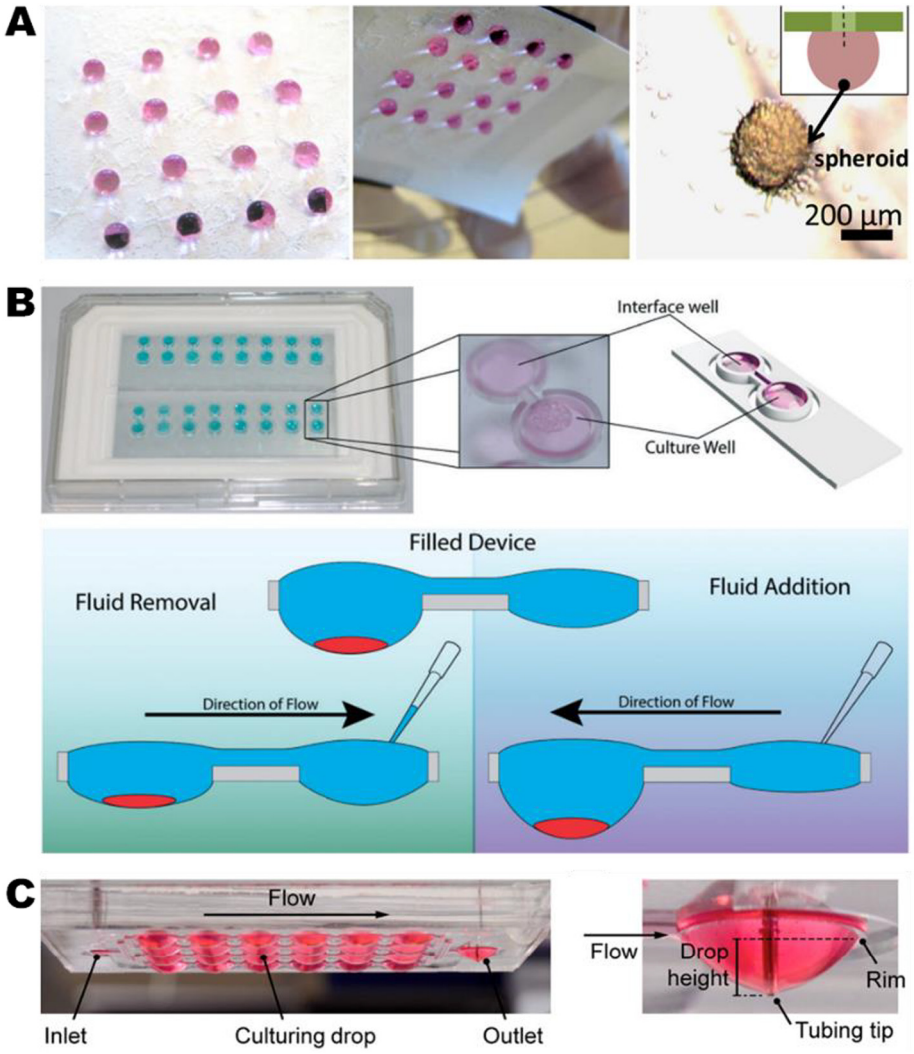

Fig. 5. (A) Images of the chips with the cell suspensions and then immediately turned upside-down. By the gravity effect, the cells in suspension aggregated, originating cell spheroids. Adapted with permission from Ref. [77]. Copyright 2014 American Chemical Society. (B) Schematic illustration of the two-well hanging droplet device and operation of a filled device containing cells (in red), when fluid is removed or added through the adjacent well to cell culture well with minimal disturbance to the culture. Adapted from Ref. [18] with permission of the Royal Society of Chemistry. (C) Image of the hanging-drop PDMS platform with circular areas for containing cell droplets and interconnected thought channels for liquid circulation. Adapted with permission from Ref. [22]. Copyright 2016 American Chemical Society. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

presented.

A careful analysis of the literature enabled the critical summarization of the main promising features and limitations associated with open fluidic systems. Main challenges include cross-contamination between samples with airborne particles or volatile compounds, cross-contamination between cells and paracrine factors while immersing whole chips under the same culture medium, and evaporation of the fluid, which can highly affect readouts in microvolumes. Acknowledging these drawbacks allows thinking of new alternatives and possible solutions for the future development and use of open fluidics devices. Controlling both the environment surrounding the open channels (with, for example, saturating agents) and the velocity of the fluid may be a good strategy to avoid undesired evaporation. A basic enclosure could be implemented to prevent airborne contaminants, lessening cross-contamination and evaporation, as well. As a future perspective, mainly in the biomedical field, open fluidics enable the application of close approaches to the *in vivo* microenvironment, where not only the 3D environment is incorporated, but also dynamic fluid and gas flows. For organ-on-chip and lab-on-chip, having representative cell co-cultures in a dynamic fluid, with contact with controlled atmospheres (with specific oxygen or carbon dioxide concentrations) is the closest one can get to recreate important disease models and to understand basic key physiological mechanisms. Moreover, the easy access to flow or static liquids/hydrogels processed on open fluidics chips may allow the straight-forward spatiotemporal control of (bio)chemical composition and the easy application of physicochemical and microscopy characterization techniques.

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