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Received March 4, 2013
Revised July 9, 2013
Accepted July 9, 2013

Review

Pumps for microfluidic cell culture

In comparison to traditional in vitro cell culture in Petri dishes or well plates, cell culture in microfluidic-based devices enables better control over chemical and physical environments, higher levels of experimental automation, and a reduction in experimental materials. Over the past decade, the advantages associated with cell culturing in microfluidic-based platforms have garnered significant interest and have led to a plethora of studies for high throughput cell assays, organs-on-a-chip applications, temporal signaling studies, and cell sorting. A clear concern for performing cell culture in microfluidic-based devices is deciding on a technique to deliver and pump media to cells that are encased in a microfluidic device. In this review, we summarize recent advances in pumping techniques for microfluidic cell culture and discuss their advantages and possible drawbacks. The ultimate goal of our review is to distill the large body of information available related to pumps for microfluidic cell culture in an effort to assist current and potential users of microfluidic-based devices for advanced in vitro cellular studies.

Keywords:
Cell / High-throughput / Microfluidic / Physiological / Pump
DOI 10.1002/elps.201300205

1 Introduction

Microfluidic devices, also known as Lab-on-a-Chip platforms, are typically polymer-based devices with micro- and nano-geometric features to hold and manipulate small volumes of fluids. Ultimately, such devices serve as tools to accurately control small volumes of liquids for a wide variety of chemical, medical, environmental, and biological applications. With small footprints, microfluidic devices provide the potential for high throughput and automated experimentation [1], enabling users to perform more experiments in a shorter period of time, at a lower cost, and with less complexity than large-scale fluidic systems. Overall, microfluidics is a relatively large area of study and a number of reviews have already been published related to the wide variety of applications and aspects of the field of microfluidics [1–6].

Beyond their diminutive size and potential for high throughput experimentation, microfluidic-based devices are an especially attractive tool for culturing cells as they enable better control over chemical and physical environments in comparison to traditional in vitro cell cultures in Petri dishes or well plates. More specifically, when cells are placed in micro-channels or chambers the cells can be exposed to flowing media to more closely resemble in vivo conditions in comparison to static cell culture in Petri dishes. This is a critical feat. In nature, cells are often found in dynamic environments with continuous or pulsatile flow, circumstances that can be straightforwardly implemented in a microfluidic device but are difficult to impose in a static cell culture [6]. Beyond utilizing microfluidic devices to mimic in vivo cellular environments, microfluidic devices can also be used to study cellular response in novel environments (i.e. conditions in which the cells are not naturally found). Such study would be useful to interrogate signaling pathway architectures or to evaluate autocrine factor response. Additionally, microfluidic devices can be employed to perform simple, periodic media replacement for experimental automation. Therefore, it is without surprise that the several advantages associated with cell culturing in microfluidic devices have led to a large number of studies for high throughput cell assays, organs-on-a-chip applications, temporal signaling studies, and cell sorting [6, 7].

A clear issue for performing cell culture in microfluidic-based devices is deciding on a technique to deliver and pump media to the cells encased in microfluidic devices that are typically no larger than the palm of a hand. While several reviews have been published on the topic of fluid manipulation and pumping in microfluidic devices [7–10], pumping techniques for perfusion cell culture in microfluidic-based devices is a subject that has been only partially examined but not separately reviewed.

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Abbreviation: DMF, digital microfluidics

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The aim of this review is to summarize the wide variety of pumping techniques that have been employed to perform perfusion cell culture in microfluidic platforms in an effort to assist current and potential users of microfluidic-based devices for advanced in vitro cellular studies. For each pumping technique, we briefly cover the underlying principle for flow generation, highlight recent examples, and discuss their strengths and weaknesses. This is followed by a look at specific applications in which particular pumping techniques were employed to achieve unique cellular studies in microfluidic devices. Applications include microfluidic devices for high throughput and multiplexed experimentation, flow recirculation, pulsed flow, and continuous flow. For clarity and brevity, we limit the range of this review to deal mainly with works that are related to pumping techniques to achieve perfusion and manipulation of cells in microfluidic-based devices. Topics such as cell isolation, separation, and cellular component analysis are only lightly touched upon in this review.

2 Background

In this section, we will briefly discuss the various pumping techniques that have been implemented for perfusion cell cultures in microfluidic devices (Fig. 1). Pumping techniques are categorized based on their driving mechanisms and are evaluated in terms of their characteristics demonstrated in the literature (flow rate, flow pattern, durations of flow, ability to recirculate fluids, and electrical power requirements) [10–58] (Table 1).

2.1 Gravity-driven flow

Gravity-driven flow is one of the simplest passive flow techniques available as it only requires two liquid reservoirs with different heights to achieve fluid propulsion from the higher reservoir to the lower one [11–19]. Generally, as the height of liquid level in the inlet reservoir decreases, the flow rate reduces until the heights of the liquid reservoirs become equal (Fig. 1A). A key advantage of gravity-driven flow is that the inlet can be filled with more liquid at any time to prolong flow and therefore this type of pumping can be considered to be an "open" system to enable long-term studies. Furthermore, it is naturally a low maintenance type of flow, requiring no moving mechanical parts. For applications that require a constant flow rate, horizontally orientated reservoirs with different heights can be used (Fig 1B). The diameter of the horizontal reservoirs are made small enough such that surface tension keeps the fluids from spilling out, and meniscus position changes that occur as liquid flows from one reservoir to the other only affects lateral positions without changing the height of the liquids [12]. The simplicity of gravity-driven flow is beneficial for high-throughput applications as it eliminates the complexity associated with fluidic interconnects/tubing and the need for off-chip active pumps (e.g. syringe pumps) that require electrical power [16]. A limitation to gravity-driven flow is that it can only produce nonpulsatile flow, rendering it unsuitable for applications in which dynamic flow is desired during cell culturing. A recent study by Sung et al. [17] introduced a periodic rocking device to tilt a plate below a microfluidic chip to virtually switch the inlet and outlet to recirculate media for cell culturing with gravity alone; however, an electrical tool for tilting was required.

2.2 Surface tension-driven flow

Another straightforward passive flow technique is known as surface tension-driven flow and works as follows. First, a big droplet is loaded at the outlet of a microchannel and is allowed to fill the channel. Then, a small droplet is introduced to the inlet of the same microchannel to induce higher internal pressure as compared to the outlet droplet (Fig. 1C). Flow occurs because the droplet introduced to the inlet displaces the droplet that was added to the outlet. Flow continues until the pressure at the inlet and the outlet equalizes. Flow based on this strategy is slightly oscillatory as flow occurs in both directions as the system stabilizes. Like gravity-driven flow, surface tension-driven flow does not require any electric power, rendering it easy to implement. Additionally, it has a small footprint, only requiring micro-liter size droplets above the inlet and outlet. While surface tension pumps are also "open" systems that enable a user to refill liquid into the inlet at any time, surface tension-driven flow is most suitable for transferring micro-liters of liquid for short-term flows and requires constant refilling for continuous flow. This restriction makes it difficult to perform long-term perfusion studies via surface tension-driven flow. Surface tension-driven flow has been widely studied and reviewed by Beebe et al. [7, 20]. This straightforward type of flow has been implemented to perform multiplexed studies [20] as well as backward flow oscillation [21]. Furthermore, surface tension-driven flow has been paired with dieletrophoresis for cell manipulation [22].

2.3 Osmosis-driven flow

Osmosis-driven flow is a third example of a passive pumping technique that has been employed for microfluidic cell cultures. This type of flow is induced by a difference in concentrations across a membrane that is only permeable to the solvent and not the solute (Fig. 1D). The flow rate generated is proportional to the difference in the osmotic pressure across the permeable membrane as well as the contact area of the membrane [23]. The most important characteristic of osmotic pumps is that they can provide very slow flow rates at almost constant flow that can last from hours to days [21–24], making it suitable for long-term cell cultures. In addition, the flow rate in osmosis-driven pumps can be easily tuned by changing the concentration of the solute. Like the other passive flow techniques, osmotic-driven flow does not require electricity; however, it does require a more involved setup than

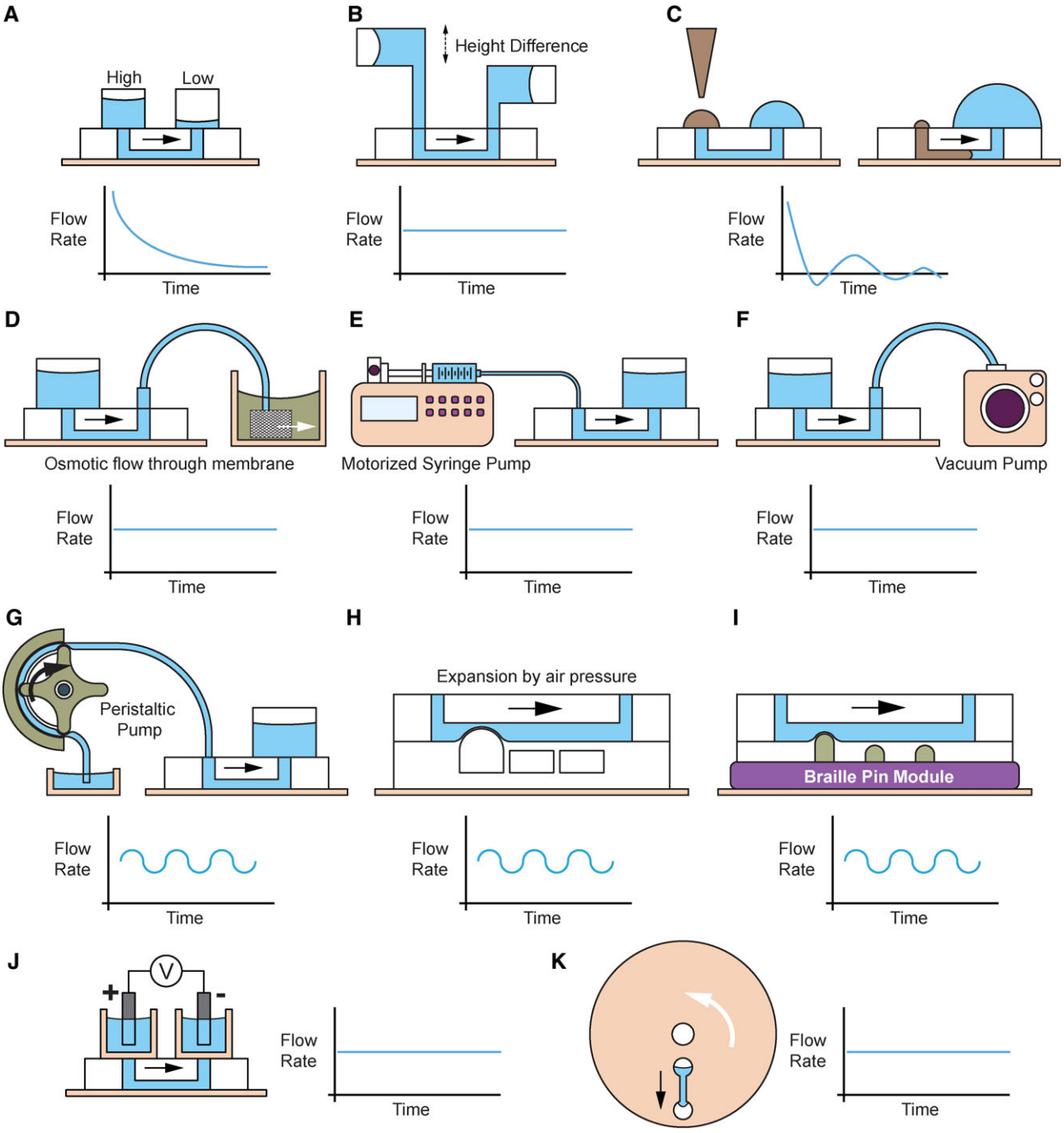

Figure 1. Schemes of types of pumps used for microfluidic cell culture devices along with their representative flow pattern characteristics. Flow rate is not plotted to scale. (A) Gravity-driven pump with vertically oriented reservoirs, (B) gravity-driven pump with horizontally oriented reservoirs, (C) surface tension-based droplet pump, (D) osmosis-based pump, (E) positive pressure syringe pump, (F) negative pressure vacuum pump, (G) general peristaltic pump, (H) peristaltic pump by pneumatic pressure, (I) peristaltic pump by Braille pins, (J) electrokinetic pump, (K) centrifugal pump of lab-on-a-disc.

gravity-driven flow or surface tension-driven flow, rendering it higher in maintenance than the other two techniques. Furthermore, osmosis-driven flow is strictly unidirectional. However, like gravity-driven flow, liquid can be refilled into the inlet of an osmosis-driven device and therefore can be considered to be "open" system.

2.4 Syringe and gas pressure pumps

While passive flow mechanisms are simple to implement, active mechanical pumps such as syringe pumps and vacuum pumps are much more widely utilized largely because of the greater control over flow they afford to the user.

Table 1. Comparison of pump characteristics for cell culture and manipulation

Driving force	Flow rate	Flow pattern	Flow duration	Electrical power requirement	Example of recirculation	Advantages	References
Gravity	8 nL/s [11], 0∼2.5 mm/s [12]	Continuous (decreasing gradient or constant)	160 min [12], 15 h [16],	No or yes [17]	Yes [17]	Simple, easy to multiplex, low cost	[11–19]
Surface tension	67 nL/s (ave.) [20]	Continuous (decreasing oscillation gradient)	2 min [20]	No	No	Simple, easy to multiplex, low cost	[20–22]
Osmosis	0.15∼15 μL/h [23]	Continuous (almost constant)	4 h∼10 days [24]	No	No	Simple, low cost, long-lasting flow	[23–26]
Syringe	0.05∼0.18 mL/h [27], 0.13, 0.2 μL/min [28]	Continuous or pulsatile [29]	8 days [28], 20 min [29]	Yes	No	Commericial, good for droplet generation	[27–29]
Vacuum	6∼20 mL/h [30] 0.1 μL/min [31]	Continuous or pulsatile [31]	4 days [32], 2 days [33]	Yes	No	Good for droplet generation, Easy to multiplex	[30–33]
Peristaltic (general)	0.3∼30 μL/min [34] 0.0∼1.1 mL/min [35]	Pulsatile	24 h [34], 24 h [35], 72 h [36]	Yes	Yes [35, 36]	Commercial, good for long-term culture and pulse stimulation	[34–37]
Peristaltic (pneumatic)	2.35 nL/s [38], 0.25 mm/s [39] 8.5∼185.1 μL/min [41]	Pulsatile	>500 h [39], 48 h [41]	Yes	Yes [39]	Easy to integrate and multiplex, good for pulse stimulation	[38–42]
Peristaltic (Braille pins)	3∼74 nL/min [43]	Pulsatile	70 min [43], 36 h [44], 96 h [45]	Yes	Yes [43–46]	Commercial, easy to integrate and multiplex, good for pulse stimulation	[43–48]
Electric (electro-kinetic)	0.054∼0.55 mm/s [49]	Continuous (constant)	Not specified	Yes	No	No moving parts, easy flow switching, pulse-free flow	[49–51]
Electric (electro-osmotic)	3.6 μL/min [52], 0.6∼1.7 μL/min [53], 1∼2 μL/min [54]	Continuous (constant)	60 min [54]	Yes	No	No moving parts, easy flow switching, pulse-free flow	[52–55]
Centrifugal (lab-on-a-disc)	<10 nL/s ∼ >100 μL/s [10]	Continuous (constant)	14 days [56], 24 h [57, 58]	Yes	No	Easy to multiplex and mix culture	[10, 56–58]

Syringe pumps use a piston to generate positive pressure to push liquids out of a liquid conduit (Fig. 1E). While on the other hand, vacuum pumps pull liquids through a conduit by inducing negative pressure (Fig. 1F).

Syringe pumps have been employed for a variety of cell culture applications [27–29] and are offered by a wide variety of vendors, enabling users to select the best pump for their desired application. A limitation of syringe pumps is that they can only pump the volume of the liquid enclosed within a single syringe, and can therefore be considered to be a "closed" system, unlike the passive pumping mechanisms described above. For applications that require large volumes of perfusion media over a long period of time, this might be a critical concern. In contrast, vacuum pumps are an "open" system as long as the inlet reservoir is continuously refilled with media. Nevertheless, both pumps have moving mechanical components, rendering them significantly higher in initial setup costs and operational requirements compared to passive pumping techniques. Several demonstrations employing syringe or vacuum pumps for cell delivery and culture have been reported [27–33].

Users may consider more sophisticated, commercially available devices in order to meet their needs. For example, the microfluidic flow control system, developed by Fluigent Company, is pneumatically operated and provides highly

stable flow controls for up to eight independent channels and can generate a wide range of flow rates.

2.5 Peristaltic pumps

The peristaltic pump is another well-known type of active pump. Peristaltic pumps are based on positive displacement in which a flexible conduit is pressed to displace the liquid inside the conduit. In a traditional peristaltic pump, a flexible tube is wrapped around a rotor that has rollers that press the tubing at a various positions and as the rotor cam rotates liquid is pushed through the tube (Fig. 1G). The peristaltic pump can be operated bi-directionally by changing the direction of rotation of the rotor, clockwise or counter clockwise. Due to the mechanism of flow generation, flow from peristaltic pumps is pulsatile and oscillatory in principle rather than constant. Peristaltic pumps can be configured to be in an "open" format in which the main fluid reservoir can be refilled or in a "closed" format in which liquids are recirculated. Peristaltic pumps have several key advantages, including the ability to produce bi-directional flow. As compared to vacuum pumps that must make direct contact with fluids, peristaltic pumps, and syringe pumps do not directly touch the fluid being propelled, making them more suitable for applications in which one wants to minimize contamination of the pump. Furthermore, when utilizing peristaltic pumps care must be taken as the pinching mechanism of the pump can cause cellular damage. Like syringe and vacuum pumps, peristaltic pumps have mechanical components that wear out over time.

A few examples exist in which a peristaltic pump was employed as a means for flow generation in microfluidic cell culture [34–36]. However, due to the large footprint associated with commercially available versions of these pumps they are not always practical to implement in microfluidic devices. A commercially available, miniaturized peristaltic pump for palm-sized cell culture systems has been developed [37]. In 2000, Quake and co-workers demonstrated pneumatically controlled microdevices in which empty microchannels, fabricated out of PDMS were expanded by air pressure to function as mixers, valves, and miniaturized peristaltic pumps [38]. Essentially, by pneumatically changing the shape of one microchannel, another underlying or overlying channel was opened or closed. Using this concept, they demonstrated that parallel channels could be sequentially expanded in a rolling fashion to achieve peristaltic-like pumping under a microchannel (Fig. 1H). This technique was used to develop one of the smallest chemostats demonstrated in the literature (16 nL of loop volume) to continuously circulate bacterial culture for mixing with newly introduced medium [39]. A fully automated cell culture microfluidic platform that utilizes a combination of monolithic microfluidic peristaltic pumps, cell culture chambers, and mixers, was also subsequently demonstrated [40].

Another example of peristaltic pumping in a microfluidic device was demonstrated by Takayama et al. [43] in which computer-controlled Braille pins were employed to push fluids in an overlying microfluidic channel (Fig. 1I). Beyond their use as a miniaturized peristaltic pump, the pins were also implemented as valves to pinch microchannels shut. The Braille pin module is compact, facilitating portability, unlike traditional bulky syringe pumps. Furthermore, Braille pin modules are commercially available [44, 45, 47]. Several applications using Braille pin pumps for flow recirculation for cell culturing in a microfluidic device have been published [43–46, 48].

2.6 Electrokinetic and electroosmotic pump

Electrokinetic or electroosmotic pumps also use electricity for active flow generation but are based on a very different principle than mechanical pumps (Fig. 1J). EOF is the movement of a polar liquid induced by an electric field applied parallel to a solid–liquid interface. Rather than generating flow with a parabolic flow profile, EOF generates plug-like flow [8, 9, 52, 54, 55]. Furthermore, electrokinetic and electroosmotic pumps are considered to be "open" systems, enabling the user to refill liquids into the inlet. In general, the pump structure is very simple as there are no moving parts.

A limited amount of work has been reported in which electrokinetic pumps were utilized for cell delivery purposes [49–51]. An important caution of electrokinetic pumps is that the electric field should be equal or less than 1 kV/cm to prevent cell lysis [50]. Moreover, EOF requires surface-ionizable materials and carries a risk of electrolysis, limiting the use of such pumps for long-term cell culture applications. By isolating the electrode from the fluids to be pumped through the use of gels or membranes, the electroosmotic pump over comes several limitations. In this fashion, the pump indirectly generates flow in a microchannel. Glawdel et al. [54, 55] have studied the use of electroosmotic pump for cell culture applications.

2.7 Centrifugal pumping

Lab-on-a-disc devices, also known as centrifugal microfluidic discs, were largely pioneered by Madou et al. [10] and are unique fluidic devices in which flow is induced by spinning the microfluidic device on a motor to generate centrifugal forces (Fig. 1K). Several applications have been reported in which cells were cultured on a lab-on-a-disc platform [10, 56–58]. Centrifugal pumping is advantageous as it is a noncontact pumping technique and enables multiplexing in a similar fashion to how a centrifuge can hold and spin many tubes. In addition, centrifugal pumping is capable of generating a wide range of flow rates, from less than tens of nL/min to more than hundreds of μL/min by controlling channel dimensions and changing rotational speeds. However, the flow generated on microfluidic discs is usually uni-directional (from the center to the outer rim), eliminating the possibility to perform flow recirculation. Unlike the case of electrokinetic or electroosmotic pump, the surface properties of disc materials are virtually irrelevant to the flow.

2.8 Other pumps

Beyond the pumping techniques for microfluidic cell culture that have been discussed above, there a variety of other types of pumps that are suitable for cell culturing applications [7–10]. For example, the diaphragm pump (also referred to as diffuser pump or membrane pump) is a pump in which a diaphragm is actuated mechanically or electrically to generate flow [7, 59–62].

Magnetic stirrer-based micropumps have been reported by a couple of research groups. Similar to macroscopic rotary pumps using mechanical propellers, continuous flow is controlled by the rotational frequency of a magnetic bar. Beebe et al. applied this technique to separate particles [63]. And Fujii et al. integrated the pump with a cell culture chip and performed long-term culture of a monolayer of intestinal cells [64].

Another type of pump that can be employed for microfluidic cell culturing is the magnetohydrodynamic pump which can produce continuous or pulsatile flow and has no moving parts [65]. A more recent pump is the non-membrane micro surface tension pump that also has no moving parts. This pump performs a sort of peristaltic pumping by the combined use of surface chemistry, channel geometry, and sequential pressurization of different channels with different gas pressure levels [66]. The advantage of this pump is the need for only one layer of channels, although a need for a defined surface chemistry and contact angle control may make the system difficult to use for pumping fluids, such as cell culture media, that can foul surfaces. Lastly, paper wicking [67] and capillary-driven passive flow [68] have the potential to be useful for microfluidic cell culture systems.

2.9 Shear stress effects on cell culture

An important concern for perfusion cell cultures in microfluidic devices is the effect of shear stress on the cells as a result of flowing media. While shear stress has been reported to positively affect cells in terms of differentiation, stimulation, and survival, stresses that are too large in magnitude can lead to cellular damage and death. This topic has been broadly dealt with by many prior studies and the effects of shear stress on both prokaryotes and eukaryotes (such as bacteria, mammalian endothelial cells, and plant cells) have already been reviewed [69–78]. Other than mechanical aspects of laminar flow, physical and chemical aspects of flow such as diffusion and chemical gradient, also affect cell status [6, 79, 80]. The surface area to volume ratio of microchannels and the substrate stiffness of microfluidic devices are additional factors for cell culture that users may take into consideration [6, 7, 79, 81, 82]. Other possible side effects of cell study in microfluidic devices have already been covered by Levchenko et al. [83].

3 Applications

This section highlights recent examples of perfusion cell cultures in microfluidic devices.

3.1 High-throughput and multiplexed experimentation

High-throughput processing (often referred to as multiplexing or high parallelism) is the concept of performing several experiments in parallel to save cost and labor. Microfluidic devices have garnered widespread interest from the cellular biology community as they offer a platform for multiplexed experimentation using a very compact footprint. Rather than setting up large-scale systems of tanks and conduits to study cells in dynamic environments, with microfluidic devices researchers are able to miniaturize their whole experimental setup into a device that typically is no larger than the palm of a hand.

A recent application was demonstrated by Yuen et al. in which they employed gravity-driven passive flow for multiplexed cell culturing and assaying in a 96-microwell plate device [16] (Fig. 2A). Through the introduction of cellulose membranes into the microchannels of the device, their platform avoided the bubble formation problem experienced by previous efforts [84, 85] that can disrupt cell perfusion and cell behavior. Another application of a multiplexed cell culturing device based on a passive flow mechanism was demonstrated by Beebe et al. [8] and was based on surface tension-driven flow (Fig. 2B). In their work, they demonstrated highly parallel compartmentalization, routing and pumping, and microfluidic cell culturing for several days. Overall, these demonstrations illustrate that with simple passive flow mechanisms relatively advanced perfusion cell cultures can be easily performed by standard life science laboratories without any prior knowledge or experience of microfluidic devices.

There are also a number of studies in which active flow mechanisms were employed to afford multiplexed cell culturing in microfluidic platforms [28, 33, 86]. For example, Wu et al. reported a high-throughput perfusion cell culture system of 30 micro-bioreactors using negative pressure (Fig. 2C) [33]. In addition, the application of positive air pressure to enable high-throughput cell culturing was reported by Hung et al. [28] and Sugiura et al. [86]. More specifically, Sugiura et al. (Fig. 2D) demonstrated a parallel cytotoxicity test of seven anticancer drugs on HeLa cells cultured in their microfluidic device. In general, active pumping techniques enable more advanced flow patterns as compared to passive pumping techniques but active pumping techniques significantly increase the complexity of the microfluidic device by requiring more peripheral hardware (e.g. computers and pump sources) and fluidic components (e.g. fluidic interconnects, tubing), serving as significant barriers to adaptation beyond the Lab-on-a-Chip community.

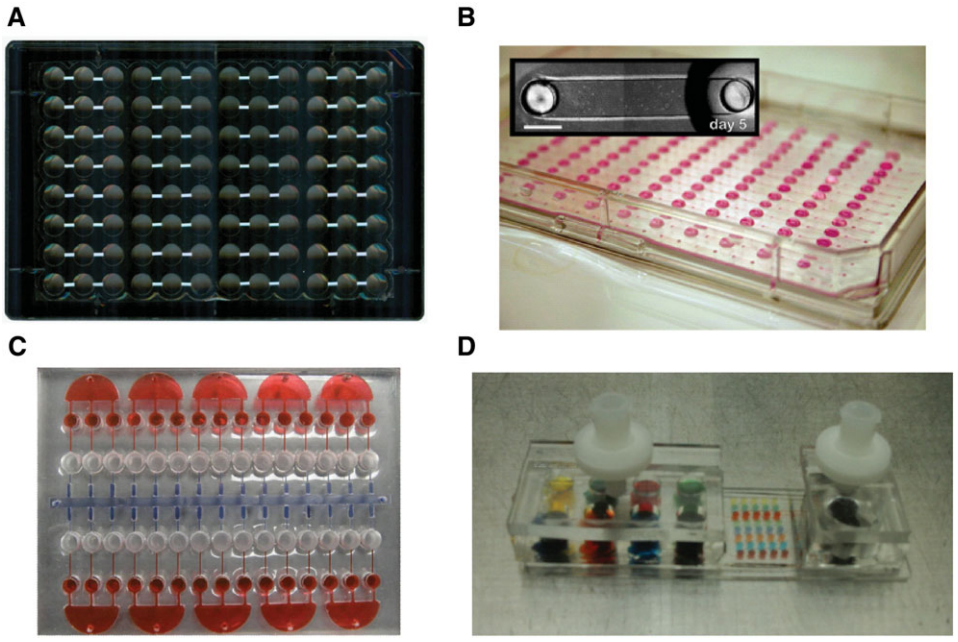

Figure 2. Examples of high-throughput microfluidic platforms for cell culture using different pumps. (A) Gravity-driven 96-well perfusion microplate for cell culture [16], (B) an array of 192 microchannels for surface tension driven flow [20], (C) 30-well perfusion 3D cell culture chip by negative pressure [33], (D) pressure-driven perfusion cell culture chip having 8 × 5 arrays of cell culture microchambers [86]. All figures are reprinted with permissions from publishers.

3.2 Flow recirculation

The ability to recirculate culture media is one of the unique advantages associated with perfusion cell culture systems compared to static cultures. Media recirculation is important to mimic in vivo cellular environments as well as for the understanding of drug absorption, distribution, metabolism, and elimination. As previously noted, media recirculation is possible with a general peristaltic pump and was demonstrated by Sung and Shuler [36]. Media recirculation is also possible with a miniaturized on-chip peristaltic pump based on pneumatically controlled microchannels and was reported by Balagaddé et al. (Fig. 3A) [38]. In the demonstration by Balagaddé et al. the miniaturized peristaltic pump was utilized to recirculate media in long-term bacteria culture for synthetic biology. Another notable example of recirculation by a miniaturized peristaltic pump was shown by Futai et al. [45] in which they employed a Braille pin-based peristaltic pump to partially recirculate autocrine factors from cultured cells (Fig. 3C). Flow recirculation in microfluidic devices can also be made possible with simple passive flow mechanisms. For example, Sung et al. [17] reported a

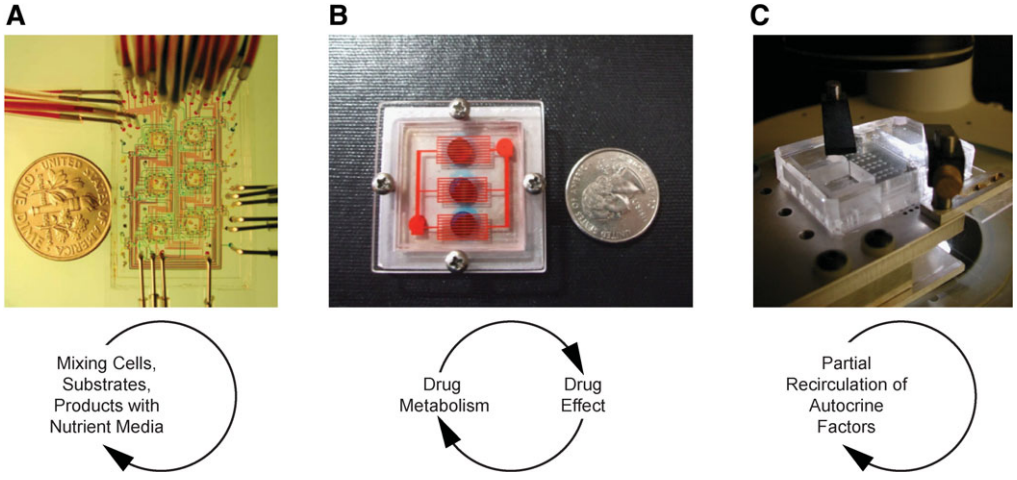

Figure 3. Top: Cell culturing chips for recirculation. Bottom: Concept schematics of recirculation for each chip. (A) Six microchemostats having circulation loop in which the medium is continuously mixed for bacterial culture [39], (B) a microscale cell culture analog having three cell culture chambers for liver, tumor, and marrow [17] with microchannel connections for gravity-based recirculation, which allows interorgan circulation of liver metabolized drugs to the tumor and marrow, (C) a portable Braille display-based microfluidic cell culture system capable of cell seeding, adhesion, and culture. The goal of recirculation here is to partially recirculate autocrine and secreted factors [45]. All figures are reprinted with permissions from publishers.

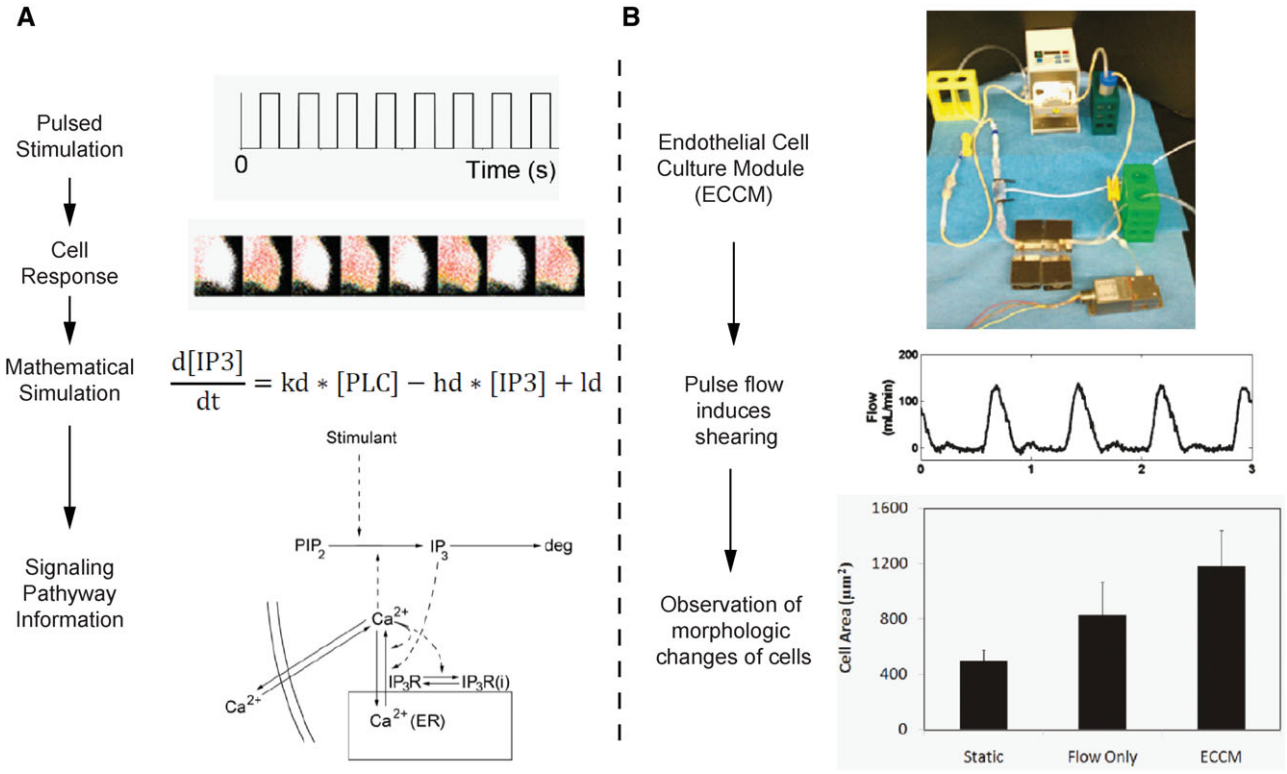

Figure 4. Applications of pulsatile flow in microfluidic cell cultures. (A) Square-wave pulses of carbachol trigger calcium responses as visualized by fluorescence resonance energy transfer based protein indicators of intracellular calcium levels. Comparison of experimental observations to predictions from mathematical models of different plausible pathway architectures enables pinpointing of the architecture of the particular cell being studied. DAG = diacylglycerol; DAG-DP = DAG-dependent protein; IP_3 = inositol triphosphate; IP_3R = IP_3 receptor; $IP_3R(i)$ = inactivated IP_3R; $Ca^{2+}(ER)$ = endoplasmic reticulum calcium [87]. (B) The endothelial cell culture module can imitate different blood flow patterns observed physiologically in blood vessels at different parts of our body. Such biomimetic pulsed flows can elicit different responses from the same types of endothelial cells just by changing flow patterns [88]. All figures are adapted with permissions from publishers.

gravity-driven recirculation system (Fig. 3B), called micro cell culture analog. This device was composed of multiple cell culture chambers that were connected to microchannels in order to reproduce interactions between organs and to screen drug toxicity.

3.3 Pulsatile flow

Many cells are naturally found in environments that have pulsatile flow, such as endothelial cells in the lumen of a blood vessel. Cells found in these environments require mechanical sheer stress to elicit physiological behavior. Therefore, to accurately mimic in vivo fluid mechanical environments, it is critical that microfluidic devices for cell culturing are powered by a source that can produce pulsatile liquid flow. Pulsed flow is also important for providing temporally patterned chemical stimulation to cells, either to mimic physiology, such as periodic release of hormones, or as a way to interrogate signaling pathway architectures. Jovic et al. [87] used the Braille pin peristaltic pump to induce a pulsed flow that was beneficial for pulsed chemical stimulation of cells (Fig. 4A). Different pattern pulses of carbachol induced different patterns of calcium responses from cultured cells. By comparing these experimental phase-locking responses to mathematical predictions from multiple plausible signaling pathway models, the most appropriate architecture of multiple pathway components within the cell being studied was determined. The usefulness of the method is its ability to use a single readout (calcium response in this case) to obtain information about multiple steps and components within a signaling pathway. Estrada et al. [88] introduced a peristaltic pump for an endothelial cell culture module (Fig. 4B) to imitate cardiac pulsed flow. In their work, they observed alignment of cytoskeletal filaments and high expression β-catenin for cultured cells in the endothelial cell culture module as compared to cells cultured in a static condition. In addition, King et al. [31] used vacuum and parallel microfluidic cell chambers to realize "flow-encoded switching" for dynamic cellular stimulation and Stocker et al. [29] employed syringes to study the effect of nutrient pulses on bacterial chemotactic behaviors.

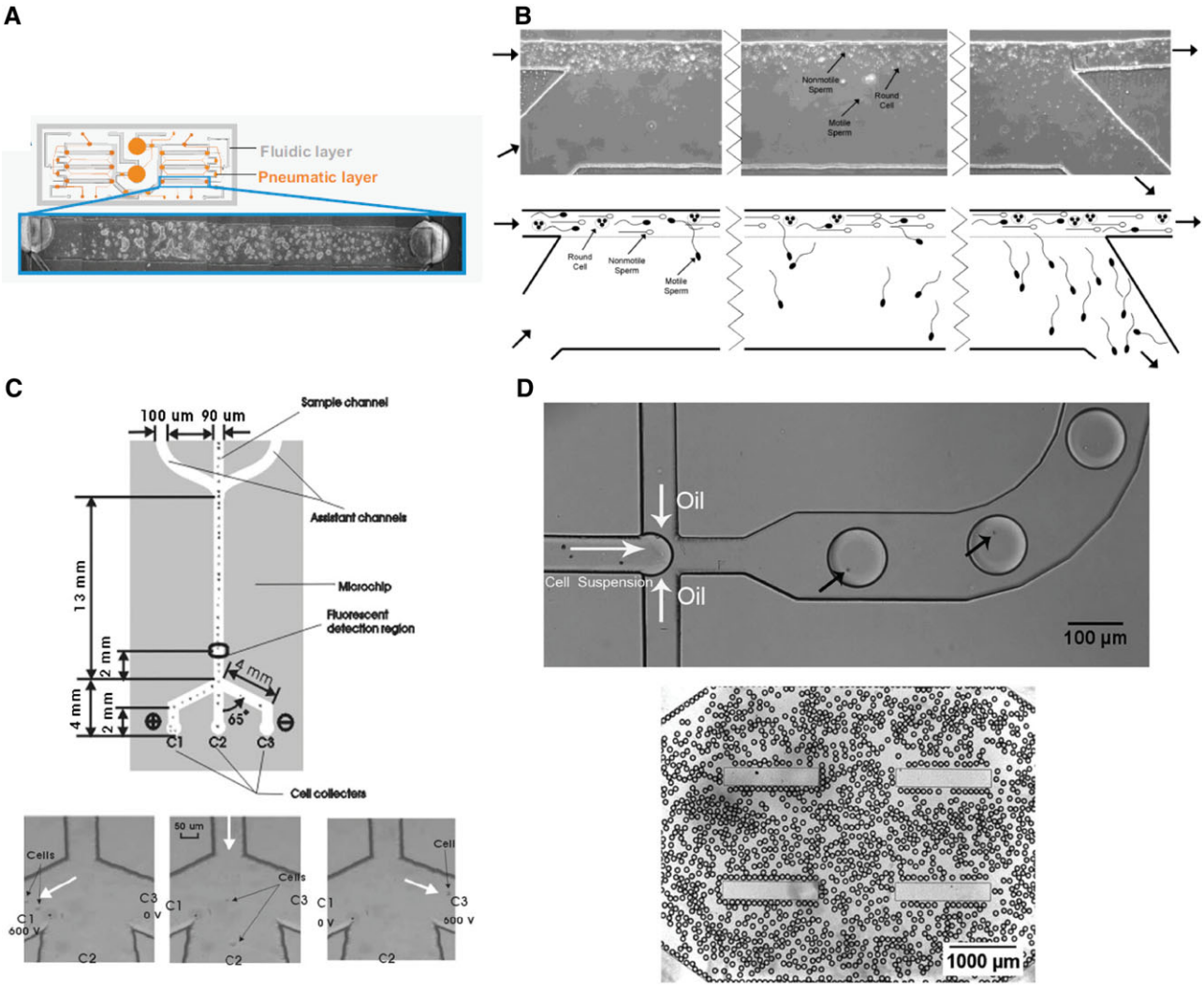

Figure 5. Examples of using continuous flows in microfluidic cell cultures. (A) Continuous media flow washes out autocrine factors from mouse embryonic stem cell culture and changed their self-renewal state [89], (B) continuous laminar flows sorts motile sperm from nonmotile cells [11], (C) gravity-driven hydrodynamic focusing flows guide HeLa cells sequentially, one-by-one through an optical interrogation zone before electrical cell sorting [90], (D) microdroplet encapsulation of single algal cell and cultivation in a separated chamber [92]. All figures are adapted with permissions from publishers.

3.4 Continuous flow

Continuous flow, which is nonpulsatile and smooth, is a basic but important type of flow with many applications in cell culture. For example, Przybyra and Voldman [89] used continuous media flow to diminish cell-secreted factors of mouse embryonic stem cells in microchambers and observed their escape from a state of self-renewal. Cho et al. [11] used a constant flow rate, gravity-driven, and multiple laminar stream microfluidic system to separate motile sperm from nonmotile components. A steady continuous flow rate was critical to ensure that the widths of the different laminar streams remained constant over the sorting period to enable consistent sorting (Fig. 5B). Continuous focusing flows of Y- or T-shape junctions in microfluidic devices are commonly used for cell cytometry to position cells within a flow stream to pass through a specific position within the cross-sectional area of a channel for precise, in-focus optical interrogation [13, 90] (Fig. 5C). With appropriate control of flow rates and interfacial tension, adjacent laminar flows of two immiscible liquids in microfluidic structures similar to Fig. 5C can generate droplets [91]. This technique has been applied to microalgae culture [92] (Fig. 5D) and to highly parallel cocultivation of bacterial communities [32].

3.5 Digital microfluidics

Pump-less microfluidic systems, such as digital microfluidics (DMF), are a recent and important technology to manipulate liquids in microfluidic devices. In these devices, the

Table 2. Required characteristics of pumps for cell culture and manipulation

Property	Gravity	Surface tension	Osmosis	Syringe	Vacuum	Peristaltic (general, pneumatic, Braille pins)	Electric (electrokinetic, electroosmotic, EWOD)	Centrifugal (lab-on-a-disc)
Stability against surface fouling	++	+	++	++	++	++	–	++
Mechanical gentleness (minimal cell squishing, not too much shear)	++	++	++	+	+	+	++	+
Chemical gentleness (minimal surfactants/toxic chemicals)	++	++	+	++	++	++	+	++
Electrical gentleness (no electrical cell damage)	++	++	++	++	++	++	–	++
Optical accessibility	++	++	++	++	++	++	++	++
Recirculation of cell culture media	+	–	–	–	–	++	+	–
Pulse flow generation	–	–	–	++	–	++	++	+
Steady flow generation	+	–	++	++	+	+	+	+

++: Recommended, +: acceptable, –: not recommended. EWOD, electrowetting-on-dielectric.

pump is essentially part of the microfluidic chip and liquid droplet manipulation is based on electrowetting-on-dielectric. The topic of DMF for digital microfluidics has been reviewed by Wheeler et al. [93]. Recent studies from the same group utilized DMF to culture various cell types, such as bacteria and mammalian cells [94–99].

3.6 Special requirements for pumps for microfluidic cell culture

There are many considerations when using pumps for cell culture in microfluidic-based devices – such as surface fouling as well as chemical (minimal use of surfactants, etc), mechanical (minimal squishing, not too much shear), and electrical (moderate electric fields) compatibility. Furthermore, additional concerns include optical accessibility, the ability to recirculate liquids, and the ability to generate pulsing/steady flows. Different pumps have unique characteristics and one must carefully select the best pump for their application. We have summarized general considerations when implementing a particular pumping technique (Table 2). In addition to considerations mentioned in Table 2, there are material considerations for on-chip cell culture when the microfluidic device is largely made of PDMS. PDMS allows oxygen permeation that can be beneficial for cell growth but also allows for evaporation of embedded liquids [47] and absorbs small hydrophobic chemicals [6, 100–104], rendering it difficult to deliver such compounds to the cells within the device.

4 Conclusions

In summary, a variety of pumps with different designs and driving mechanisms are available for short- and long-term cell culture in microfluidic devices. Each has unique characteristics, requirements, and advantages. Simple, passive flow pumps are easy to use and are advantageous for high-throughput assays. As they require very little equipment for implementation, they are more likely to be adapted by users outside of the Lab-on-a-Chip community. However, as passive flow pumps produce simple flows, they are not suitable for cell culture situations in which dynamic flow patterns are desired. Therefore, the importance of active pumps cannot be undervalued when it is necessary to perform complex and demanding experiments, such as "organ-on-a-chip" or "body-on-a-chip," to mimic in vivo tissue environments and to interorgan chemical communication. An area of opportunity and need is the development of active pumps that are user-friendly, compact, have low power requirements, and are capable of producing a wide range of flow rates and flow patterns. Or alternatively, passive pumps that allow for a higher degree of flow control and programing. Devices that combine multiple different pumping techniques can also be useful and enable unique fluidic functions. For example, the work of Goral et al. [16] applied gravity-driven flow and membrane wicking together. Peng's work of micro surface tension pump [68] can be regarded as a combination of pneumatic pressure and surface tension. Overall, the main goal of this review was to distill out key concepts from the large body of information available related to pumps for microfluidic cell cultures. We hope that this article will assist current and potential users of microfluidic-based devices for advanced in vitro cellular studies.

The authors were partly supported by WCU (World Class University) program (R322008000200540), and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A6A3A01040672).

The authors have declared no conflict of interest.

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