CHAPTER

1 Viable cell culture in PDMS-based microfluidic devices

Melikhan Tanyeri*, Savaş Tay[†,‡,1]

*Biomedical Engineering Program, Duquesne University, Pittsburgh, PA, United States
†Institute of Molecular Engineering, University of Chicago, Chicago, IL, United States
‡Institute of Genomics and Systems Biology, University of Chicago, Chicago, IL, United States
[1]Corresponding author: e-mail address: tays@uchicago.edu

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Abstract

Microfluidics has played a vital role in developing novel methods to investigate biological phenomena at the molecular and cellular level during the last two decades. Microscale engineering of cellular systems is nevertheless a nascent field marked inherently by frequent disruptive advancements in technology such as PDMS-based soft lithography. Viable culture and manipulation of cells in microfluidic devices requires knowledge across multiple disciplines including molecular and cellular biology, chemistry, physics, and engineering. There has been numerous excellent reviews in the past 15 years on applications of microfluidics for molecular and cellular biology including microfluidic cell culture (Berthier et al., 2012; El-Ali, Sorger, & Jensen, 2006; Halldorsson et al., 2015; Kim et al., 2007; Mehling & Tay, 2014; Sackmann et al., 2014; Whitesides, 2006; Young & Beebe, 2010), cell culture models (Gupta et al., 2016; Inamdar & Borenstein, 2011; Meyvantsson & Beebe, 2008), cell secretion (Schrell et al., 2016), chemotaxis (Kim & Wu, 2012; Wu et al., 2013), neuron culture (Millet & Gillette, 2012a, 2012b), drug screening (Dittrich & Manz, 2006; Eribol, Uguz, & Ulgen, 2016; Wu, Huang, & Lee, 2010), cell sorting (Autebert et al., 2012; Bhagat et al., 2010; Gossett et al., 2010; Wyatt Shields Iv, Reyes, & López, 2015), single cell studies (Lecault et al., 2012; Reece et al., 2016; Yin & Marshall, 2012), stem cell biology (Burdick & Vunjak-Novakovic, 2009; Wu et al., 2011; Zhang & Austin, 2012), cell differentiation (Zhang et al., 2017a), systems biology (Breslauer, Lee, & Lee, 2006), 3D cell culture (Huh et al., 2011; Li et al., 2012; van Duinen et al., 2015), spheroids and organoids (Lee et al., 2016; Montanez-Sauri, Beebe, & Sung, 2015; Morimoto & Takeuchi, 2013; Skardal et al., 2016; Young, 2013), organ-on-chip (Bhatia & Ingber, 2014; Esch, Bahinski, & Huh, 2015; Huh et al., 2011; van der Meer & van den Berg, 2012), and tissue engineering (Andersson & Van Den Berg, 2004; Choi et al., 2007; Hasan et al., 2014). In this chapter, we provide an overview of PDMS-based microdevices for microfluidic cell culture. We discuss the advantages and challenges of using PDMS-based soft lithography for microfluidic cell culture and highlight recent progress and future directions in this area.

Below, we present an overview of material properties of PDMS and its implications for microfluidic cell culture.

1 PDMS: MATERIAL PROPERTIES

PDMS (polydimethylsiloxane, $[C_2H_6OSi]_n$) is a synthetic polymer with a backbone consisting of repeating chain of Si-O molecules with two methyl groups attached to silicon. In the presence of a curing agent containing a catalyst molecule (typically platinum), PDMS polymers crosslink through a thermally driven polymerization

Table 1 Material Properties of PDMS (Mark, 2009)

Density	0.965 g/mL
Refractive Index	1.4
Elastic Modulus (Wang, Volinsky Alex, & Gallant Nathan, 2014)	0.57–3.7 MPa
Contact Angle (Mata, Fleischman, & Roy, 2005)	109–115°
Gas Permeability @ 35° [O_2 and CO_2] (Merkel et al., 2000)	34 and 22×10^{-6} cm^2/s
Thermal Conductivity	0.15 W/m K
Dielectric Constant	2.3–2.8
Electrical Conductivity	4×10^{13} Ωm

process forming a solid transparent elastomeric material. For microfluidics research, RTV-615 from Momentive Performance Materials and Sylgard 184 from Dow-Corning are the most common two-component PDMS kits available in the market consisting of a base and a crosslinker agent. The material properties dictate specific advantages and limitations of using PDMS for biological applications. Some of the material properties of PDMS are provided in Table 1.

1.1 TWO-COMPONENT ELASTOMER

First and foremost, the initial two-component PDMS polymer kits offer an ideal platform for soft lithography. In soft lithography, the PDMS base and crosslinker solution (typically mixed at a 10:1 base:crosslinker ratio) is cast over a master mold—fabricated by photolithography or micromachining—and uncrosslinked PDMS conforms to the 3D topology of the mold, inverse-replicating the features at the micro- and nanoscale. Low temperature (70–80°) thermal driven polymerization process is convenient for most biological and chemical applications and even curing at room temperature is possible for sensitive applications.

Unique material properties of PDMS played a key role in making soft lithography a powerful tool for biological applications. Soft lithography enables fabrication of complex structures including a network of channels, reservoirs, pumps and mixers in a single device (Unger et al., 2000). Soft lithography allows for creating multilayer devices by adding multiple layers of PDMS containing functional device units. Each layer can be fabricated using a separate master mold and then combined to form a monolithic device (Unger et al., 2000). Multiple device layers can be combined by activating each layer surface via oxygen plasma and bonding the layers by aligning the features on each layer. Alternative methods for combining device layers include using an off-ratio polymerization and partial curing. In off-ratio polymerization, each device layer is fabricated using a base:crosslinker ratio where one layer is fabricated with excess base and the other with excess crosslinker. The two device layers are then aligned and brought into conformal contact to extend the thermal polymerization process between the two layers due to the availability of excess uncrosslinked polymers and catalyst molecules, forming a monolithic device at the end. In partial curing, the polymerization process for two device layers is interrupted prior to

completion and the two layers are aligned and brought into conformal contact to drive the polymerization process to the end to obtain a monolithic device. Each of these three methods has its own advantages and limitations for biological applications. Ideally, at the end of the polymerization process, the amount of uncrosslinked PDMS polymers should be minimized as these may leach into microchannels and adversely affect cell culture assays. Therefore, oxygen plasma bonding or a partial curing method might provide the best results for cell culture assays in microfluidic devices fabricated by multilayer soft lithography.

1.2 OPTICAL PROPERTIES

PDMS is transparent with a refractive index of 1.4 which enables imaging cell cultures. Multiple imaging modalities are available including brightfield, fluorescence, phase contrast and DIC. Detection, imaging, and tracking at single molecule and single cell level have been realized using PDMS-based microfluidic devices (Taniguchi et al., 2010; Tay et al., 2010; Taylor et al., 2009). While there are no major obstacles for high-resolution brightfield imaging even at high frame rates (Otto et al., 2015; Tse et al., 2013), some caution should be taken with fluorescence imaging as PDMS exhibits autofluorescence with excitation in the near-UV range (300–400 nm). Excitation and imaging through thick slabs of PDMS will result in low signal-to-noise ratio hampering high-resolution fluorescence imaging. The refractive index of PDMS is different than that of glass ($n = 1.518$) which will result in a less than optimal performance for phase contrast and DIC imaging of cells and cellular components. Nevertheless, based on the success of a decade of studies on utilizing microfluidic systems for cell imaging and tracking, we can conclude that the benefits of microfluidic devices for cell-based assays vastly exceed the limitations pertaining to imaging.

1.3 STIFFNESS/COMPLIANCE

PDMS has an elastic modulus of ~0.6–3.7 MPa (Wang et al., 2014) which is much lower than materials commonly used for cell culture such as glass (~50 GPa) and thermoplastics (e.g., polystyrene ~3 GPa). Due to its low elastic modulus, PDMS is highly compliant, facilitating replica molding to create the microfluidic structures in PDMS via soft lithography. High compliance of PDMS allows for easy removal of PDMS slabs from master molds and helps attain optimal conformal contact with additional device layers for efficient and reliable bonding. Furthermore, fabrication of flexible microstructures in PDMS has enabled novel studies in cellular mechanobiology to investigate cell adhesion on surfaces, cell-generated forces, mechanotransduction, mechanosensing and cell signaling (Jansen et al., 2015; Kim et al., 2009). By using different blends of PDMS (e.g., Sylgard 184 and Sylgard 527), it is possible to tune the stiffness of PDMS in a wider range (5 kPa to 1.72 MPa) (Palchesko et al., 2012) essentially matching the stiffness of a variety of human tissues such as brain (1.4–1.9 kPa), cardiac muscle tissue

(10–15 kPa), cartilage (0.5–0.9 MPa), and bone (7–30 GPa) (Wells, 2008). It is well known that matrix stiffness affects stem cell differentiation, cell fate and lineage (Engler et al., 2006), and scientists have recently taken advantage of PDMS and hydrogels to precisely control matrix stiffness and direct stem cell differentiation on polymeric substrates (Park et al., 2011; Wen et al., 2014). While compliant and flexible nature of PDMS opens up a variety of unique applications, it also imposes certain restrictions for particular experiments. PDMS microchannels may bulge under high pressure leading to errors in fluid flow velocities which may prove important in experiments based on flow-induced shear stress or chemical gradient generation. Low aspect ratio microchannels (typically <1:4 height: width) may lead to sagging of channel ceilings specifically in multilayer devices which may lead to inaccurate estimates of microchannel and chamber volumes. However, these drawbacks can be often overcome by careful design of the microfluidic device, controlling pressure drop across the device features, avoiding pressure build-up, and adding support structures as needed.

1.4 SURFACE PROPERTIES

PDMS is a hydrophobic material with a contact angle of 109–115° (Mata et al., 2005). Polystyrene, the most common material for cell culture substrates, is also inherently hydrophobic and therefore unsuitable for cell culture in its native form (Curtis et al., 1983). To render polystyrene suitable for cell culture, the polystyrene surface is typically treated with plasma or corona discharge to make it more hydrophilic and further treated with gamma irradiation for sterilization (Barker & LaRocca, 1994). Specifically, hydroxyl groups formed on the surface promote attachment of cells partially by facilitating adsorption of adhesion proteins (e.g., fibronectin) in serum included in the cell growth media (Curtis et al., 1983; Ryan, 2008). For some cell types (e.g., primary neurons, glial cells), positively charged surfaces—which can be obtained by coating surfaces with synthetic polymers such as poly-D-lysine—can enhance cell attachment, growth and differentiation, especially in serum-free and low serum conditions (Ryan, 2008). Polystyrene has been used for cell culture since mid-1960s (Curtis et al., 1983); surface properties and modifications essential for cell culture have been extensively studied and commercial solutions for culturing a variety of cell lines and growth medium conditions have been developed. PDMS has only been used for cell culture for less than two decades and while numerous studies have been conducted on its compatibility with cell culture (Lee, Jiang, Ryan, & Whitesides, 2004; Regehr et al., 2009), academic and commercial processes for improving its biocompatibility for cell culture are not yet as established as polystyrene. Nevertheless, PDMS substrates and microfluidic devices have been successfully used to culture some of the most sensitive cell lines such as neurons (Millet & Gillette, 2012b; Millet et al., 2007; Park et al., 2006; Shi et al., 2013), primary cells (Srigunapalan et al., 2012), stem cells (Chung et al., 2005; Kim et al., 2006; Lecault et al., 2011; Zhang & Austin, 2012), and organoids (Au et al., 2014; Vadivelu et al., 2017), suggesting that PDMS is

compatible with sustained growth of most—if not all—cell lines, assuming that PDMS surface treatments are optimized for each cell culture. In the past 15 years, there have been several studies for characterization and modification of PDMS surfaces for biomedical applications (Mata et al., 2005; Zhou, Ellis, & Voelcker, 2010) and surface treatment protocols similar to that of polystyrene have been developed and applied to PDMS substrates and microfluidic devices to render them suitable for cell culture. One potential drawback of PDMS is the hydrophobic recovery (Bodas & Khan-Malek, 2007; Eddington, Puccinelli, & Beebe, 2006; Lee et al., 2004; Regehr et al., 2009), where surface plasma treatments do not yield long-term, stable hydrophilic surfaces as polystyrene does, since low-molecular-weight uncrosslinked PDMS oligomers diffuse from the bulk to the surface, thereby returning the substrate to its hydrophobic state. Microfluidic cell culture assays should be performed within a short period after the plasma treatments to render the surfaces hydrophilic and suitable for cell adhesion and proliferation.

1.5 GAS PERMEABILITY

Oxygen and carbon dioxide are two indispensable components of cell culture to sustain growth, proliferation, and maintain a physiological pH. Besides the direct effect on cell viability, dissolved oxygen concentration has a profound effect on cell behavior, morphology and differentiation (Simon & Keith, 2008). Carbon dioxide levels are maintained at a specific level to stabilize cell culture pH with media containing carbonate-based buffers. Therefore, it is essential to precisely control gas exchange and, in specific, oxygen and carbon dioxide levels in cell culture medium. Oxygen and carbon dioxide permeability of PDMS is 34 and 22×10^{-6} cm^2/s (3400 and 2200 $\mu m^2\ s^{-1}$ @ 35°) (Merkel et al., 2000), respectively, which is very similar to that in growth media (2520 and 2400 $\mu m^2\ s^{-1}$ in water @ 35°). Highly porous structure of PDMS ensues high gas permeability and allows for quick gas exchange. In practice, thick PDMS slabs serve as reservoirs enabling diffusion and constant supply of vital gases (O_2 and CO_2) for cell culture. If the thickness of the PDMS layer over the cell culture is comparable to that of the media in a typical flask or petri dish (1–4 mm), then the gas exchange and the oxygen concentrations will be similar to that of conventional cell culture performed on macroscopic cell culture platforms such as flasks and petri dishes. In this case, passive permeation of oxygen and carbon dioxide through PDMS is generally sufficient to sustain aerobic respiration and provides adequate medium buffering to maintain a physiological pH. Thinner PDMS slabs or membranes, especially 50 μm and below, would allow faster diffusion of oxygen which may lead to hypoxic microenvironment and devices involving such geometries either should be avoided or the oxygen concentration and delivery should be diligently controlled.

PDMS is permeable to water vapor (1700 $\mu m^2\ s^{-1}$ @ 25°, Watson & Baron, 1996) which necessitates precise control of humidity over the microfluidic culture. The surface to volume ratio in microfluidic cell culture systems is typically much higher compared to conventional macroscopic (petri dish/flask-based) cell cultures.

Therefore, control of evaporation proves to be more important as small amounts of evaporation can result in substantial shifts in ion and reagent concentrations and thereby inducing unfavorable changes in osmolarity, and pH of the growth medium (Heo et al., 2007). In cases where the humidity of the microfluidic system is not well-controlled, evaporation may lead to bubble formation which will ultimately cause changes in fluidic resistance and blockage of fluid flow. Air bubbles in microfluidic channels may interact with the cell membrane which typically mechanically disrupts the membrane structure leading to lysis. Conducting the microfluidic cell culture experiments within an environmental chamber which controls temperature, gas concentrations (e.g., CO_2) and humidity generally offers the best solution for long-term or sensitive cell culture studies. Incorporating on-chip isosmotic media baths adjacent to but physically separated from the culture chambers also help alleviate evaporation, osmolarity and pH related problems (Lecault et al., 2011). On-chip gas exchangers to regulate oxygen and carbon dioxide content in microfluidic channels may help develop standalone solutions for some microfluidic cell culture applications (Thomas, Raghavan, & Forry, 2011).

1.6 THERMAL CONDUCTIVITY

Thermal conductivity of PDMS (0.15 W/mK) is significantly lower than that of water (0.591 W/mK) suggesting that PDMS walls minimize heat dissipation, thereby maintaining the cell culture at a stable temperature. As long as the microfluidic device is in thermal equilibrium with its environment and evaporative cooling is eliminated by controlling the ambient humidity, PDMS-based devices help eradicate temperature fluctuations which will otherwise have an impact on cell growth, enzyme activity and protein expression.

1.7 ELECTRICAL CONDUCTIVITY

PDMS is an electrical insulator with very high electrical resistance (4×10^{13} Ωm). Low conductivity of PDMS facilitates integration of electrodes into microfluidic devices. For instance, a microfluidic patch clamp device was developed to record ion channel activity from single cells (Pantoja et al., 2004); electrodes embedded in PDMS microfluidic devices were used to measure electrical properties of single cells (Yuan et al., 2016); and in microfluidic cell sorting (Sciambi & Abate, 2015).

2 CELL DENSITY AND PERFUSION

Microfluidic cell culture is notably different in size, dimensions, media volume and number of cells in comparison to conventional cell culture performed in petri dishes and flasks (Gomez-Sjoberg et al., 2007). In Table 2, we provide a comparison of macroscopic and microfluidic cell culture based on typical cell density and media replenishment requirements. There are two distinct features about the microfluidic cell

Table 2 A Comparison of Macroscopic (Dish, Culture Plate, Flask) and Microfluidic Cell Culture Based on Typical Cell Density and Media Replenishment Requirements Highlighting Some of the Fundamental Differences Between the Two Approaches (Thermo Fisher Scientific, 2018)

	Surface Area (cm^2)	Seeding Density	Cells at Confluency[a]	Growth Medium (mL)	Cells Per Medium volume (#/mL)	Media Replenishment (h)
Dish (35 mm)	9	0.3×10^6	1.2×10^6	2	0.6×10^6	48–96
Culture plate (12-well)	4	0.1×10^6	0.4×10^6	1–2	$0.2 - 0.4 \times 10^6$	48
Flask (T-25)	25	0.7×10^6	2.8×10^6	3–5	$0.5 - 0.9 \times 10^6$	48–96
Microfluidic chip[b]	0.09	3000	12,000	0.36×10^{-3}	33.3×10^6	0.5–2

[a] *Assuming HeLa cells may vary depending on cell type.*
[b] *Assuming the microfluidic chip has 100 chambers with dimensions $300 \times 300 \times 40$ μm (width × length × height).*

culture: (1) the small surface area entails a low number of cells cultured in a single experiment and (2) number of cells per medium volume is significantly higher (~30–150×) which better simulates in vivo cell density while necessitating frequent replenishment of culture medium. While continuous or periodic perfusion of culture medium delivers vital nutrients and removes waste products, care should be taken in the delivery method or frequency ensuring that the cell growth and proliferation is not disrupted. High cell density per unit volume in microfluidic cultures imposes that the media exchange should be typically carried out every 0.5–2 h as compared to 48–96 h in conventional dish/well/flask-based cell cultures. Endogenous growth factors and signaling molecules need to reach to a critical concentration and be maintained at that level following cell seeding for healthy culture growth. Small volume chambers in microfluidic cultures provide a major advantage over conventional cell cultures as it allows faster accumulation of growth factors and signaling molecules to stimulate culture growth (Yu, Alexander, & Beebe, 2007). Therefore, medium exchange rates need to be optimized to ensure replenishment of nutrients and removal of waste while maintaining to a steady concentration of cell signaling and growth factors in culture medium (Giulitti et al., 2013). Therefore, designing microfluidic culture chambers and feeding mechanisms enabling efficient, homogenous perfusion of fresh culture media is essential for cell viability and behavior (Mata et al., 2005). Culture chamber and perfusion system designs which enable indirect (via diffusion), partial, and periodic exchange of culture media help nurture a minimally disruptive microenvironment for cell growth while eliminating any flow-induced shear stress on cells during media exchange (Gomez-Sjoberg et al., 2007; Kellogg & Tay, 2015; Kellogg et al., 2014; Kolnik, Tsimring, & Hasty, 2012; Su et al., 2013; Tay et al., 2010).

3 ADVANTAGES OF PDMS-BASED MICROFLUIDIC CELL CULTURE

3.1 LENGTH SCALES COMPATIBLE FOR CELL STUDIES

The dimensions of microfluidic channels, typically tens to hundreds of micrometers along each dimension, are well suited to the physical scale of biological cells and enable precise control of the cell microenvironment at relevant length and time scales (Hung et al., 2005). Small channel and chamber volumes offer a convenient platform to work with limited samples such as primary patient samples (Nagrath et al., 2007).

3.2 RAPID PROTOTYPING FOR CUSTOMIZED EXPERIMENTAL DESIGN

The first bio-related applications of microfluidic devices were implementations of capillary electrophoresis as microfluidic channels provide a convenient platform for separation of biomolecules such as proteins and nucleic acids (Harrison et al., 1993). The materials choice for these first-generation microfluidic devices was mainly silicon and glass, as microfabrication techniques were previously established for these materials from microelectronics applications (Duffy et al., 1998).

While polystyrene (PS) is the dominant materials choice for conventional molecular and cellular biology, its application in microfluidics has been limited due to the unavailability of rapid, low-volume, low-cost prototyping methods (Berthier, Young, & Beebe, 2012; Halldorsson et al., 2015). Many of these limitations are linked to the microfluidic device fabrication steps such as mold fabrication, access port integration and bonding device layers (Berthier et al., 2012). Furthermore, due to its rigid structure, PS microfluidic devices need integrated or external pumping mechanisms to drive fluids within the microfluidic device.

Soft lithography based on PDMS has enabled rapid prototyping of microfluidic devices with complex functionality. Rapid and low-cost prototyping capability provides flexibility in system design. Facile integration of device components such as pumps, mixers, and reservoirs leverages complete analytical control over the experiments. Researchers have been empowered by the rapid, iterative design and fabrication processes which help create customized PDMS-based microfluidic devices for a specific biomedical application within a reasonable time and budget. PDMS remains a leading option for microfluidic cell culture because of its convenience, reliability, and its unmatched versatility (Mehling & Tay, 2014).

3.3 HIGH-THROUGHPUT ASSAYS VIA PARALLELIZATION, INTEGRATION AND AUTOMATION

Miniaturization also facilitates high-throughput studies via parallelization, integration and automation of assay steps (Balagaddé et al., 2005; Gomez-Sjoberg et al., 2007; Hung et al., 2005; Tay et al., 2010). Furthermore, small volumes inherent to microfluidic systems help reduce consumption of reagents, proteins, enzymes, etc., thereby presenting an attractive platform for high-throughput applications with expensive sample and reagents (Brouzes et al., 2009).

3.4 PRECISE CONTROL OVER CELL MICROENVIRONMENT

Microfluidic systems offer unparalleled spatial and temporal control over cellular microenvironments. Using basic principles of fluid flow and diffusion, spatiotemporally-controlled chemical or thermal gradients can be generated within microfluidic devices, allowing for previously inaccessible studies in immune response, metastasis, chemotaxis, cell migration, embryogenesis, and wound healing (Chung & Choo, 2010; Kim, Kim, & Jeon, 2010). Automation of microfluidic devices enables mimicking cell microenvironments via controlled inputs such as continuous perfusion and chemical stimulation (Kellogg et al., 2014).

3.5 HIGH SPATIOTEMPORAL RESOLUTION MEASUREMENTS

Microfluidic cell culture devices enable studies of small cell populations with single cell resolution, making it possible to capture heterogeneity in cell response which is inherently hindered by ensemble averaging in population-based studies using

conventional cell cultures (Yin & Marshall, 2012). For instance, cell migration (Chen et al., 2015; Chung et al., 2009a; Schwarz et al., 2016) and cell signaling (Cheong, Wang, & Levchenko, 2009; Chung et al., 2011; Junkin et al., 2016; Taylor et al., 2009) studies can be performed at the single cell level in the presence of spatiotemporally controlled external stimuli revealing mechanisms as to how cells simultaneously communicate, process information and respond to countless external cues.

3.6 BIOCOMPATIBILITY

Fully cured PDMS is biologically inert and is used in a variety of biomedical applications. Similar to thermoplastics used in cell culture, PDMS requires surface modification to improve its biocompatibility in adherent cell culture; as the hydrophobic surface properties either inhibit or severely disrupt adhesion and growth for most cell lines (Ramsey et al., 1984; Ryan, 2008; Yamada et al., 2003). However, surface treatments rendering PDMS surfaces more hydrophilic enable cell adhesion and proliferation for various cell lines. The biocompatibility of PDMS has been debated in the microfluidics community, but a clear consensus on the subject has not been reached (Berthier et al., 2012; Halldorsson et al., 2015; Mukhopadhyay, 2007; Sackmann, Fulton, & Beebe, 2014; Toepke & Beebe, 2006; van Midwoud et al., 2012). Viable culture of some of the most sensitive primary cells has been shown in PDMS-based microfluidic devices (Junkin et al., 2016; Kaestli, Junkin, & Tay, 2017; Kellogg & Tay, 2015; Lecault et al., 2011; Taylor et al., 2009; Zhang et al., 2017b). Studies investigating impact of PDMS on cell culture and behavior have also been reported (Eddington et al., 2006; Lee et al., 2004; Regehr et al., 2009; Toepke & Beebe, 2006; Yu et al., 2007); hydrophobic recovery, absorption of small molecules, and leaching emerge as potential problems in microfluidic cell culture applications (Berthier et al., 2012). Nevertheless, the ever-growing body of literature on microfluidic cell culture, which overwhelmingly relies on soft lithography-based (PDMS) microdevices, suggests that researchers were able to come up with viable solutions to culture and study a wide range of cell/tissue types including some of the most sensitive ones such as neural cells (Majumdar et al., 2011; Millet & Gillette, 2012b; Millet et al., 2007; Park et al., 2006; Shi et al., 2013; Taylor et al., 2005), primary cells (Srigunapalan et al., 2012), stem cells (Chung et al., 2005; Kim et al., 2006; Lecault et al., 2011; Zhang & Austin, 2012), and organoids (Au et al., 2014; Vadivelu et al., 2017).

4 CHALLENGES OF PDMS-BASED MICROFLUIDIC CELL CULTURE

4.1 CELL ADHESION

As discussed above, microfluidic devices require specific surface chemistries to promote cell adhesion and proliferation, and mediate cell behavior (Ramsey et al., 1984). PDMS surfaces are typically treated with oxygen plasma and subsequently

coated with adhesion-promoting proteins such as fibronectin, laminin, and collagen to render them suitable for cell culture (Zhou et al., 2010). Most cell lines prefer a relatively hydrophilic surface for cell adhesion and proliferation (Chinn, Horbett, & Ratner, 1994); however, there is an important aspect that needs to be taken into consideration for surface treatment of PDMS. Highly hydrophilic surfaces exhibit antifouling properties precluding cell culture on such surfaces (Zhang & Chiao, 2015). Therefore, selective treatment of PDMS surfaces is required for microfluidic cell culture. Culture chambers where cell growth and proliferation would take place should be treated to render them relatively more hydrophilic, whereas the rest of the device should be treated to render them very hydrophilic minimizing small molecule absorption and undesired protein/enzyme adsorption (Gomez-Sjoberg et al., 2007; Kellogg et al., 2014).

4.2 ABSORPTION/ADSORPTION OF MOLECULES

Due to its permeability and hydrophobicity, PDMS is prone to absorption/adsorption of small molecules and biomolecules (Toepke & Beebe, 2006). Depletion of small molecules due to absorption by PDMS may have a profound effect on cell culture applications due to the critical role of secreted soluble factors on cell signaling, behavior and function. Methods to compensate for small molecule absorption include surface coating of PDMS with low permeability materials such as parylene and wax (Ren et al., 2010; Sasaki et al., 2010), pretreatment of surfaces with sol-gel methods (Abate et al., 2008; Gomez-Sjoberg et al., 2010; Orhan et al., 2008), polymer films (Ausri et al., 2018; Patel, Choi, & Meng, 2010), and surfactants such as pluronic (Wu, 2008; Wu & Hjort, 2009; Wu et al., 2006). Furthermore, supplementing the cell culture with fresh media and stimulants as needed may alleviate some of the drawbacks associated with absorption of molecules by PDMS. Surfactant molecules such as pluronic have diblock or triblock copolymer structures with a mix of hydrophobic and hydrophilic moieties. When PDMS microchannels are treated with pluronic and filled with aqueous solutions, the hydrophilic moieties in pluronic tend to migrate to the PDMS/water interface while the hydrophobic moieties stay partly embedded in PDMS. In this manner, pluronic-treated PDMS surfaces yield a very hydrophilic, anti-fouling surface resistant to non-specific absorption/adsorption of proteins and enzymes (Wu, 2008; Wu & Hjort, 2009).

4.3 LEACHING

PDMS in incompletely cured form contains residual polymer chains that are not crosslinked and free to diffuse within the bulk material. When in contact with solution, these free oligomers can leach out into the solution (Regehr et al., 2009). There are several measures that can be taken to minimize potential leach out of uncrosslinked oligomers. First, for sensitive cell culture applications, microfluidic devices should be fabricated using a 10:1 (base:crosslinker) ratio, and any off-ratio mixtures

should be avoided. In this case, multilayer soft lithography can be performed by adhering the layers using plasma activation or partial curing protocols rather than relying on off-ratio base:crosslinker formulations. The PDMS slabs should be baked at least for 24 h to ensure the curing process is complete. In addition, the PDMS slabs can be autoclaved to drive the polymerization process to a completion. Furthermore, the PDMS slabs can be treated with various solvent extraction methods to leach out uncrosslinked oligomers from the bulk regions (Lee, Park, & Whitesides, 2003). Finally, the fabricated devices can be incubated with growth media overnight to facilitate leaching out of undesirable chemicals within bulk PDMS slab.

PDMS is not the only polymeric material prone to leaching. Thermoplastics commonly used in biological research laboratories, such as polystyrene and acrylic, are also susceptible to leaching. For instance, some bioactive contaminants used in the manufacturing process of disposable laboratory plasticware have been shown to leach into aqueous buffer (McDonald et al., 2008).

4.4 HYDROPHOBIC RECOVERY

PDMS surface is inherently hydrophobic, which poses a challenge to its applications in the life sciences where most biological systems are aqueous-based. Oxygen plasma treatment is a fast and efficient method for transforming the hydrophobic PDMS surface to hydrophilic. Nonetheless, the effect of plasma treatment on PDMS is short-lived as hydrophobic recovery is observed within a short period (Bodas & Khan-Malek, 2007; Eddington et al., 2006; Fritz & Owen, 1995; Occhiello et al., 1992). The rapid hydrophobic recovery is commonly attributed to the migration of low-molecular-weight species from the bulk to the surface (Bodas & Khan-Malek, 2007; Eddington et al., 2006). To offset this adverse effect, researchers may either use microfluidic devices immediately following oxygen plasma treatment or utilize additional surface treatment methods such as coating surfaces with adhesion-promoting proteins or poly-D-lysine to render them suitable for cell culture (Hattori, Sugiura, & Kanamori, 2011; Wang et al., 2009; Zhou et al., 2010).

4.5 POROUS NATURE OF PDMS

Besides absorption of small molecules and leaching of uncrosslinked polymers, porous nature of PDMS is also the key reason for its gas permeability. As discussed above, gas permeability of PDMS is an asset for microfluidic cell culture; however, if the ambient gas concentration (primarily CO_2 and O_2) and humidity in the vicinity of the microfluidic device are not adequately controlled, unexpected deviations in the pH and osmolarity of the medium may arise. Therefore, for efficient implementation of microfluidic cell culture, researchers should strictly control the cell microenvironment. Effective exchange/delivery of oxygen and carbon dioxide ensures that the cell culture is supplied with sufficient oxygen and the culture medium is maintained at a constant pH. Active control of humidity negates the impact of evaporation, thereby

minimizing changes in osmolarity and deter bubble formation. Bubbles inadvertently introduced into growth medium can be eliminated by external bubble traps or filter structures integrated into the microfluidic device.

4.6 CHALLENGES SPECIFIC TO SINGLE (ISOLATED) CELL CULTURE

There are specific challenges pertaining to isolation, manipulation and long-term observation of individual cells in microfluidic devices. Multifunctional quantitative single cell studies in microfluidic devices provide more in-depth information on cell state and behavior by allowing simultaneous monitoring of a larger number of parameters (gene expression, protein modification, etc.), however, their throughput is typically limited compared to conventional methods such as flow cytometry (Junkin et al., 2016). In order to increase the throughput, highly parallel manipulation of cells and their environments are required, which is typically achieved by automated control of thousands of modular units on the microfluidic device. Such device architectures involve high-density integration of membrane valves and multiplexing manifolds to generate precisely defined biochemical inputs, immanently imposing a tighter margin of error in device design and fabrication (Zhang et al., 2017b). Each modular unit should be engineered to perform multiple tasks such as isolation, culture, perfusion, stimulation and harvesting to enable versatile, multifunctional assays. Crosstalk and contamination should be eliminated by entirely isolating individual chambers from one another. Long-term culture of single cells requires more stringent control of cell microenvironment in comparison to conventional microfluidic cell culture. Primarily, the maintenance of sufficiently high humidity levels is imperative. Second, cell culture chambers should not be treated with surfactant molecules (e.g., pluronic), which are used to minimize non-specific absorption/adsorption of biomolecules, as they also inhibit cell adhesion and proliferation. In some applications where cell chambers are exposed to such treatments, surfaces should be rinsed extensively to minimize the adverse effects. Finally, for weakly adherent or non-adherent single cell studies, the microfluidic chambers should be designed such that feeding and stimulation of cells are performed efficiently to allow for long-term cell imaging/tracking while preventing undesired cell displacement (Lecault et al., 2011; Zhang et al., 2017b).

5 APPLICATIONS

While initial work on the biological applications of microfluidics was focused on proof-of-concept demonstrations showcasing technical capabilities and potential applications in biology, the field has matured considerably since then, extending to studies focused on providing novel insights in many fields of biology including systems biology, developmental biology, tissue engineering, stem cells, cell signaling, and cell migration. Below, we provide some examples of PDMS-based microfluidic cell culture, the list is by no means exhaustive and only provides a brief overview of some of the recent developments:

5.1 3D AND CO-CULTURES

Much of our understanding of biological mechanisms has been garnered from studying cells cultured on 2D surfaces. However, living organisms are composed of cells growing and interacting on 3D extracellular environments. Therefore, in the past 10 years, researchers have focused on developing in vitro 3D biomimetic environments to achieve a fundamental understanding of processes underlying cell structure, adhesion, mechanotransduction and signaling in order to capture physiological behavior of cells. To this end, many microfluidic platforms have been developed to create such controlled 3D microenvironments for cell culture (Baker & Chen, 2012; Meyvantsson & Beebe, 2008; van Duinen et al., 2015). For instance, Huang et al. engineered a microfluidic device which allows discrete constructs of 3D cell-laden hydrogels for real-time imaging of interactions between multiple cell types exposed to autocrine and paracrine signaling molecules (Huang et al., 2009). Kim et al. (2013) devised a microfluidic device for the formation of perfusable 3D microvascular networks by mimicking physiological angiogenic processes in order to study the formation and function of blood vessels and their responses to various biochemical and biophysical cues (Fig. 1). Similarly, Van Der Meer et al. designed and fabricated a 3D construct of vascular tissue inside a PDMS microchannel by injecting a mixture of human umbilical vein endothelial cells (HUVECs), human embryonic stem cell-derived pericytes and rat tail collagen I. The cells organized themselves into a single long tubular structure similar to a blood vessel along the microfluidic channel within 12 h (van der Meer et al., 2013). Sudo et al. developed a microfluidic co-culture system using 3D culture of rat hepatocytes and rat/human microvascular endothelial cells (MVECs) to investigate the heterotypic interaction between hepatocytes and MVECs during capillary morphogenesis (Sudo et al., 2009). By integrating a 3D hepatocyte culture and an in vitro angiogenesis model, they created a co-culture model which recapitulates processes underlying liver regeneration.

Booth et al. developed a microfluidic blood-brain barrier (μBBB) by co-culturing b.End3 endothelial cells with C8-D1A astrocytes and demonstrated the validity of their μBBB model by observing strongly expressed tight junctions between endothelial cells, maintenance of high electrical resistance through trans-endothelial electrical resistance (TEER) measurements, and assessing barrier selectivity using permeability assays (Booth & Kim, 2012). Majumdar et al. developed a microfluidic platform for in vitro studies of central nervous system interactions where they successfully co-cultured hippocampal neurons and glia for more than 3 weeks (Majumdar et al., 2011). They observed that co-culturing neurons with glia obviated the need for supplying neurons with pre-conditioned glia media and increased the transfection efficiency of neurons. Kim et al. developed a microfluidic model to develop a commensal biofilm of enterohemorrhagic *E. coli* (EHEC) along with an epithelial cell monolayer (HeLa cells) to test the effect of commensal microenvironment on pathogen virulence, infection, and colonization in the gastrointestinal tract (Kim, Hegde, & Jayaraman, 2010). Bersini et al. developed an in vitro tri-culture model to study breast cancer metastasis to bone tissue (Bersini et al., 2014). Specifically, they created a bone-like microenvironment consisting

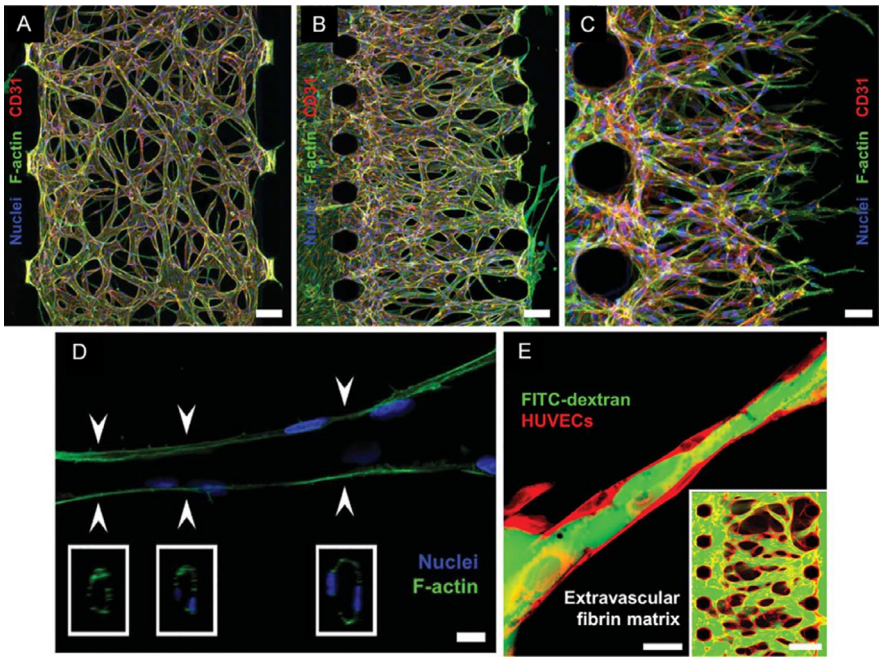

FIG. 1

Engineered 3D microvessels: Immunofluorescence imaging of overall architecture of vascular networks established by (A) vasculogenic (B) angiogenic processes grown over 4 days (scale bar, 100 μm). (C) Displays angiogenic sprouts grown over 2 days (scale bar, 50 μm). (D) Cross-sectional images of a blood vessel showing a hollow lumen enclosed by endothelial cells (scale bar, 10 μm). (E) Demonstration of perfusable microvascular network by introducing a small fluorescent molecule, FITC-dextran, into the artificial blood vessel (scale bars, 20 μm, insert scale bar, 200 μm).

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of a collagen gel with osteo-differentiated human bone marrow-derived mesenchymal stem cells (hBM-MSCs) lined with endothelium and studied the extravasation of highly-metastatic MDA-MB-231 human breast cancer cells into this artificial bone tissue.

5.2 ORGAN ON CHIPS

Organ-on-chip technology integrates microfluidic technologies with 2D and 3D cell cultures and tissue-like structures to study human physiology in organ-specific context and to develop physiologically-relevant in vitro disease models (Bhatia & Ingber, 2014; Huh, Hamilton, & Ingber, 2011). Organ-on-chip systems enabled formation of cell microenvironments with features reminiscent of living organs to

simulate tissue- and organ-level physiology, by supporting tissue differentiation, recapitulating tissue-tissue interfaces, incorporating spatiotemporal signaling gradients, and mechanotransduction mechanisms. To this end, numerous organ/tissue-on-chip models have been developed to date including lung (Huh et al., 2010, 2012), heart (Agarwal et al., 2013; Grosberg et al., 2011), kidney (Jang & Suh, 2010; Jang et al., 2013), liver (Kane et al., 2006; Lee Philip, Hung Paul, & Lee Luke, 2007; Nupura et al., 2016; Toh et al., 2009), gut (Kim et al., 2012a), spleen (Rigat-Brugarolas et al., 2014), bone-marrow (Torisawa et al., 2014), female reproductive tract (Xiao et al., 2017), nerve (Majumdar et al., 2011; Shi et al., 2013), muscle (Grosberg et al., 2012), skin (Wufuer et al., 2016), blood-brain barrier (Booth & Kim, 2012; Griep et al., 2013), retina (Dodson et al., 2015), and artery (Yasotharan et al., 2015). Researchers have also built microfluidic devices mimicking physiological processes and disease models such as cancer-on-a-chip (Zhang, Zhang, & Zhang, 2017), tumor-on-a-chip (Albanese et al., 2013), and thrombosis-on-a-chip (Costa et al., 2017; Zhang et al., 2016). In addition, there is a recent, noteworthy academic and commercial effort on developing "human-on-a-chip" models consisting of interconnected departments, each representing a different organ, linked through a microfluidic circulatory system (Baker, 2011; Esch, King, & Shuler, 2011; Luni, Serena, & Elvassore, 2014; Maschmeyer et al., 2015; Skardal, Shupe, & Atala, 2016; Wagner et al., 2013; Zhang et al., 2009). One of the first demonstrations of organ-on-a-chip applications was the human gut-on-a-chip developed by Ingber lab (Kim et al., 2012a). The biomimetic microdevice was composed of two microfluidic channels separated by a porous flexible membrane coated with extracellular matrix and lined by human intestinal epithelial (Caco-2) cells that mimics the complex structure and physiology of the gut (Fig. 2). When they introduced flow-induced shear stress over the microchannels and exerted a cyclic strain mimicking physiological peristaltic motions, a columnar epithelium developed by polarizing rapidly, growing spontaneously into folds that recapitulate the structure of intestinal villi, and forming a high integrity barrier to small molecules. Furthermore, they were able to co-culture a gut microbe (Lactobacillus rhamnosus GG) for over a week on the luminal surface of the cultured epithelium without compromising epithelial cell viability.

5.3 STEM CELLS

Stem cells hold remarkable promise in tissue engineering and regenerative medicine due to their self-renewal potential, and inherent ability to differentiate into specific cell types. In the past 15 years, researchers have utilized precise microenvironment control, a hallmark advantage of microfluidic systems, to investigate mechanism of proliferation, differentiation, stimulation, (re)programming and evolution of embryonic, adult (mesenchymal, hematopoietic, neural), and cancer stem cells (Huang et al., 2011; Karimi et al., 2016; van Noort et al., 2009; Wu, Lin, & Lee, 2011; Zhang & Austin, 2012). The proliferation and differentiation of the stem cells can be well controlled by manipulating the chemical environment in a microfluidic

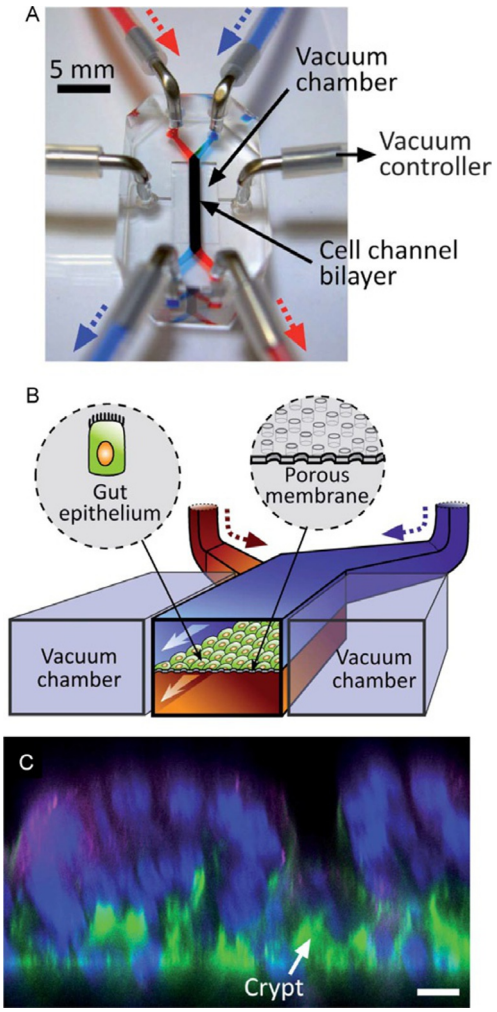

FIG. 2

Gut-on-a-chip: (A) An image of a PDMS-based gut-on-a-chip device with fluidic connections. (B) A schematic of the gut-on-a-chip device consisting of a microfluidic channel in the middle separated by a flexible porous ECM-coated membrane lined by gut epithelial cells. This channel is sandwiched between two neighboring vacuum chambers which is used to exert cyclic mechanical strain on the epithelial cells, mimicking the peristaltic motions in the gut. (C) A confocal fluorescence image of a vertical cross section of a region of the undulating epithelium at 170 h confirming the presence of intestinal villi lined by consistently polarized columnar epithelial cells labeled with F-actin (green), basal nuclei (blue), and apical mucin expression (magenta) separated by a crypt (scale bar, 20 μm).

Reproduced from Kim, H. J., et al. (2012). Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab on a Chip, 12*(12), 2165–2174, with permission from the Royal Society of Chemistry.*

system. One of the earliest reports of PDMS-based stem cell culture was by Mohr, de Pablo, and Palecek (2006). Using a PDMS microwell array featuring physical and extracellular matrix patterning constraints to limit colony growth, they maintained undifferentiated human embryonic stem cell cultures for 2–3 weeks without passaging, while retaining pluripotency and self-renewal. Ungrin et al. reported a similar method based on PDMS microwell-arrays to obtain spatially and temporally synchronized human embryoid bodies (hEBs) by precisely controlling the differentiation and aggregation of human embryonic stem cells (Ungrin et al., 2008). Occhetta et al. designed a microfluidic platform to investigate processes involved in mesenchymal progenitor cells differentiation (Occhetta et al., 2015). They cultured 3D micromasses of human bone marrow-derived mesenchymal stromal cells under continuous perfusion and exposed them to well-defined concentrations of morphogens (Wnt3a, FGF2 and TGFβ3) involved in the first phases of embryonic limb development. As compared to traditional pellet culture methods, microfluidic devices yielded a more uniform and robust micromass response to morphogens, and a low TGFβ3 concentration for proliferation and induction of chondrogenic differentiation. Lecault et al. devised a microfluidic platform to study hematopoietic stem cell (HSCs) proliferation at the single-cell level (Lecault et al., 2011). Their device design allowed them to capture nonadherent cells, such as HSCs, culture them by isosmotic perfusion without major disturbance while retaining their functional properties, and study the role of a cytokine (SF) in regulating the survival of cytokine-activated HSCs. Chung et al. engineered a gradient-generating microfluidic platform which optimizes proliferation and differentiation of neural stem cells (NSCs) by exposing them to a concentration gradient of growth factors under continuous flow, thereby minimizing autocrine and paracrine signaling (Chung et al., 2005).

5.4 CELL SIGNALING

Microfluidic platforms have also been utilized for examining cell signaling and communication. For instance, using microfluidic single-cell measurements, our group has shown that NF-κB dynamics in fibroblasts synchronize with oscillating TNF signal and become entrained, leading to significantly increased NF-κB oscillation amplitude and mRNA output compared to non-entrained response, indicating that synergy between oscillation and noise allows cells to achieve efficient gene expression in dynamically changing signaling environments (Kellogg & Tay, 2015). Recently, we developed an automated microfluidic system to quantitatively probe single-cell input-output dynamics (Junkin et al., 2016). Specifically, we isolated single immune cells (macrophages) and exposed them to an inflammatory stimulant (bacterial lipopolysaccharide, LPS) and measured cytokine secretion transcription factor activity (Fig. 3). We studied temporal single cell response under brief (single pulse), repeated (pulse-train) and chronic (continuous) exposure to stimulant (LPS) by measuring cytokine secretion (primarily TNF) using an on-chip bead-based fluorescence sandwich assay. Our device design allowed us to simultaneously measure a

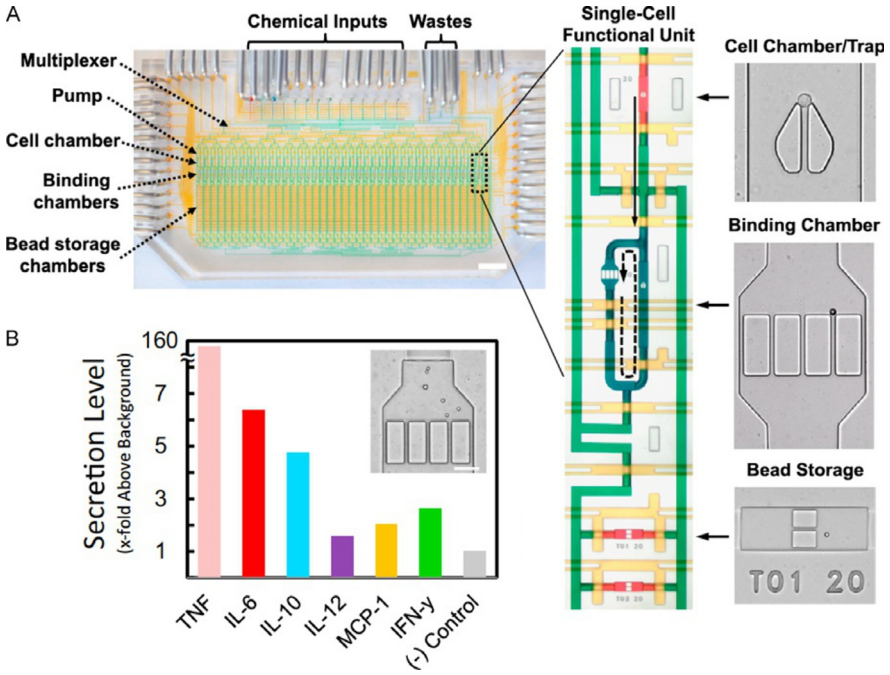

FIG. 3

High-content single-cell immune dynamics: (A) Automated microfluidic device for studying single-cell input-output dynamics. The device is capable of capturing and culturing single-cells in 40 isolated nanoliter-sized chambers, exposing cells to dynamic stimuli, and determining cell response by measuring cytokine secretion through an on-chip bead-based fluorescence sandwich assay (scale bar, 5 mm). (B) Multiplexed detection of cytokine release from single macrophages in response to continuous exposure of lipopolysaccharides, an inflammatory stimulant.

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number of cytokines secreted from single cells, capturing dynamic immune response at the single cell level and revealing highly heterogeneous response to inflammatory inputs. Previously, Taylor et al. developed a high-throughput microfluidic single-cell analysis platform to investigate a mitogen-activated protein kinase (MAPK) signaling network in *Saccharomyces cerevisiae* to uncover the combined effect of gene deletions and changing stimulant conditions on the mating response (Taylor et al., 2009). Similarly, Chung et al. devised a microfluidic platform for high-throughput capture and imaging of hundreds of single cells (Chung et al., 2011). Using a dense array capable of capturing and simultaneously imaging 800 cells, they measured heterogeneity in calcium oscillatory behavior in Jurkat cells and monitor increase in intracellular calcium concentration in response to ionomycin stimulation.

5.5 DRUG SCREENING

Microfluidic platforms provide several benefits for drug screening/discovery studies such as high-throughput, reduced reagents, and integration of physiologically-relevant 3D cell cultures and biomimetic models of human tissues (Chi et al., 2016). Sung et al. developed a microfluidic device consisting of multiple cell culture chambers that are connected with fluidic channels to mimic multi-organ interactions and test drug toxicity in a pharmacokinetic-based manner (Sung, Kam, & Shuler, 2010). Specifically, they cultured three cell lines representing the liver, tumor and marrow and tested the toxicity of an anticancer drug, 5-fluorouracil. They found out that each cell type exhibited differential responses to the drug, and the responses in the microfluidic environment were different from those in static environment. Xu et al. designed a microfluidic platform for 3D co-culture of lung cancer and stromal cell lines to perform drug sensitivity tests (Xu et al., 2013). Using the microfluidic platform, they tested cell response to different cancer drugs (gefitinib, paclitaxel, cis-platin and gemcitabine), determined the optimal dose for each drug and also studied cell response to combined drug therapy. Similarly, Kim et al. developed a microfluidic cell array for screening and optimizing combinatorial drug treatments for cancer therapy (Kim et al., 2012b). The microfluidic chip consists of a cell culture array and an on-chip module which can generate pairwise concentration combinations. Using this microfluidic chip, they exposed PC3 prostate cancer cells to combinations of sensitizer drugs (doxorubicin or mitoxantrone) and a cancer drug (TRAIL) and studied their synergistic relationship in TRAIL-induced apoptosis. Agarwal et al. designed a heart-on-a-chip device for evaluating the in vitro response of engineered cardiac microtissues to pharmacological agents (Agarwal et al., 2013). The device comprises anisotropic cardiac microtissues recapitulating the in vivo tissue architecture of the heart ventricle. Using this device, they tested a non-selective beta-adrenergic agonist (isoproterenol) dose response on cardiac contractility.

5.6 ADHESION, SPREADING AND MIGRATION ASSAYS

Cell niche is a complex microenvironment often involving gradients of physical or biochemical cues implicated in cell signaling and regulation of cell function and behavior. Facile generation of precise physical and biochemical gradients using microfluidic devices enabled researchers to design novel chemotactic (cell motility due to chemical gradients) (Kim & Wu, 2012; Wu, Wu, & Lin, 2013), durotactic (cell motility due to substrate stiffness gradient) (Isenberg et al., 2009; Vincent et al., 2013), and haptotactic (cell motility due to gradient of surface-bound ligands) (Sundararaghavan, Masand, & Shreiber, 2011) assays. For instance, as one of the very early successful applications of microfluidics, Jeon et al. developed a microfluidic device consisting of a network of microfluidic channels that can generate spatially and temporally controlled gradients of chemotactic factor (IL-8) to study neutrophil chemotaxis, and observed strong directional migration of neutrophils toward increasing concentrations of IL-8 (Li Jeon et al., 2002). Furthermore,

employing a microfluidic method to fabricate hydrogels with stiffness/compliance gradients ranging between 1 and 80 kPa, Isenberg et al. studied the durotactic behavior of vascular smooth muscle cells on these hydrogels by analyzing cell morphology, polarization and orientation (Isenberg et al., 2009). In addition, Chung et al. developed a microfluidic platform to study endothelial cell migration under co-culture conditions. By using an interconnected tri-channel geometry, they characterized migration of human dermal microvascular endothelial cells (HMVEC) under growth factor stimuli (VEGF), and under co-culture with two types of cancer cells (MTLn3 or U87MG) (Chung et al., 2009b). Hong et al. developed a microfluidic co-culture platform capable of maintaining and tracking single-cell pair interactions. Using this platform, they cultured and tracked stem cell-fibroblast pairs for several generations and found out that migration patterns of paired cells depend on their initial distance from each other and heterotypic pairing led to distinct proliferation patterns in comparison to homotypic co-culture (Hong, Pan, & Lee, 2012).

6 OUTLOOK

Here, we provided a brief overview of PDMS-based microfluidic devices for cell culture applications. Microfluidics field is rapidly evolving combining advances in science and engineering to address some of the vital challenges in biomedical sciences. Microfluidic techniques continue to play an ever-expanding role in commercial bioanalytical and biomedical devices. There are already commercial microfluidic cell culture products available for biologists. While microfluidics offers a powerful toolbox for cell culture applications, it is yet to reach its full potential. PDMS is likely to continue its central role in microfluidics for applications in biomedical sciences due to its rapid, low-cost prototyping capability. As the field advances, standards for device design principles, surface modification processes, common experimental protocols, and a list of troubleshooting solutions will emerge, making microfluidics more accessible to the broader scientific community.

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