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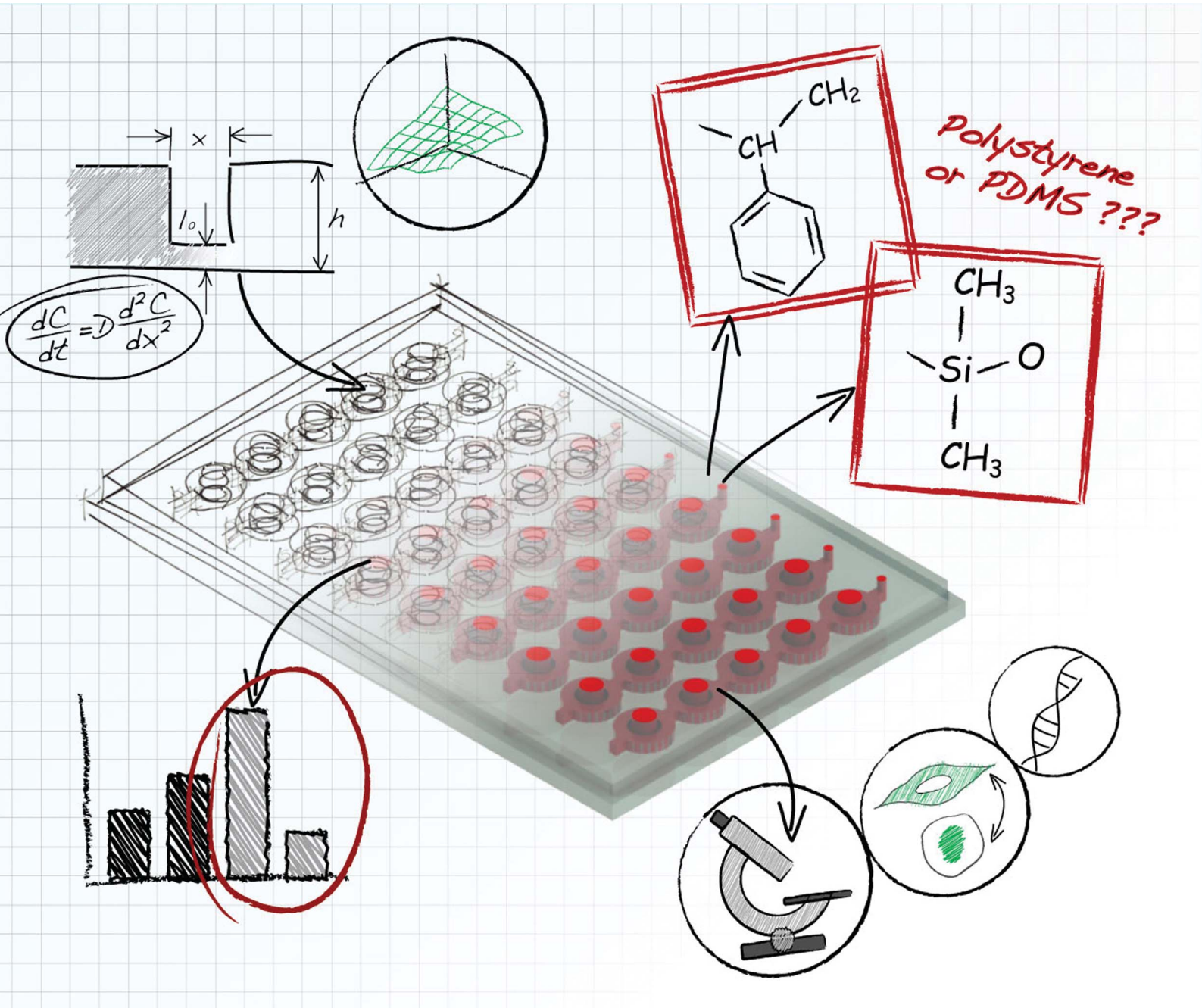

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CRITICAL REVIEW

Engineers are from PDMS-land, Biologists are from Polystyrenia

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As the integration of microfluidics into cell biology research proceeds at an ever-increasing pace, a critical question for those working at the interface of both disciplines is which device material to use for a given application. While PDMS and soft lithography methods offer the engineer rapid prototyping capabilities, PDMS as a material has characteristics that have known adverse effects on cell-based experiments. In contrast, while polystyrene (PS), the most commonly used thermoplastic for laboratory cultureware, has provided decades of grounded and validated research conclusions in cell behavior and function, PS as a material has posed significant challenges in microfabrication. These competing issues have forced microfluidics engineers and biologists to make compromises in how they approach specific research questions, and furthermore, have attenuated the impact of microfluidics on biological research. In this review, we provide a comparison of the attributes of PDMS and PS, and discuss reasons for their popularity in their respective fields. We provide a critical evaluation of the strengths and limitations of PDMS and PS in relation to the advancement and future impact on microfluidic cell-based studies and applications. We believe that engineers have a responsibility to overcome any challenges associated with microfabrication, whether with PS or other materials, and that engineers should provide options and solutions that assist biologists in their experimental design. Our goal is not to advocate for any specific material, but provide guidelines for researchers who desire to choose the most suitable material for their application, and suggest important research directions for engineers working at the interface between microfabrication technology and biological application.

1. Introduction

The development of microscale systems for both fundamental and applied research in cell biology has experienced significant growth and advancement in recent years.[1–3] The rapid progress in this area can be partially attributed to increased functionality combined with the small scale of these systems, which offers the advantage of lower volumes, reduced consumption of reagents and potential for increased throughput. For cell-based applications specifically, microscale systems offer a physical scale well suited for studying cells in spatially and temporally controlled microenvironments.[4–7] This has importantly led to new advanced methods for probing and understanding cells and their properties, including cell morphology and motility, biomechanical forces, and complex biophysical and biochemical cell-cell interactions (Fig. 1).[8–12] In particular, the size of microscale systems are naturally suited for studying cells of limited availability, such as from primary patient samples.[13–15] Microscale systems have thus provided new insights into cell behavior and function, and have enabled us to tackle complex biological questions that were once inconceivable with conventional platforms.

The current pace of research in the development and application of microscale systems for cell biology has also been facilitated by an increasing number of collaborations between engineers and biologists that are vital to the scientific research enterprise. The interdisciplinary nature of this field necessitates collaborative efforts to advance ideas from concept to assay development and validation, and ultimately to implementation and application (Fig. 1). While many of these collaborations have been successful in advancing science and technology, microscale systems, to a large extent, have not penetrated the cell biology research community, and have yet to become widely accepted platforms for studying cell biology. This is partly attributable to the fact that communication between biologists and engineers continues to be somewhat hindered by subtle yet critical differences between their respective research cultures. The natural tendency of researchers to choose conventional and proven methods rather than novel and potentially more effective methods, which may contain a degree of uncertainty, amplifies this divide.

In the case of microfluidic cell-based systems, this "clash of cultures" has occurred between biologists and engineers over material selection for microdevice fabrication. Microfluidics

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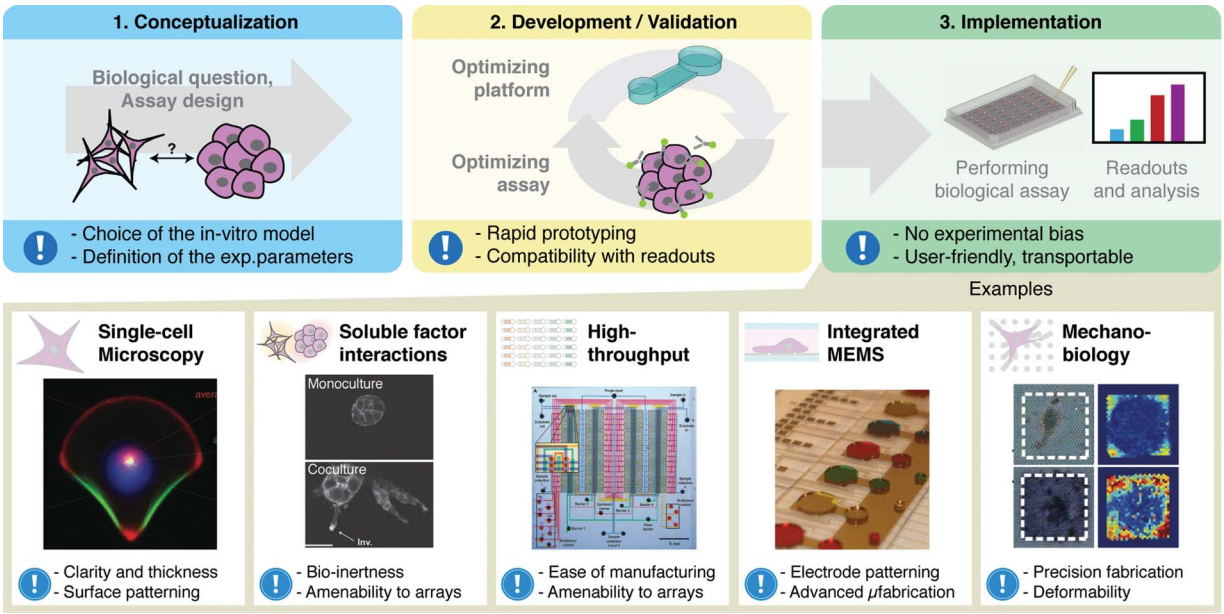


Fig. 1 Integration of microscale technologies and cell biology requires collaboration between biologists and engineers from conceptualization to development and validation and finally to implementation. When properly implemented, state-of-the-art microfluidic cell-based systems can drastically enhance our ability to examine the behavior and function of cells. Exclamation mark icon represents key considerations for each section. Examples of these systems have enabled the observation of intracellular organization (reprinted from ref. 8 with permission of the National Academy of Science), the study of multi-cellular soluble factor communication (reproduced in part from ref. 9 with permission of the Royal Society of Chemistry), the creation of high-throughput microfluidic systems (from ref. 10; reprinted with permission from AAAS), the creation of integrated droplet based cell-culture systems (reproduced in part from ref. 11 with permission of the Royal Society of Chemistry) and the measure of mechanical forces exerted by cells (reprinted by permission from Macmillan Publishers Ltd: Nature Methods ref. 12, copyright (2010)).

engineers have become accustomed to using polydimethylsiloxane (PDMS) for rapid prototyping of designs and for fabrication of microdevices because of its many attractive properties over other materials, including its low cost, ease of use, and high compliance. However, a growing number of reports have begun to increase awareness of potential spurious effects associated with culturing and studying cells in PDMS microdevices.[16,17] In contrast, biologists have relied heavily in the past fifty years on platforms such as Petri dishes, culture flasks, and microtiter well plates made mostly from polystyrene (PS). The majority of research data collected on biological cells *in vitro* has been based on cell behavior on PS surfaces. Thus, from the perspective of a biologist, microscale systems would be more attractive if devices were available in more popular and widely used laboratory materials such as PS. Unfortunately, microfabrication in PS and other plastics is considerably more challenging compared to microfabrication in PDMS, limiting the availability of plastic-based microscale systems to those with access to dedicated equipment and expertise.

This contrast in research cultures regarding material selection raises a number of important questions that need to be addressed to further enhance collaborations between engineers and biologists and allow microfluidics research to continue at or above the current pace of discovery. How significant are the adverse effects of PDMS on microscale cell biology studies? What current challenges do engineers face that prevent or limit them from fabricating more in other materials, particularly PS, and how do we overcome these challenges? Importantly, how will increased availability and accessibility of PS microdevices further enhance the integration between microfluidics technology and cell biology?

In this review, we address these questions by directly comparing PDMS and PS in the context of developing and fabricating microscale cell-based systems, specifically for biological studies. The goal is to enhance the collaborative relationships between engineers and biologists, and further accelerate integration between microfluidics and cell biology. First, we provide a historical perspective on the independent developments of cell culture and microfluidics over the past century, and review the recent efforts to integrate these fields for the benefit of interdisciplinary research. Such a historical account will partially help to explain the distinct contrast in research cultures between the respective fields, and potentially help guide future research directions. Second, we discuss the major issues associated with PDMS for microfluidic cell-based systems, their significance, and methods to circumvent these issues. We then review PS fabrication methods and highlight recent advances that may help to alleviate bottlenecks in the process workflow for rapid prototyping in PS. Finally, we address questions raised by both engineers and biologists on the issue of material selection, giving consideration to their respective needs. This review, which discusses topics ranging from microfabrication to cell culture, is intended for integrative bioengineers who aim to create tools that can be translated to biology laboratories. The goal of this review is not to advocate for PDMS, PS, or any other material, but instead to stimulate interest and further dialogue in this important topic of material selection, and allow researchers from the two sides to overcome differences in research culture and come to

a mutual understanding. Ultimately, bringing this topic to the forefront will further promote collaborations that will lead to continued progress in research and development of microfluidic cell-based technologies.

2. Historical perspective

The use of PS for *in vitro* biology and PDMS for microfluidics happened through a series of historic events that provided the right environment for their emergence. To understand how PS and PDMS became the materials of choice in the respective areas of cell culture and microfluidics, it is informative to first examine these landmark events that have helped shape the separate fields. Doing so provides us with the necessary background to understand why these materials became popular and which properties of the material contributed to their rise in popularity. Here we provide a brief historical account of the major scientific events over the last century (Fig. 2), and offer insight into how they shaped the research cultures of each field. This insight will hopefully instruct us on how to address necessary compromises in material selection when working at the interface between microfluidics and cell biology.

2.1. Timeline of cell culture

In 1907, Ross Harrison marked the beginning of cell (or tissue) culture and *in vitro* biology when he first demonstrated outgrowth of nerve fibers from a frog embryo on a glass dish.[18] While this type of culture is considered routine today, such a feat – the ability to sustain life at the cellular level outside the whole of an organism – was in fact, at the time, viewed with both amazement and guarded skepticism.[19] In the next three decades, Alexis Carrel shaped much of the tissue culture field by introducing aseptic methods, designing the canted-neck culture

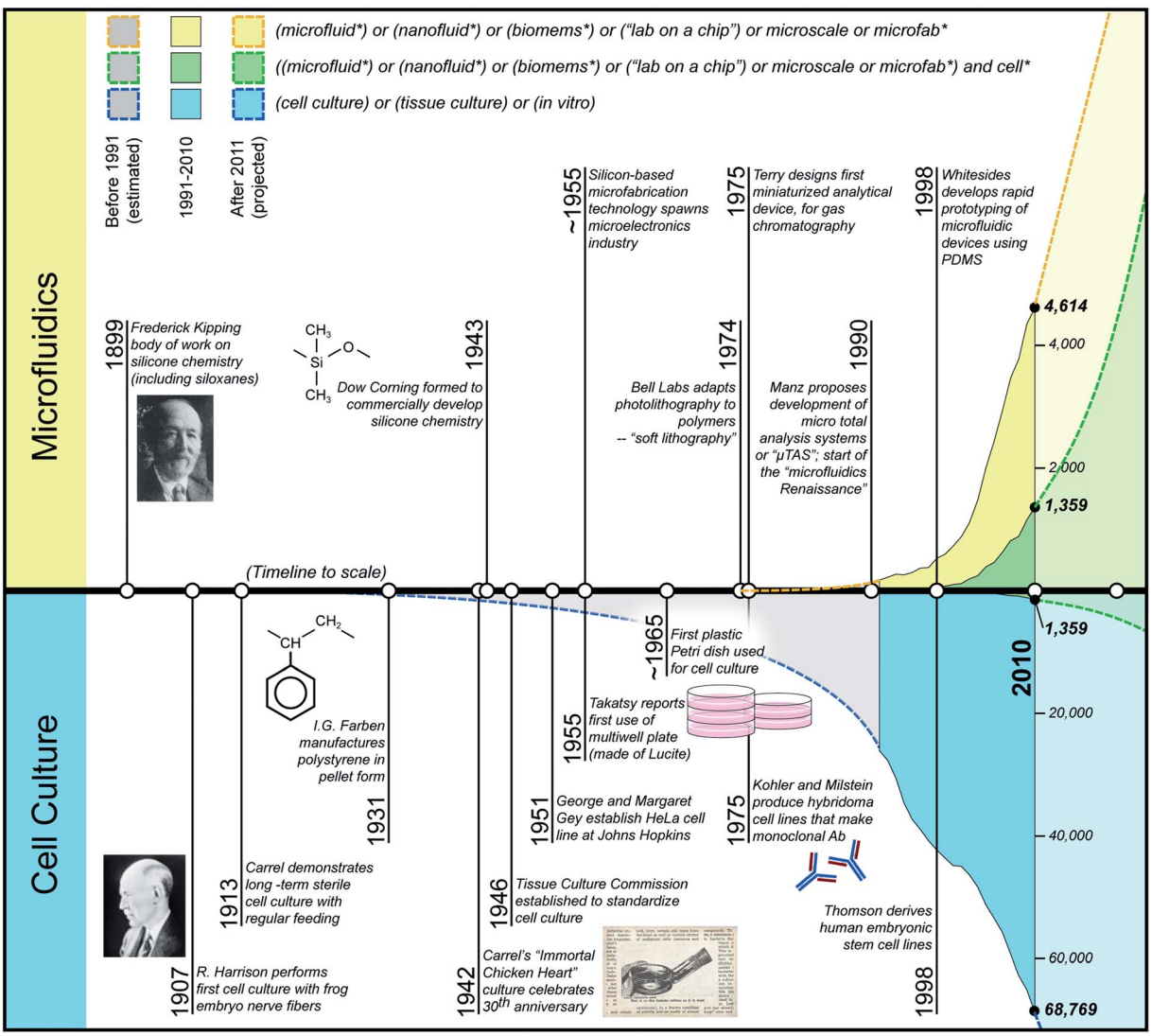


Fig. 2 Timelines of major historic events in microfluidics and cell culture. Filled curves represent number of publications found per year, using the Web of Science search terms noted in the figure legend (yellow: microfluidics; blue: cell culture; green: integration of microfluidics and cell culture; search performed September 2011). Note that blue and yellow curves have different scales, and that the green curves are identical, mirror images of each other to show proportion within the independent fields of research.

glassware, and optimizing cell culture techniques. One of the most publicized achievements in this era was perhaps the demonstration of the "immortal chicken heart", in which Carrel showed that routine replenishment of nutrients and removal of cellular waste products was sufficient and necessary to sustain a culture for thirty years.[20]

The mid-1940s through the 1950s marked an important turning point in the field of cell culture.[19] During this period, the potential impact of cell culture, both as a tool for basic research in biology and as a vehicle for mass production of biological substances, began to fully emerge. First, the Tissue Culture Commission (now the Society for In Vitro Biology) was established to standardize cell culture techniques and provide materials and references on cell culture, marking the official scientific recognition of cell culture as a major research field. Second, important immortal cell lines were developed, first by Earle, who established the L929 mouse fibroblast cell line, followed by George and Margaret Gey, who established the well-known HeLa cell line,[21] thereby allowing the establishment of cells as standard biological instruments for research and biotechnology. The ability to store, ship, and exchange the cell lines was enabled by the development of cryopreservation techniques, further fueling the expansion of the field. Finally, one of the most impactful developments was the work by Enders and Salk in 1954 that led to the mass production of the polio vaccine using cultured cells as instruments for massive expansion of the vaccine.[22]

As the field received increasing attention, the exclusive use of glass cultureware became a limiting factor, and alternative materials such as plastics began receiving more consideration as culture surfaces. Replacing glass with plastics helped reduce labor-intensive cleaning processes, lower operational costs, and improve durability of culture equipment, further promoting cell culture as a practical and accessible field of science. Coincidentally, the plastics industry was flourishing, and companies such as Dow Corning and Falcon Plastics developed processes that allowed a significant decrease in the cost of plastic fabrication, particularly PS, thereby enabling its use in myriad applications including household appliances, building materials and electronics equipment.[23] Because of these advances, as well as a number of attractive properties (*e.g.*, optical clarity, mechanical strength, durability, and low cost), PS became a reasonable choice as a material for commercial disposable cell culture labware. The final step in the transition to PS as a cell culture substrate was achieved with the development of oxygen plasma surface treatments by Falcon Plastics, which rendered PS more hydrophilic and "glass-like", and ultimately improved its ability to promote cell adhesion and proliferation.[24,25]

PS ultimately became the dominant material from the 1960s onward, presumably due to increasing availability provided by the major plastics manufacturing firms.[24] While other materials such as poly(methylmethacrylate) (PMMA or "Lucite") were also used at the time, such as for the first "well plate",[26] their commercial availability eventually declined due to the increased pressure from PS. Over the subsequent fifty years, PS labware became the standard for *in vitro* experiments, leading to an exponential increase in the number of studies and publications in *in vitro* biology (Fig. 2). Because of its ubiquitous use, coupled with the accumulation of trusted data over decades of research, it has become difficult for other materials to establish themselves as materials of choice for biological research platforms and become more widespread.

2.2. Timeline of microfluidics

While microfluidics has a much shorter history than cell culture, similarities exist in their paths of development. In the mid-1970s, several key technical advances were reported that laid important groundwork in microfluidics, namely the first miniaturized analytical device designed for gas chromatography,[27,28] and the development of soft lithography at Bell Laboratories.[29] However, the developments in microfluidics were slow in the subsequent fifteen years, and focused mainly on microfabrication techniques in silicon for various device components.[30–35]

The turning point for microfluidics occurred in the early 1990s with the idea of creating micro total analysis systems (μTAS), or "lab on a chip" devices, that were capable of reducing conventional laboratory equipment onto miniaturized platforms.[36] During this "Renaissance" era of microfluidics,[37] research focused mostly on applied chemistry and physics at the microscale,[38–40]with only a small number of pioneering reports demonstrating integration of microfluidics with biology[41] and even fewer reports using microfluidics specifically for cell biology research.[42–44] The popular material choices for these "first generation" microdevices included silicon and glass, mostly because microfabrication techniques were already established for these materials from microelectronics applications. Thermoplastics such as PMMA and polycarbonate (PC) were also prominent in the early developments of microfluidics because of their low cost and ease of manufacturing. Yet, despite growing interest, research in microfluidics was restricted to laboratories equipped with specialized equipment for performing silicon, glass, and thermoplastic microfabrication. The most significant advancement in microfluidics was the development of soft lithography and the use of PDMS as a material for rapid prototyping of microscale devices.[45] In addition to possessing a number of attractive properties (*e.g.*, optical clarity, low cost, reproducibility) that made PDMS competitive with glass and thermoplastics, its most significant advantage was its ease of use, specifically the ability to fabricate devices without the need for expensive equipment, which allowed rapid prototyping and testing of microscale designs, immediate widespread adoption in research laboratories, and an explosion in the number of research teams and publications in the field of microfluidics (Fig. 2).

The accessibility and ease of use of PDMS are the main reasons for the enormous expansion of cell-based microfluidics research. These properties have enabled many laboratories, including those with modest resources and technical expertise, to quickly set up fabrication processes and rapidly develop novel experimental tools for basic and applied scientific research. This has been especially important in cell biology research because the complexity of cells and cellular systems requires significant optimization of the tools used to probe them, which can only be achieved efficiently with a design process that allows rapid iteration through many designs. While many of the early reports on cell-based microfluidics (as well as some current reports) were focused on proof-of-concept demonstrations showcasing technical capabilities and the wide range of potential applications in

biology,[46–51] the field has since matured considerably, extending to studies focused on providing novel insights in fundamental cell biology.[9,52–56]

2.3. Summary

The emergence of PS and PDMS marked major turning points in the histories of cell culture and microfluidics, leading to immense growth for both fields, as can be seen by the sharp increases in publications (Fig. 2). In both cases, these materials emerged from a list of alternatives based on two main practical considerations: accessibility and availability. Indeed, it can be argued that there are currently no other notable materials that can rival the accessibility and availability of PS and PDMS in their respective disciplines over the same breadth of applications. Yet, while both fields have had similar major turning points followed by similar paths, there are also differences in their histories that will likely contribute to their distinct future trends. The most notable difference is the length of their histories: cell culture has more than a century of development while microfluidics spans a mere three decades of sharp progress. Importantly, these histories are linked respectively to two contrasting research cultures that further distinguish the nature of these fields. While technological fields like microfluidics are inherently more dynamic and transient, areas of basic science like biology necessarily build their strength from the gradual process of collecting empirical data and experiences, resulting in a solid foundation of scientific evidence and knowledge that gradually becomes fortified in the literature after years of rigorous testing. While this is a simplified view, given these two disciplines, microfluidics has the fluidity to adapt, evolve, and conform to the more rigid landscape in basic science and biology.

In general, while PDMS and PS will likely coexist and continue to play significant roles within their distinct fields, a challenge arises at the interface of these areas: Which material do we use when we do research that integrates microfluidics and cell biology? This important question must be addressed for continued progress in this interdisciplinary research area. Our focus in this review, on discussing the suitability of PDMS and PS for cell-based microfluidics applications, is succinctly summarized in the following quote, which ironically comes from a book on PS:

"*Unfortunately the selection of a suitable material, and elimination of unsuitable ones is not so easy. If the designer or fabricator does not consider carefully the properties of the material in terms of what is required of the finished article, he* [*or she*] *may make a poor choice*."

——— Teach and Kiessling (1960).[23]

3. PDMS *versus* PS

PS and PDMS have many attractive qualities well suited for their separate fields. Yet, these materials also have a number of important limitations that have raised some concerns regarding their usage as the material of choice at the intersection of these fields with respect to cell-based microfluidics applications. In this section, we change our focus from a historical perspective to the technical aspects of these fields, and discuss the main issues with using PS and PDMS at the interface of microfluidics and cell biology, both in terms of *in vitro* experimentation and microfabrication, and review the current approaches used to overcome these issues.

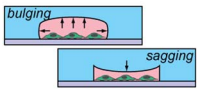
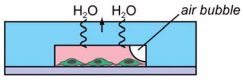
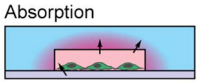
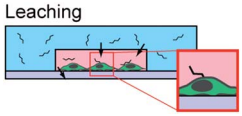
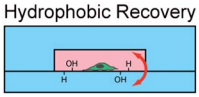
3.1. PDMS for cell-based assays

PDMS has been central to the development of microfluidics in the last fifteen years due to a number of attractive properties. Nevertheless, a number of limitations have recently been cited that raise concerns about PDMS as an appropriate material for cell-based studies. Here we discuss five important properties of PDMS that potentially have adverse effects for microscale cell studies: deformation, evaporation, absorption, leaching of uncrosslinked oligomers, and hydrophobic recovery (Table 1).

3.1.1. Deformation. An often-cited advantage of PDMS is its high compliance, or low stiffness. PDMS has an elastic modulus ~1–3 MPa, four orders of magnitude lower than glass (~50 GPa) and three orders of magnitude lower than thermoplastics like PS (~3 GPa). High compliance allows PDMS to be easily deformable, facilitating removal from master molds and conformity to other surfaces for reliable bonding. PDMS deformation has been exploited during operation of the device to enable sorting and trapping events,[57] to measure cell traction forces[58] or biological responses to substrate deformation,[59] and to actively apply cell stretching in microsystems in a manner similar to conventional Flexercell systems.[60] The compliance of PDMS can also be leveraged when combined with glass or thermoplastics to create hybrid microdevices with dual advantages.[61] However, the most significant development resulting from the high compliance of PDMS has been the ability to create integrated valves and pumping systems that permit high-density multiplexing and fluidic control.[10,62–65] For these applications where deformation of the material is integral to device functionality, PDMS is a superior choice compared to stiffer materials.

The compliance of PDMS, however, can become a limitation in applications where the deformation of microchannels and other microfeatures are not desirable. This is particularly true for cell culture experiments that require precise control over shear forces, such as shear stress studies on endothelial cell monolayers.[6,51–53,56,66,67] Under certain pressure-driven flow conditions, for instance, microchannels may bulge due to the high pressure acting against compliant PDMS,[68] or sag at the ceiling under its own weight when the aspect ratio is too low, causing variations in the expected shear rates. While cross-sectional deformations also occur in thermoplastic devices, typically after thermal bonding (see Section 2.2.3), the issue with PDMS is that deformation occurs dynamically during operation, and changes with varying pressure. Inability to account for these deformations can lead to erroneous estimation of shear stress, and can contribute to bias in data analysis and interpretation. This issue can be partially mitigated by changing PDMS curing parameters (*e.g.*, mixing ratio, curing temperature and time) to achieve higher PDMS stiffnesses.[68,69] However, this approach reduces deformation at the cost of introducing additional variability between PDMS devices made with different parameters, further complicating comparisons of biological results, particularly across different laboratories.

Table 1 Limitations of PDMS for microfluidic cell-based systems

Problem	Cause	Applications Affected	Solution	References
Deformation 	- High compliance (low stiffness) - Low aspect ratio	- Endothelial cell response to shear	- Modify curing parameters - Avoid low aspect ratios - Avoid high pressure flows	- Gervais *et al.* (2006)
Evaporation 	- Permeability to water vapor	- Static no-flow experiments - Osmolarity-sensitive experiments - Cell death from bubble propagation	- Coat with Parylene - Ensure environments humidified - Incorporate media baths or sacrificial liquid reservoirs	- Verneuil *et al.* (2004) - Heo *et al.* (2007) - Berthier *et al.* (2008) - Lecault *et al.* (2011)
Absorption 	- High permeability of material	- Soluble factor signaling studies involving small hydrophobic molecules	- Coat with Parylene - Coat with paraffin wax	- Toepke & Beebe (2006) - Regehr *et al.* (2009) - Ren *et al.* (2010)
Leaching 	- Uncrosslinked oligomers	- Protein trafficking across membrane - Signaling through membrane-bound receptor proteins	- Coat with Parylene - Soxhlet extraction	- Regehr *et al.* (2009)
Hydrophobic Recovery 	- Surface diffusion of low molecular weight chains	- Unstable surface treatment or functionalization	- Use surface-treated device as soon as possible after treatment - Use hybrid devices with non-PDMS culture surface	- Eddington *et al.* (2006)

3.1.2. Evaporation. Gas permeability of PDMS has been cited as an important advantage for cell culture applications because of the need for sufficient O_2 and CO_2 diffusion from cells through the PDMS, particularly for long-term cultures (*i.e.*, days to weeks). Oxygen permeability through PDMS, based on reported diffusion coefficients, is ~2,000 to 4,000 $\mu m^2\ s^{-1}$,[70,71] while that through PS is ~2 $\mu m^2\ s^{-1}$,[72] three orders of magnitude lower than PDMS. Thus, microchannels made from PDMS may be better suited for cell culture. A brief analysis of oxygen diffusion rates, however, shows that PDMS-based microfluidic systems may in fact produce a hyperoxic microenvironment that can cause cell stress.[73,74] This is because the diffusion rate of oxygen in bulk PDMS and in media are similar (*i.e.*, ~3000 $\mu m^2\ s^{-1}$), and thus the oxygen supply through a thin microfluidic device, (*e.g.*, ~100 μm media and ~200 μm PDMS for a total height of ~300 μm in materials) can be much higher than that in cell culture flasks or Petri dishes that typically contain a height of media ranging between ~1–4 mm.

Permeability can also become a detriment in various microfluidic applications because of the issue of evaporation. Whereas PS and COP have low water vapor permeability (~43 $\mu m^2\ s^{-1}$ and 0.86 $\mu m^2\ s^{-1}$, respectively),[72] PDMS is extremely permeable to water vapor (~1,000–6,000 $\mu m^2\ s^{-1}$).[75,76] Evaporation is inherently present in regular macroscale cell cultures as well, but the phenomenon becomes more dominant at the microscale where small amounts of evaporation can significantly shift volumes, concentrations, chemical balances, critical gradients and other factors.[77–79] In certain cases, evaporation can be exploited at the microscale to reveal new techniques or methods, such as evaporation-induced sample concentration,[80] or evaporation-driven continuous flow.[81] However, evaporation more commonly leads to important adverse effects in microfluidic cell-based systems, such as osmolarity shifts that affect cell differentiation.[75] A particularly challenging mode of evaporation occurs through the bulk PDMS material because of its relatively high water vapor permeability, even though the entire fluidic volume may be contained. While evaporation rates are slow in closed systems, they can cause significant loss of liquid volume, which can lead to bubbles that propagate, block flow, and lyse cells in microchannels, causing dramatic, detrimental effects that can lead to loss of data or result in experiments ending prematurely. In "open" systems where liquid surfaces are exposed to air, such as open-channel devices,[82] EWOD-based digital microfluidic platforms,[11,83] and passive pumping-based systems,[50,84] evaporation may still occur through bulk PDMS layers, but occurs at a higher rate at exposed vapor-liquid interfaces, such as surfaces of open wells and droplets. Since this higher rate of evaporation is due to open microfluidic designs, it can be avoided by simply designing closed microfluidic systems when open design is not necessary for device function.

Methods have been developed to alleviate the adverse effects of PDMS permeability and evaporation. Parylene coating of PDMS surfaces was demonstrated as an effective method of preventing or significantly mitigating evaporation,[75] but it requires additional fabrication procedures and equipment, and may hinder subsequent bonding or surface treatment steps. Recently, a report showed that a large isoosmotic media bath helped to maintain desired osmolarity in cell culture chambers that were adjacent to but physically separated from the bath by a PDMS membrane.[85] Both these methods, however, are circumventions that are needed because of the use of PDMS.

3.1.3. Absorption. PDMS is a permeable material prone to bulk absorption of hydrophobic compounds,[86] and is notably

different from materials like thermoplastics and glass that are only subject to surface adsorption. Small molecule absorption by PDMS is an important issue for cell culture applications because of the integral role of soluble factor signaling on cell behavior and function. Regehr *et al.* provided evidence of the biological impact of small molecule absorption by showing that PDMS significantly depleted estrogen levels in the culture media, leading to inhibition of activator protein-1 activation *via* estrogen signaling.[16] Additionally, differences were found in the responses of transfected human embryonic kidney (HEK) cells to fluoxetine (Prozac®) when cultured in PDMS rather than PS microchannels, where the effectiveness of the drug in PDMS microchannels was significantly abrogated, presumably due to absorption of drug.[17] Thus, while microscale platforms offer improved sensitivity compared to macroscale systems,[4,84] gains in sensitivity may, in some cases, be tempered by comparable depletion of soluble factors because of absorption by PDMS. This can affect not only fundamental biology studies, but also drug discovery and high-throughput screening applications that rely on platform materials that are inert to absorption. While it may be possible to leverage absorption to reduce undesirable soluble factors (*e.g.*, growth inhibitors), this may prove to be challenging to control precisely. Coating with parylene[87] or with paraffin wax[88] have recently been demonstrated as methods of circumventing PDMS absorption. Regardless, in cases where cell signaling is the focus of the research, PDMS may inherently bias results, and alternative materials may prove to be better options. The complexity of cell-based studies, however, makes it challenging to predict the overall impact of small hydrophobic molecule absorption. From our experience, this can sometimes be a contentious issue among biologists who are eager to collaborate with a microfluidics lab, but are hesitant about the known absorption issues of PDMS-based devices.

3.1.4. Leaching. PDMS in cured form contains residual uncrosslinked polymer chains that can freely diffuse within the bulk material. When in contact with solution, these uncrosslinked oligomers can leach out of the bulk and into solution. Regehr *et al.* demonstrated the leaching of uncrosslinked oligomers and showed that in cell culture studies, these oligomers can incorporate into the membranes of culture cells.[16] Soxhlet extraction with ethanol (or other organic solvents) over multiple cycles can effectively reduce the amount of uncrosslinked oligomers in the bulk material,[89] resulting in less deposition on cell membranes. The issue of leaching has not received much attention to date. Its importance, however, is expected to become more evident as microfluidic systems become more popular for studies of cell membranes (*e.g.*, signaling through membrane-bound receptors, protein trafficking through cell membranes). Similar to the issue of absorption, leaching is difficult to monitor and control. Thus, the actual impact of leaching in cell biology experiments is unclear, but potentially important.

3.1.5. Hydrophobic recovery. A common laboratory method involves using oxygen plasma treatment to convert natively hydrophobic surfaces like PDMS to hydrophilic surfaces in order to facilitate bonding between dissimilar materials, permit surface functionalization, and facilitate microchannel filling as well as cell culture. PDMS, based on its silicon backbone, offers similar surface chemistry to glass, and thus is an attractive material for surface modification. However, PDMS polymer chains also have the ability to diffuse from the bulk to the surface, thereby replacing hydroxyl groups on the surface that had been produced *via* oxygen plasma. This issue of hydrophobic recovery[90] raises concerns related to practicality and accessibility. First, PDMS devices that are rendered hydrophilic for operation have a limited shelf life after plasma treatment because of the gradual reversal of hydrophilicity. In academic laboratory settings, shelf life is an issue that has mostly been overlooked, and in our experience can add unnecessary strain and workload to collaborations with biologists. For example, because of the concerns related to hydrophobic recovery, PDMS-based devices with surface functionalizations have to always be prepared anew – often within the hour of an experiment - and this limits the engineers' ability to mass-produce devices beforehand. Furthermore, this issue limits the ability to form collaborations with others in more distant locations because of the inability to prepare devices and ship without surface deterioration. For commercialization of PDMS-based devices, the issue of shelf life is a major limitation, and is a strong reason why thermoplastic materials still attract substantial interest. Second, experimentation is significantly inconvenienced because of the need to overcome hydrophobic resistance in the filling of aqueous solutions, and the fact that cells cultured on the surface may experience dynamic changes in hydrophilicity that ultimately affect cell adhesion strength and subsequent integrin-mediated signaling and mechanotransduction. This issue can be avoided by using hybrid microscale systems composed of a thermoplastic or glass bottom substrate and PDMS-based microchannels, provided that the PDMS does not negatively affect the experiments due to one of the other limitations discussed previously.

3.2. PS in microfabrication

While PDMS and soft lithography have enabled the rapid expansion of microscale technologies and helped accelerate the advancement of microfluidic platforms, the inherent limitations of PDMS described above have raised concerns over its suitability in certain cell-based applications. Because most of these limitations are specific to PDMS and generally do not apply to thermoplastics (except for leaching of plasticizers, which has also been reported in PS),[91] there has been renewed interest within the research community to develop methods and platforms in plastic materials. In this context, PS is particularly attractive not only because it is unaffected by PDMS-specific drawbacks, but also because of its importance in biology and reputation as a ubiquitous material for tissue culture plasticware. Furthermore, plastics continue to garner special attention in applications that require microscale devices that are manufacturable in large quantities and disposable.[92] Therefore, PS has potential to help bridge the current gap between the state-of-the-art in microfluidics technology and the needs of biologists.

Although microfabrication in thermoplastics has been well studied,[93–106] thermoplastics continue to be less popular than PDMS as device materials for microscale cell-based systems. This trend can be attributed to challenges associated with achieving reliable and repeatable fabrication processes, and to the higher costs associated with thermoplastic microfabrication

compared to soft lithography, especially for low production volume applications that are typically found in academic laboratories. PS microfabrication suffers from multiple bottlenecks along the typical process workflow, including the need to (1) make molds capable of resisting high temperatures and pressures that are commonly found in hot embossing processes, (2) create inlet and outlet access ports for world-to-chip interfacing, and (3) overcome challenges associated with bonding thermoplastic materials. Recently renewed interest in thermoplastic materials for microfluidics has triggered the development of improved fabrication methods to alleviate these bottlenecks, as well as other novel methods that may contribute to increasing their popularity. In this section, we review these recent developments, and provide evidence that the microfluidics community has recognized a growing need to develop platforms more immediately suitable to the needs of the biologist.

3.2.1. Mold fabrication. The first major step in fabricating microfluidic devices in thermoplastics such as PS involves creating the desired patterns and features in the plastic. Different approaches have been traditionally used for this task, but the two most popular methods are injection molding[107–109] and hot embossing (Fig. 3).[61,110,111] While injection molding is reliable, reproducible, and low cost in high volume production cases, hot embossing is more accessible and flexible for low to medium production, and is less expensive to set up, making it the more practical choice for academic labs. Typically, molds for hot embossing and injection molding are fabricated by CNC or precision laser machining, which can be time consuming and laborious, but can also be affordably outsourced when a final design has been chosen. However, molds made with these processes have significant surface roughness because of the fabrication process, which can affect subsequent bonding, or more importantly interfere with optical microscopy and imaging of cells. Molds can be polished to alleviate this issue, but this adds cost and labor, and severely limits our ability to perform rapid prototyping of designs during development.

Recent work has begun to demonstrate more practical, low-cost methods for making molds, and have done so by relying on soft lithography methods as a framework, and adapting the process to meet the needs of thermoplastic fabrication. To produce low-cost micromolds, high strength epoxies have been used in place of metal molds to eliminate the need for micromachining and polishing. Positive relief epoxy micromolds for hot embossing can be easily created by casting the epoxy into negative-relief PDMS mold that were in turn originally cast from SU8-silicon molds.[61] Use of composite aluminum filled epoxies has since improved mold durability at high temperatures and pressures.[110] PS has also been directly embossed from a positive-relief PDMS mold (Fig. 3A),[111] which takes advantage of the compliance of PDMS to facilitate demolding. The use of compliant PDMS molds was also demonstrated in a method where PS was dissolved in solvent, poured into a PDMS mold, heated on a hot plate to evaporate the solvent, and then finally removed from the PDMS mold, producing high aspect ratio features (Fig. 3C).[112] Additionally, polyurethane plastic molds have also been cast directly from existing PDMS devices to re-create rigid masters.[113] These methods only add one extra step to the mold fabrication process, and do not require the use of additional equipment beyond a heated press or hot plate.

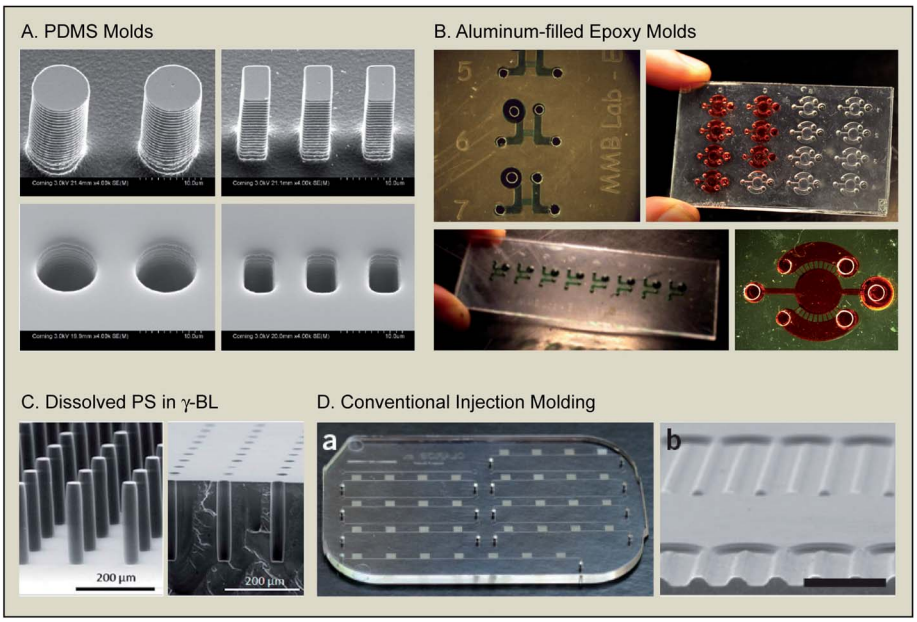


Fig. 3 New wave of PS-based microfabrication. (A) The use of PDMS molds to directly emboss into PS. Reproduced in part from ref. 111 with permission from the Journal of Micromechanics and Microengineering (IOP Publishing, Ltd.). (B) The use of aluminum-filled epoxy molds and through-hole embossing to produce arrayed microfluidic cell-based systems in PS. Reprinted with permission from ref. 110. Copyright (2011) American Chemical Society. (C) Fabrication of high-aspect ratio PS features by dissolving solvent into PS before casting into compliant PDMS molds. Reproduced in part from ref. 112 with permission of The Royal Society of Chemistry. (D) PS device fabricated by conventional injection molding. The device shown has been used for clinical diagnostics in the third world, demonstrating the progress made in the application of plastic microfluidic devices. Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, ref. 109, copyright (2011).

Epoxy molds have several important advantages including significantly lower cost, faster turnaround times, and smoother surfaces compared to micromachined metal molds, and can be easily integrated with existing soft lithography methods and workflow, facilitating rapid prototyping in PS. In terms of disadvantages, epoxy molds have lower mechanical strength and durability compared to tool steel, and as a result, fewer devices per mold are produced before mechanical failure of the mold. This is more acceptable in an academic setting than in an industrial setting because designs are frequently modified during development, and additional epoxy molds can be quickly created from the original negative-relief PDMS mold. Epoxy molds are, however, limited in the aspect ratio and density of features that can be achieved because of the demolding process. Therefore, rigid molds typically require draft angles on the vertical faces of the mold features that are challenging to fabricate when relying only on soft lithography methods. Nevertheless, process workflow for thermoplastic fabrication has become sufficiently streamlined that rapid prototyping is no longer prohibitive, making PS fabrication more accessible to academic labs, especially in the design and assay development phases (Fig. 1).

3.2.2. Access ports and interfacing. One particular challenge with using hard thermoplastics instead of more compliant PDMS is the interfacing of the microfluidic system with external fluid dispensing equipment such as syringe pumps and pipettes.[114] Traditional hot embossing techniques require subsequent manual drilling of access ports using a drill press.[115,116] Laser ablation has recently been used to rapidly create microwells with some automation, but while such microwells can be adapted as access ports, significant meltback of the material due to laser power substantially limits precision.[117] From our experience, this meltback can ultimately affect bonding, uniformity of cell seeding, and other downstream device operations. The issue of world-to-chip interfacing and rapid creation of access ports is magnified for high-throughput applications in hard thermoplastic materials because individually addressable channels with an inlet and outlet port must be fabricated instead of using multiplexing and integrated pneumatic valves.[10] For tubeless microfluidic systems, such as those using passive pumping,[50] large arrays of individually addressable microfluidic channels necessitate creation of tens to hundreds of access ports, which are readily achieved with PDMS as ports can be directly fabricated at the embossing stage,[118] but quickly become impractical in plastics if manual drilling is required. In these cases, injection molding becomes attractive and practical for commercial product manufacturing, but setup costs remain a barrier for modest microfluidic labs. Double sided hot embossing to create through-holes in a PS part directly has also been demonstrated, but requires expensive and technically challenging molds.[119] Recently, a method that can create through-holes in PS with a single hot embossing step has been adapted from previous methods demonstrated for plastics with low glass transition temperatures (T_g).[119] The method relies on differences in T_g of two different but adjacent thermoplastics to allow the mold to penetrate one layer (lower T_g) while only indenting the second layer (higher T_g), thereby creating through-holes in one step and removing a bottleneck in the thermoplastic workflow (Fig. 3B).[110] However, this method is limited to moderate aspect ratios and feature heights mainly because of challenges with demolding from a rigid mold. From our experience, the thickness of the through-hole embossed part is limited to ~1 mm, and this has implications on overall mechanical rigidity of the part. Thus, in cases where thicker parts are necessary for rigid devices or where special features have high aspect ratios, other approaches such as micromachining, injection molding, and access port drilling may still be preferable.

3.2.3. Bonding. An additional challenge in thermoplastic fabrication is the reliable and repeatable bonding of multiple parts fabricated from hot embossing or injection molding. An excellent review on this specific topic is available in the literature.[106] While bonding of thermoplastics like PMMA and PC have been well documented in the microfabrication literature, PS specifically has received much less attention, partly because PS can deform considerably below its T_g, thus making it more difficult to maintain feature fidelity during temperature-assisted bonding. The manufacturability of PMMA and PC is likely why they have thus far been used more than PS for many non-cell-based microfluidic applications. Of the methods that have been reported for bonding PS, solvent bonding and thermal diffusion bonding are the two most commonly used methods for bonding PS and other thermoplastic microdevices. Solvent bonding relies on the application of organic solvents such as methanol, acetonitrile, and dimethyl sulfoxide (DMSO) that react chemically to solubilize the surfaces of parts to be bonded.[97,106,120,121] Although strong bonds are produced, solvent bonding can cause significant deformation in the microfluidic channel geometries, particularly in the height of the channels, which can have significant impact on surface-to-volume ratios and thus the concentration of soluble and secreted factors. Additionally, this method may leave traces of organic solvents on microchannel surfaces, which may potentially affect culture cells and bias results, even with concentrations in the picomolar range.[122] Because of the difficulty in predicting or measuring the impact of these trace quantities, this issue has created uncertainty regarding the appropriateness of using this method for cell-based applications.

A second popular method is thermal diffusion bonding, which relies on heat and pressure to promote reorganization, diffusion, and physical entanglement of polymeric chains at the interface of bonded surfaces.[106,110] Although it is simple to implement and circumvents solvent-related issues, thermal bonding becomes increasingly difficult with larger device dimensions because of the challenge of maintaining uniform interfacial contact over large areas using high enough pressure to permit thermal diffusion, but low enough pressure so that microfeatures are not deformed. Low temperature bonding has recently been demonstrated, though it requires activation of the surfaces with ultraviolet or ozone treatment.[101]

Other methods, such as laser welding and ultrasonic welding, have also been developed, but are not as frequently found in an academic setting due to the need for expensive specialized equipment (for more details, the reader is referred to the aforementioned review).[106] Notably, some interesting methods have been attempted to alleviate some of the problems mentioned. These include the use of permeant-assisted diffusion,[123] or the use of two similar thermoplastics with slightly different T_g, in which a thin layer (<10 μm) of the low T_g plastic is applied on a layer with a higher T_g, creating an "adhesive" bonding layer.[124]

In summary, there are many methods for achieving bonding and sealing of plastic microdevices. The overarching issue is that the bonding process for thermoplastics is significantly more challenging than for PDMS, which can easily be bonded (reversibly or irreversibly) to many other surfaces. While progress has been made, bonding is still regarded as one of the main bottlenecks in the thermoplastic fabrication workflow. Therefore, more pragmatic, reliable, and easy to implement solutions, which are also compatible with cell culture, are necessary to develop a workflow for thermoplastic microfabrication that can be competitive with that of PDMS.

3.2.4. Surface chemistry and functionalization. Microscale cell-based assays often require surface modifications to enhance or improve fabrication and functionality. This ability is particularly important for cell cultures that require specific surface chemistries to promote cell adhesion and proliferation, and mediate cell behavior.[125,126] Furthermore, the development of user-friendly microfluidic systems also requires simple, reliable filling methods to simplify device preparation. PDMS allows a wide range of surface modification methods that are mostly inherited from techniques developed for glass, and as such is an attractive material for microfabrication engineers. In contrast, methods for surface modification of PS, besides oxygen plasma treatment,[127,128] are less well known. Several strategies for tethering functional molecules to organic polymers have been developed based on comb polymer chemistry.[129]

Surface hydrophilization also facilitates preparation of microscale cell-based systems, allowing capillary flow-mediated filling while minimizing bubble generation. This aspect is important because it ultimately reflects on the ease-of-use of the device, particularly by users without engineering or microfluidic training. Despite limited options for surface functionalization, PS remains advantageous compared to PDMS because hydrophobic recovery is minimal in comparison,[130] thereby enabling the fabrication of devices well in advance of experiments. The ability to prepare devices in advance, ship, and store them is an attractive characteristic that is likely to further enhance collaboration that typically involves coordinating multiple researchers from different laboratories.

4. Discussion and outlook

It has become increasingly clear based on trends in scientific research that microscale cell-based systems have had – and will likely continue to have – a significant role in cell biology research. However, there also exists a significant gap in cultures between engineering and biology research that has somewhat hindered the widespread integration and adoption of microscale cell-based systems by the biology community. One particularly important example of the existence of this gap, as argued in the current review, is in device material selection where PDMS and PS have independently become the popular and standard choices in microengineering and cell biology, respectively. To enhance integration of microfluidic systems in biology, further facilitate collaborations between bioengineers and cell biology researchers, and ultimately enable accelerated advancement of *in vitro* biology, it is imperative that we examine the critical issues related to how we choose between PDMS and PS to fabricate our systems. Here, we reviewed both the historical trajectories of microfluidics and cell culture, and the practical and technical issues associated with material selection, and argued that the choice of material can have significant impact on the long-term adoption of microfluidic systems by the biological research community.

First, a historical perspective revealed differences in how engineers and biologists typically approach the adoption of new technologies. On one hand, cell biology has been built mostly from incremental advances that stem from hypothesis-driven experimentation, and have only adopted technologies gradually when it was either necessary (*e.g.*, the transition from glassware to plasticware in cell culture), or exceptionally enabling (*e.g.*, the development of Transwell inserts, or the development of the Zigmond chamber). In contrast, cell-based microscale engineering is a relatively nascent field marked inherently by frequent disruptive developments in technological advancement (*e.g.*, emergence of PDMS-based soft lithography, dynamic substrates[131]) caused mainly by the natural and constant need for innovation. Because of this difference in research cultures, the integration of new engineered technologies into the biology community is inherently challenging. In this context, the use of PS for microdevice fabrication can potentially help to ease the transition of microfluidics into biology by presenting the technology within the more familiar setting of PS-based formats. While a brief look at our history has proven useful, we also recognize that history and culture alone do not sufficiently explain the limited adoption of microscale cell-based systems in biology. Indeed, the biology community in the past has shown a willingness to embrace other enabling platforms when they have proven to be impactful. We believe that an open discussion on the differences in research culture, within the context of material selection for microfluidic systems, will lead to a recognition that PS-based formats are critical to facilitate and enhance integration, and will likely help reduce existing barriers to widespread adoption.

Second, and perhaps more importantly, we discussed practical and technical issues related to the use of PDMS and PS, and argued that the choice of material can have significant biological implications for many microscale cell-based applications. While the properties of PDMS, such as its compliance and permeability, have been leveraged to add a number of important functionalities to microscale systems,[132] issues of microchannel bulging, sagging, and evaporation have also been linked to negative effects on cell-based studies.[68,77,78] Mounting evidence suggests more subtle yet fundamental interactions between PDMS and cells in microscale culture due to absorption of small hydrophobic molecules and incorporation of leached PDMS monomers into cellular membranes.[16] While the extent and significance of these interactions have yet to be fully explored, these concerns add uncertainty and experimental bias that contribute to hindering widespread adoption of PDMS-based microdevices. Our current understanding suggests these biases are particularly present in studies involving cell signaling, cell-cell communication, and cytokine and drug induction where concentration and function of proteins, small molecules, and growth factors can be affected. While PS and other plasticware may also suffer from some material biases based on recent reports that are bringing this issue to light,[91] these biases appear

to be much less dramatic in their impact on experiments compared to PDMS-derived issues. We acknowledge that many biological applications, such as PCR, cell sorting, cell capture, and short-term (*i.e.*, <1 h) cell culture studies are not as significantly affected by PDMS-derived issues as cell signaling studies, and as such can be performed equally well in PDMS-based systems. However, we contend that in order to pursue increasingly complex cell biology questions from concept through development to implementation and analysis (Fig. 1), we must become increasingly aware of all such material biases as they pertain to our application of interest. This awareness will likely dictate how we design and validate systems, analyze data, interpret results, and make conclusions, and ultimately determine how successful we will be at integrating technology with basic science.

While the underlying focus of this review has been on device fabrication within an academic setting, an additional confounding factor yet to be discussed is that of manufacturability and its importance on commercialization and integration on a broader scale. Academic laboratories typically serve as the initial sources of newly developed technological platforms, benefiting from the freedom of a flexible research environment to explore innovative ideas and iterate through designs *via* rapid prototyping. In such a setting, PDMS-based microfabrication remains a useful and powerful methodology because of its accessibility, while in contrast PS-based fabrication is still more challenging despite recent reports aimed at achieving rapid prototyping of PS-based devices (Fig. 3).[109–112] From the perspective of commercialization, however, PDMS becomes a major limiting factor because manufacturing costs and production volumes do not scale up favorably with soft lithography, and shipping, packaging, and storage of surface-treated PDMS microdevices remains challenging given that hydrophobic recovery tends to revert surface treatments to their original state. Thus, when manufacturability is factored into design, PS and other plastics have a distinctive edge over PDMS because manufacturing costs for plastics decrease significantly for high production volumes, and plastics do not degrade during long-term storage.

Based on all these perspectives, it is evident that the issue of material selection is far from trivial. Material selection has implications on experimental research, and ultimately on the ease of adoption by collaborating scientists. While our focus has been on comparing PDMS to PS, it should be recognized that the issue of material selection in general is far broader given that a growing number of reports are demonstrating advantages of various other materials, including cyclo-olefin copolymer (COC),[17,61,101,106,133] and Teflon.[134] Additionally, interesting developments have also been made with the use of paper in microfluidics.[135–140] Essentially, choosing which material is appropriate for your cell-based application depends on several key aspects: (1) manufacturability or ease of fabrication, (2) effects on the biology of cultured cells, and (3) suitability of the material for the desired analyses and readouts (Fig. 4). Even with a growing list of alternatives, PDMS remains a leading option because of its convenience, reliability, and its ability to enable unmatched functionalities including flexible valves, deflectable microposts, and cell-stretching membranes. However, for more advanced biological investigations such as long-term cultures involving cell signaling, the PDMS biases must be either circumvented or completely avoided by choosing another material. In these more biologically oriented applications, PS stands clearly above other alternatives due to the wealth of knowledge accumulated on PS over the years and the fact that PS does not suffer from the same key limitations as PDMS. Moreover, as mentioned its manufacturability is superior to that of PDMS, even though its ease of fabrication in low production volume, rapid prototyping settings is less attractive. This is where microfabrication engineers have refocused their research, and continued progress in this area will further close the gap between PDMS and PS on the convenience of fabrication. In

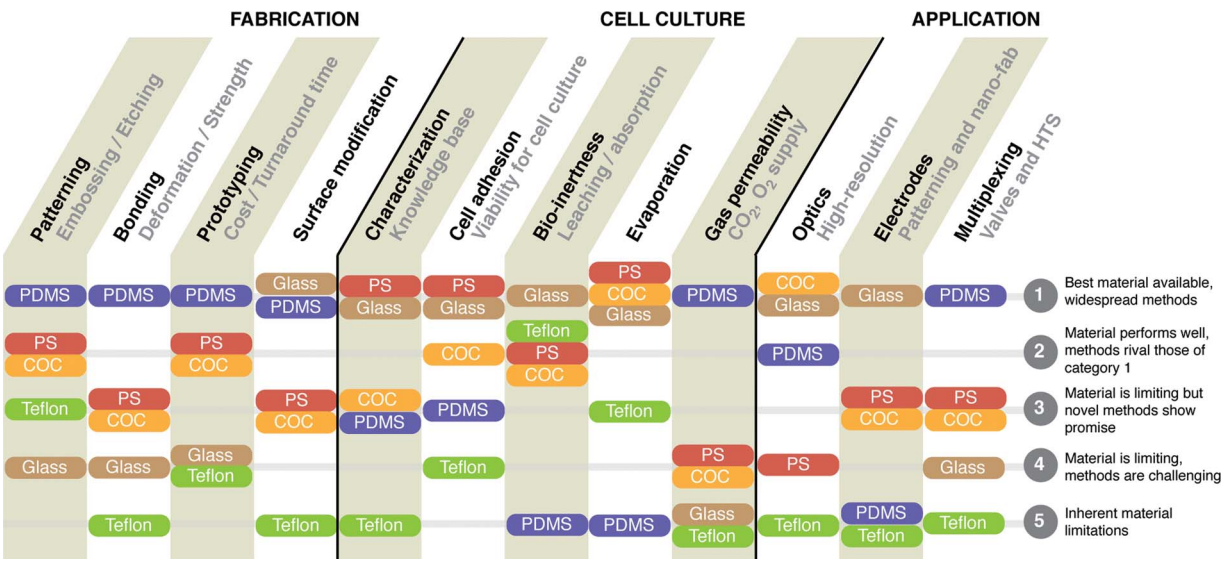


Fig. 4 Comparative strengths and weaknesses of materials used for microfluidic cell-based device fabrication. The materials are ranked for their abilities for various properties important in cell-based experiments, which are grouped under three general categories: The ability to fabricate the microsystems, the ability to perform controllable cell-based experiments, and the potential for integrated micro-engineering applications.

the case of other specific applications, such as high-resolution microscopy, alternative materials such as glass and COC are highly recommended. For this reason, COC has also been gaining popularity because most fabrication methods developed for PS are easily translatable to COC. Glass, having the advantage of being the first material used in both microfluidics and *in vitro* biology, offers well-grounded knowledge, chemical inertness, high quality optics, and the ability to integrate patterned electronics. However, the significantly higher price for microsystems made out of glass, as well as the necessity to re-utilize them, make it a very specialized material. Finally, the most recent addition to our list of material alternatives is Teflon,[134] which comprises a unique set of properties that give it potential in niche applications, but has not yet been used widely enough to be competitive.

Ultimately, adoption of microscale technologies by biologists hinges on two main aspects: (1) successful collaborations between engineers and biologists in academic settings where the technology is first conceived, and (2) the establishment of standard platforms that, because of manufacturability, are widely accessible and available to the biology community at large. The responsibility to develop an acceptable platform relies partially on the engineer to adapt and integrate materials and technologies in a manner that suits the needs of the biologist. This task can and will be facilitated by an expanded repertoire of fabrication methods for different materials, particularly those that can compete with PDMS. Specifically, for cell-based applications, increasingly accessible PS fabrication methods are beginning to emerge. More work in this research area is still required to ease the transition for biologists toward microscale cell-based systems. It is our hope that the increased exposure of the issue of material selection in cell-based studies can further lead to new and improved fabrication methods suited for enabling applications.

In conclusion, material selection, especially between PDMS and PS, needs to be considered more frequently and more seriously by engineers and biologists alike. The choice of material is particularly important when new technology is being introduced into a well-grounded and complex discipline such as cell biology that involves many unknown interactions and biases. While PDMS is currently the overwhelming choice for microfluidic engineers, developments in PS fabrication methods have begun to improve its accessibility to the point where PS warrants consideration as the new material of choice for certain cell-based applications. The ability for microfluidics engineers to offer platforms in both PS and PDMS may play an important role in achieving further integration between microfluidics and cell biology, and further promote fruitful collaborations between engineers and biologists. Indeed, material considerations constitute only one aspect contributing to limited adoption of microsystems for cell-based research. Other aspects, such as the challenge of integrating with existing infrastructure,[50] and the lack of a killer application,[141] also deserve some attention from the microfluidics community, especially those involved in cell biology applications. An increased focus on thermoplastic fabrication will hopefully bring new solutions for making PS fabrication more accessible and reliable, expand our choices for microfabrication, and, ultimately stimulate collaborations between biologists and engineers.

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