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REGULAR PAPER

A Review of Fabrication and Applications of Confined Microchannels for Cell Migration Assay

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Abstract

Cell migration is an essential process in a number of physiological and pathological events, and known to be modulated by external microenvironment because cells may sense physical and chemical signals from the microenvironment and collectively respond to these signals. Over the past two decades, a lot of efforts have been made to study how external microenvironment can affect cell migration behaviors. Cells often migrate through confined environments in vivo, such as extracellular matrices in tissues and capillary vessels. Understanding how cells move in these constrained spaces is crucial to clarify various biological processes. For instance, during embryonic development, cells migrate through specific pathways to form tissues and organs. In wound healing, cells migrate to repair damaged tissues. In cancer, tumour cells migrate to invade surrounding tissues and metastasize to distant sites. Recent advances of bio-MEMS technologies have enabled to characterize cell mechanics and to control local cellular environment at micro-scale. In order to study cell migration under confinement, microchannels have been widely fabricated and used due to their directionality and compatibility. Thus, this study reviews recent work on fabrication of microchannels and their applications to investigate cell migration behaviors, ranging from straight channels to tortuous structures. Challenges and limitations associated with studying cell migration in microchannels are also discussed. Reviewing cell migration in confined environments may provide valuable insights into the underlying mechanisms of cell migration and aid in developing strategies for therapeutic interventions.

Keywords Cell migration · Device fabrication · Microchannels · PDMS devices · Confined environments

1 Introduction

Cell migration plays a pivotal role in in both physiological and pathological contexts. For instance, in order for cells to migrate to the site of injury for tissue repair and regeneration, cell migration is essential to wound healing [1]. For the immune system to initiate an effective response, cell migration in the immune system enables immune cell trafficking to areas of infection or inflammation [2]. On the other hand, in cancer, cell migration plays a crucial role in metastasis, or the spread of cancer cells to distant organs, which has a substantial effect on the course of the disease and the effectiveness of treatment [3]. Moreover, cell migration coordinates the appropriate arrangement of cells and tissues during development and tissue regeneration, which is necessary for creating complex structures and healing injured tissues [4]. Furthermore, knowing the mechanisms underlying cell migration can help design tailored medication delivery systems and diagnostic tools for a range of illnesses, such as autoimmune disorders, cancer, and cardiovascular diseases [5, 6]. Thus, knowing the complexities of cell migration promises to advance our knowledge of the mechanisms behind disease as well as the creation of novel therapeutic approaches.

There are several important approaches for studying the dynamics of cell migration, which includes the Boyden chamber assay [7], scratch assay [8], and microchannel techniques [9] as show in Fig. 1. The Boyden chamber assay

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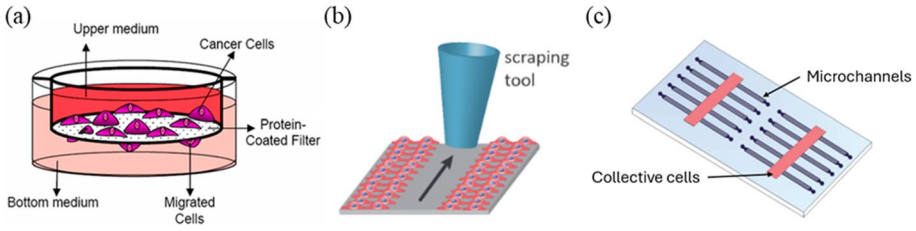


Fig. 1 Approaches for studying cell migration, **a** Boyden chamber assay [7], **b** scratch assay [8], and **c** microchannel techniques (modified from the reference [9]). Images reproduced with permission from references [7–9]

involves seeding cells on a porous membrane inside a chamber and putting a chemoattractant in the lower chamber to cause migration. Migratory reactions to various stimuli are elucidated through the quantification of cells that traverse the membrane [10]. An easy approach to watch collective cell migration and wound closure is by the scratch experiment, which entails making a "scratch" or gap in a confluent cell monolayer and tracking the migration of cells to fill the gap over time [11]. By creating controlled settings, microchannel techniques allow cells to migrate in response to different stimuli. Researchers can replicate the limited spaces that cells in microchannels experience [12].

The microchannel or microfluidic techniques first appeared in the latter half of the twentieth century, they provided accurate manipulation and control over fluid flow at the micrometer scale [13]. To investigate how cells behave in small spaces, scientists started adding microchannels to cell migration tests in the early 2000s [14]. Microchannel arrays and high-throughput screening devices were made possible by later developments in microfabrication techniques [15]. Studying cell migration in microchannels holds significant importance in unravelling fundamental cellular behaviours within controlled environments. Microchannels serve as versatile platforms that mimic physiological conditions, enabling precise observations of cell migration dynamics. This research avenue contributes invaluable insights across various biomedical domains. For instance, Mazalan et al. [16] investigated how geometric curvature influences collective cell migration in tortuous microchannels, providing insights into adaptive migration modes and offering guidance for the design of tissue scaffolds. Additionally, microchannels aid in understanding cancer cell invasion [17, 18], immune cell trafficking [19], wound healing [20, 21], barotaxis, and chemotaxis [22], and other physiological processes. Insights gained from these studies enhance our understanding of disease mechanisms and offer a basis for developing therapeutic interventions. The utilization of microchannels in modelling vascular mimicry further exemplifies their versatility in simulating complex microenvironments. Altogether, the collective body of research underscores the pivotal role of microchannels in advancing our understanding of cell migration processes and their implications for health and disease.

Since 1990's, the applications of microchannels for studying cell migration have increased as depicted in Fig. 2 because they offer a sophisticated platform to investigate and understand the complexities of cellular behaviours. One key advantage is the ability to precisely control the microenvironment for in vitro cell culture models, recapitulate physiological conditions, observe migration dynamics in confined spaces, and investigate the impact of physical constraints on cell behaviours [23]. Microchannels allows for tailoring dimensions, surface properties, and geometries, enabling the manipulation of parameters crucial for studying specific aspects of cell migration [24]. Microchannels also enable high spatial and temporal resolution, facilitating real-time imaging and tracking of individual cells. The confined space provides detailed insights into cell movements, interactions, and responses to stimuli [25]. Another advantage lies in the ability to mimic physiological conditions, such as tissue barriers and capillary vessels, enhancing the relevance of findings to in vivo scenarios [26]. The controlled nature of microchannels also facilitates quantitative analysis, enabling standardized measurements of migration parameters. Moreover, microchannels contribute to cost-effectiveness by requiring smaller quantities of cells, reagents, and materials compared to traditional assays, aligning with ethical considerations in research practices [27]. Collectively, these advantages position microchannels as powerful tools for advancing our understanding of cell migration processes.

This study reviews various microchannel designs and fabrication of microchannels, cell migration mechanisms, and the recent applications in biomedical field. After a review of recent research on cell migration in microchannels, this study also discusses challenges and limitations associated with studying cell migration in microchannels.

2 Microchannel Designs, Materials and Fabrication Methods

In this section, the various of microchannel designs, materials and fabrication methods employed to investigate the complex phenomenon of cell migration is reviewed. The microchannels, recognized as a crucial tool in cellular studies, are utilized as a controlled environment where migratory behaviours of cells can be observed and analysed. Ranging from simple straight channels to complex serpentine configurations, each design imparts unique insights into how

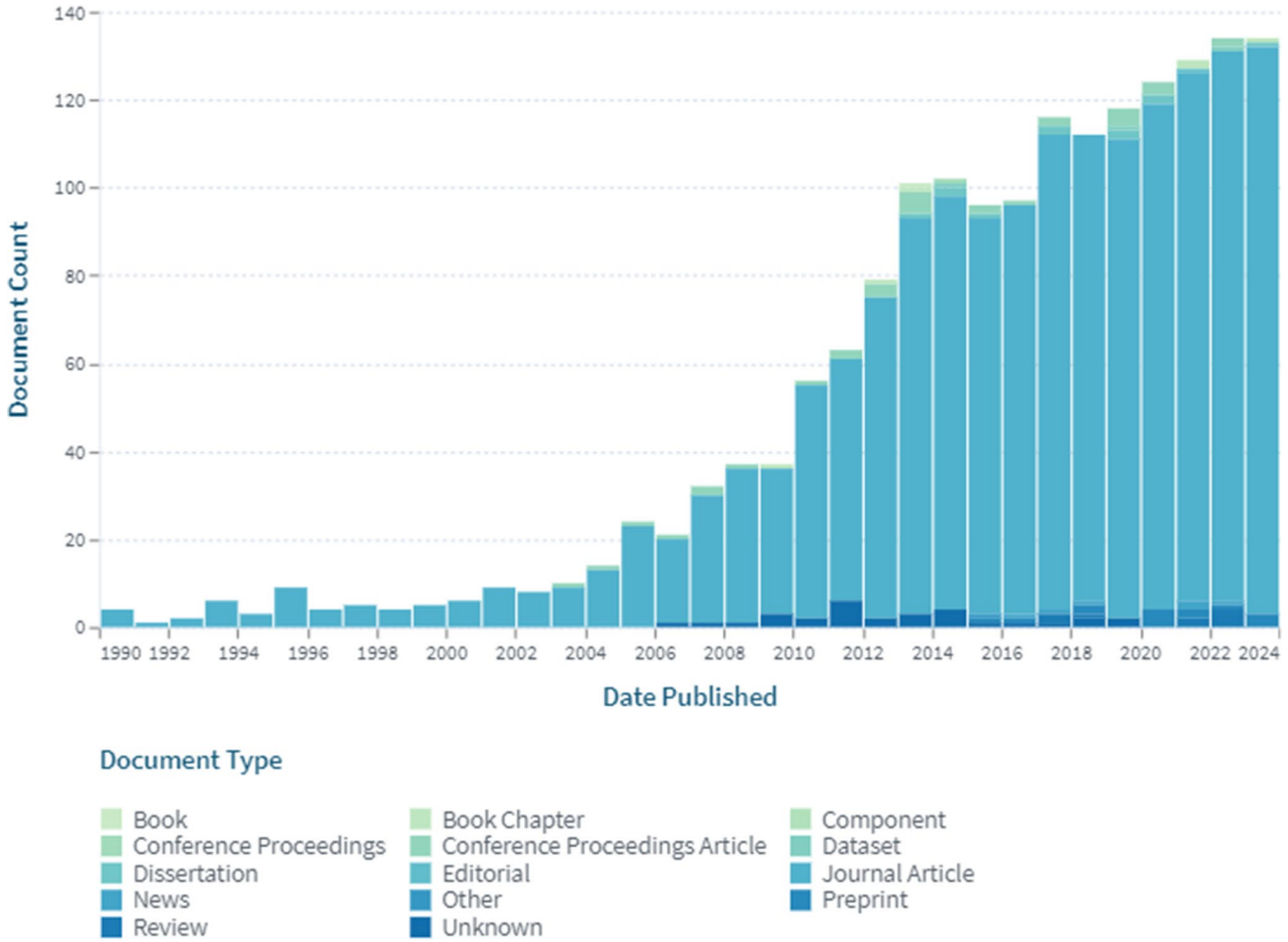


Fig. 2 Scholarly work on cell migration study using microchannel overtime by www.lens.org with keywords of cell migration and microchannels [28]

cells respond to different spatial and geometrical cues. Additionally, various fabrication methods are examined, allowing for the precise creation of these microchannels, ensuring reproducibility and control in experimental setups. This section contributes to a foundational understanding of the instrumental role played by microchannels in advancing our comprehension of cell migration dynamics.

2.1 Microchannel Designs

The designs of microchannels play a pivotal role in shaping the cellular landscape and influencing key aspects of the cell migration process. The microscale confinement in microchannels closely mimics physiological environments encountered by cells in vivo, providing researchers with a powerful platform to study cell behaviour in a controlled and realistic setting. The designs of microchannels including physical dimensions such as widths, heights, and shapes, can profoundly impact cell migration mechanisms. Narrow channels may induce confined migration, leading to altered cytoskeletal dynamics and migration rates, reminiscent of in vivo tissue structures. Moreover, microchannels offer precise control over gradients of signalling cues, allowing researchers to investigate how spatial constraints influence chemotaxis and haptotaxis. Understanding how microchannel dimensions influence cell migration not only enhances our basic knowledge of cellular responses but also holds significant implications for disease modelling, regenerative medicine, and the development of targeted therapeutic strategies. Thus, the importance of microchannel dimensions in cell migration process lies in their ability to recapitulate physiological conditions, enabling a deeper understanding of cellular behaviours and fostering advancements in biomedical research and applications.

So far, there are various microchannel designs offer unique insights into cell migratory behaviours. The straightforward approach of a simple straight microchannel serves as a fundamental design for guiding cells along a linear path. This design allows researchers to observe the basic migration behaviours and cellular responses

to specific cues in a controlled environment. For example, Park and Doh [29] developed a technique to create straight microchannels that are tightly packed with T cells as depicted in Fig. 3a. To aid in T cell sedimentation near microchannel entry, trapezoid-shaped reservoirs were sandwiched between microchannel arrays that varied in width (15–80 µm) and had fixed height (4 µm) and length (1.5 mm). The effects of microchannel width and medium tonicity on T cell motility within cell dense microenvironments were quantitatively assessed by utilizing velocity field information to further examine various motility parameters. They showed that two important factors influencing T cell motility in constrained, cell-dense microenvironments were probably weak contacts between nearby T cells and dynamic polarization with significant shape change.

On the other hand, tortuous-shaped microchannels (Fig. 3b), characterized by winding paths, replicate the intricate conditions encountered by cells within tissues. This more complex and dynamic setting enables the exploration of adaptive migration modes and how cells respond to varying geometrical constraints. Microchannels with strategically placed obstacles present physical barriers for cells to navigate (Fig. 3c and 3d), mimicking challenging tissue conditions. This obstacle-laden design aids researchers in exploring how cells adapt their migration strategies in the presence of impediments. For instance, to simulate different intra-tumor settings of epithelial-like cancer cells (ELCs) and highly invasive cancer cells (HICs) and investigate the invasive potentials of different subpopulations, Shin et al. created an in vitro microchannel device of heterogeneous cancer cell subpopulation growth in

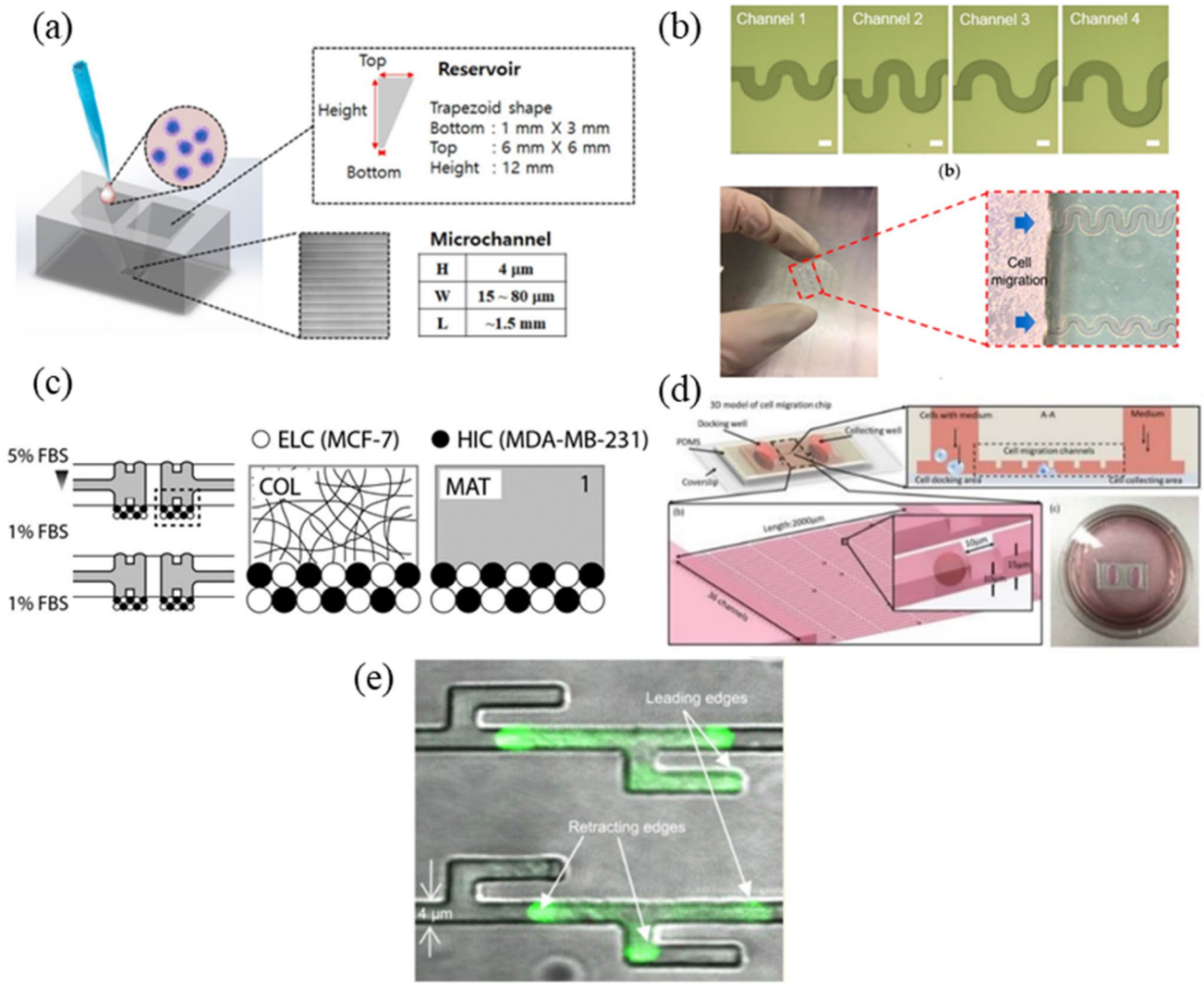


Fig. 3 Various microchannel designs. **a** Straight-shaped microchannel [29], **b** tortuous-shaped microchannel [16], **c** straight microchannel with microtracks [30], **d** microchannel with vertical micro-construction structures [31], **e** branching microchannels [32]. Images reproduced with permission from references [16, 29–32]

stack [30]. An ECM microenvironment that is physiologically relevant for cells was created by building a particular microchannel cell culture apparatus that incorporates the ECM scaffold of Matrigel (MAT) and collagen type 1 (COL) as shown in Fig. 3c. Additionally, microchannels featuring permeable membranes facilitate controlled interactions between cells in adjacent compartments [33]. This permeable membrane design is valuable for studying paracrine signalling, allowing researchers to investigate how cells communicate across a semi-permeable barrier, influencing their migration patterns in a physiological context. Each of these microchannel designs contributes to a comprehensive understanding of cell migration dynamics in diverse environments.

In the realm of microchannel designs for cell migration, the serpentine microchannel stands out with its snake-like, zigzagging configuration [34]. This unique design introduces continuous changes in direction and curvature, creating an environment where cells must navigate through twists and turns. Researchers leverage this configuration to investigate how cells respond to frequent alterations in their migration path, gaining valuable insights into adaptive migratory behaviours. Circular microchannels, in contrast, form closed-loop structures that enable cells to move in a circular fashion [35]. This design is particularly valuable for studying rotational migration patterns and examining how cells interact within a continuous circular environment. By observing cells navigating through circular paths, researchers gain insights into distinct migratory behaviours shaped by the circular configuration. Branching microchannels as shown in Fig. 3c, characterized by patterns that create multiple paths for cells to choose from, reflect the branching structures commonly found in tissues [32]. This design allows researchers to observe cell decision-making processes as cells navigate through various branches. It simulates bifurcations or junctions within physiological environments, providing a platform to explore how cells respond and adapt to branching structures. The branching microchannel design contributes to a deeper understanding of cell migration dynamics in scenarios that mimic the complexities of natural tissue architectures.

2.2 Materials and Fabrication Methods

Fabricating microchannels for cell migration involves careful selection of materials and precise microfabrication techniques to create controlled environments that mimic in vivo conditions. Materials commonly used for microchannel construction include biocompatible polymers such as polydimethylsiloxane (PDMS) [27, 36], polymethyl methacrylate (PMMA) [37, 38], and hydrogel [39, 40]. PDMS, in particular, is widely employed due to its optical transparency, flexibility, gas permeability and cost-effectiveness as a silicone-based polymer, making it suitable for cell studies. It possesses overall inert, non-toxic, and non-flammable properties. Microfabrication techniques like soft lithography are commonly utilized for PDMS-based microchannels, involving the replica molding of a master template onto the polymer. Photolithography and etching processes are employed with materials like glass or silicon to create more rigid and precise microchannels. The choice of material and fabrication technique influences key factors such as surface properties, roughness, and mechanical flexibility, which, in turn, impact cell adhesion, migration dynamics, and overall experimental outcomes. Selecting appropriate materials and microfabrication techniques is crucial in ensuring the creation of functional microchannels that faithfully reproduce physiological conditions for accurate investigations into cell migration processes.

The fabrication process for PDMS microfluidic devices follows a standard protocol involving three main steps, illustrated in Fig. 4 [41]. Firstly, the microfluidic network is devised and formed through traditional microfabrication methods, producing a negative mold. Following this, liquid PDMS is cast onto the mold and solidified within a temperature range of 60 to 80˚C. After solidification, the PDMS membrane is meticulously detached, uncovering open channels on its surface. To finalize the device, the microchannels are sealed by adhering the PDMS membrane onto a flat surface. Although this procedure inherently configures the

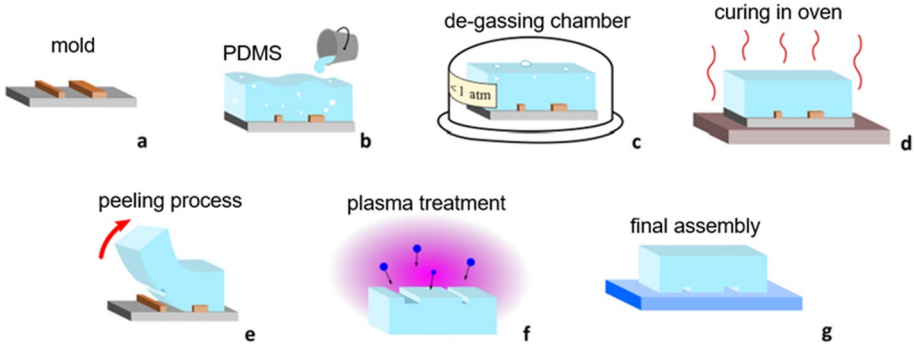


Fig. 4 Fabrication steps of PDMS-based microchannel (modified from the reference [41]). **a** A negative mold. **b** PDMS casting. **c** Deaeration. **d** PDMS cure. **e** Peeling off. **f** Plasma treatment. **g** A sealed PDMS device. Images reproduced with permission from the reference [41]

microchannels in a planar layout, the creation of 3D fluidic networks is achievable through a multilayer configuration or sacrificial layer approach. Notably, after sealing on a transparent substrate, the migration area becomes optically accessible, enabling the use of a standard objective for live imaging of cell migration.

Pathak and Kumar [42] created polyacrylamide (PA) microchannels, denoted as μPACs (depicted in Fig. 5), for investigating cell migration in a system where the stiffness of the extracellular matrix (ECM) and the degree of confinement could be independently controlled. In their fabrication methodology, they employed a combination of photolithography techniques and controlled polymerization of PA hydrogels to produce microchannels with varying widths embedded in PA gels of specific stiffness.

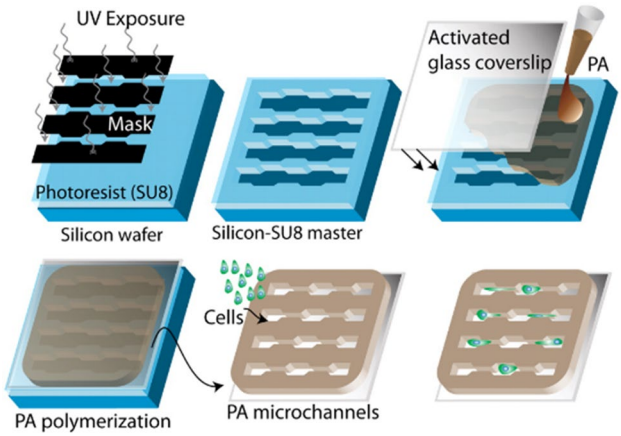


Fig. 5 Fabrication steps including fabrication of a silicon master, formation of a PA hydrogel, separation of the PA hydrogel from the master, and seeding of cells [42]. Images reproduced with permission from the reference [42]

Additionally, femtosecond laser micromachining (FLM) has been employed in the fabrication of microchannel devices tailored for cell assays, allowing for the development of biochips with intricate 3D geometries using materials like glass or polymers, as illustrated in Fig. 6 [43]. This method achieves precision at the micrometer and even nanometer scale, offering a level of robustness that proves challenging to attain with PDMS and hydrogel approaches. The FLM process entails focusing a femtosecond laser beam within a material transparent to the laser wavelength. The high intensities at the focal point induce non-linear absorption phenomena, resulting in a lasting modification of material properties. Specifically in glasses, controlled irradiation conditions lead to a localized increase in the etching rate, facilitating the creation of embedded microfluidic channels through subsequent chemical etching. This capability is leveraged to fabricate 3D microfluidic devices in glasses such as fused silica or Foturan, eliminating the necessity for a clean room or post gluing/bonding procedures.

Recently, Poskus et al. [18] assessed the cytocompatibility of several resins and characterized the minimum feature size for the moulding of PDMS-based microfluidic platform for cellular studies, using a low-cost, commercially available liquid crystal display (LCD)-based 3D printer. The workflow of the fabrication process is shown in Fig. 7. In the fabrication process, a commercially accessible LCD-based Phrozen Sonic Mini 4 K resin printer was utilized to produce 3D-printed moulds.

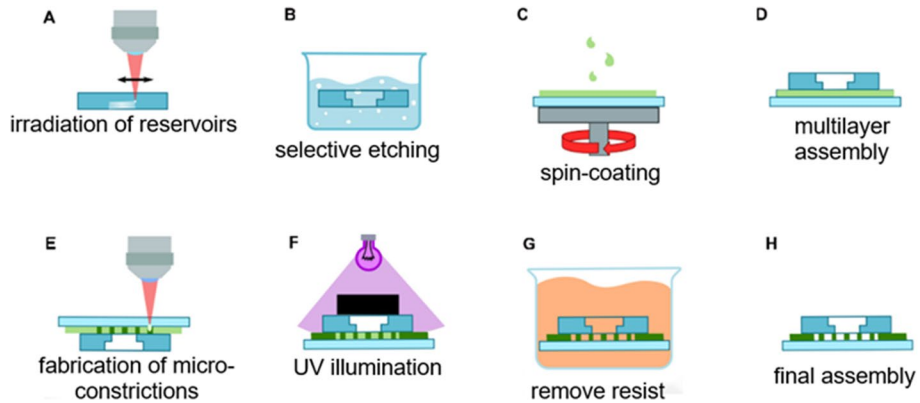


Fig. 6 Procedure for fabricating a multilayer device schematic using femtosecond laser micromachining (FLM) (modified from the reference [43]). **A** Irradiation of reservoirs inside the fused silica layer using fs-laser; **B** Selective etching of the irradiated structures in an HF solution; **C** Application of resist via spin-coating on the glass coverslip; **D** Assembly of the multilayer structure; **E** Fabrication of micro-constrictions using 2PP; **F** Sealing the device with UV illumination and masking; **G** Removal of unpolymerized resist by a solvent bath; **H** Final assembly of the device. Images reproduced with permission from the reference [43]

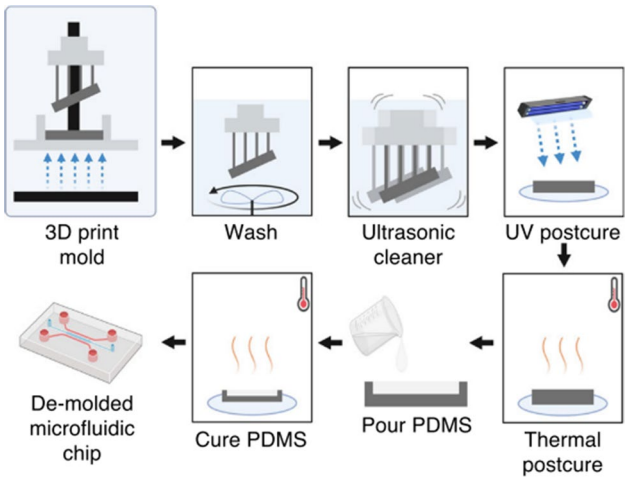


Fig. 7 Fabrication of microchannel using LCD 3D printing [18]

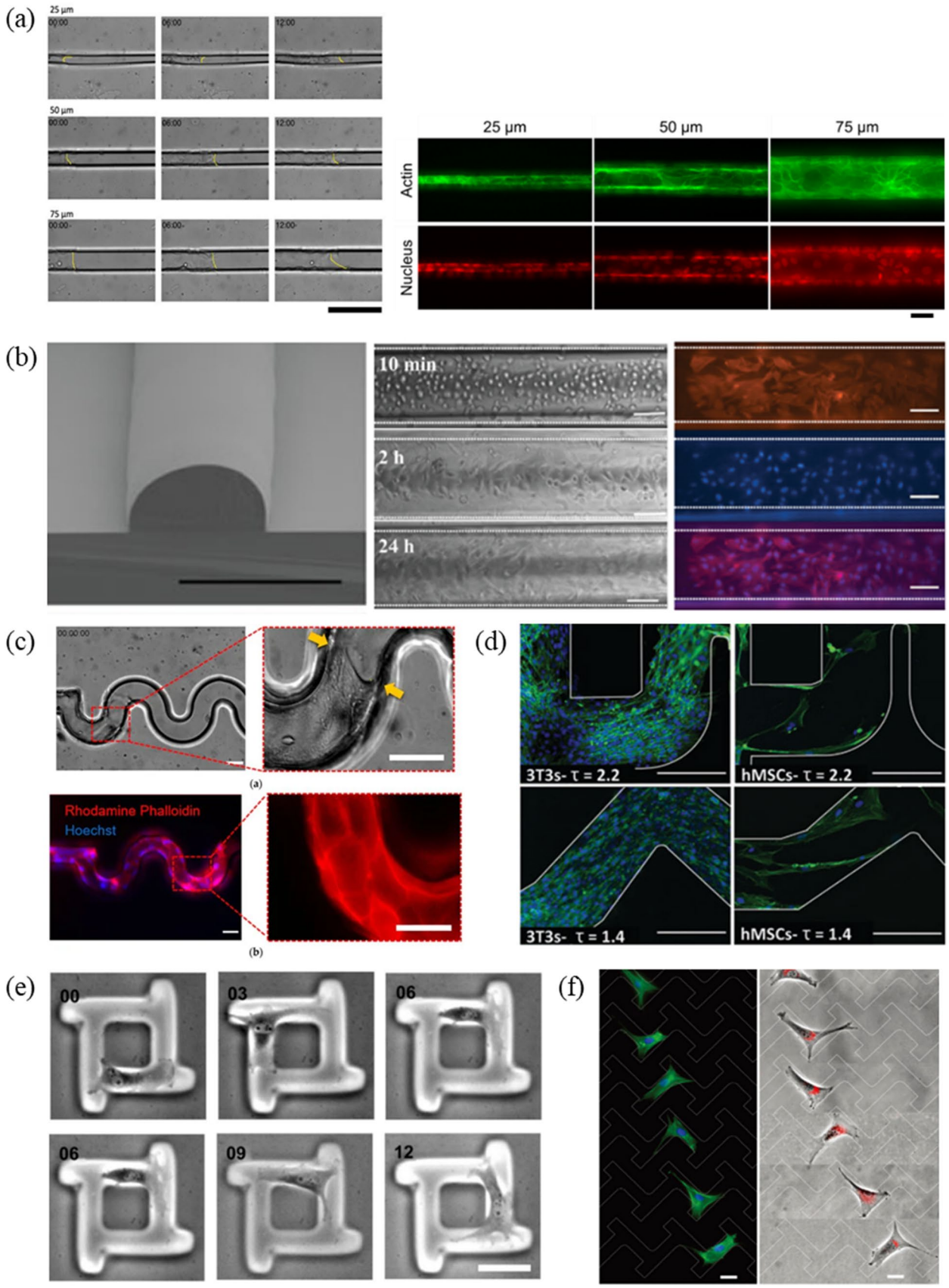
3 Cell Migration Behavior in Straight-Shaped, Curve-Shaped Microchannels and Different Stiffness of Substrate

Physical confinement induces alterations in the cytoskeletal structure, commonly identified by the alignment of cytoskeletal elements along the direction of cell migration. When confined within narrow microchannels, cells often exhibit actin accumulation around the cell cortex, the inhibition of stress fibers, and alignment of phosphorylated myosin light chain (MLC) along the migration axis, irrespective of substrate stiffness.

Ramlan et al. [44] fabricated PDMS straight microchannels (Fig. 8a) with three different widths using PDMS to study behaviour of collective cell migration. In their results, cells migrated with high velocities in wider microchannels, and the cells and nuclei were not elongated in the middle regions of the microchannels. On the other hand, in the regions where cells contact to the walls, cells were orientated along the walls and in the narrow microchannels cells slowly migrated. They showed that collective cell migration changed depending on the position of the cells from the walls due to geometric constraints, i.e., the contact guidance effect. Choi et al. [45] effectively cultivated primary HUVEC within the circular PDMS microchannel (Fig. 8b), achieving a coverage of 74%. Both cell alignment along the channel direction and sustained cell viability over a span of 3 days might be due to the in vivo-like cell microdevices could provide a more reliable and practical platform than the conventional 2D-based cell assays.

Additionally, Mazalan et al. [16] developed a device with tortuous microchannels as shown in Fig. 8c, featuring geometric variations to simulate the pore curvature encountered by cells within 3D-engineered tissue scaffolds. Geometrical constraints were manipulated using microfabrication technology to create 3D structures presenting varying radii of curvature and channel amplitude to the constituent cells. The response to varying radii of curvature and channel amplitude was observed in both leading and trailing cells within the tortuous microchannels. Alterations to collective migratory behaviour, including changes in cell velocity, morphology, and turning angle, were identified. Nevertheless, these findings contradicted the earlier investigation conducted by Mills et al. [46] (Fig. 8d), where they noted no differences in the migration rate of the leading edge of 3T3s and hMSCs within tortuous channels with a curvature index ranging from 1.1 to 2.2. The inconsistency could be attributed primarily to the fact that the width of the tortuous microchannel was 200 µm, which is four times greater than the width of the tortuous microchannel device utilized by Mazalan et al. [16]. While the study by Ko et al. [34] showed that uniform width microchannels with an overhanging zigzag design can induce the polarization of various types of cells (Fig. 8e), even those with altered intracellular signals that promote random movement. In the same paper, Ko et al. [34] reported that the HUVEC extends broader, but shorter lamellipodia compared to fibroblasts, and this difference likely affects the efficacy of the overhangs in rectifying cell migration as shown in Fig. 8f. Valuable insights into the adaptive migration modes of collective cells were provided by the findings, revealing how they respond to mechanical cues presented in the form of varying geometrical constraints. The study emphasized the significance of mechanical signals, specifically geometric variations in the microenvironment, in influencing collective cell migration. This understanding contributes to the overall comprehension of cell migration mechanisms, particularly for the optimization of tissue scaffold designs in tissue engineering applications.

Pathak and Kumor [42] found that, upon conducting morphometric analysis on these cells, the projected cell area rose as the stiffness of the matrix increased. Cells failed to contact the most compliant ECMs and spread widely on the stiffest ECMs. Interestingly, however, they also found that cell polarization showed a biphasic response on ECM stiffness, similar to migration speed, while spreading area rose monotonically with ECM stiffness. On the softest and hardest matrices, cells specifically took on isotropic morphologies, but on the matrices with intermediate stiffness, they exhibited a highly polarized, spindle-like shape.


(a)
25 μm
50 μm
75 μm
Actin
Nucleus
(b)
10 min
2 h
24 h
(c)
Rhodamine Phalloidin
Hoechst
(d)
3T3s- τ = 2.2
hMSCs- τ = 2.2
3T3s- τ = 1.4
hMSCs- τ = 1.4
(e)
00
03
06
06
09
12
(f)

◂**Fig. 8** Cell migration under straight-shaped microchannel. **a** MDCK cells leading edge (yellow line) in the 3 different widths of microchannels for 12 h of migration (scale bar: 200 μm), and fluorescent images of actin (green) and nucleus (red) inside 25 μm, 50 μm and 75 μm microchannels (scale bar: 50 μm) [44] and **b** HUVEC culture in the complete circular microchannel and fluorescence staining images of the actin filament and nuclei (scale bars 100 μm) [45]. Cell migration under curve-shaped microchannel. **c** Fluorescence images show the actin filament and nucleus (stained in red by Rhodamine Phalloidin and in blue by Hoechst, respectively) of cells in a tortuous microchannel device (scale bars: 50 μm) [16] and **d** fluorescent images of 3T3s and hMSCs within t = 1.4 and 2.2 tortuous channels (scale bars: 200 mm) [46]. **e** Cyclic connected rectangular wells with overhangs direct migration of NIH3T3 fibroblast in the preset clockwise direction (time in hours) (scale bar: 50 μm) (modified from the reference [34]) and **f** HUVEC migrated on two-dimensional zigzag microchannel (scale bars: 100 μm) [34]. Images reproduced with permission from references [16, 34, 45, 46]

4 Applications of Microchannels in Biomedical Research and Disease Modelling

The application of microchannels facilitates the investigation of cell migration in a controlled, reproducible, and dynamic manner. By mimicking the complex in vivo microenvironments, microchannels allow researchers to observe and manipulate key factors influencing cell migration, including substrate stiffness, chemical gradients, and geometrical constraints. This level of control is essential for unravelling the underlying mechanisms governing cell migration and its implications in various health-related processes. In this context, microchannels serve as powerful tools for studying diseases where aberrant cell migration is a hallmark, such as cancer metastasis. The ability to create microenvironments that replicate the conditions encountered by migrating cells enables researchers to gain insights into the factors influencing the invasive behaviour of cancer cells. Additionally, microchannels are employed to model immune cell trafficking, providing a platform to understand how immune cells migrate and interact during inflammatory responses. The integration of microchannels in biomedical research not only advances our fundamental understanding of cell migration but also holds promise for applications in drug discovery, tissue engineering, and personalized medicine. By utilizing microchannels, researchers can screen drug candidates based on their impact on cell migration, design biomimetic scaffolds for tissue regeneration, and tailor interventions for diseases associated with aberrant cell movement. Moreover, microchannels have proven instrumental in modeling cancer cell invasion, allowing researchers to replicate the complex microenvironments encountered in tissues. In a specific case study focusing on breast cancer, microchannels were employed to investigate the invasion patterns of cancer cells through confined spaces, providing valuable insights into metastatic potential and identifying factors influencing invasion [30].

Moreover, the investigation conducted by Jacobelli et al. [47] delved into the regulation of T cell motility during tissue trafficking using various microchannel arrays, as illustrated in Fig. 9a. T cells lacking Myosin IIA displayed increased adherence to high-endothelial venules, diminished interstitial migration, and ineffective lymph node recirculation. Through spatiotemporal analysis within microchannels, it was revealed that the optimization of motility rate depended on the level of confinement and the presence of Myosin IIA, rather than integrin adhesion. This optimized motility was characterized by a Myosin IIA-dependent rapid 'walking' mode, involving multiple small and simultaneous adhesions to the substrate, effectively preventing unwanted and prolonged adhesions. The role of Myosin IIA in adhesion discrimination emerged as critical for facilitating efficient T cell navigation through intricate tissues.

In the realm of wound healing, Gupta et al. [48] conducted a study with a specific focus on quantitatively examining the migration of fibroblast cells within the context of the wound-healing process. Employing a microfluidic-based wound-healing assay, the research dynamically replicated traumatic wounds on fibroblast cell monolayers. The microfluidic chip featured a transparent silk film, establishing conditions for 3D cell culture that closely resemble the extracellular matrix (ECM) environment. This innovative setup facilitated continuous real-time observation of cells throughout the entire wound-healing process. A computational investigation was conducted to assess the effects of dynamic medium-induced shear stress on the base and wall of the microchannel, with the objective of optimizing inlet flow conditions and identifying stress-prone areas. Scaffolds were strategically positioned in these identified areas to evaluate the impact of shear forces on the migration of fibroblast cells. Subsequently, an in vitro microfluidic system was employed to investigate cell migration under external shear forces during the wound-healing process. The findings revealed that optimal shear stress significantly expedites wound healing within a 24-h timeframe, whereas surpassing a defined threshold hinders the process by dislodging fibroblast cells from the substrate. Importantly, these observed phenomena were consistent in both coplanar microfluidic surfaces (utilizing a multichannel interlinked model) and transitional microfluidic channels.

Aoun et al. [22] investigated leukocyte transmigration, a crucial step in the immune response. The research utilized microfluidic transwells to examine the transition of human effector T lymphocytes from 2 to 3D migration and assess longitudinal forward-thrusting forces in smooth microchannels (Fig. 9b). The forward-thrusting force refers to the force exerted in the forward direction by cells during cell migration. It involves the dynamic interplay of cellular adhesion,

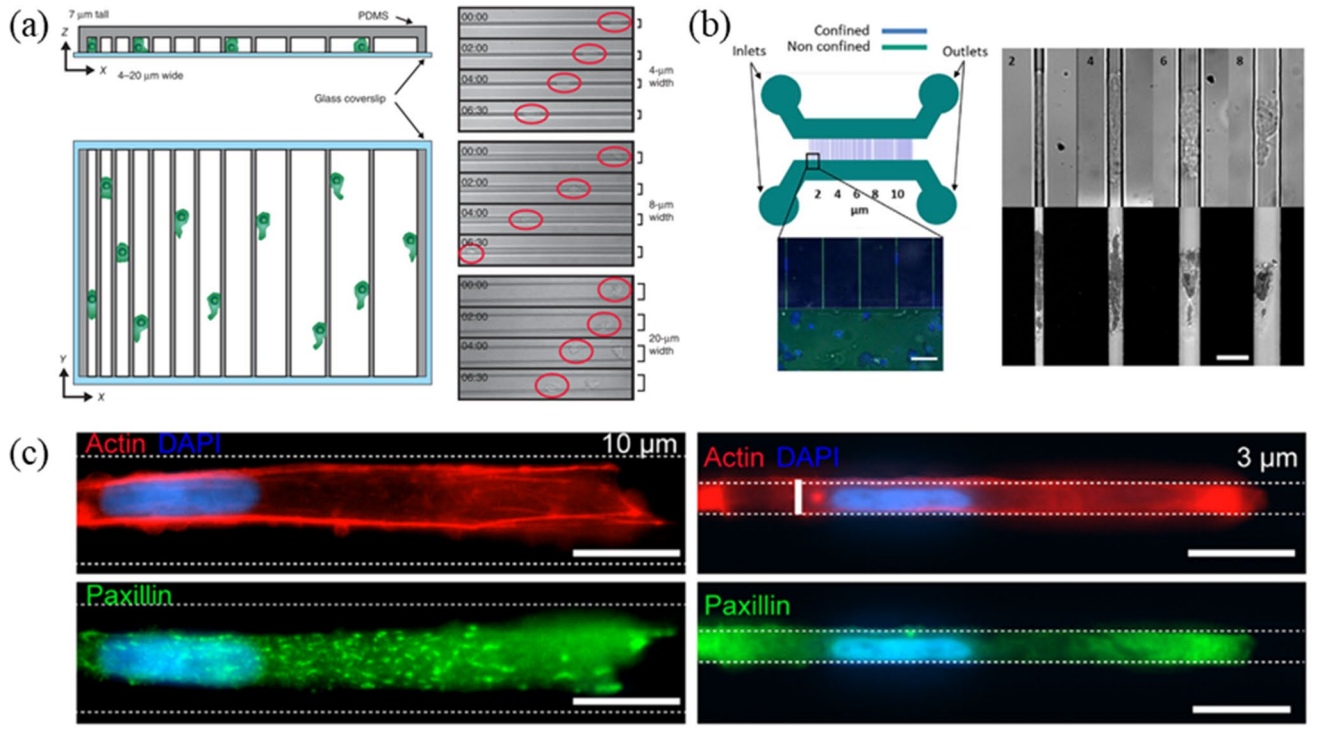


Fig. 9 **a** Variable microchannel array assembly and brightfield time-lapse microscopy of vehicle-treated CD8 + T cells 4–5 days after activation, red circles outline T cell positions; numbers in top right corners indicate time (in min:s) [47]. **b** Microfluidic transwell device for transmigration and 3D migration and bright-field images show T cells migrating in channels of variable widths [22]. **c** Immunofluorescence staining in fixed cells reveals punctate focal adhesions in cells transiting 10 μm channels (left), but a loss of paxillin expression and organization in cells transiting 3 μm channels (right). Scale bars = 10 μm [17]. Images reproduced with permission from references [17, 22, 47]

contractility, and motility to propel the cell in the desired direction. In the study, it was observed that integrins LFA-1 played a significant role in transmigration without chemotactic cues. Adhesion and contractility were crucial for overcoming nucleus penetration, but surprisingly dispensable in topographically smooth microchannels. Qualitatively consistent with treadmilling and squeezing mechanisms, 3D migration in smooth channels revealed the impact of adhesion on limiting migration under stress conditions. Stalling conditions, assessed by pressure drops, indicated that adhesion controls stalling under pressure rather than force, shedding light on the compression-induced perturbations in cell polarization. This microfluidic transwell approach provides valuable insights into 2D and 3D migration, barotaxis, and chemotaxis in Leukocytes.

The study reported by Holle et al. [17] utilized PDMS microchannels to investigate cancer cell invasion through the ECM as shown in Fig. 9c. The research focused on the transition from mesenchymal to amoeboid invasion, crucial for overcoming physical barriers. In narrow channels, migration was faster, even without cell-binding ECM proteins. Cells in these channels exhibited characteristics of amoeboid invasion, such as blebbing and smooth leading-edge profiles. Live cell labelling revealed a mechanosensing period where cells attempted mesenchymal-based migration before transitioning to an amoeboid phenotype. Inhibition experiments highlighted the dynamic interplay between Rho/ROCK and Rac pathways, demonstrating cancer cells' ability to adapt invasion strategies based on the physical constraints of the ECM.

These diverse applications, as summarized in Table 1, showcase the versatility of microchannels in modelling various physiological conditions related to cell migration, contributing significantly to our understanding of disease processes and potential therapeutic interventions.

5 Challenges and Future Perspectives

The exploration of cell migration in microchannels presents several challenges that influence the reliability and comparability of research outcomes. One notable hurdle is the heterogeneity in microchannel designs, encompassing variations in geometry, size, and surface properties. This diversity complicates the establishment of universal standards, introducing inconsistencies in findings across studies. Therefore, the common frameworks and guidelines for microchannel design need to be established for ensuring consistency and

Table 1 Comparison of previous research on cell migration using microchannels

Authors	Design of microchannel	Material of microchannel	Fabrication methods	Cell types	Results	Applications
Ramlan et al. [44]	Straight microchannels	PDMS	Photolithography	MDCK	The velocities of collective cell migration were higher with increasing the width of the microchannels	Cancer cells therapies
Mazalan et al. [16]	Tortuous microchannels	PDMS	Photolithography	MDCK	Cells move slower at the curve area as compared to straight path	Tissue engineering
Mills et al. [46]	Tortuous microchannels	PDMS	Photolithography	NIH-3T3 mouse fibroblast & HMSCs	3T3s and the hMSCs migration did not display any variations in cell wave front migration rate within any of the tortuous channels	Tissue engineering
Ko et al. [34]	Microchannels with an over-hanging zigzag design	PDMS	Photolithography	NIH3T3 and human umbilical vein endothelial cell (HUVEC)	The continuous zigzag microchannels can drive cell movement even in cells with changed intracellular signals that promote random movement	Tissue engineering
Yang et al. [49]	Microchannels with varying diameters of about 100, 200, and 400 μm	Bacterial nanocellulose and saponified cellulose acetate	Laser aided punching	BMSCs of rat	The microchannels favours cell proliferation and promote cell migration	Tissue engineering and regenerative medicine
Aoun et al. [22]	Microchannel with variable widths (h = 2 or 4 μm; w = 2, 4, 6, 8, and 10 μm)	PDMS	Photolithography	T lymphocytes	The compression of the cell's leading edge in microchannels appears to control adherent cell arrest under stress	Transmigration of other immune and cancer cells
Mossu et al. [19]	Microchannel with nanoporous silicon nitride (NPN) membranes	PDMS	Photolithography	CD34 + cells were isolated from human umbilical cord blood (UCB)	A novel human in vitro model of the BBB from human ECs derived from cord blood CD34 + hematopoietic stem cells	Cerebrovascular pathologies

comparability across studies. Additionally, the standardized protocols, variations in fabrication techniques, and differences in experimental conditions should be ensured to be reproduced.

The selection of materials for constructing microchannels is crucial, posing challenges in achieving both material biocompatibility and alignment with experimental objectives. The potential induction of unwanted cellular responses by certain materials can impact the precision of migration studies. Future research should focus on developing innovative materials that offer optimal biocompatibility while addressing the specific needs of diverse cell types.

The standardization in microchannel experiments is crucial to overcome the difficulties brought about by variations in design and experimental setups. Setting uniform frameworks and standards for microchannel geometry is essential. These should cover dimensions such channel width, length, and height in addition to the configuration and form of microstructures inside the channels [50]. Researchers can guarantee uniformity in experimental setups and make cross-study comparisons easier by using standardized geometry. The creation of procedures for creating microchannels is another topic of interest. To ensure repeatability and dependability, standardized protocols offer detailed instructions for creating microchannels utilizing a variety of methods, such as photolithography or soft lithography [15].

Microscale observation of cell behaviours within microchannels requires advanced imaging techniques, but limitations arise in real-time visualization, especially in 3D microenvironments. Acquiring high-resolution images while preserving cell viability over extended periods poses technical challenges. These high-resolution images could be benefit in therapeutic development in three-dimensional microenvironments, where researchers can determine which drugs modulate migratory pathways or disrupt metastatic processes, evaluate the impact of pharmacological agents on migration behaviour, and suggest candidates for additional preclinical study by imaging cells in real time after drug treatment. This method makes it possible to quickly screen sizable compound libraries and develop tailored treatments for conditions like cancer metastasis that involve abnormal cell migration. Therefore, future directions should involve advancements in imaging technologies, especially in high-resolution live-cell imaging. Another critical challenge lies in the limited understanding of the in vivo relevance of microchannel findings. Cells in microchannels may exhibit distinct behaviours compared to their behaviour in native tissues, raising concerns about the direct translatability of results to in vivo scenarios. Thus, engaging researchers from fields such as tissue engineering, biomechanics, and systems biology can provide a holistic perspective, bridging the gap between microchannel studies and native tissue environments. Integrating microchannel research with organ-on-a-chip technologies may offer more physiologically relevant platforms for studying cell migration.

Looking ahead, emerging trends may include the incorporation of machine learning (ML) in data analysis, enabling more robust interpretation of complex migration patterns. For instance, ML models have the ability to forecast different facets of cell migratory behaviour, including the possibility of directed migration, the possibility of cancerous metastasis, and the reaction to a certain therapy. Thus, it can provide precise predictions and support clinical decision-making or drug discovery initiatives by combining various datasets and understanding intricate correlations between input variables and migration outcomes.

Additionally, exploring the integration of microchannels with microfabrication techniques like 3D printing could lead to the development of more sophisticated and customizable microenvironments. 3D printing also enables rapid prototyping of microenvironments with complex geometries and features, eliminating the need for time-consuming and labor-intensive fabrication processes associated with SU-8 photolithography. Researchers can design and iterate 3D-printed scaffolds more quickly, accelerating the development of novel experimental models for studying cell migration dynamics.

Lastly, aforementioned conventional microfabrication techniques involve photolithography using standard photoresists such as SU-8. However, the use of the standard photoresists is associated with the intense use of toxic and climate-active chemicals. To reduce the heavy environmental burdens, dry film photoresists (DEPs), which has a low environmental impact, has recently been proposed as a low-cost and greener technology to replace photolithography using the standard photoresists [51].

6 Conclusions

In conclusion, the future of microchannel-based cell migration research lies in addressing current challenges through standardization, material innovation, enhanced visualization, interdisciplinary collaborations, and the incorporation of emerging technologies. By overcoming these hurdles, the field can advance towards more reliable, reproducible, and clinically relevant insights into cell migration dynamics. Through an examination of cell migratory pathways, scientists able to help clinicians to pinpoint possible targets for preventing metastasis and creating anti-cancer treatments. For patients with chronic wounds or impaired healing, it is especially crucial to comprehend the specific mechanisms of cell migration in wound healing since this can lead to the development of novel therapeutics to promote faster and more effective wound closure.

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Declarations

Competing interests The authors declare no competing interests.

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