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CELL SCIENCE AT A GLANCE

SPECIAL ISSUE: RECONSTITUTING CELL BIOLOGY

Reconstitution of cell migration at a glance

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ABSTRACT

Single cells migrate in a myriad of physiological contexts, such as tissue patrolling by immune cells, and during neurogenesis and tissue remodeling, as well as in metastasis, the spread of cancer cells. To understand the basic principles of single-cell migration, a reductionist approach can be taken. This aims to control and deconstruct the complexity of different cellular microenvironments into simpler elementary constrains that can be recombined together. This approach is the cell microenvironment equivalent of *in vitro* reconstituted systems that combine elementary molecular players to understand cellular functions. In this Cell Science at a Glance article and accompanying poster, we present selected experimental setups that mimic different events that cells undergo during migration *in vivo*. These include polydimethylsiloxane (PDMS) devices to deform whole cells or organelles, micro patterning, nano-fabricated structures like grooves, and compartmentalized collagen chambers with chemical gradients. We also outline the main contribution of each technique to the understanding of different aspects of single-cell migration.

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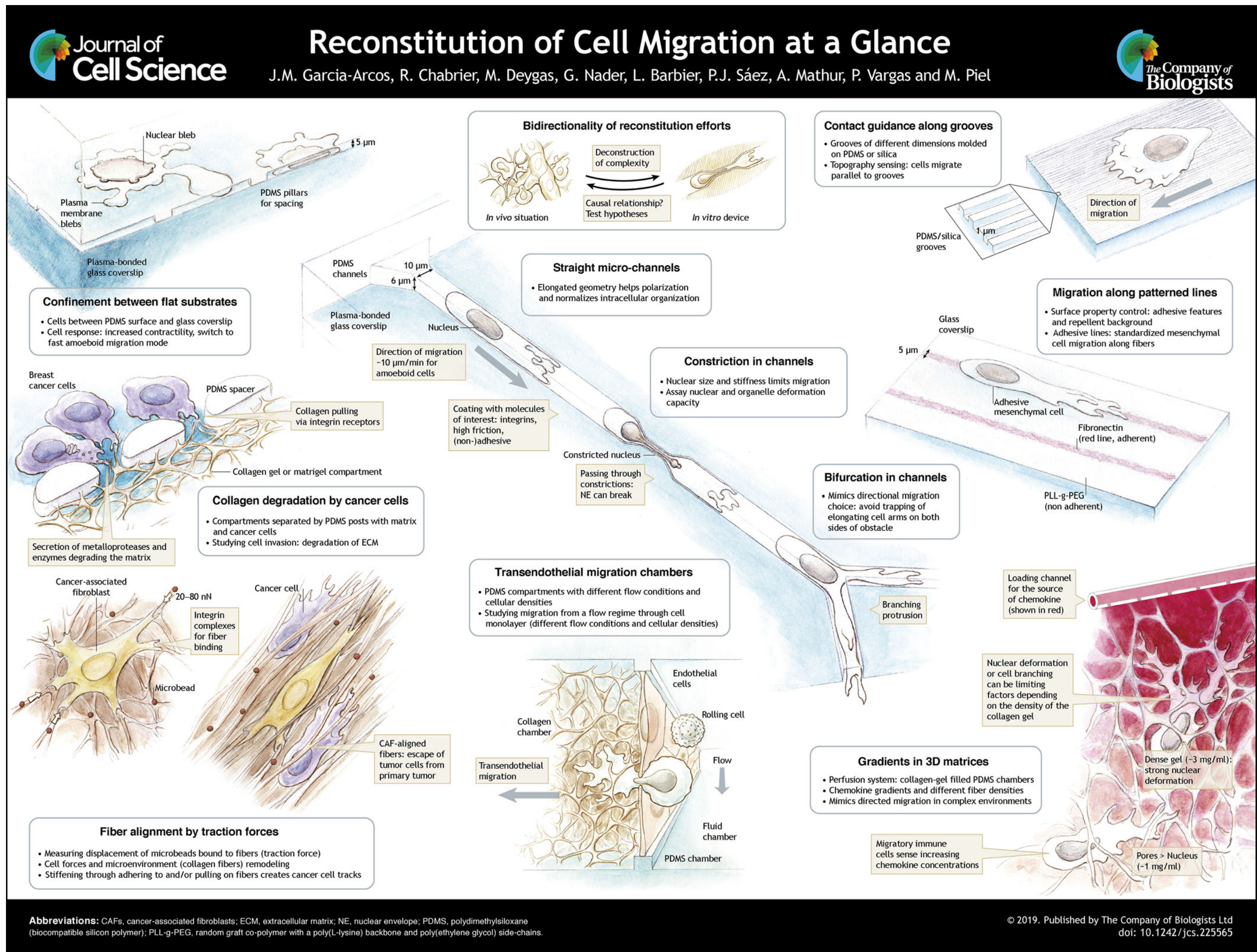
KEY WORDS: Microfabrication, Cell migration, Microfluidics, Micropatterning, *In vitro* assay



Journal of Cell Science (2019) 132, jcs225565. doi:10.1242/jcs.225565

Introduction

Cell migration in tissues depends on a balance between propulsive forces produced by the cell and physical tissue constraints, such as stiffness and pore size of the extracellular matrix (ECM), which present physical challenges to the moving cell. Additionally, organelle size and deformability constitute limiting factors for cells migrating through dense matrices (Calero-Cuenca et al., 2018; Ruprecht et al., 2015). Microfluidics and microfabrication have been shown to constitute a valuable platform to deconstruct the variety of constrains cells encounter as they move in a complex tissue, and are able to identify specific mechanisms cells use to overcome these constrains (Barnhart et al., 2011; Thiam et al., 2016). This is achieved through *in vitro* cell migration assays, most of which use microfabrication to mold polydimethylsiloxane (PDMS) chambers, a widely used biocompatible and optically transparent elastomer, which can be coupled to glass for observation. Other elastomers and hydrogels, including biopolymers like collagen or agarose gels, and synthetic ones like polyacrylamide or poly(methyl methacrylate) (PMMA), can be similarly molded but this is not discussed here (see Carlborg et al., 2011; Liu et al., 2015). We also do not elaborate on the fabrication procedures, which have been described many times and have a multitude of variants depending on their precise applications. The standard procedure, made popular by the chemist George Whitesides (Harvard University), is called soft lithography and was derived from standard methods used in microelectronics: a photosensitive resin is spin-coated onto a silicon wafer and then patterned by UV light exposure through a photomask bearing transparent and opaque features. The parameters of the spin coating can be modulated to control the thickness of the layer. A 2D design of the device is printed on a photomask. Features that can be as small as a micron can be microfabricated without requiring more equipment than a spin-coater, a hot plate and a collimated UV lamp (Heuzé et al., 2011). The process can be repeated to make more than one layer to build chambers with multiple heights. Here, we will introduce a selection of *in vitro* cell migration assays and highlight the different biological questions that they address.

Confinement between flat substrates

A simple addition to the classical 2D flat migration substrate is a coverslip placed on top of the cells at a distance that confines them without killing them. The simplest version of this assay has been used for immune cells for decades, because they migrate better in these conditions (Irimia et al., 2007; Malawista and De Boisfleury Chevance, 1997). It provided the first proof that confined cells can migrate without specific adhesion onto a matrix, a process called chimneying in reference to a rock-climbing technique (Heuzé et al., 2011; Lämmermann et al., 2008; Malawista and De Boisfleury Chevance, 1997; Renkawitz et al., 2009). Recent improvements of the method, using a microfabricated coverslip bearing micro-pillars of defined height, allows application of a precise confinement height down to 1.5 µm (Irimia et al., 2007; Jacobelli et al., 2010; Le Berre et al., 2012) (see poster). An alternative method that requires less specialized equipment relies on the use of spacer beads (Logue et al., 2018; Tozluoğlu et al., 2013; Vargas et al., 2016). Confinement improves migration of weakly adhesive cells, such as immune cells, which can then reach the same range of speed they achieve in a tissue (Heuzé et al., 2013; Prentice-Mott et al., 2016). It also induces non-motile cells, such as zebrafish embryonic germ cells, to display exceptionally fast and directional movements (Kraning-Rush et al., 2013; Ruprecht et al., 2015). In addition to the degree of confinement, the level of adhesion also has a large impact on cell motility. Substrates can be treated with various ratios of poly-L-lysine-graft-polyethylene glycol (PLL-g-PEG) copolymer, which makes surfaces non-adhesive, to pLL-g-PEG-RGD [Arg-Gly-Asp (RGD) integrin-binding peptide], which makes surfaces adhesive. This results in surfaces with tunable adhesiveness (Barnhart et al., 2011; Kirby and Lammerding, 2018). With the combination of high confinement (3 µm) and low adhesion (using purely the PLL-*g*-PEG copolymer), slow mesenchymal cells switch to an amoeboid morphology and display fast migration with a speed increase of up to 100-fold, reminiscent of the mesenchymal to amoeboid transition of invading cancer cells (Friedl et al., 2011; Liu et al., 2015). In such conditions, almost non-motile HeLa cervical cancer cells can match the speed of fast neutrophil-like cells. This indicates that the mechanisms that govern cell motility in flat confined and unconfined microenvironments is different, and highlights the capacity of cells to adapt their migration to the physical properties of their landscape.

Migration inside straight micro-channels

Micron-sized channels that are fabricated with the aid of biocompatible material like PDMS have been extensively used to study confined cell migration, especially for immune cells (Davidson et al., 2015; Irimia et al., 2007; Thiam et al., 2016). In such channels, cells also move at the speed they achieve in tissues, but with an elongated morphology that is more reminiscent of what is observed in dense matrices. Being able to precisely control the height and width of the channels, one can span the regimes of migration from confinement between flat substrates, with larger channels, to thin elongated cells, with smaller dimensions (see poster). Channels are created by molding PDMS in a microfabricated wafer (by means of classical UV lithography, see Introduction) and binding this PDMS to a glass coverslip. Channels can also be coated with various ECM molecules, like fibronectin and collagen, or anti-adhesive synthetic molecules like PLL-*g*-PEG, providing control over adhesiveness of the substrate (Denais et al., 2016; Heuzé et al., 2011; Raab et al., 2016). Cells only move back and forth inside the channel. This makes it straightforward to quantify cell speed and persistence. The elongated shape of cells inside thin channels also normalizes internal organization and distributes organelles along the cell length, possibly helping continuous cell polarization. This simplifies the quantitative comparison of the subcellular architecture of migrating cells (Alexander et al., 2013; Giri et al., 2013; Irimia et al., 2007; Jacobelli et al., 2010) and allows the use of averaged localization maps for dynamic structures such as actin networks, which is similar to what can be achieved using 2D micropatterns (see below). Comparison of such localization maps has revealed that dendritic cells switch migration modes as they mature upon encounter of pathogen-associated signals (Elliott et al., 2015; Lämmermann et al., 2009; Vargas et al., 2016) and a role for microtubules in directional memory (Ambravaneswaran et al., 2010; Paul et al., 2016; Prentice-Mott et al., 2016). Recently, a technique for fabrication of channels inside collagen gels has been developed, allowing the topographic control of more physiological matrices (Kraning-Rush et al., 2013; Prentice-Mott et al., 2013). Overall, microchannels allow the study of cell migration in landscapes with normalized morphology, in which the speed and cellular organization at the single-cell level can be studied in large samples, supporting experimental reproducibility.

Organelle deformation – micro-channels with constrictions

Organelle size and deformability often constitute limiting factors for cells migrating through dense matrices. In order to understand better

Journal of Cell Science

Journal of Cell Science (2019) 132, jcs225565. doi:10.1242/jcs.225565

the cell response to physical challenges that are encountered *in vivo*, controlled *in vitro* experiments can be used to reproduce specific scenarios, such as subcellular deformations. During cell migration, intracellular forces are transmitted across the cytoskeleton to the nucleus (Kirby and Lammerding, 2018; Mak and Erickson, 2014). The nucleus is the largest and stiffest cellular organelle; consequently, its deformability limits the ability of the cell to pass through tight spaces (Frantz et al., 2010; Friedl et al., 2011). Our group and others recently published examples of the application of micro-fabricated devices to explore how migrating cells behave when deforming their nucleus through small holes (Cukierman et al., 2001; Davidson et al., 2015; Thiam et al., 2016), causing nuclear envelope deformation, rupture and DNA damage (Denais et al., 2016; Raab et al., 2016; Shoulders and Raines, 2009). Such devices consist either of channels that vary their dimension along their length, or of pillars with small spacing, introducing constrictions that are smaller than the cell nucleus (see poster). These findings have raised new specific questions on organelle repair and the DNA damage response in migrating cells, such as what the long-term consequences of nuclear envelope transient ruptures (Shah et al., 2017) in the context of cancer development or immune responses could be. Furthermore, they have stressed the importance of expanding the cell migration toolbox to study the multifaceted process of single-cell migration in complex 3D micro-environments.

Cell branching and directional choice – micro-channels with bifurcations

During interstitial migration, cells encounter a complex and irregular meshwork of ECM and integrate a wide variety of physical and chemical cues that guide their migration. The observation of migrating cells *in vivo* revealed that, while following a path, they constantly face complex crossings. These are formed, for example, by collagen fibers, and the migrating cells extend their cytoplasmic ‘arms’ in many directions (Alexander et al., 2013; Giri et al., 2013; Vargas et al., 2017). In such a context, the ability of the cell to choose a single ‘arm’ is essential to allow its efficient migration (Elliott et al., 2015; Lämmermann et al., 2009; Weber et al., 2013). Channels with a Y-shaped bifurcation constitute a minimal system to study the choice of cells between two extending protrusions (Ambravaneswaran et al., 2010; Paul et al., 2016; Vargas et al., 2017) (see poster). The physical and chemical properties of each path can be well controlled and independently tuned. Consequently, research on directed migration of leukocytes and tumor cells pointed to sensitivity of the directional choice to very small force imbalances, such as the one introduced by hydraulic resistance (Moreau et al., 2018 preprint; Aizel et al., 2017; Cheng et al., 2007; Chung et al., 2009; Clark et al., 2018; Polacheck et al., 2011; Prentice-Mott et al., 2013; Sáez et al., 2018a) and the importance of the microtubule cytoskeleton in symmetry breaking and cell polarization towards the chosen path (Leithner et al., 2016; Mak and Erickson, 2014; Vargas et al., 2016).

Directed migration in complex environments – chemokine gradients in 3D collagen matrices

Cell migration in the interstitial space of tissues is a complex process that requires the adaptation of cells to the structure of their microenvironment. The interstitial space is mainly composed of fibers of ECM that arrange in a 3D network containing collagen, fibronectin, elastin, laminin and other filamentous proteins (Bretou et al., 2017; Frantz et al., 2010; Sáez et al., 2018b, 2017). Pioneering work that addressed how cells move in such a microenvironment opened the way to understand motility in complex microenvironments (Cukierman et al., 2001; Harrison, 1911). The use of extracellular matrix components in *in vitro* assays allows the control of the microenvironment, where concentration, stiffness, alignment of the fibers and composition of the matrix can be modified. Among the different fibers, collagen is the most prevalent component of the extracellular matrix in tissues (Elkhatib et al., 2017; Schumann et al., 2010; Shoulders and Raines, 2009). Collagen fibers can crosslink through their extremities, named telopeptides, which results in a 3D meshwork. In order to efficiently localize in the organism, cells need to move in this complex matrix and at the same time sense chemotactic factors that drive their movement in the tissue (Guetta-Terrier et al., 2015; Vargas et al., 2017). This is particularly important during immune responses, in which the directed migration of leukocytes through the interstitial space permits the development of proper immune reactions (Doyle et al., 2009; Weber et al., 2013). Among leukocytes, directed migration of neutrophils towards an injured tissue and of dendritic cells towards the lymphatic vessels are key functions that involve the coordination between mechanosensing of the microenvironment and sensing of chemical cues (Théry, 2010; Vargas et al., 2017). To study chemotaxis through complex environments, it is possible to combine a 3D collagen meshwork and a well-controlled chemokine gradient (Aizel et al., 2017; Cheng et al., 2007; Chung et al., 2009; Clark et al., 2018; Maiuri et al., 2012; Polacheck et al., 2011; Sáez et al., 2018a). For example, a microfabricated chamber contains a central compartment with a collagen gel, open to a side chamber in which chemokines can be injected and diffuse through the gel (see poster). Controlled chemokine gradients in collagen gels have been used to show that actin that is nucleated by the actin-related protein (Arp)2/3 complex at the cell front is required for protrusion-mediated space exploration, but dispensable for directional migration (Leithner et al., 2016; Maiuri et al., 2015; Vargas et al., 2016). Furthermore, they helped to explore the role of Ca^{2+} and Ca^{2+} channels in directed dendritic cell migration (Bretou et al., 2017; Doyle et al., 2009; Sáez et al., 2017, 2018b). Future efforts using these systems might help determine how cells integrate the simultaneous sensing of physical and biochemical cues to optimize their migration in 3D landscapes. Such an integration is likely needed for efficient displacement and positioning of cells in tissues/organs, in which multiple signals might impact on the path they follow as they move.

Adhesion-limited migration – migration of cells along patterned lines

Migrating single cells, especially mesenchymal cells like fibroblasts and some tumor cells, can move along thin fibers (Harrison, 1911; Infante et al., 2018) and other tracks in tissues and in reconstituted matrices (Doyle et al., 2009; Elkhatib et al., 2017; Schumann et al., 2010). This phenomenon can be mimicked with minimal systems such as suspended fibers (Guetta-Terrier et al., 2015; Janson and Putnam, 2015) or by using thin printed lines of adhesive molecules on a 2D substrate (Clark and Vignjevic, 2015; Doyle et al., 2009) (see poster). In general, micropatterning is often used to control the shape of adherent cells in 2D cultures (Provenzano et al., 2006; Théry, 2010). The standardized cell migration conditions that micropatterning provides made it possible to organize a ‘World Cell Race’ (Maiuri et al., 2012; Provenzano et al., 2006), which produced a large dataset to compare motility across many different adherent cell types. This experiment revealed a conserved mechanism coupling cell speed and persistence (Harrison, 1911; Maiuri et al., 2015). This technique has also been used to study the polarity of migrating cells. For example, in migrating epithelial

Journal of Cell Science

 Journal of Cell Science (2019) 132, jcs225565. doi:10.1242/jcs.225565

cells, the Golgi and the microtubule-organizing center localize to the back of the cell when lines are thin enough, resembling the organization of amoeboid cells (e.g. immune cells). In contrast, on wider lines, they occupy a location at the front of the nucleus, as previously reported for mesenchymal cells (Bettinger et al., 2009; Doyle et al., 2009). The functional significance of this polarity switch is still largely unknown, but might be relevant in cancer cells to allow matrix degradation in front of the nucleus (Infante et al., 2018; Kim et al., 2010). Interestingly, migration on thin lines has been shown to promote a motility mode similar to the one observed in 3D microenvironments, indicating that this system could be used as a simplified tool for the study of mechanisms controlling cell migration in more complex microenvironments (Doyle et al., 2009; Karuri et al., 2004; Teixeira et al., 2003).

Topographical effects on migrating cells – contact guidance by nano-grooves

In vivo, cells constantly pull, align, cut and rearrange the ECM, generating specific topographical features at various scales down to tens of nanometers (Janson and Putnam, 2015; Kim et al., 2009). This is particularly relevant in the context of cancer, where the tumor microenvironment plays a very important role in the dissemination of the disease (Clark and Vignjevic, 2015; Holle et al., 2017). The structure of the ECM in stroma surrounding the tumor guides the invasion of cancer cells (Kwon et al., 2012; Provenzano et al., 2006). A major rearrangement causes collagen fibers to be radially aligned, promoting the invasion of cancer cells into the stroma (Driscoll et al., 2014; Provenzano et al., 2006). The topographical effect on cell migration and polarity falls into the general phenomenon of contact guidance. The first observations of this phenomenon date back more than a century ago (Bettinger et al., 2009; Harrison, 1911). It can be recapitulated *in vitro* using microfabricated grooves or aligned fibers (Bettinger et al., 2009; Park et al., 2016) (see poster). In most cases, cells migrate parallel to the grooves, which biases the orientation of focal adhesions and actin fibers. Examples of cells that undertake such motility include rat myocytes (Kim et al., 2010; Pieuchot et al., 2018), SV40-immortalized or primary human corneal epithelial cells (Clark and Vignjevic, 2015; Karuri et al., 2004; Teixeira et al., 2003), NIH 3T3 fibroblasts (Kim et al., 2009; Takata et al., 2007) and human pancreatic cancer cells (Blaha et al., 2017; Holle et al., 2017). Interestingly, contact guidance has also been observed in amoeboid migrating cells such as confined T cells (Gaggioli et al., 2007; Kwon et al., 2012) and the amoeba *Dictyostelium discoideum* (Driscoll et al., 2014). The dimensions of the grooves determine whether cells fall inside grooves as in a channel, or if they cover many groves. In the latter case, contact guidance is related to the dynamics of actin that polymerizes on the cell edges, but the exact mechanism is still a subject of debate (Bettinger et al., 2009). Interestingly, gradients in the topography of the microenvironment have been shown to direct cancer cell motility in a process named topotaxis (Park et al., 2016). In general, groove depth, rather than periodicity or density, seems to play a major role in modulating the cell response (Pieuchot et al., 2018).

Matrix remodeling by migrating cells – matrix degradation and fiber alignment

Deregulated migration and invasion of the extracellular matrix are critical steps in the escape from the primary tumor and the colonization of new tissues by metastatic tumor cells (Clark and Vignjevic, 2015). Cells have two strategies to penetrate dense matrices: (1) expanding the matrix openings through physical remodeling and proteolytic degradation of the ECM, (2) contorting their shape to accommodate the available space (see section on constrictions). Imaging of 3D migration and invasion can be achieved by viewing cells in collagen and gelatin gels. In such assays, cells are either platted on, embedded inside or sandwiched between gels (Takata et al., 2007). A novel microfluidic matrix invasion model (see poster) was recently employed to image cell–cell and cell–matrix interactions driving metastatic cancer cell invasion (Blaha et al., 2017). It includes a cell-loading compartment and a collagen compartment, separated by micropillars – invading cells insert between the pillars while degrading the gel, mimicking the escape from a primary tumor with a basal membrane.

Independently of matrix degradation, physical ECM remodeling can influence cell migration through phenomena such as contact guidance by aligned fibers (see above). In particular, cancer-associated fibroblasts (CAFs) can alter ECM architecture and physical properties, generating tracks that cancer cells follow (Gaggioli et al., 2007). In order to study the impact of CAF remodeling of the ECM on cell migration, several studies have used ECM that was produced and deposited by CAFs (Calero-Cuenca et al., 2018; Erdogan et al., 2017; Goetz et al., 2011; Gopal et al., 2017). Fibronectin deposition and assembly into aligned fibers by CAFs guides single or collective migration of cancer cells in a 2D environment or in tumor spheroids (Attieh et al., 2017; Calvo et al., 2013; Thiam et al., 2016). The mechanism behind this fibronectin-based parallel-fibers assembly by CAFs requires cell contractility and adhesion to the matrix (Attieh et al., 2017; Carlborg et al., 2011; Erdogan et al., 2017). Since fibronectin overexpression is correlated with poor prognosis, and alignment of fibronectin fibers is a prominent feature in cancer, ECM remodeling by CAFs represents a potential common mechanism to guide cancer cell invasion. To study the mechanism by which cells pull on fibers to align them, the displacement of microbeads bound to the matrix can be monitored and coupled to mathematical modeling to extract the traction forces (Hall et al., 2016; Legant et al., 2010; Malawista and De Boisfleury Chevance, 1997; Polacheck and Chen, 2016; Roca-Cusachs et al., 2017) (see poster). These observations indicate that cancer cell invasion can be highly influenced by the activity of the cells as well as the properties of their microenvironment. The use of controlled microfabricated systems can help to dissect the contribution of both phenomena to the migration of cancer cells in complex microenvironments.

Cell migration through the endothelium – mimicking a complex *in vivo* system with multiple compartments and cell types

One of the main events that is needed for the migration of cells between tissues is their entry in (and exit from) the lymphatic or blood vessels. These systems connect distant organs in the body and facilitate cell distribution through the organism. Cell entry in the vasculature occurs through the endothelium, a cellular layer that separates the solid 3D reticular configuration of the interstitial space from the flowing fluid inside the vessels (Lämmermann et al., 2008; Malawista and De Boisfleury Chevance, 1997; Renkawitz et al., 2009; Vargas et al., 2017). Cell passage through the endothelium has been classically studied using endothelial monolayers cultured on top of membranes or plastic dishes in which flow can be applied (Barzilai et al., 2017; Le Berre et al., 2012; Shulman and Alon, 2009; Stroka et al., 2013). However, these systems fail to recapitulate the complexity of transendothelial migration observed *in vivo*. Recently, this complexity has been mimicked by using a series of microfabricated devices that combine sequential chambers

Journal of Cell Science

containing 3D extracellular matrix, endothelium monolayers and a fluid compartment in which in most cases flow can be applied (Chen et al., 2017; Han et al., 2012; Jeon et al., 2015; Logue et al., 2018; Tozluoğlu et al., 2013; van Duinen et al., 2017; Wong and Searson, 2014). These systems allow the study of cell passage through the endothelium in both directions, mimicking intravasation and extravasation and the consequent transition between complex microenvironments in which the stiffness of the extracellular matrix and the speed of the flow can be precisely tuned (see poster).

Concluding remarks

In the past two decades, a profusion of cell migration assays has appeared, exploiting the possibilities that are offered by microfabrication techniques. Here, we presented a sample of them, illustrating how they allow researchers to tackle the question of how single cells migrate through complex environments with a reductionist approach. By deconstructing this complexity into simpler modules, mimicking a specific type of constrain or phenomenon, these devices also provide standardization and reproducibility, which is important for example for drug screening (Heuzé et al., 2013; Regnault et al., 2018). In addition, the precision in fabrication techniques opens the possibility to fine-tune key environmental parameters and perform quantitative experiments to foster the development of physical models of cell migration. We explain, for each device, the *in vivo* situation that was mimicked; however, the rationale for developing a specific device could also emerge from a biological or a biophysical question, exploiting the capacity to design specific geometries. The devices can then be used to test and validate some hypotheses that have a broader significance and can apply to cells migrating in complex environments that are not restricted to the simplified devices presented here. This inverted logic (starting from the design of the device rather than from mimicking a specific *in vivo* situation) not only allows establishing clear causal relationships, but also has the potential to open new questions that could not emerge from *in vivo* studies.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area is supported by Centre National de la Recherche Scientifique (CNRS), Institut Curie, Institut National de la Santé et de la Recherche Médicale (INSERM), PSL research University, Paris Saclay University and Paris Descartes University.

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at
http://jcs.biologists.org/lookup/doi/10.1242/jcs.225565.supplemental

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