Journal of Laboratory Automation
17(1) 59–65

DOI: 10.1177/2211068211426550
http://jala.sagepub.com
SAGE

Advances in Wound-Healing Assays for Probing Collective Cell Migration

Reza Riahi[1,*], Yongliang Yang[1,*], Donna D. Zhang[2], and Pak Kin Wong[1,3]

Abstract

Collective cell migration plays essential roles in a wide spectrum of biological processes, such as embryogenesis, tissue regeneration, and cancer metastasis. Numerous wound-healing assays based on mechanical, chemical, optical, and electrical approaches have been developed to create model "wounds" in cell monolayers to study the collective cell migration processes. These approaches can result in different microenvironments for cells to migrate and possess diverse assay characteristics in terms of simplicity, throughput, reproducibility, and multiplexability. In this review, we provide an overview of advances in wound-healing assays and discuss their advantages and limitations in studying collective cell migration.

Keywords

wound healing, collective cell migration

Introduction

Collective cell migration is a highly regulated cellular activity involved in numerous normal physiological processes, such as embryogenesis, tissue repair, angiogenesis, and wound healing.[1,2] Recently, collective cell migration also has been suggested to play essential roles in invasion and metastasis of malignant tumors.[3] During collective migration, cells often form integrated monolayers and interact mechanically and biochemically through cell–cell adhesions. Cell proliferation, cell–microenvironment interaction, and cell signaling are also involved in this complex process. To study the dynamic collective cell migration process, various in vitro techniques, such as wound healing assays, Boyden chambers, and sprouting assays, have been developed.[4,5] Among these techniques, wound-healing assays are some of the most common approaches in studying collective cell migration due to their simplicity and ability to visualize the cells during cell migration.

In a typical wound-healing assay, a cell-free region is introduced in a cell monolayer by physically removing cells in the monolayer. The introduction of cell-free region induces various cellular responses, such as cell growth and cell migration.[6] The dynamics of collective cell migration toward the cell-free region can then be observed using time-lapse microscopy, and the migration rate and interactions between the cells can be analyzed. This is one of the earliest methods to study cell migration in vitro and has been applied in various cell types. Although widely used, there are several limitations in the conventional wound-healing assay. First, the scraping speed and the geometry of the wounded region can vary among different experiments. These factors could potentially make it difficult to compare between experiments. Second, the scraping process involves mechanical injury to the cells, and the cellular contents can be released into the surroundings. The extent of cell injury is difficult to control and could complicate the cell migration process. Third, modifying the surface using extracellular matrix (ECM) coatings is challenging because the scratching process might break the coating. Furthermore, the throughput, reproducibility, and multiplexability of the conventional wound-healing assay can often be limited.

With recent advancements in micromachining, optics, chemistry, and electronics in the past decades, researchers

[1]Department of Aerospace and Mechanical Engineering, The University of Arizona, Tucson, AZ, USA
[2]Department of Pharmacology and Toxicology, The University of Arizona, Tucson, AZ, USA
[3]Agricultural & Bioseystems Engineering, Biomedical Engineering and Bio5 Institute, The University of Arizona, Tucson, AZ, USA
*These authors contributed equally to this work.

Received Jul 27, 2011.

Corresponding Author:
Pak Kin Wong, PhD, Department of Aerospace and Mechanical Engineering, University of Arizona, P.O. Box 210119, Tucson, AZ 85721, USA
Email: pak@email.arizona.edu

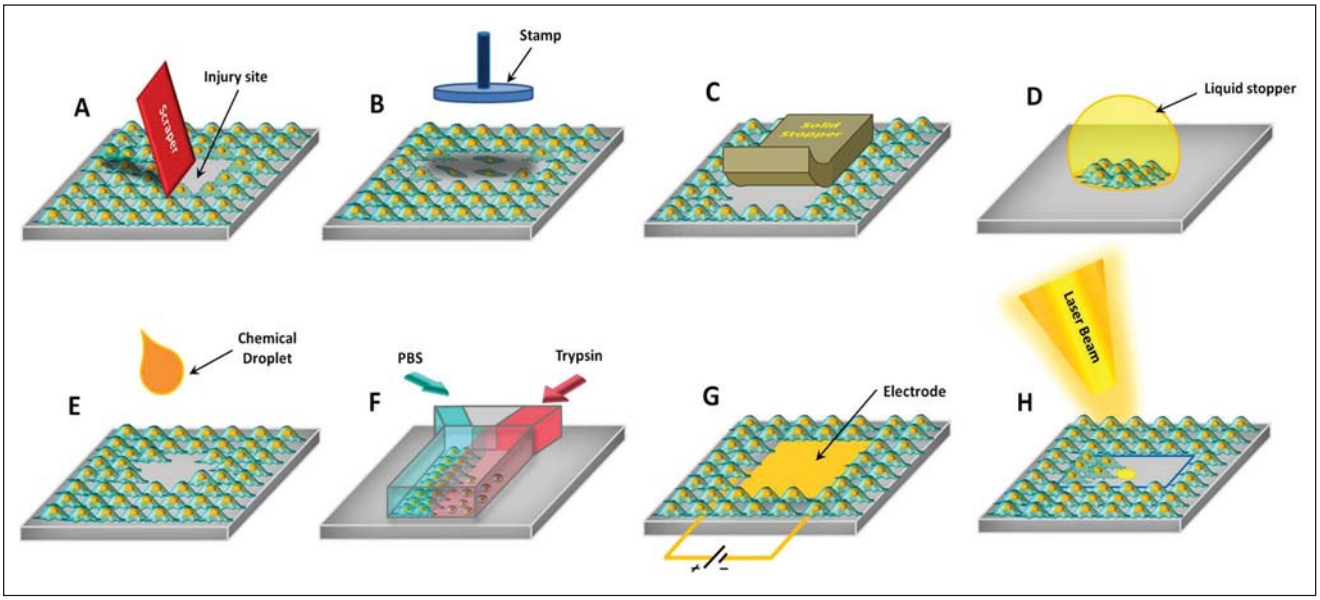

Figure 1. Wound-healing assays based on (A) scratching, (B) stamping, (C) solid barrier, (D) liquid barrier, (E) droplet chemical assay, (F) microfluidic chemical assay, (G) electrical assay, and (H) laser ablation.

have developed novel approaches to implement the wound-healing assay in a more controllable manner. In this review, we describe the advances in wound-healing techniques for studying collective cell migration. These assays are categorized according to their wounding principles. In particular, mechanical approaches, including scraping and physical blocking, are first described. Then, wounding techniques based on nonmechanical methods such as chemical, electrical, and optical approaches are reviewed. Finally, key issues that should be considered when implementing a wound-healing assay for a collective cell migration study are discussed.

Mechanical Wounding Assay

The most common method for studying collective cell migrations is mechanical wounding because of its simplicity and cost-effectiveness. In a mechanical wounding assay, cells are disrupted physically, and the collective cell migration process is observed. Mechanical wounding assays can be categorized into mechanical scratching and stamping assays.

In a typical mechanical scratching assay, cells are allowed to grow until they reach confluence, and a mechanical wound is created by physical scraping, as shown in **Figure 1A**. Mechanical scraping can be performed using a pipette tip, needle, bladder, razor, rubber policeman, cotton bud, or Teflon spatula for creating a single scratch or a plastic hair comb or wounder for creating multiple scratches simultaneously.[7-13] To standardize the process and increase the throughput, the scratching assay can be implemented using a robotic system.[14] To improve the reproducibility and minimize the surface damage, a drill press assay has been developed.[15,16] In this method, a stabilized, rotating, silicone-tipped drill press is used to create uniform circular lesions in an intact cell monolayer. In addition, the ECM (e.g., Matrigel) can be incorporated with the assay to create a pseudo-matrix barrier on the cell-free surface or wounding sites. Another reproducible scratching method for cell–substrate interaction studies is the hydrophilic polydimethylsiloxane (PDMS) slab assay.[17,18] This creates a wounded cell layer near the boundary and minimizes cell debris and surface damage in the free region of the substrate. In this method, a short hydrophilic PDMS slab with a desired shape is embedded onto the substrate precoated with the material of interest, allowing cells to grow on top of both substrate and PDMS. After a confluent cell monolayer is formed, the removal of PDMS wounds reproducibly the cells near the boundary and allows cells to migrate onto the debris-free surface.

Another approach for mechanical wounding is the stamping assay (**Fig. 1B**). In this method, a confluent cell monolayer is punched by a stamp to form a reproducible and uniform pattern. Advantages of stamping assays include creation of wounds with arbitrary shapes and study of cell migration in the presence of cell debris. For example, a stamping assay has been reported to study phagocytosis of cell debris in the wound site during wound healing.[19] In this assay, a PDMS mold pattern is placed on the cell monolayer to punch cells and creates wounded cell areas. Because of the presence of damaged cells and cell debris in the wound site, chemotactic reagents can be released locally, which influences the cell migration in directional studies. Another stamping assay for studying cell regeneration is the stamp-sliding assay.[20]

In this method, a cell monolayer is stamped by a Neoprene pattern under pressure control followed by rotary or lateral movement of the stamp to create desired cell patterns, such as circles and islands. The method is able to make uniform and narrow cell bands, which expedites the activation of the entire remaining cell population for collective migration.

Physical Barrier Assay

A physical barrier is another method to create cell-free regions for collective cell migration. Instead of removing cells in the monolayer, a physical barrier is applied before cell seeding to block cell attachment, and the removal of the physical barrier initiates the cell migration process. The repeatability and standardization of the physical barrier assay are more achievable compared with the scratch assay. The use of a physical barrier also minimizes injury to the cells and keeps the cell-free region intact for ECM deposition. This could allow investigation of different molecular mechanisms and signaling pathways underlying cell migration and elucidation of the specific functions of ECM components and growth factors. Using physical barriers to minimize cell damage is likely to be important for studying collective migration processes that do not involve cell injury, such as cancer metastasis, inflammation, and angiogenesis. The barrier assays can be divided into solid barriers and liquid barriers.

Solid barriers have been used for decades to study cell migration, as shown in **Figure 1C**.[21] A solid barrier is typically composed of biocompatible materials. One of the early solid barriers for cell migration studies is the Teflon fence.[22] In this assay, a Teflon fence is placed in a tissue culture dish, and cells are plated at high density. After forming a confluent cell monolayer, migration and proliferation of cells are studied after the release of the Teflon fence. Other solid stoppers include the Flexiperm disc[23,24] and microstensil.[25] These solid barriers are made of silicone or PDMS with desired shapes. These materials can adhere to smooth surfaces without glue and are able to create multiple wounding sites. Their biocompatibility, ease of use, and high adhesiveness render them useful in numerous tissue culture applications. In addition, the barrier can have a closed or open shape.[21] In the closed shape, the stopper has no opening holes and acts like a solid barrier.[26] Cells are seated around the stopper and grown until confluence, after which the stopper is removed. The open-shape barriers, such as circular rings, consist of inner and outer compartments in which cells can be seated either inside or outside of the barrier.[27-29] The open-shape assay can have two different inside and outside coatings based on the experimental requirements.

Another physical barrier assay for collective cell migration studies is called a "detachable substrate."[30,31] This method consists of two complementary parts composed of PDMS and polystyrene and fabricated by replica molding. In this setup, the PDMS substrate has a convex edge that is complementary to the concave edge of the polystyrene substrate, allowing two parts to be attached and detached repeatedly. After seeding cells and forming a cell monolayer on one substrate, the intact complementary substrate is attached and allows cells to migrate onto the untouched surface for cell migration studies. This method can also be used for studying cell–cell interactions. In this case, two different cell types are being cultured in each substrate separately until they become confluent. Then two parts are joined in discrete configurations such that two cell types are adjacent to one another—for the dynamic manipulation of cell–cell adhesion and investigation of heterotypic cell interactions.

Liquid stoppers are another barrier assay enrolling liquid or gel barriers in outward cell migration and proliferation studies in which less substrate and cellular damages are required near the boundary (**Fig. 1D**). One of the earliest liquid stoppers is agarose gel.[32] Cells are introduced to agarose gel after being centrifuged into pellets. To isolate cell suspension, a droplet of the agarose gel–cell suspension is delivered to the microwell plate surface and cooled down until the gel solidifies. The medium is then gently added to wells and is incubated for cell migration studies. To study cell migration on the various surface coatings without cellular damage, a multichannel migration device, which takes advantage of surface tension, can also serve as a liquid stopper in microchannels.[33] In this technique, cells are first seated in the main chamber to establish a confluent cell monolayer. The fluid is prevented from entering the side microchannels connected to the main chamber due to surface tension controlled by appropriate channel dimensions and material properties. After forming a confluent cell layer, the fluid in the main chamber enters the microchannels when they are backfilled with a syringe, and this is done to investigate the impact of different ECM coatings on collective cell migration inside the microchannels. Another method to study sheet migration on a defined surface is the oil droplet migration assay.[34] This is optimized for cell migration and proliferation studies due to minimum disturbance on seated cells. In this method, an aqueous drop of medium–cell suspension is pipetted into a light mineral oil, and the drop falls on the matrix surface due to the density difference. After a confluent monolayer is formed, cells are released from the liquid stopper by aspirating the oil out of the dish. In addition, a method called the bullseye[35] is served as a wound-healing assay to observe cell behavior within the gelled ECM. In this method, cells suspended in the collagen introduced to the central bore of a disc shape Teflon support. Then, cell-free collagen is pipetted over Teflon support, and the completed assay peels off the support after almost a 1-h incubation for radial cell migration in vitro. This technique provides a useful approach for studying the polymeric ECM encountered by cells in gel-based materials.

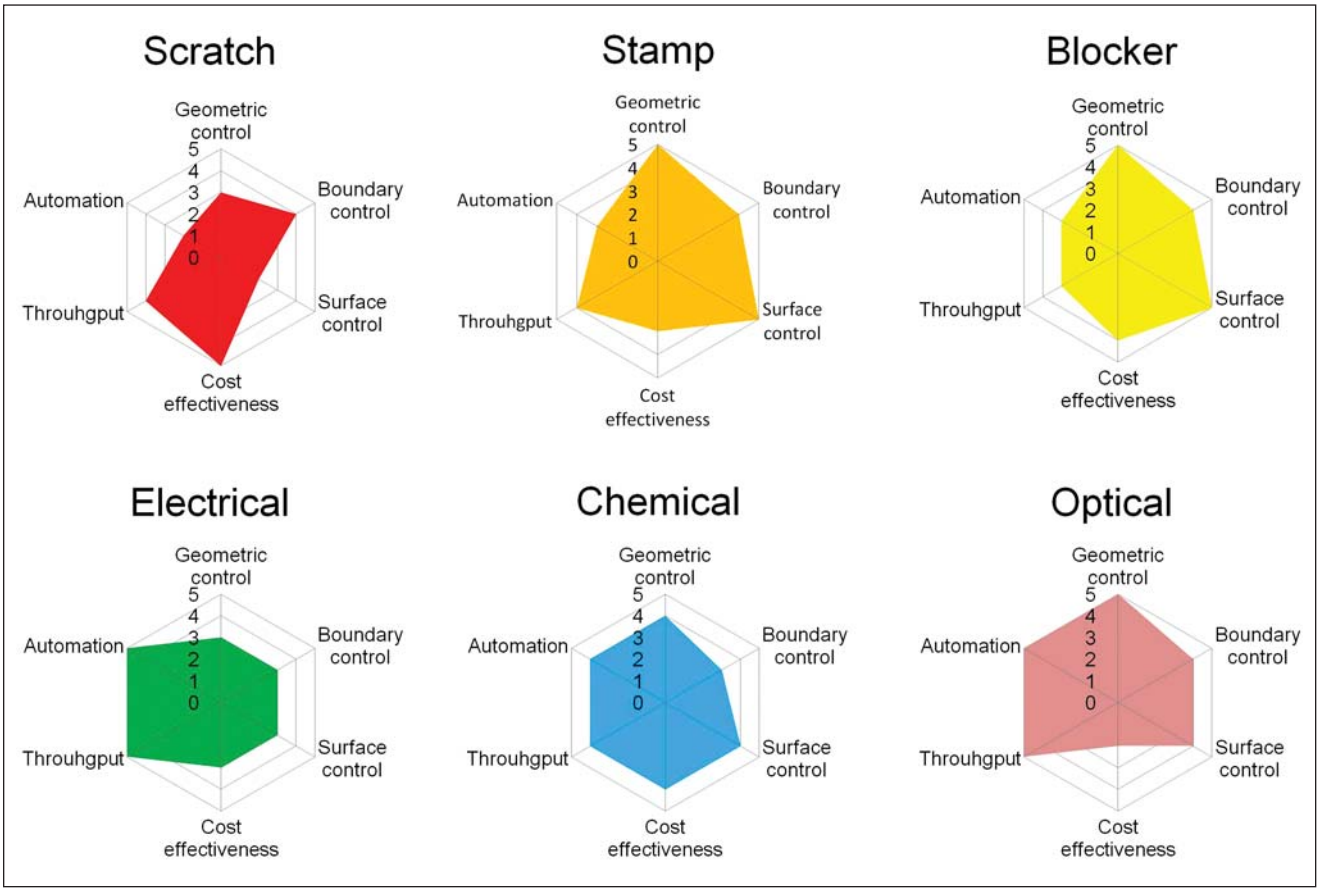

Figure 2. Characteristics of wound-healing assays.

Chemical Methods

The cell monolayer can also be wounded using chemical methods. For instance, sodium hydroxide has been applied to lyse cells and create model wounds chemically.[36] In this approach, as shown in **Figure 1E**, a small droplet of sodium hydroxide is pipetted onto the cell monolayer to selectively remove cells in contact with the droplet. The size of the wound is controlled by the volume of the chemical applied. To control the location of cell removal, the chemical wounding method can also be implemented using microfluidics. The flow in a microchannel is generally laminar due to the low Reynolds number of fluid flows in the microscale, which does not mix across different streams. Based on the laminar nature of a low Reynolds number flow, a microfluidic device has been developed to selectively remove cells in a microchannel.[37] The concept of this assay is presented in **Figure 1F**. The channel has three inlets, which are responsible for cell seeding, wounding, and wound-healing processing. During the experiment, trypsin solution is applied in one or two inlets depending on the size and location of the cell-free region. To improve the throughput of this method, a fully integrated microfluidic system is used for the wound-healing assay. This system is composed of a 24-well dish and computer controlled. The wounding and observation are fully automated, and the fluids are precisely controlled.[38] The microchannel also allows cell stimulation mechanically or chemically during the wounding process. In particular, fluid flow can be applied to investigate the role of shear stress in wound healing, and chemical gradients can be created in the microchannel to study their chemotactic potentials.

Electrical Methods

Compared with mechanical wounding methods, electrical wounding methods, based on the electrical cell–substrate impedance sensing (ECSI) technique, can create wounds with different geometries and measure the electric impedance data of the cell monolayer, which indicates the cell movement. The principle of this method is shown in **Figure 1G**. The electrical wound-healing system typically is composed of a working electrode for cell adhesion and a counter-electrode. By applying a relatively large voltage between the electrodes, the cells on the electrode can be electropermeablized permanently to generate a model wound in the

monolayer.[39] After wounding, the variation of impedance on the cell–adhesion electrode is recorded to monitor the cell migration. Because of the insulating property of the cell plasma and membrane, the cell movement can be characterized by measuring the electric impedance of the cell monolayer. The wounding and measurement processes can be strongly influenced by the electric signals applied between the electrodes. For example, the polarity of the cell–adhesion electrode determines the wounding characteristics if a direct-current (DC) pulse is applied.[40] If the cell adhesion electrode is positive, the cell wounding is extended beyond the electrode. If the polarity inverses, it will result in a higher uncertainty in the impedance measurement. A high-frequency AC signal can be applied to improve the electrical wounding method. With AC electric potential, the cells permanently electroporated can be confined on the electrode. Besides the electroporation principle, the electrical wound methods can also be combined with the physical blocker principle to generate a cell-free area for collective cell migration. In addition, a self-assembled inhibitive monolayer can block the cell from settling onto the electrode after cell seeding.[1] When the cell sheet is confluent and the cell edge forms around the electrode, a DC signal is applied to remove the inhibitive monolayer through an electrothermal effect. After that, the cells are able to migrate onto the cell-free electrode. Compared with electropermeablization, this method can exclude the cell debris in the cell-free region. A major advantage of the electrical method is the ability to automate the process for high-throughput study. An array of wounds can be monitored simultaneously and automatically based on the impedance. This can significantly increase the throughput compared with manual optical characterization with a microscope.

Optical Methods

Wounding of the cell monolayer can also be performed using optical methods. A laser can mediate photothermal, photochemical, and photomechanical effects on cells.[41] The laser-mediated effects can be used to wound the cell monolayer, as shown in **Figure 1H**. In this method, the cells in the predefined wound region were eliminated by laser ablation based on the photomechanical effect.[42] The motion of the laser pulse and the microscopy observation can be computer controlled, and thus this automated method is suitable for multiwell assays with high efficiency. Other advantages of optical wounding methods include the ability to create repeatable wounds, create wounds with arbitrary shapes, and perform automated microscopic observation using the optical interface.

Discussion

Cell migration is an essential component of various pathological and physiological processes, including wound healing, cancer metastasis, embryonic development, and tissue regeneration. The requirements of collective cell migration studies may vary according to the biological question of interest. Thus, the selection of an appropriate cell migration assay depends on the various features, including wound geometry, surface properties, and other method limitations. These important criteria are summarized in **Figure 2**. When cell injury is involved, such as the wound-healing study, a scratch assay is commonly used. To examine the influence of cell injury at the boundary in the collective cell migration process, a scratch wound-healing assay can be performed in conjunction with noninjury assays, such as the physical barrier assay.[21,40] The scratch assay is easy to perform and cost-effective, but the result can vary between different laboratories. Other wound-healing assays such as stamp, laser, and electrical assays might be used when reproducibility and geometric control are major concerns. The geometry of the wounded region in these assays can be well defined and executed in a reproducible manner. Furthermore, the optical and physical barrier assays with proper surface modification provide useful approaches to investigate the roles of ECM proteins in the collective migration process.[33,42] When cells are introduced to a free region without injury, such as those in vitro applications dealing with tumor invasion and tissue regeneration, a barrier assay may eliminate the effect of cell injury.[43] This method is also useful for those research topics concerning the surface coating effects on collective cell migration. When the geometry of wounds is of interest, the solid barrier, optical wounding, and electrical blocker assays are suitable choices to control the wounding geometry. Nevertheless, a liquid barrier assay is a good choice where confluent cells need to migrate onto an intact substratum of endogenous ECM or purified matrix component, such as in embryonic development and in angiogenesis during tumor growth.[34,35] The electrical blocker wounding method can also be considered in this situation. Moreover, the microfluidic method will benefit the cell migration study involving shear stress and a chemical gradient, such as in angiogenesis and osteogenesis. The fluid in the microchannel exerts a shear stress on the cell monolayer, which mimics the in vivo condition. To better represent the physiological environment and study the role of the ECM, the bullseye method can be used to avoid rigid substrate for cell migration in vitro.

In addition to the biological considerations, the assay characteristics, such as the repeatability, throughput, and cost, should also be considered when selecting a wound-healing assay. Considering the throughput and repeatability, automated wound-healing assays are often good choices. For instance, the scratch assay can be performed automatically by incorporating robotic techniques to provide assays with high throughput and repeatability.[14] With the high resolution of lasers and the microfabrication of electrodes, the optical and electrical methods are automated assays with precise control and repeatability. For

the electrical assay, cell movement can be measured through the changes of the impedance of the cell-covered region. Multiple wounding assays can be performed and measured in parallel to increase the throughput. The cost of the assay should be taken into consideration. The scratch, blocker, and chemical assays are some of the cost-effective choices for research applications. Although the optical and electrical methods are generally more expensive, they can provide high resolution, high throughput, and flexibility. A general summary of these considerations is provided in **Figure 2**.

Future Perspective

Numerous ongoing research directions have been performed to develop novel wound-healing assays for studying collective cell migration. For instance, research has shown that cells can behave differently in 2D and 3D conditions. Research has shown that cells can behave differently in 2D and 3D conditions. To mimic the physiological condition of cells, researchers are developing 3D wound-healing assays. A wound-healing assay in 3D will significantly benefit the investigation of angiogenesis. In addition, the particle image velocimetry and other cell motion tracking methods should be further developed to analyze the characteristics of the collective cell migration process in a systematic manner. On the other hand, many mechanical and biochemical factors play important roles in the dynamics of collective cell migration, such as the stress among the cell in the monolayer, the intracellular gene expression, and the mechanical interaction between the cells and the substrate. Wound-healing assays with novel sensing and actuation modules should be developed to investigate these aspects of collective cell migration. In addition, the micro- and nano-techniques are versatile for manipulating the cell microenvironment.[44] The incorporation of novel micro- and nano-techniques in a wound-healing assay can potentially further enhance the resolution and controllability of wound-healing assays. For example, lithographic techniques, such as soft lithography and plasma lithography, can be used to pattern cell monolayers in defined geometry,[45] enabling researchers to study the geometric control of collective cell migration.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research and/or authorship of this article: R.R. is partially supported by the University of Arizona TRIF Imaging fellowship. This work is supported by the National Institutes of Health Director's New Innovator Award (1DP2OD007161-01), the National Science Foundation (0855890), and the James S. McDonnell Foundation.

References

1. Friedl, P.; Gilmour, D. Collective Cell Migration in Morphogenesis, Regeneration and Cancer. *Nat. Rev. Mol. Cell. Biol.* **2009**, *10*, 445–457.
2. Rorth, P. Collective Cell Migration. *Annu. Rev. Cell. Dev. Bi.* **2009**, *25*, 407–429.
3. Deisboeck, T. S.; Couzin, I. D. Collective Behavior in Cancer Cell Populations. *Bioessays* **2009**, *31*, 190–197.
4. Chen, H. C. Boyden Chamber Assay. *Methods Mol. Biol.* **2005**, *294*, 15–22.
5. Hasan, J.; Shnyder, S. D.; Bibby, M.; Double, J. A.; Bicknel, R.; Jayson, G. C. Quantitative Angiogenesis Assays In Vivo: A Review. *Angiogenesis* **2004**, *7*, 1–16.
6. Todaro, G. J.; Lazar, G. K.; Green, H. The Initiation of Cell Division in a Contact-Inhibited Mammalian Cell Line. *J. Cell. Comp. Physiol.* **1965**, *66*, 325–333.
7. Burk, R. R. Factor from a Transformed Cell Line That Affects Cell Migration. *Proc. Natl. Acad. Sci. U. S. A.* **1973**, *70*, 369–372.
8. Coomber, B. L.; Gotlieb, A. I. In Vitro Endothelial Wound Repair: Interaction of Cell Migration and Proliferation. *Arteriosclerosis* **1990**, *10*, 215–222.
9. Fronza, M.; Heinzmann, B.; Hamburger, M.; Laufer, S.; Merfort, I. Determination of the Wound Healing Effect of Calendula Extracts Using the Scratch Assay with 3T3 Fibroblasts. *J. Ethnopharmacol.* **2009**, *126*, 463–467.
10. Li, M.; Firth, J. D.; Putnins, E. E. An In Vitro Analysis of Mechanical Wounding-Induced Ligand-Independent KGFR Activation. *J. Dermatol. Sci.* **2009**, *53*, 182–191.
11. Matsubayashi, Y.; Razzell, W.; Martin, P. 'White Wave' Analysis of Epithelial Scratch Wound Healing Reveals How Cells Mobilise Back from the Leading Edge in a Myosin-II-Dependent Fashion. *J. Cell Sci.* **2011**, *124*, 1017–1021.
12. Yue, P. Y. K.; Leung, E. P. Y.; Mak, N. K.; Wong, R. N. S. A Simplified Method for Quantifying Cell Migration/Wound Healing in 96-Well Plates. *J. Biomol. Screen.* **2010**, *15*, 427–433.
13. Zhou, W.; Grandis, J. R.; Wells, A. STAT3 Is Required but Not Sufficient for EGF Receptor-Mediated Migration and Invasion of Human Prostate Carcinoma Cell Lines. *Br. J. Cancer* **2006**, *95*, 164–171.
14. Simpson, K. J.; Selfors, L. M.; Bui, J.; Reynolds, A.; Leake, D.; Khvorova, A.; Brugge, J. S. Identification of Genes That Regulate Epithelial Cell Migration Using an siRNA Screening Approach. *Nat. Cell. Biol.* **2008**, *10*, 1027–1038.
15. Daniel, T. O.; Liu, H.; Morrow, J. D.; Crews, B. C.; Marnett, L. J. Thromboxane A(2) Is a Mediator of Cyclooxygenase-2-Dependent Endothelial Migration and Angiogenesis. *Cancer Res.* **1999**, *59*, 4574–4577.
16. Kam, Y.; Guess, C.; Estrada, L.; Weidow, B.; Quaranta, V. A Novel Circular Invasion Assay Mimics In Vivo Invasive Behavior of Cancer Cell Lines and Distinguishes Single-Cell Motility In Vitro. *BMC Cancer* **2008**, *8*, 198.

17. Fong, E.; Tzlil, S.; Tirrell, D. A. Boundary Crossing in Epithelial Wound Healing. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 19302–19307.
18. Nikolic, D. L.; Boettiger, A. N.; Bar-Sagi, D.; Carbeck, J. D.; Shvartsman, S. Y. Role of Boundary Conditions in an Experimental Model of Epithelial Wound Healing. *Am. J. Physiol. Cell. Physiol.* **2006**, *291*, C68–C75.
19. Lee, J.; Wang, Y. L.; Ren, F.; Lele, T. P. Stamp Wound Assay for Studying Coupled Cell Migration and Cell Debris Clearance. *Langmuir* **2010**, *26*, 16672–16676.
20. Lan, R.; Geng, H.; Hwang, Y.; Mishra, P.; Skloss, W. L.; Sprague, E. A.; Saikumar, P.; Venkatachalam, M. A Novel Wounding Device Suitable for Quantitative Biochemical Analysis of Wound Healing and Regeneration of Cultured Epithelium. *Wound Repair Regen.* **2010**, *18*, 159–167.
21. van Horssen, R.; ten Hagen, T. L. M. Crossing Barriers: The New Dimension of 2D Cell Migration Assays. *J. Cell. Physiol.* **2011**, *226*, 288–290.
22. Pratt, B. M.; Harris, A. S.; Morrow, J. S.; Madri, J. A. Mechanisms of Cytoskeletal Regulation: Modulation of Aortic Endothelial Cell Spectrin by the Extracellular Matrix. *Am. J. Pathol.* **1984**, *117*, 349–354.
23. Iwazaki, R.; Watanabe, S.; Otaka, K.; Ota, K.; Ono, Y.; Sato, N. The Role of the Cytoskeleton in Migration and Proliferation of a Cultured Human Gastric Cancer Cell Line Using a New Metastasis Model. *Cancer Lett.* **1997**, *119*, 191–199.
24. Ohtaka, K.; Watanabe, S.; Iwazaki, R.; Hirose, M.; Sato, N. Role of Extracellular Matrix on Colonic Cancer Cell Migration and Proliferation. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 346–352.
25. Poujade, M.; Grasland-Mongrain, E.; Hertzog, A.; Jouanneau, J.; Chavrier, P.; Ladoux, B.; Buguin, A.; Silberzan, P. Collective Migration of an Epithelial Monolayer in Response to a Model Wound. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 15988–15993.
26. Kroening, S.; Stix, J.; Keller, C.; Streiff, C.; Goppelt-Struebe, M. Matrix-Independent Stimulation of Human Tubular Epithelial Cell Migration by Rho Kinase Inhibitors. *J. Cell. Physiol.* **2010**, *223*, 703–712.
27. Fischer, E. G.; Stingl, A.; Kirkpatrick, C. J. Migration Assay for Endothelial-Cells in Multiwells: Application to Studies on the Effect of Opioids. *J. Immunol. Methods* **1990**, *128*, 235–239.
28. van Horssen, R.; Galjart, N.; Rens, J. A. P.; Eggermont, A. M. M.; ten Hagen, T. L. M. Differential Effects of Matrix and Growth Factors on Endothelial and Fibroblast Motility: Application of a Modified Cell Migration Assay. *J Cell. Biochem.* **2006**, *99*, 1536–1552.
29. Walters, A.; Hulkower, K. I.; Gehler, S. Comparison of the Oris™ Cell Migration Assay to the Scratch Assay. http://www.platypustech.com/ApNote_Platypus_Oris_Scratch.pdf
30. Hui, E. E.; Bhatia, S. N. Micromechanical Control of Cell-Cell Interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 5722–5726.
31. Kaji, H.; Yokoi, T.; Kawashima, T.; Nishizawa, M. Controlled Cocultures of HeLa Cells and Human Umbilical Vein Endothelial Cells on Detachable Substrates. *Lab Chip* **2009**, *9*, 427–432.
32. Varani, J.; Orr, W.; Ward, P. A. A Comparison of the Migration Patterns of Normal and Malignant Cells in Two Assay Systems. *Am. J. Pathol.* **1978**, *90*, 159–172.
33. Doran, M. R.; Mills, R. J.; Parker, A. J.; Landman, K. A.; Cooper-White, J. J. A Cell Migration Device That Maintains a Defined Surface with No Cellular Damage during Wound Edge Generation. *Lab Chip* **2009**, *9*, 2364–2369.
34. Cai, G.; Lian, J.; Shapiro, S. S.; Beacham, D. A. Evaluation of Endothelial Cell Migration with a Novel In Vitro Assay System. *Methods Cell Sci.* **2000**, *22*, 107–114.
35. Vernon, R. B.; Gooden, M. D. New Technologies In Vitro for Analysis of Cell Movement on or within Collagen Gels. *Matrix Biol.* **2002**, *21*, 661–669.
36. Legrand, C.; Gilles, C.; Zahm, J. M.; Polette, M.; Buisson, A. C.; Kaplan, H.; Birembaut, P.; Tournier, J. M. Airway Epithelial Cell Migration Dynamics: MMP-9 Role in Cell–Extracellular Matrix Remodeling. *J. Cell. Biol.* **1999**, *146*, 517–529.
37. Nie, F.-Q.; Yamada, M.; Kobayashi, J.; Yamato, M.; Kikuchi, A.; Okano, T. On-Chip Cell Migration Assay Using Microfluidic Channels. *Biomaterials* **2007**, *28*, 4017–4022.
38. Conant, C. G.; Nevill, J. T.; Schwartz, M.; Ionescu-Zanetti, C. Wound Healing Assays in Well Plate–Coupled Microfluidic Devices with Controlled Parallel Flow. *J. Assoc. Lab. Automation* **2010**, *15*, 52–57.
39. Noiri, E.; Hu, Y.; Bahou, W. F.; Keese, C. R.; Giaever, I.; Goligorsky, M. S. Permissive Role of Nitric Oxide in Endothelin-Induced Migration of Endothelial Cells. *J. Biol. Chem.* **1997**, *272*, 1747–1752.
40. Keese, C. R.; Wegener, J.; Walker, S. R.; Giaever, I. Electrical Wound-Healing Assay for Cells In Vitro. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 1554–1559.
41. Koller, M. R.; Hanania, E. G.; Stevens, J.; Eisfeld, T. M.; Sasaki, G. C.; Fieck, A.; Palsson, B. Ø. High-Throughput Laser-Mediated In Situ Cell Purification with High Purity and Yield. *Cytometry* **2004**, *61A*, 153–161.
42. Zordan, M. D.; Mill, C. P.; Riese, D. J.; Leary, J. F. A High Throughput, Interactive Imaging, Bright-Field Wound Healing Assay. *Cytometry* **2011**, *79A*, 227–232.
43. Wang, L.; Zhu, J.; Deng, C.; Xing, W.-L.; Cheng, J. An Automatic and Quantitative On-Chip Cell Migration Assay Using Self-Assembled Monolayers Combined with Real-Time Cellular Impedance Sensing. *Lab Chip* **2008**, *8*, 872–878.
44. Kim, D.-H.; Wong, P. K.; Park, J.; Levchenko, A.; Sun, Y. Microengineered Platforms for Cell Mechanobiology. *Annu. Rev. Biomed. Eng.* **2009**, *11*, 203–233.
45. Junkin, M.; Wong, P. K. Probing Cell Migration in Confined Environments by Plasma Lithography. *Biomaterials* **2011**, *32*, 1848–1855.