

Video Article

A Customizable Chamber for Measuring Cell Migration

Aniqa N. Chowdhury¹, Huu Tri Vo¹, Sharon Olang¹, Elliott Mappus¹, Brian Peterson¹, Nora Hlavac¹, Tyler Harvey¹, Delphine Dean¹

¹Department of Bioengineering, Clemson University

Correspondence to: Delphine Dean at finou@clemson.edu

URL: <https://www.jove.com/video/55264>

DOI: [doi:10.3791/55264](https://doi.org/10.3791/55264)

Keywords: Bioengineering, Issue 121, cell migration, chemoattractants, concentration gradient, diffusion, Boyden chamber assay, microchannel

Date Published: 3/12/2017

Citation: Chowdhury, A.N., Vo, H.T., Olang, S., Mappus, E., Peterson, B., Hlavac, N., Harvey, T., Dean, D. A Customizable Chamber for Measuring Cell Migration. *J. Vis. Exp.* (121), e55264, doi:10.3791/55264 (2017).

Abstract

Cell migration is a vital part of immune responses, growth, and wound healing. Cell migration is a complex process that involves interactions between cells, the extracellular matrix, and soluble and non-soluble chemical factors (e.g., chemoattractants). Standard methods for measuring the migration of cells, such as the Boyden chamber assay, work by counting cells on either side of a divider. These techniques are easy to use; however, they offer little geometric modification for different applications. In contrast, microfluidic devices can be used to observe cell migration with customizable concentration gradients of soluble factors^{1,2}. However, methods for making microfluidics based assays can be difficult to learn.

Here, we describe an easy method for creating cell culture chambers to measure cell migration in response to chemical concentration gradients. Our cell migration chamber method can create different linear concentration gradients in order to study cell migration for a variety of applications. This method is relatively easy to use and is typically performed by undergraduate students.

The microchannel chamber was created by placing an acrylic insert in the shape of the final microchannel chamber well into a Petri dish. After this, poly(dimethylsiloxane) (PDMS) was poured on top of the insert. The PDMS was allowed to harden and then the insert was removed. This allowed for the creation of wells in any desired shape or size. Cells may be subsequently added to the microchannel chamber, and soluble agents can be added to one of the wells by soaking an agarose block in the desired agent. The agarose block is added to one of the wells, and time-lapse images can be taken of the microchannel chamber in order to quantify cell migration. Variations to this method can be made for a given application, making this method highly customizable.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55264/>

Introduction

In order for vital processes such as wound healing, immune responses, and embryonic development to occur, cell migration must take place. Cell migration involves the interaction between cells and neighboring cells, the extracellular matrix, and soluble chemical cues (attractants or repellants). As an example, in the process of wound healing, fibroblasts play an integral role in fibrogenesis and wound contraction, where the cells are recruited to the site of injury to synthesize collagen in order to form the extracellular matrix³. Numerous mechanisms behind the migration of fibroblasts to a wound site have been studied, and they include different mechanical, physical, electrical, and chemotactic factors⁴. Fibroblasts respond especially well to different concentration gradients of growth factors. These different growth factors work together to optimize tissue regeneration⁵. While observing the chemotactic response of fibroblasts to growth factor concentrations, one can study the pattern of directional migration of fibroblasts and how they orient themselves around physical obstacles in order to reach their destination. Therefore, the goals of this study were to first develop a system in which fibroblast growth could be tracked under guidance by physical barriers and to secondly model the growth of fibroblasts as they navigate through the system.

Currently, the Boyden chamber assay is the most widely used system to measure the migration of cells⁶. The Boyden chamber consists of a two-chamber multi-well plate where each well may contain medium with or without chemoattractants⁷. A filter membrane provides a porous interface between the two chambers in each well; this creates a barrier so that cells cannot pass through unless it is by active migration. Typically, for the Boyden chamber, a chemoattractant is added to the lower chamber, and the system is allowed to equilibrate to form a gradient between the upper and lower wells⁴. One problem with the Boyden chamber assay is that steep gradients end up forming along a single axis perpendicular with the surface of the membrane. This causes the difference in the chemoattractant concentration between the upper and lower wells to be a lower than what was originally expected. Due to this constraint, the Boyden chamber assay makes it hard to correlate specific cell responses with particular gradient characteristics, such as the slope and the concentration difference. Without these measurements, it is hard to study multi-gradient signal integration.

To address some of the constraints of the traditional Boyden chamber assay, microfluidic assays have been developed to form customizable concentration gradients¹. Standard methods for creating microfluidic systems require clean rooms for lithographic techniques. These techniques can be difficult to learn especially in a standard classroom setting. Thus, we have designed a chamber system for measuring cell migration that can be made without using a clean room. Using our system, the wells of the assay can be adjusted to a preferred size, and a linear concentration

gradient of customized slope can be produced. This allows for accurate measurement of chemotaxis from random movement. The design is an inexpensive and easy-to-use system to model cell growth in response to different chemical stimuli.

Protocol

1. Producing a Microchannel Chamber to Create a Concentration Gradient

1. Cutting an acrylic mold piece

1. Obtain a piece of acrylic of the desired width. Typically use 1/16-inch-thick acrylic sheets. Thicker sheets are difficult to cut well and very thin sheets do not have the requisite mechanical strength, causing them to break or warp during the process.
 2. Create a CAD file with the desired shape of the acrylic piece to produce a cavity within the polydimethylsiloxane (PDMS). Adjust the channel width and length to achieve the desired gradient relevant to individual experimental protocols.
 1. Here, use an acrylic piece of the following dimensions: two squares (0.254 cm x 0.254 cm) connected by a 0.127 cm wide channel (0.254 cm long) (**Figure 1**).
 3. Import the CAD file into the laser cutting apparatus and place the acrylic piece in a laser cutter according to the manufacturer's protocol.
- NOTE: Alternatively, 3D-print the piece from the CAD design. The advantage of this method is that alternate materials can be used for the mold; however, the resolution and reproducibility can be decreased with 3D printing compared to laser cutting.

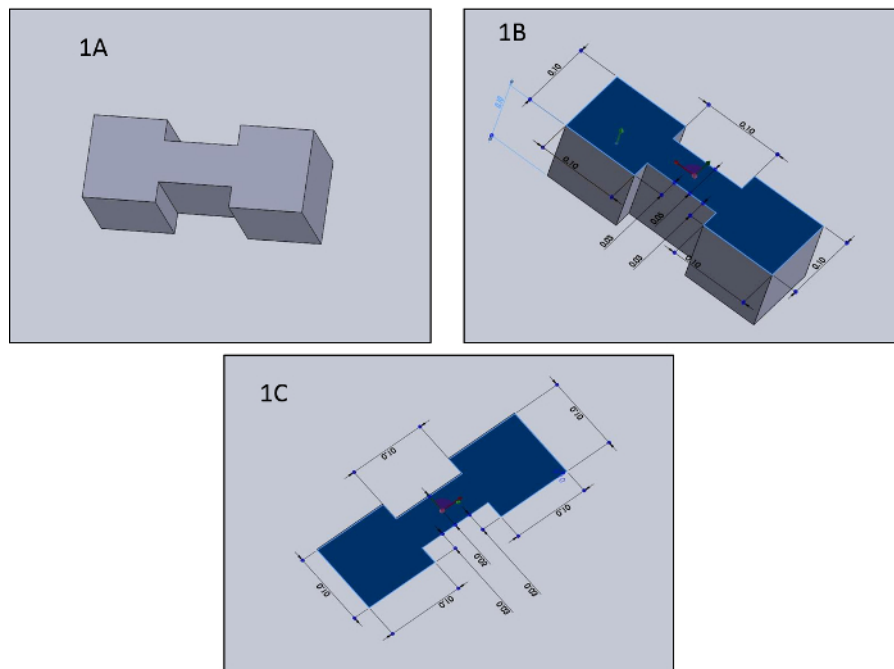


Figure 1: Computer-Aided Design (CAD) Representation of Microchannel Chamber. This image depicts the CAD drawing of the acrylic insert needed to create the microchannel chamber. **1A)** Top view of CAD drawing, **1B)** Side view of CAD drawing with dimensions in inches, **1C)** Top view of CAD drawing with dimensions in inches [Please click here to view a larger version of this figure.](#)

2. Pouring the polydimethylsiloxane (PDMS)

1. In a weigh boat, mix 10 parts by weight of commercial Elastomer Base (e.g. Sylgard 184) with 1 part by weight of commercial Elastomer Curing Agent (e.g. Sylgard 194). Typically, mix 10 g of Elastomer Base to 1 g of Elastomer Curing Agent.
2. Stir to combine the base and curing agent with a micropipette for 5 min.
3. Set up a vacuum bell and place the weigh boat with PDMS in the vacuum for 30 min to remove trapped air bubbles.
4. Turn off the vacuum and remove the weigh boat. Pour the PDMS on top of the acrylic cut-out in a small Petri dish. Make sure that the PDMS completely covers the insert.
5. Allow the PDMS to cure overnight (at least 18 h) at room temperature.

3. Removing the insert from PDMS

1. After curing the PDMS, remove the insert by carefully cutting the PDMS around the acrylic piece with a scalpel.

2. Plating the Cells in a Microchannel Chamber

1. Sterilizing the chamber for plating cells.

NOTE: While PDMS can be sterilized using ethylene oxide or other techniques, a quick UV treatment is fast, inexpensive, and sufficient for cell culture studies. The short UV treatment duration did not impair the structure or mechanical integrity of the PDMS piece.

1. In a standard cell culture cabinet, completely cover the chamber with 70% ethanol.
2. Put the Petri dish in laminar flow hood with the lids off.
3. Shut the hood and turn on the UV light. Expose to UV light for 1-2 h.
4. Open the laminar flow hood and wait 15 min to reestablish flow.
5. Remove the 70% ethanol.
6. Wash the chambers twice with sterile phosphate buffered saline (PBS). Use 1 mL of PBS for each wash. Remove PBS and allow chambers to dry overnight with the lids off.

NOTE: Alternatively, instead of using a laminar flow hood, use a UV chamber box for sterilization if one is available. Make a UV box by placing a UV light source in the back of a cardboard box lined with aluminum foil. The aluminum foil reflects the light to allow for even illumination of the sample.

2. Plating Cells in Chamber

1. Count cells using Trypan blue and a hemocytometer.
 1. Add 100 μ L of the cell suspension to 400 μ L of 0.4% Trypan blue and mix gently. Apply 100 μ L of this solution to the hemocytometer by gently filling both chambers under the glass coverslip.
 2. Use a microscope with a 10X objective to focus on the grid on the hemocytometer. Use a hand tally counter to count the live unstained cells within one set of 16 squares on the hemocytometer.
 3. Count cells in all 4 sets of 16 squares. Take the average cell count from the 4 sets of 16 squares and multiply by 5×10^4 . This final number is the number of cells/mL in the cell suspension.
2. Add 2,500 cells to one well in each chamber. This translates to a near-confluent cell density of $\sim 30,000/\text{cm}^2$.
3. Allow cells to sit in the chamber for 60 min before adding media (Dulbecco's Modified Eagle's Medium, 10% fetal bovine serum, 1% penicillin-streptomycin).

3. Dextran Soaked Agarose Blocks

1. Creating an agarose block

1. Pour 3% agarose into the 3D printed mold (2 mm x 2 mm x 2 mm).
2. Allow the agarose to solidify in a vacuum chamber for 30 min to remove any bubbles.
3. Remove the agarose block from the mold.

NOTE: Alternatively, pour 3% agarose into small Petri dish at a thickness of 2 mm. Cut a 2 mm x 2 mm x 2 mm block using a scalpel or other cutting tool. This is not as precise as the mold system but it can be a little easier to do.

2. Completely submerge the agarose block in a solution of the desired concentration of the chemotactic factor. To assess the concentration gradient formation, flush the chamber first with fluorescent dextran. The concentration and molecular weight of dextran should match that of a soluble factor or drug of interest.
3. Soak the agarose block overnight.

4. Time-lapse of the Dextran Diffusion to Assess the Soluble Factor Concentration Gradient

1. Place the dextran soaked agarose block in a small square well of the microchannel chamber.
2. Place the microchannel chamber in a cell imaging system or use another fluorescent microscope with a time-lapse capability. Begin the time-lapse according to manufacturer's instructions.

NOTE: Results shown are taken with GFP filter, 50% light, 4/10 pH, and 4X magnification.

5. Time-lapse of Cell Growth

1. Plate cells in the microchannel chamber at one end of the chamber. Here, use 3T3 fibroblasts. Add 2,500 cells to one well in the microchannel chamber. Count cells as described in 2.2.1.
 1. Allow cells to sit in the chamber for 60 min before adding media (Dulbecco's Modified Eagle's Medium, 10% fetal bovine serum, 1% penicillin-streptomycin). Keep the microchannel chamber with plated cells at 37 °C in 5% CO₂. Cell migration through the channel can be viewed with a microscope.
 2. Add a marker or use a microscopic defect in the chamber as a marker in order to serve as a starting place to quantify the distance the cell front moves.
2. Establish a gradient by placing an agarose gel (8 mm³ block) containing the concentrated growth factor, chemo-attractant, drug, or other factor being tested (here, 40% FBS is being used as an example) at one end of the microchannel chamber.
3. Take time-lapse images of the moving cell front. Here, the results show images of the cell front that were taken every 12 h using an imaging system.
4. Use pictures of the cell front to quantify cell growth rate. Calculate the growth rate by first using the MATLAB polyfit function (roipoly) to create a polygonal mask defined by the cell front, the channel walls, and the reference marker. The dimensions of this mask yield the migration distance of the front from which average cell front velocity is calculated. Other studies have utilized a similar method⁹.

NOTE: Compare the edge of the cell growth front on each frame to the concentration of the soluble factor at that same distance. The soluble factor concentration gradient is calculated using a 1D diffusion model approximation.
5. Take fluorescent images of the cells using Phalloidin and DAPI staining.
 1. Fix cells before staining with Phalloidin.

1. Warm 4% paraformaldehyde at 37 °C.
2. Add enough 4% paraformaldehyde to cover cells. Keep cells in the paraformaldehyde for 10 min.
3. Rinse twice with PBS for 15 min each time. Use enough PBS to cover the cells.
2. Remove PBS from cells and add enough permeabilizing solution (0.1% Triton X-100 in PBS) to cover cells. Leave solution for 10 min.
3. Wash twice with PBS for 5 min each time. Use enough PBS to cover the cells.
4. Remove PBS from cells and add blocking solution (1% bovine serum albumin in PBS) for 20 min.
5. Remove blocking solution and wash 2 times with PBS for 5 min each time. Use enough PBS to cover the cells.
6. From this point, protect cells from light with aluminum foil when not being used. Remove PBS from cells and add Phalloidin 488 diluted 1:50 in PBS for 1 h.
7. Wash 2 times in PBS for 5 min for each wash. Use enough PBS to cover the cells. Then remove PBS from cells.
8. Add DAPI (4',6-diamidino-2-phenylindole) diluted 1:1,000 in PBS to cells for 15 min.
9. Remove DAPI and wash cells twice with PBS for 5 min for each wash.
10. Remove last wash and add enough PBS to cover cells. Cells can now be imaged with a fluorescence microscope.

Representative Results

Figure 2 shows the movement of the cell front across the channel in response to a gradient of fetal bovine serum placed at the opposite end of the channel from where the cells are plated. The cell front is shown at 48 h (**Figure 2A**), 72 h (**Figure 2B**), 96 h (**Figure 2C**), and 120 h (**Figure 2D**) post plating. The movement of the cell front was tracked with these time-lapse images, and the migration distance and growth rate were calculated by the MATLAB polyfit function. From this, the average cell front velocity was found to be 13.4 $\mu\text{m}/\text{h}$ (**Figure 3**). **Figure 4** shows the fluorescent gradient established by placing a dextran soaked agarose block at one end of the channel and allowing dextran to diffuse through the channel for 12 h. A time-lapse image was taken at every hour, and the fluorescence across the distance of the channel was measured and plotted as shown in **Figure 4** and compared to a 1D diffusion model.

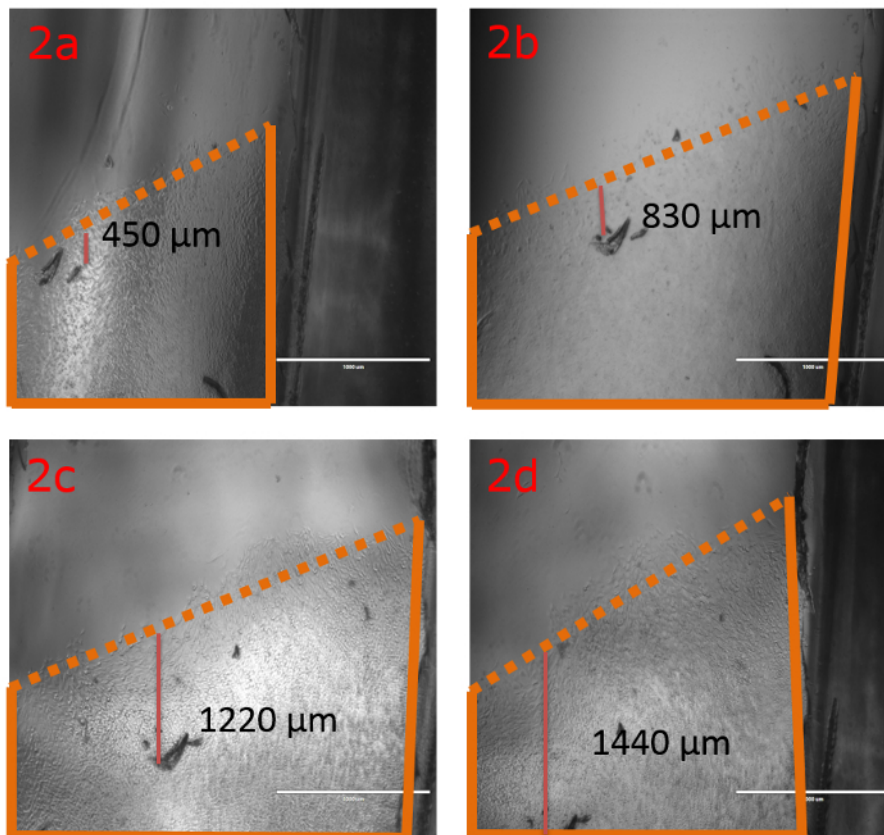


Figure 2: Cell Front Movement. Time-lapse images of the cell front in the microchannel chamber moving through the channel. The cell front marked in orange lines, and the migration distance is included. This migration distance was measured from the marking on the plate to the front of the cell front as shown. A 1 mm scale bar is shown in white. **A)** 48 h post plating, **B)** 72 h post plating, **C)** 96 h post plating, **D)** 120 h post plating. [Please click here to view a larger version of this figure.](#)

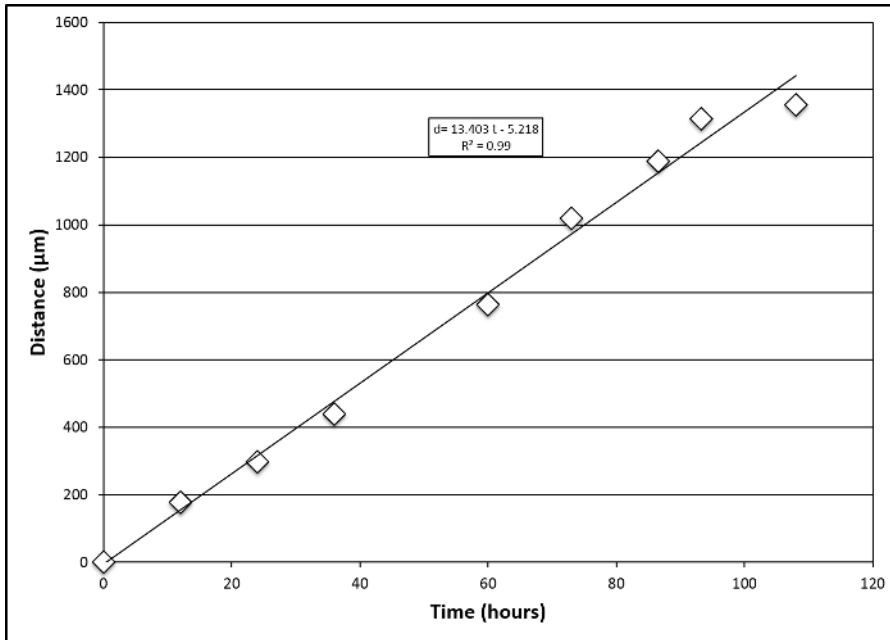


Figure 3: Growth Rate Calculation. Cell front movement tracked with time-lapse pictures. Growth rate was calculated using the MATLAB polyfit function (roipoly) to yield average cell front velocity of 13.4 $\mu\text{m}/\text{h}$. [Please click here to view a larger version of this figure.](#)

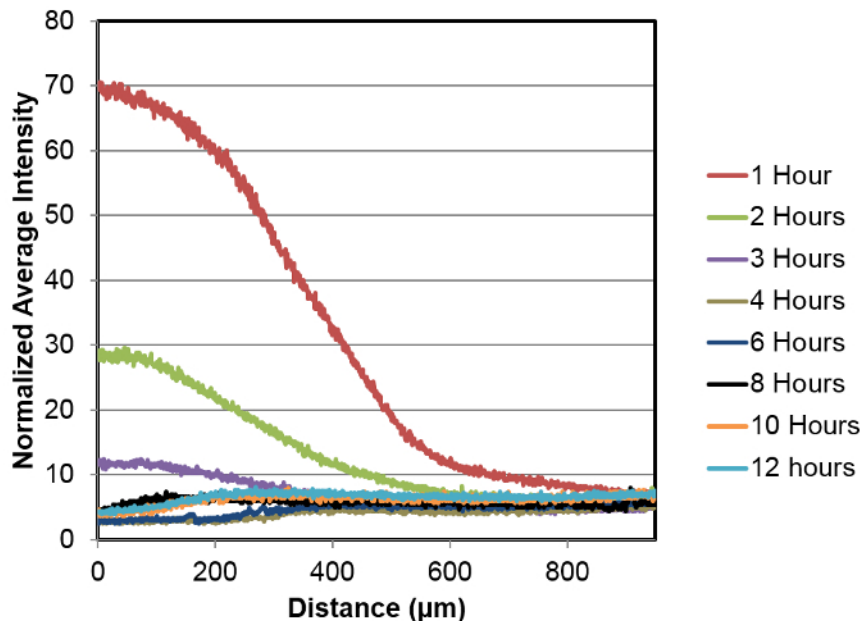


Figure 4: Fluorescent Gradient with Dextran. Fluorescent intensity measured across the distance of the channel where each line represents the gradient at a particular hour. ImageJ was used to calculate the fluorescence intensity. This can be done using the Analyze | Measure tool. First, choose Set Measurements under Analyze and select intensity. Then, choose Measure under Analyze in order to get average pixel intensity. This can be plotted versus distance in microns as shown in this figure. [Please click here to view a larger version of this figure.](#)

Discussion

Our microchannel chamber may be used for a multitude of purposes, including determining the cell migration rate in response to growth factors and chemoattractants and measuring the diffusion rate of a drug from a polymer matrix. It is possible to utilize our microchannel chamber to grow cells and place a chemoattractant at one end of the chamber. The cells grow in response to the chemoattractant, and the cell migration can be quantified by taking time-lapse images that may be analyzed in order to determine the cell migration rate. Representative results of this method are shown in **Figure 3**, where the growth rate was determined to be 13.4 $\mu\text{m}/\text{h}$ using the MATLAB polyfit function.

Another usage of our microchannel chamber is to measure the diffusion rate of a drug out of a polymer matrix. A fluorescently tagged dextran of a similar molecular weight to the drug of interest may be used to model the diffusion of the drug of interest. It is important to note that the

samples must be handled carefully as a large amount of convection will disrupt the gradient. The representative results of this method are shown in **Figure 4**. An advantage of this model in comparison to the Boyden chamber assay is that it is more applicable to adherent cell types because of the flat surface; whereas, the cells must fall through a small tube in the Boyden chamber before adhering. Another limitation of the Boyden chamber assay is that it is an endpoint assay; thus, chemotaxis cannot be visually observed. In contrast, with our method, chemotaxis is observed through the time-lapse images⁹. The use of micropipettes containing chemoattractants have also been used a method of observing cell migration. However, the main drawback of this method is low throughput¹⁰. The Dunn chemotaxis chamber is another assay that is used in conjunction with time-lapse imaging like the method detailed here^{11,12}. The chamber consists of two concentric circles carved on the face of the glass slide in order to create an inner and outer well, and a bridge separates the two wells. The chemoattractant is placed in the outer well, and the cells are allowed to migrate from the inner well to the outer well. This migration is quantified using time-lapse images that are analyzed using image analysis software. Similar to the Boyden assay, one of the limitations of the Dunn chamber assay is that it cannot be easily modified to create custom soluble factor concentration gradients.

Another simple technique for assessing cell migration is an *in vitro* scratch¹³. This method is fast and inexpensive as it only requires creating a scratch in the cell monolayer and capturing of time-lapse images of cell migration close to the previously created scratch. However, the major disadvantage of this method is that it is not suitable to measure responses to a chemical factor concentration gradient. Our method offers the potential to create a chemical gradient and to quantify cell migration relative to the chemical gradient. Our method may be combined with the scratch method as a scratch may be created in the cell monolayer, while a chemotactic factor is present in one of the wells in the microchannel chamber. This allows for the quantification and modeling of wound healing. Another potential application of our device is to determine the response of cancer cells to agents that inhibit cell growth.

Other microfluidics based systems have also been used for cell migration studies^{14,15}. These studies utilize lithographic techniques with clean rooms to create master molds in silicon and/or laser machining to create microfluidic channels in silicon wafers. Silicon wafer micro machining can be expensive in comparison to our method that utilizes cheaper materials (PDMS and acrylic) to manufacture a microchannel chamber. Our device overcomes the limitation of other PDMS based microfluidic devices in which it was not possible to create small 3D channels¹⁵. With our method, we have successfully created PDMS based device with small diameter channels. Other studies have used a PDMS-based microfluidic device with two layers of microchannels and a multi-channel gas mixer that is computer controlled in order to create arbitrary gradients of oxygen to study the migration of cells under different gradients of oxygen¹⁶. There is potential for our device to be used in a similar fashion with the addition of a gas mixing chamber at one end of the microchannel chamber.

Microfluidic devices are often made using soft lithographic techniques; these can be adapted to create cell migration chambers. These methods involve printing a mask with channel patterns with computer-aided design (CAD). These masks are then projected on a master mold coated with a photosensitive resist through optical lithography. After the master mold is made, PDMS is poured on the master and allowed to harden in order to create a PDMS mold that can be used for microfluidic studies^{17,18,19}. Chemoattractant or chemorepulsive compounds are placed in the chamber in high concentration in one of the reservoirs to create gradients¹⁷. While these techniques are similar to our set up, they can be expensive due to the high cost of creating the master mold for the system and the use of clean rooms. In addition, these methods can be difficult for students to learn in a class based setting. The inserts used in our set-up to create the channels can be created with a variety of methods, including 3D printing and laser cutting. Thus, our system allows for the creation of a wide variety of molds for relatively low cost particularly as 3D printing becomes more affordable and widely available.

PDMS based microfluidic devices for measuring cell migration and growth have been used for a wide range of applications. For instance, in neuroscience research studies²⁰, devices were fabricated using soft lithography techniques in which the PDMS component was placed on a tissue culture dish or glass in order to form two compartments that were separated by a physical barrier with micron-sized grooves to allow neurons to grow across the compartments. Time-lapse images were taken to show neurons crossing the barrier. The method of micropatterning used to produce this device is complex, while the production method for our device is simple and can be used by researchers at all levels. Another study also used an easy low-cost method for producing a similar PDMS microfluidic device using a master mold created from scotch tape in order to create an impression in the PDMS to produce microchannels. An advantage of our approach over the one described in this method is that the channels in our method are fabricated in one step, while this protocol requires adhesion between two PDMS layers which creates problems with medium flow to the cells²¹.

In conclusion, our microchannel chamber method is customizable and may be used to obtain different results. The method allows for the creation of a concentration gradient in order to suit the user's needs. Furthermore, our device offers an advantage over existing devices due to its relatively low cost and ease of use for researchers at all levels.

Disclosures

The authors have nothing to disclose.

Acknowledgements

The authors acknowledge Clemson University's Creative Inquiry program and NSF CBET1254609 for providing funding for this project.

References

1. Sia, S. K., & Whitesides, G. M. Microfluidic devices fabricated in poly (dimethylsiloxane) for biological studies. *Electrophoresis*. **24** (21), 3563-3576 (2003).
2. Lin, F., et al. Generation of dynamic temporal and spatial concentration gradients using microfluidic devices. *Lab Chip*. **4**(3), 164-167 (2004).
3. Darby, I., & Hewitson, T. Fibroblast Differentiation in Wound Healing and Fibrosis. *Int Rev Cytol*. **257**, 143-179 (2007).
4. Thampatty, B. P., & Wang, J. H.-C. A new approach to study fibroblast migration. *Cell Motil Cytoskel*. **64**, 1-5 (2007).

5. Discher, D., Mooney, D., and Zandstra, P. Growth Factors, Matrices, and Forces Combine and Control Stem Cells. *Science*. **324**, 1673-1677 (2009).
6. Zengel, P., *et al.* μ -Slide Chemotaxis: A new chamber for long-term chemotaxis studies. *BMC Cell Biol.* (2011).
7. Chen, H. Boyden Chamber Assay. *Methods Molec Biol.* **2**, 15-22 (2005).
8. Safferling, K., *et al.* Wound healing revised: A novel reepithelialization mechanism revealed by in vitro and in silico models. *JCB*. **203** (4), 691-709 (2013).
9. Saadi, W., *et al.* Generation of stable concentration gradients in 2D and 3D environments using a microfluidic ladder chamber. *Biomed Microdevices*. **9**, 627-635 (2007).
10. Cammer, M., & Cox, D. Chemotactic Responses by Macrophages to a Directional Source of a Cytokine Delivered by a Micropipette. *Methods Mol Biol.* 125-135 (2014).
11. Zicha, D., Dunn, G. A., & Brown, A. F. A new direct-viewing chemotaxis chamber. *J Cell Sci.* **99**(4), 769-775 (1991).
12. Wells, C. M., & Ridley, A. J. Analysis of cell migration using the Dunn chemotaxis chamber and time-lapse microscopy. *Methods Mol Biol.*, 31-41 (2005).
13. Liang, C.C., Park, A.Y., Guan, J.L. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat. Protoc.* **2**, 329-333 (2007).
14. Chen, Y. C., *et al.* Single-cell migration chip for chemotaxis-based microfluidic selection of heterogeneous cell populations. *Sci. Rep.* **5** (2015).
15. Wright, G. A., *et al.* On-chip open microfluidic devices for chemotaxis studies. *Microsc. Microanal.* **18**(04), 816-828, .doi:10.1017/S1431927612000475 (2012).
16. Adler, M., Polinkovsky, M., Gutierrez, E., & Groisman, A. Generation of oxygen gradients with arbitrary shapes in a microfluidic device. *Lab Chip.*, **10** (3), 388-391 (2010).
17. Huang, Y., Agrawal, B., Sun, D., Kuo, J. S., & Williams, J. C. Microfluidics-based devices: New tools for studying cancer and cancer stem cell migration. *Biomicrofluidics*. **5**(1), 013412, .doi:10.1063/1.3555195 (2011).
18. Taylor, A. M., Rhee, S. W., & Jeon, N. L. Microfluidic chambers for cell migration and neuroscience research. *Microfluid Tech: Rev Protoc.* 167-177 (2006).
19. Tang, S. K., & Whitesides, G. M. Basic microfluidic and soft lithographic techniques. *Optofluidics: Fundamentals, Devices and Applications*. Y. Fainman, L. Lee, D. Psaltis, and C. Yang, eds. (McGraw-Hill, 2010), (2009).
20. Taylor, A. M., *et al.* Microfluidic multicompartiment device for neuroscience research. *Langmuir.*, **19** (5), 1551-1556 (2003).
21. Aidelberg, G., Goldshmidt, Y., & Nachman, I. A Microfluidic Device for Studying Multiple Distinct Strains. *JoVE*. (69) (2012).