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Measuring spontaneous neutrophil motility signatures from a drop of blood using microfluidics

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Abstract

Neutrophils play an essential role in the protection against infection, as they are the most numerous circulating white blood cell population and the first responders to injury. Their numbers in blood are frequently measured in the clinic and used as an indicator of ongoing infections. During inflammation and sepsis, the ability of neutrophils to migrate is disrupted, which may increase the risk of infection, even when the neutrophil count is normal. However, measurements of neutrophil migration in patients are rarely performed because of the challenges of performing the migration assays in a clinical setting. Here, we describe a microfluidic assay that measures the spontaneous neutrophil migration signatures associated with sepsis. The assay uses one droplet of patient's blood in a microfluidic device, which circumvents the need for neutrophil isolation from blood. This assay may also be useful for the study of the effect of various immune modulators on neutrophil migration behavior from healthy volunteers and patients.

1 INTRODUCTION

The study of innate immune responses in patients, particularly those of neutrophils, is under increasing investigation. Neutrophils constitute the major circulating white blood cell population and are the first responders to tissue injury. Neutrophil count is part of the standard blood analysis in clinical laboratories. Neutropenia is an abnormally low neutrophil count. It can be inherited or acquired and increases susceptibility to bacterial or fungal infections and impairs the resolution of these infections. Neutrophilia, on the other hand, is an increased number of neutrophils, most often caused by ongoing inflammatory and infectious diseases. In some cases, such as burn injuries (Lavrentieva et al., 2007) the neutrophil count alone is not an accurate indicator of immune status. Recently, neutrophil functional competence has been proposed as more valuable in a broad range of conditions, such as sepsis.

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). If not diagnosed early and managed promptly, sepsis can lead to shock, multiple organ failure, and death. Despite advances in healthcare, existing epidemiologic studies suggest that sepsis remains a considerable burden which affects more than 30 million people worldwide every year (Fleischmann et al., 2016) and is increasing at

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a rate of 1.5% annually (Angus & Van der Poll, 2013). In addition to its high mortality rate, this global crisis places a significant clinical and economic burden on the healthcare system. Sepsis is the leading cause of hospitalization in the US costing more than \$23 billion each year (Torio & Moore, 2016).

Early diagnosis of sepsis is critical, as mortality is decreased by 7.6% per hour when antibiotics are administered early to patients (Kumar et al., 2006). However, early diagnosis remains a challenge as the pathophysiology of sepsis is complex and remains incompletely understood. There are several biomarkers of sepsis, including C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 and reactive oxygen species but none proved to be reliable and clinically effective (reviewed by Liu et al., 2016). A major obstacle to accurate diagnosis of sepsis is the differentiation between sepsis and systemic inflammatory response syndrome (SIRS).

In the context of sepsis, neutrophil dysfunction likely contributes to both weak immune responses and off-target organ damage (Brown et al., 2006). Neutrophils from septic patients lose the ability to respond appropriately to chemotactic signals (Jones et al., 2014) and have altered antimicrobial activity (Solomkin, 1990).

Neutrophil migration has been monitored in several studies as a potential indicator of inflammatory status or infections (Hoang et al., 2013). However, standard migration assays such as Boyden chambers (Boyden, 1962), Dunn and Zigmond chambers (Zicha et al., 1991; Zigmond, 1977) and micropipette techniques (Gerisch & Keller, 1981) have significant limitations in quantifying the dynamic nature of the migration process. Moreover, these assays are not adequate for a clinical setting as they are time-consuming and require large volumes of blood. The development of microfluidic-based assays compatible with direct patient blood analysis is addressing many of these limitations and is increasing the accuracy of the measurements. A recent study measured the CD64 expression by neutrophils in blood from 450 patients, using a microfluidic device that enabled the prediction of patient sepsis prognosis (Hassan et al., 2017). In another study, a microfluidic assay using a P-selectin-coated surface was developed to purify neutrophils from whole blood and study chemotaxis (Sackmann et al., 2012). Moreover, the direct measurement of neutrophil motility in a droplet of blood, in the presence of other blood cells and serum factors, without the requirement for any neutrophil purification, has also been described (Ellett et al., 2018). Direct characterization of neutrophil migration using microfluidic devices can be applied to multiple donor species, and a recent study has revealed significant differences among migration counts, velocity, and directionality among neutrophils from mice, rats, and humans (Jones et al., 2016).

Our group has previously developed a microfluidic device that identified a sepsis-specific spontaneous migration signature displayed by isolated neutrophils originating from septic patients, which enabled the prediction of septic patients with 80% sensitivity and 77% specificity (Jones et al., 2014). Inspired by these results, our group has recently engineered a microfluidic device that measures, from one droplet of diluted blood, the spontaneous migration behaviors of neutrophils in the context of sepsis (Ellett et al., 2018). From 42

patients, the use of whole blood increased the performance of the assay to 97% sensitivity and 98% specificity for sepsis.

In this chapter, we describe in detail the fabrication steps of this microfluidic device, the experimental and analytical procedures used to investigate septic patient blood samples. We also employ the device to test the effect of various immune modulators on the spontaneous neutrophil migration behavior from healthy volunteers.

2 MICROFLUIDIC DEVICE

Here we describe the fabrication and preparation of a microfluidic assay that enables measurement of spontaneous neutrophil motility from a diluted blood sample. This method can be used to investigate and diagnose patient blood such as septic patients (Ellett et al., 2018) or study the effect of various immune modulators on spontaneous neutrophil migration behavior by spiking whole blood from healthy volunteers.

This protocol is dedicated to the measurement of spontaneous neutrophil migration only, as no chemotactic gradient is generated within these devices prior sample loading.

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Each microfluidic device is composed of eight migration mazes, two on each side of a square whole blood-loading chamber. Each neutrophil migration maze is composed of a red blood cell (RBC) filter, a series of migration channels and a maze (Fig. 1).

2.1 MICROFLUIDIC DEVICE FABRICATION

1. Design a device using AutoCAD and print chrome masks for photolithography.
2. Fabricate the master mold on a silicon wafer, in a clean room, using standard photolithographic techniques. Spin-coat the silicon wafer, with a first 4- μ m thick epoxy-based negative photoresist layer (SU-8, Microchem, Newton, MA) to define the migration channels and a second 45- μ m epoxy layer to define the whole blood-loading chamber.
3. Pattern the wafer by sequential ultraviolet light exposure through two photolithographic masks and process according to the manufacturer's instructions.
4. Use the patterned wafer as a mold to cast polydimethylsiloxane (PDMS, Sylgard 184, Ellsworth Adhesives, Wilmington, MA) device. Mix vigorously PDMS (20g) with curing agent (2g) and pour carefully onto mold.
5. Use a vacuum desiccator for at least 1h to degas PDMS.
6. Bake and cure PDMS microfluidic device for at least 12h in an oven set to 75°C.
7. Cut the PDMS from the master mold.

8. Punch the delivery port using a 1.2-mm punch (Harris Uni-core, Ted Pella).
9. Punch the entire device out of the PDMS using a 5-mm puncher (Harris Uni-core, Ted Pella).
10. Remove particles from device surfaces using adhesive tape.
11. Oxygen plasma treat a 35-mm glass-bottom multiwell plate (P06G-1.5–20-F, MatTeK Co., Ashland, MA) or a single small glass-bottom dish (P35G-0–20-C, MatTeK Co., Ashland, MA) twice; once alone for 35s and then again, along with the devices (face up) for another 35s.
12. Using tweezers, bond devices by carefully placing them face down on the glass-bottom multiwell plate or Petri dish. Apply slight pressure on the device to evacuate any air trapped between the device and the glass.
13. Bake plate with bonded devices on a hot plate set to 75°C for 10min.

Note: Devices bonded on glass-bottom multiwell plates (e.g., 6-well plates) can be used in a confocal microscope and standard inverted fluorescent microscope. The devices bonded on glass-bottom small Petri dishes are used for the CytoSMART imaging system (Fig. 2).

2.2 MICROFLUIDIC ASSAY PREPARATION

1. Prime devices with 50µL Iscove's modified Dulbecco's medium (IMDM, ThermoFisher Scientific) containing 20% fetal bovineserum (FBS, Life Technologies) by pipetting in, on top, and around the device (Fig. 3).
2. Vacuum devices for 10min and allow to equilibrate for another 10min until all channels are filled. By applying a vacuum to the device, the solution is instilled into the channels of the device as gases in the channels diffuse into the de-gassed PDMS.
3. Confirm wetting of device channels under microscope. No bubbles should be present within the device.
4. Fill the well containing the device with fresh IMDM containing 20% FBS until the top of the device is completely submerged under liquid.

Note: These devices can be prepared and stored at 4°C up to 1 month before use.

3 SAMPLE PREPARATION AND LOADING

Here we describe the possibility of performing two different types of experiments using human blood samples. First, by acquiring a patient blood sample, this assay can enable in a short amount of time early-diagnosis of septic condition (Ellett et al., 2018). Second, by collecting peripheral or capillary blood from a healthy donor, this assay can be employed to study the effect of immune modulators on the induction of neutrophil motility and thus attempt to recapitulate sepsis-like neutro-phil phenotype by spiking blood.

3.1 HUMAN NEUTROPHILS FROM PATIENT PERIPHERAL BLOOD

1. Collect or order peripheral blood sample from patients in heparin-coated vacuum tubes (Vacutainer, Becton Dickinson), preferably from indwelling lines. Use blood sample within 1 h after collection. (*Patient samples must be obtained after written informed consent and through procedures approved by an Institutional Review Board.*)
2. Prepare 50 μ L IMDM with 20% FBS stained with Hoechst 33342 dye (ThermoFisher) at 32 μ M (Staining is necessary for fluorescent microscopy).
3. Add 50 μ L whole blood sample to 50 μ L stained media (1:1 dilution).
4. Mix by gently pipetting once.
5. Incubate the blood and Hoechst stain for 5–10min at room temperature to allow for fluorescent staining of cell nuclei.
6. Extremely gently, pipette 1.5 μ L of stained blood into the loading chamber using a gel-loading tip (Eppendorf), taking care to draw the tip out of the device while dispensing.

3.2 HUMAN NEUTROPHILS FROM HEALTHY PERIPHERAL BLOOD

1. For spiking experiments, collect or order peripheral blood from healthy volunteers aged 18years or older in heparin-coated vacuum tubes and use as soon as possible, within 1h after collection. A sample volume of 0.01–1mL of blood is usually sufficient.
2. Prepare the immune modulators solution in IMDM with 20% FBS at twice the target modulator concentration stained with Hoechst (32 μ M).
3. Add 50 μ L whole blood sample to 50 μ L immune modulator in stained media (1:1 dilution).
4. Mix by gently pipetting once.
5. Incubate spiked blood sample for at least 15min at room temperature.
6. Load the blood sample as previously described.

Note: Hoechst stain is necessary for only for fluorescent microscopy.

3.3 HUMAN NEUTROPHILS FROM HEALTHY CAPILLARY BLOOD

1. For spiking experiments, prepare 1mL of IMDM with 20% FBS in a 2mL Sodium Heparin BD Vacutainer. Mix vigorously.
2. Prepare the immune modulator solution in previous IMDM with 20% FBS (with Sodium Heparin) at twice the target concentration stained with Hoechst (32 μ M).
3. From a consenting volunteer aged 18years or older, disinfect one finger using an alcohol swab and let dry. Promptly prick side of finger with a 1.5mm 30G contact-activated lancet (BD Microtainer). Wipe away the first drop of blood with a sterile gauze pad.

4. Coax blood out of pricked site by gently pressing around the area. Using a pipette tip, collect blood and pipette into a 1.5mL Eppendorf tube.
5. Mix blood 1:1 with previously immune modulators solution.
6. ~ 20–50µL is usually the maximum amount of blood collected from a finger prick. The volumes used for the 1:1 dilution step should be prepared accordingly.
7. Mix by gently pipetting once.
8. Incubate spiked blood sample for at least 15min at room temperature.
9. Load the sample as previously described.

Note: Use Hoechst stain only if using fluorescent microscopy. For controls, use the Heparin containing media without immune modulator.

Tips

- Loading too much blood or pressing the pipette too strongly may lead to RBCs entering the mazes, causing blockage and preventing neutrophils from migrating into the side channels. At the end of loading, there should be a uniform circle of whole blood in the center of the device, not yet in contact with the side channels and mazes.
- Load devices when near microscope to avoid shaking dishes during transportation.
- Do NOT overload the wells with media, as this will cause media to spill out the sides when the lid is placed on (Fig. 4).
- Make sure devices are fully covered by media prior to loading. Under-filling the well will cause the sample to flow through and out the device.

4 MICROSCOPY

4.1 BRIGHTFIELD MICROSCOPY—CytoSMART LIVE-CELL IMAGING

The CytoSMART LUX2 system (Eindhoven, The Netherlands) is a compact and incubator-proof inverted microscope for brightfield live-cell imaging. It is composed of a 10 × fixed objective, 5MP CMOS Sensor, and has a 2.4 × 1.5mm field of view.

1. Connect CytoSMART microscope to a PC tablet and place device in an incubator or warm room at 37°C.
2. Open CytoSMART software and choose between “zoom in” and “zoom out” option in the menu tab depending on how many migration mazes is desired in the field of view. *The “zoom out” option allows two mazes to be imaged but may make it harder to see the migrating neutrophils. Using the “zoom in” option allows only one maze in the field of view, but the higher magnification enables easier tracking of the moving neutrophils.*

3. Align device (glass-bottom Petri dish) by manually adjusting position and make sure microscope is focused on the maze(s) using the “focus bar” on the bottom of the screen.
4. Press “start experiment” and enter experiment name.
5. Choose snapshot interval for 10s (this setting may require a software updated from CytoSMART if not already implemented).
6. Hit “start” and the microscope will automatically acquire images for 4h.

Note: The field of view of the CytoSMART system allows one to collect data from a maximum of two mazes out of eight mazes present in one device. Determination of the final “Sepsis score” requires multiplying the score obtained from two mazes by a factor 4 to reflect the whole device (Fig. 5).

4.2 TIME-LAPSE FLUORESCENT MICROSCOPY

1. Alternatively, one could record the spontaneous neutrophil migration into mazes with a fully automated time-lapse fluorescent Nikon TiE inverted wide-field microscope (10 × or higher) with a biochamber heated to 37°C with 5% CO₂ (or equivalent microscope).
2. *Important:* Place the multiwell plate onto microscope stage to set up and save points BEFORE loading any diluted blood samples into devices. Each microfluidic device provides eight fields of views, each containing one migration maze. Each field is imaged every 2min to enable accurate tracking of cell motility, for 4h.
3. Remove the multiwell plate and load the blood sample into each device, as previously described.
4. Place the multiwell plate back onto microscope stage and make sure the plate is in the exact same location as when setting points. This can be checked by looking at the live view of the maze at the first x/y point. Start experiment as soon as possible, ideally within minutes after loading the sample.

Note: Images of the loading chamber can be recorded as well and employed to approximate the total number of neutrophils in the device and to calculate the percentage of neutrophils migrating (Fig. 6).

5 DATA ANALYSIS

Neutrophil spontaneous migration can be tracked using ImageJ/Fiji analysis software, from either brightfield images (acquired using a CytoSMART microscope) or fluorescent time-lapse images (acquired using a Nikon TiE microscope). Neutrophil tracking is key for quantifying the various spontaneous migration parameters necessary for the calculation of the sepsis score.

The experiments conducted with CytoSMART are saved in a local folder containing a compilation of each snapshot in a JPG format, which is automatically saved under the

Browse tab under “Menu.” The experiments using a fluorescent Nikon TiE and the Nikon NIS-Elements software are saved in ND2 format in a local folder. Both formats are compatible with ImageJ/Fiji analysis software.

5.1 BRIGHTFIELD MANUAL TRACKING

Image pre-processing

1. Import “image sequences” using ImageJ/Fiji. For the CytoSMART JPG files, select “virtual stack” when prompted.
2. Crop images to get one migration maze and rotate images such that whole blood chamber is on the left edge of the field of view (optional).
3. Adjust brightness and contrast.
4. Subtract background by selecting “Process/Subtract background” commands. Decrease rolling ball radius to 20 pixels to increase visibility of cells (optional).

Manual tracking

1. Open “tracking” then “manual tracking” in the “Plugins” menu.
2. Press “show parameters,” fill in the appropriate “time interval” and “x/y calibration” information. *Important:* For the CytoSMART using low magnification (“zoom out”), the calibration is 1.945µm/pixel. If using other microscopes the calibration will be different. To perform the calibration, use the known length of the maze (650µm) and divide by the measured length of the maze in pixels.
3. Press “add track.” On the image sequence, scroll through time until observing a neutrophil passing through the RBC filter and entering the main channel. Press on the neutrophil. A new “result” window will appear showing track number, distance, velocity, and other parameters. Every time a new point is pressed, the following image in the sequence appears. Follow neutrophil migration until the end of the experiment while taking note of possible migration phenotypes (arrest, oscillations, and retrotaxis). When finished, press “end track” and follow another neutrophil by pressing “add track,” which will start back at the beginning of the sequence of stacks.
4. After tracking every neutrophil entering the maze, select, copy and export tracking results in Excel (MS Office) or equivalent software. The Results window can also be directly saved in an XLS format which can open in Excel.
5. Track neutrophils in all mazes of the same device/condition.
6. Tracking in instances of numerous neutrophil migration to ensure complete tracking of all cells:
 - i. Once the first track is completed select “overlay lines” or “overlay dots” under “drawing.” This will produce a new stack with a traced track of the cell as lines or dots, depending on which option is selected.

- ii. Each time a new overlay stack is produced, the previous overlay stack may be deleted. Check to make sure that the last track that was overlaid is on the new overlay stack before deleting the previous.
- iii. Select “add track” with the new overlay stack and track another cell.
- iv. A new “result” window will pop up with the previous track(s) and the current track.
- v. *Important:* Make sure to select “overlay lines” or “overlay dots” after completing each cell’s track. Always keep the most recent “results” window with the latest number of tracks.
- vi. After the last cell is overlaid, the stack sequence will show all the tracked cells on one stack, which can be saved and used to count any of the above described phenotypes used to calculate the sepsis score (Fig. 7).

Note: Whenever an error is made when tracking a cell, use the “delete last point” function in the tracking menu. This will remove the last point clicked. To delete an entire track: select track number (which is found under the “delete last point” tab) and select “delete track.”

Sepsis score calculation

1. Sum the individual cell’s traveled distances in both mazes and find the average distance traveled of all neutrophils in that device. Divide this by four.
2. Determine the number of migrated neutrophils from the results window. From the final overlain stacks of each maze determine the total number of phenotypes observed in both mazes of the same device.
3. Calculate the score for two mazes:

$$\text{Sepsis score} = N * (O + P + R + AD) / 10^3$$

where N, is the total number of migrated neutrophils, O, the total number of oscillation phenotype, defined as “the total number of cells that switch direction twice in a channel and migrate for more than 15µm in each segment,”

P, the total number of arrest phenotype, defined as “a cell with zero velocity,” R, the total number of retrotaxis phenotype, defined as “any cell that leaves the maze back into the central chamber,”

AD, average distance of all neutrophils traveled in the two mazes, divided by four.

4. As each device is composed of eight mazes in total, to calculate the sepsis score (Ellett et al., 2018), the score obtained above is multiplied by 4.
5. A blood sample with a sepsis score over 30 is considered septic (Fig. 7).

5.2 FLUORESCENT AUTOMATED TRACKING—TRACKMATE (IMAGEJ/FIJI)

Image pre-processing

1. On ImageJ/Fiji, open ND2 file (or another image sequence).
2. In “BioFormat Import Options,” make sure to open the file as a “Hyperstack” and that “Specify range for each series” is the only box checked in the memory management setting. In color options, select “composite” color mode.
3. Select Series (each of them is one x/y point): select one series to analyze.
4. In “BioFormats Range Option,” use default settings.
5. Rotate image such that the blood-loading chamber is on the left (optional).
6. Crop the images selecting the area containing migration channels and maze only (exclude the RBC filter channels).
7. Adjust brightness and contrast for both channels.
8. Select the Hoechst/Blue channel and scroll to a time point in which migrating cells are visible.

Tracking using TrackMate

1. In “Plugins,” open “tracking” then “TrackMate.”
2. Select “Log detector.”
3. Select the “channel segment number” corresponding to Hoescht/Blue channel (2, if using brightfield and Hoescht channels only). Estimate Blob Diameter: 10–15µm with a threshold of 5µm. Preview to verify accuracy. If identifying object other than cells, increase the threshold until not recognized.
4. Initial Thresholding: select all.
5. Select a view: “Hyperstack Displayer.”
6. Select filter on spots: “Uniform Color.”
7. Select a Tracker: “LAP tracker.”
8. Set the tracking parameters. Select the appropriate time interval. For images acquired at 2min time interval, select the “linking distance” at 150–250µm, the “gap-closing” distance is 50–100µm and the “gap-closing max frame gap” at 2µm. Select “feature penalties” for “Quality” and “X” to a value of 1 for both linking distances and gap-closing. This prevents horizontal tracking between two channels. Note: Y penalties should be used instead of X penalties if the direction of the main channels runs horizontal.
9. Scroll through time and verify that the track matches cell migration trajectory. If gaps are visible, return to “Settings for tracker” to increase “Linking distance” until gap disappears. If tracker joins tracks that are from two different cells, decrease “linking distance.”

10. Set filter on tracking: “Track ID.” Additional filters can be added to enhance tracks such as “Track displacement” which eliminates small tracks that are not from neutrophils.
11. Display Options: select “Analysis.” Three windows open with different tracking data.
12. Select, save and export set of data displaying distances for each track (cell) present in the maze.
13. Close all windows and start from the beginning with the next series.
14. Sepsis score or neutrophil migration phenotypes calculations can then be performed as described previously.

6 CONCLUSION

Neutrophils are an important component of the innate immune system. Obtaining a deeper understanding of neutrophil biology in the context of homeostasis, infection, and inflammation will provide a valuable resource to both clinical and research communities. Microfluidic devices provide an opportunity to study neutrophil biology in the context of small volumes of minimally processed whole blood, enabling studies in the presence of other blood cells and plasma, and greatly reducing the costs traditionally associated with isolation. Here, we provide detailed protocols for the use of recently-described microfluidic devices, for studying spontaneous neutrophil motility in the context of disease, and stimulation by candidate immune modulators.

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REFERENCES

- Angus DC, & Van der Poll T (2013). Severe sepsis and septic shock. *New England Journal of Medicine*, 369(9), 840–851. [PubMed: 23984731]
- Boyden S (1962). The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *Journal of Experimental Medicine*, 115(3), 453–466. [PubMed: 13872176]
- Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, & Treacher DF (2006). Neutrophils in development of multiple organ failure in sepsis. *The Lancet*, 368(9530), 157–169.
- Ellett F, Jorgensen J, Marand AL, Liu YM, Martinez MM, Sein V, et al. (2018). Diagnosis of sepsis from a drop of blood by measurement of spontaneous neutrophil motility in a microfluidic assay. *Nature Biomedical Engineering*, 2(4), 207.
- Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. (2016). Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *American Journal of Respiratory and Critical Care Medicine*, 193(3), 259–272. [PubMed: 26414292]
- Gerisch G, & Keller HU (1981). Chemotactic reorientation of granulocytes stimulated with micropipettes containing fMet-Leu-Phe. *Journal of Cell Science*, 52(1), 1–10. [PubMed: 7037797]

- Hassan U, Ghonge T, Reddy B, Jr., Patel M, Rappleye M, Taneja I, et al. (2017). A point-of-care microfluidic biochip for quantification of CD64 expression from whole blood for sepsis stratification. *Nature Communications*, 8, 15949.
- Hoang AN, Jones CN, Dimisko L, Hamza B, Martel J, Kojic N, et al. (2013). Measuring neutrophil speed and directionality during chemotaxis, directly from a droplet of whole blood. *Technology*, 1(1), 49–57. [PubMed: 24809064]
- Jones CN, Hoang AN, Martel JM, Dimisko L, Mikkola A, Inoue Y, et al. (2016). Microfluidic assay for precise measurements of mouse, rat, and human neutrophils chemotaxis in whole blood droplets. *Journal of Leukocyte Biology*, 100(1), 241–247. [PubMed: 26819316]
- Jones CN, Moore M, Dimisko L, Alexander A, Ibrahim A, Hassell BA, et al. (2014). Spontaneous neutrophil migration patterns during sepsis after major burns. *PLoS One*, 9(12), e114509. [PubMed: 25489947]
- Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. (2006). Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical Care Medicine*, 34(6), 1589–1596. [PubMed: 16625125]
- Lavrentieva A, Kontakiotis T, Lazaridis L, Tsotsolis N, Koumis J, Kyriazis G, et al. (2007). Inflammatory markers in patients with severe burn injury: What is the best indicator of sepsis? *Burns*, 33(2), 189–194. [PubMed: 17215085]
- Liu Y, Hou JH, Li Q, Chen KJ, Wang SN, & Wang JM (2016). Biomarkers for diagnosis of sepsis in patients with systemic inflammatory response syndrome: A systematic review and meta-analysis. *SpringerPlus*, 5(1), 2091. [PubMed: 28028489]
- Sackmann EK, Berthier E, Young EW, Shelef MA, Wernimont SA, Huttenlocher A, et al. (2012). Microfluidic kit-on-a-lid: A versatile platform for neutro-phil chemotaxis assays. *Blood*, 120(14), e45–e53. [PubMed: 22915642]
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. (2016). The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*, 315(8), 801–810. [PubMed: 26903338]
- Solomkin JS (1990). Neutrophil disorders in burn injury: Complement, cytokines, and organ injury. *The Journal of Trauma*, 30(12 Suppl), S80–S85. [PubMed: 2254997]
- Torio C, & Moore B (2016). National inpatient hospital costs: The most expensive conditions by payer. <https://www.hcup-us.ahrq.gov/reports/statbriefs/sb204-Most-Expensive-Hospital-Conditions.jsp>. Accessed 5 June 2018.
- Zicha D, Dunn GA, & Brown AF (1991). A new direct-viewing chemotaxis chamber. *Journal of Cell Science*, 99(4), 769–775. [PubMed: 1770004]
- Zigmond SH (1977). Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *The Journal of Cell Biology*, 75(2), 606–616. [PubMed: 264125]

FIG. 1. A microfluidic device to assay spontaneous neutrophil motility. (A) Macroscopic image of the microfluidic device indicating the loading chamber and one of the eight migration mazes. Right, a magnified view (dashed box) showing a detailed diagram of the neutrophil migration maze. (B) Still images extracted from a time-lapse video obtained from a sample from a patient with sepsis, showing examples of behavior identification from neutrophil tracks. The time stamps is in h:min.

FIG. 2.

Bonded microfluidic device. (A) Device bonded to 35-mm glass-bottom 12-well plate (MatTeK Co., Ashland, MA). (B) Device bonded to a single small 35-mm glass-bottom Petri dish (MatTeK Co., Ashland, MA). (C) Magnified view of microfluidic device composed of a loading chamber in the center and two mazes on each side (eight mazes in total per device).

FIG. 3. Microfluidic assay preparation. (A, B) Priming device with IMDM and 20% FBS by pipetting in, on top and around the device. (C) Microfluidic device submerged in media.

FIG. 4. Microscopic view of a loaded microfluidic chip. 10 × Objective (Olympus CKX41). The scale bar is 250μm.

FIG. 5. Brightfield microscopy—CytoSMART live-cell imaging. (A) CytoSMART mini-microscope connected to tablet, imaging a microfluidic device. (B) Microscopic brightfield image obtained from CytoSMART showing spontaneously migrating neutrophils (white circles). The scale bar is 100μm.

FIG. 6. Spontaneous neutrophil migration observed after spiking healthy blood with immune modulator. Fluorescent microscopic image showing nucleus of migrating neutrophils stained with Hoechst (32μM). Several red blood cells (RBCs) can be observed inside the migration channels. The scale bar is 100μm.

FIG. 7. Manual tracking of migrating neutrophils using ImageJ/Fiji. Microscopic images were obtained using the CytoSMART mini-microscope. Tracks of different colors represent distinct neutrophils. The scale bar is 100μm.