# Neutrophil Chemotactic Response to Chemokine Gradients in a Microfluidic Device

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## Abstract:

**Neutrophils play an important role in the immune system by both degrading foreign bacteria and releasing mediators that contribute to the inflammatory response. Chemokines released from an injury site create a gradient of signaling molecules that guides neutrophil movement, or chemotaxis, towards the site. The human immune system employs a variety of different chemokines to achieve this task. In an effort to better understand how each of these chemokines affects chemotaxis, our group has fabricated a microfluidic device that allows us to expose neutrophils to a time-constant, controllable gradient of chemokines and monitor chemotactic responses of neutrophils to the gradient. Preliminary results suggest that a gradient of n-formyl-methionine-leucine-phenylalanine (fMLP) affects neutrophil chemotaxis.**

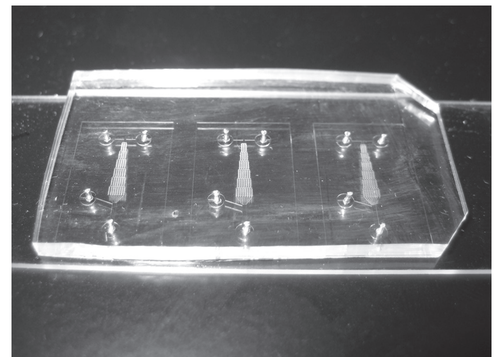

*Figure 1: The completed device.*

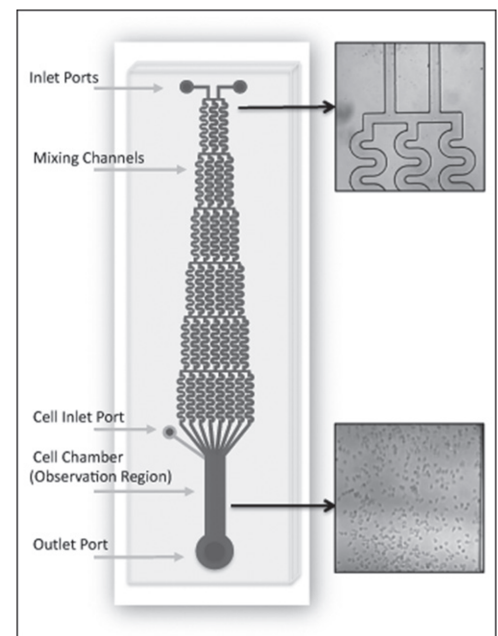


*Figure 2: Device schematic.*

## Background:

During neutrophil chemotaxis, chemokine molecules are released from the tissue near an injury. These chemokines form a concentration gradient; chemokines are most concentrated at the injury site and less concentrated far from the injury. Neutrophils are able to detect this gradient, orient themselves along it, and move in the direction of increasing chemokine concentration. The goal of this research was to fabricate a device capable of exposing neutrophils to a steady, controllable chemokine gradient and to determine neutrophil response to a range of different chemokines at various concentrations.

## Experimental Procedure:

Previous research in this area was limited by the inability to create a chemokine gradient that did not diffuse and change with time. The device design, based on work by the Jeon lab at the University of California at Irvine, overcomes this hurdle using microfluidics. Traditional photolithography techniques were used to pattern a polydimethylsiloxane (PDMS) layer with the desired device channels, and the layer was plasma-bonded channel-side-down to a glass slide to create the final device (Figure 1). A syringe pump flushed a chemokine solution and a buffer solution into the device through two separate inlet ports, which were systematically separated and mixed in a series of serpentine channels. After several rounds of this mixing, the device blended the original solutions into eight separate channels, each containing slightly different proportions of the chemokine and buffer (pictured in Figure 2). These channels filtered into

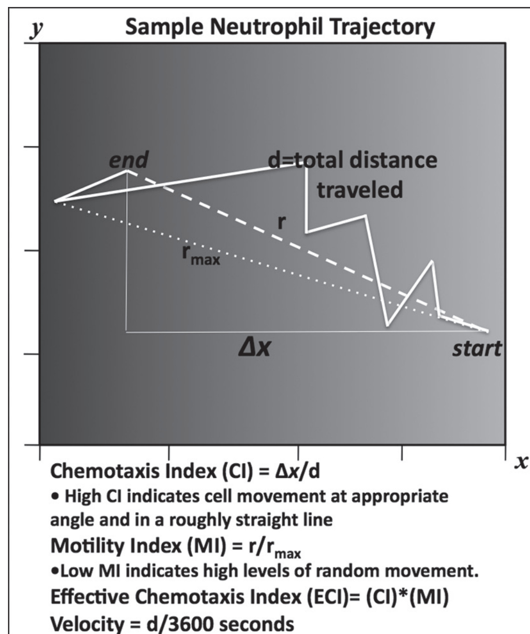


*Figure 3: Chemotaxis parameters.*

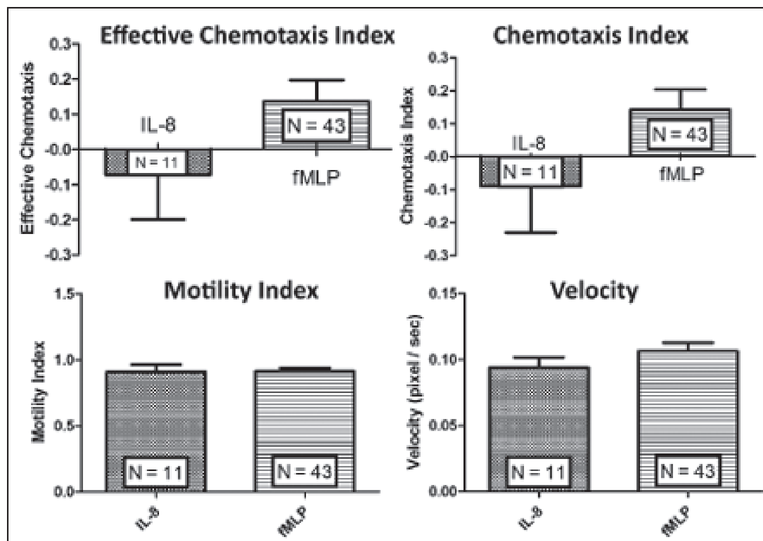


*Figure 4: Data collected.*

a cell collection chamber, producing an approximately smooth concentration gradient from the pure chemokine solution to pure buffer. Neutrophils were introduced into the cell chamber through a separate inlet port. Images of the cell chamber were captured every thirty seconds for one hour and combined to form a time lapse video of cell movement in response to the gradient in the device.

Cell trajectories were analyzed using several key parameters (Figure 3). The chemotaxis index, which describes the efficiency of the cell in orienting and traveling toward a higher chemokine region, and the motility index, which indicates whether or not a high chemotaxis index is due to non-chemotactic random motion, form the basis of our analysis. Multiplying these parameters yields the effective chemotaxis index, which acts as a rough indicator of the degree to which the cell exhibited chemotaxis. Velocity data was also collected.

## Results and Conclusions:

Preliminary trials were completed with two well-known chemokines. IL-8, a chemokine involved in the inflammatory response, was chosen for its prominence in previous neutrophil chemotaxis research. A bacterially-derived chemokine, fMLP, was chosen as an example of chemokines derived from alternate sources. One trial was run with each of the two chemokines, and trajectories gathered in each trial were analyzed.

The data, pictured in Figure 4, displays some unexpected results. The effective chemotaxis index for the fMLP trial was positive, indicating that neutrophils oriented in the appropriate angle towards increasing chemokine concentration and traveled along a fairly straight path in that direction. The effective chemotaxis index for IL-8, however, was negative, indicating that neutrophils moved, on average, away from the IL-8 source. The motility index and velocity for IL-8 were comparable to that of the fMLP trials, indicating that this unusual result was not due to random, non-directional neutrophil movement. As chemotaxis toward IL-8 is a well-documented phenomenon, however, we expect that these results are due to either a small sample size or contamination of the IL-8 solution. In addition, all cells for a given trial are drawn from the same donor, increasing the chance of atypical results due to individual variation.

## Future Work:

Although our research to date includes only one chemokine in any given trial, biological chemokine gradients often involve multiple chemokines at once. To fully understand the role of chemotaxis in disease states, we must investigate how chemotaxis changes in multi-chemokine systems. The Haynes Lab aims to develop a hierarchy of chemokines, describing which are the most powerful mediators of chemotaxis and which provide more subtle, weaker signaling in multi-chemokine situations.

This research will be conducted by introducing a second chemokine to the microfluidic device through the inlet port previously containing buffer solution. Doing so will create a smooth gradient from one chemokine to another, exposing neutrophils to a set of competing signals. By observing which of the two chemokines the neutrophils chemotax towards, we will determine which chemokine dominates movement. Single-chemokine research conducted previously will serve as a control and comparison point for multi-chemokine trials.

## Acknowledgments:

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