# Traction Forces of Neutrophils Migrating on Compliant Substrates

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ABSTRACT Proper functioning of the innate immune response depends on migration of circulating neutrophils into tissues at sites of infection and inflammation. Migration of highly motile, amoeboid cells such as neutrophils has significant physiological relevance, yet the traction forces that drive neutrophil motion in response to chemical cues are not well characterized. To better understand the relationship between chemotactic signals and the organization of forces in motile neutrophils, force measurements were made on hydrogel surfaces under well-defined chemotactic gradients created with a microfluidic device. Two parameters, the mean chemoattractant concentration ($C_M$) and the gradient magnitude ($\Delta c/\Delta x$) were varied. Cells experiencing a large gradient with $C_M$ near the chemotactic receptor $K_D$ displayed strong punctate centers of uropodial contractile force and strong directional motion on stiff (12 kPa) surfaces. Under conditions of ideal chemotaxis—cells in strong gradients with mean chemoattractant near the receptor $K_D$ and on stiffer substrates—there is a correlation between the magnitude of force generation and directional motion as measured by the chemotactic index. However, on soft materials or under weaker chemotactic conditions, directional motion is uncorrelated with the magnitude of traction force. Inhibition of either $\beta_2$ integrins or Rho-associated kinase, a kinase downstream from RhoA, greatly reduced rearward traction forces and directional motion, although some vestigial lamellipodium-driven motility remained. In summary, neutrophils display a diverse repertoire of methods for organizing their internal machinery to generate directional motion.

## INTRODUCTION

Relating observations of cell motion to underlying mechanical processes is a major aim in the field of cell migration. During migration, adherent cells generate traction forces at the cell-substrate interface that enable net displacement. Traction forces can vary significantly in magnitude, location, orientation, and timescale, depending on the cell type and its physiological function. In addition, chemical and mechanical conditions in the local microenvironment surrounding a cell can influence force generation. Initial studies of cellular forces focused on anchorage-dependent cells such as fibroblasts, endothelial cells, and epithelial cells and demonstrated that these cell types are capable of applying large traction forces on the order of 10,000 nN on their underlying substrates during adhesion and chemokinesis (1–7). Simultaneous imaging of traction forces and fluorescently tagged adhesion proteins within individual cells have shown that forces are associated with focal adhesions and that adhesions at the leading edge of the cell are the sites of active force generation and propulsion (8,9). A frontal towing model of motility has been proposed to explain the spatial and temporal distribution of traction forces at the leading edge in anchorage-dependent cells (9).

Although the process of cellular force generation in anchorage-dependent cells has been under intense investigation for more than three decades, force measurements of fast-moving cells such as keratocytes, *Dictyostelium*, metastatic tumor cells, and immune cells such as neutrophils have been elusive until recently (7,10–16). Studies of these cells types have revealed that the highest traction forces in highly motile cells are located at the rear of the cell relative to movement (12,13,16,17). Average forces in these high-speed cells are approximately two orders of magnitude lower than those observed for anchorage-dependent cells, as expected based on the previously established inverse correlation between speed and force (18). The presence of higher traction forces at the rear during motion suggests that rapidly-migrating amoeboid cells generate forward propulsion using a system of forces and molecular assemblies distinct from that of anchorage-dependent cells. To further assess the core mechanical principles that govern the motility of rapidly migrating cells, we examined the traction forces of neutrophils in response to chemoattractant signals.

Neutrophils are key players in the inflammatory response to injury and pathogens and their activation by chemoattractants leads to firm adhesion and migration (19). The response of neutrophils to chemoattractants during chemokinesis and chemotaxis has been studied extensively on stiff glass substrates and is well characterized (20). More recently, we and others have examined neutrophil chemokinesis and chemotaxis on hydrogel substrates (16,17). Under a linear gradient $\Delta c/\Delta x$ of chemoattractant c(x), where the mean concentration of chemoattractant is $C_M$ at the cell center, neutrophils display increased directional migration with increasing $\Delta c/\Delta x$ and respond most effectively when $C_M$ is near the $K_D$ of chemotactic ligand binding for the receptor (18,22). Chemoattractant gradients rapidly induce neutrophil polarity and the formation of distinct pseudopodial and uropodial regions. In the presence of uniform

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stimulation, morphologic polarity is also observed, although the cell will move in random directions (23). The formation of neutrophil polarity begins with chemoattractant receptor-activated G-proteins that initiate divergent signaling through Rho GTPases and the formation of spatially segregated frontness and backness signaling modules (24). At the leading edge, accumulation of Rac leads to F-actin polymerization and the extension of pseudopods. At the sides and rear, RhoA activity stimulates contractility through p160-ROCK and myosin II (25). To date, the spatial patterns of these molecules have not been correlated with the spatial pattern of traction stresses in these cells.

The rapid motility of neutrophils combined with their well-defined responses to chemical signals makes them an ideal system for investigating the relationship between signaling, motility, and force generation in amoeboid cells. Using traction force microscopy and microfluidic gradients, we measured neutrophil forces on compliant hydrogel substrates in uniform and gradient solutions of the chemoattractant fMLP (Fig. 1). In a previous study, we and others demonstrated that cellular forces depend on integrin adhesion and that integrin adhesion can trigger RhoA activity through force-dependent pathways (26). To examine how neutrophils generate traction forces on substrates with different mechanical properties, we examined the effect of inhibiting signals through $\beta 2$ integrins and RhoA. We found a direct correlation between chemotactic signal and the magnitude and polarity of traction stresses on stiff surfaces and under strong chemotactic signals. On stiffer materials, smaller values of the gradient, $\Delta c/\Delta x$, or changes in $C_M$ away from the $K_D$ of receptor binding led to less intense force generation, lower root-mean-squared forces, and a smaller chemotactic index. Under chemokinesis (in a uniform field of chemoattractant), cells exerted lower root-mean-squared forces and moved randomly. We also found that the substrate plays a role in controlling directional motion: stiffer substrates allow cells under the same chemoattractant gradient to organize more efficiently and generate larger traction stresses. On soft materials or weak chemoattractant signals, cells can display directional motion that is uncorrelated with traction stress. Our data provide insight into the coordination of neutrophil forces that is critical for understanding how these cells migrate through tissue compartments to hunt pathogens and respond to inflammation.

## MATERIALS AND METHODS

### Reagents

Adhesive ligands were Protein G (Pierce Biochemicals, Rockford, IL), human ICAM-1 Fc, and E-Selectin Fc (R&D Systems, Minneapolis, MN). TS1/18 $\beta 2$ integrin antibody was used at 10 $\mu$g/mL to block adhesion (Pierce Biochemicals) (27), neutrophils were activated with fMLP chemoattractant peptide (Sigma-Aldrich, St. Louis, MO), and cell contractility was inhibited using 10 $\mu$m Y-26732 ROCK inhibitor (Calbiochem, San Diego, CA).

### Isolation of neutrophils

Whole blood was obtained from healthy human donors by venipuncture and collected in BD Vacutainer tubes containing citrate-EDTA anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Blood was layered onto

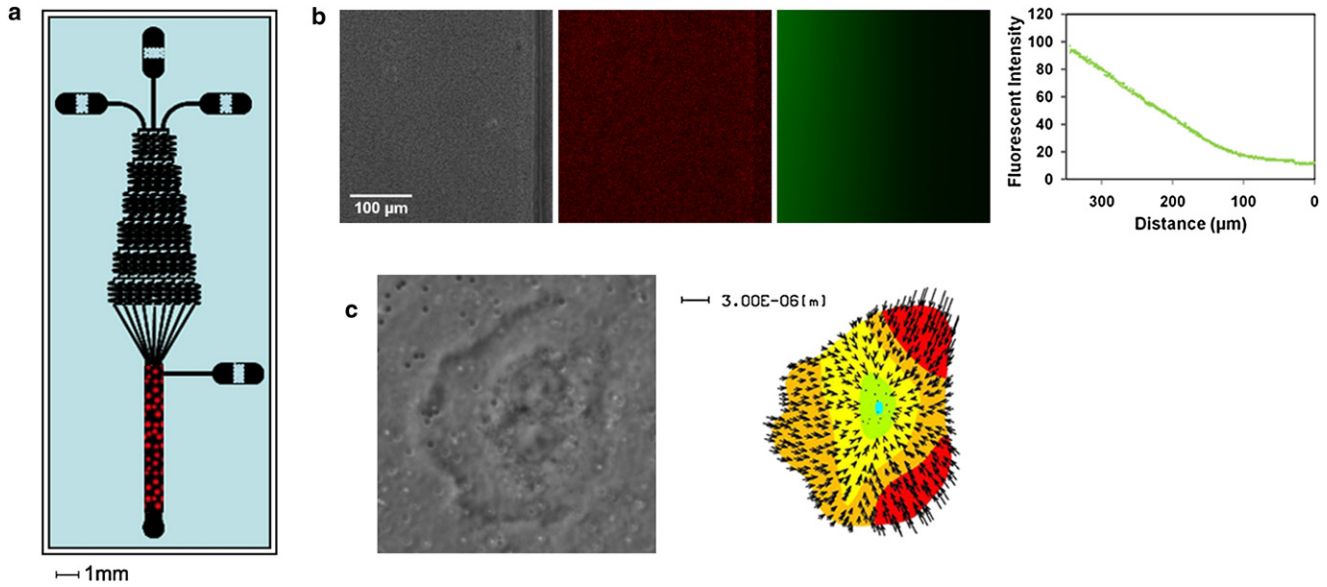


FIGURE 1 Overview of microfluidic traction force chamber. (*a*) A microfluidic gradient generator was used to create linear gradients over a traction force gel embedded in the main channel area of the device. Red dots represent the region where the traction force gels are placed and cells are observed. (*b*) Phase-contrast (*left*) and fluorescent-bead images (*middle*) of a traction force gel within the main channel of a microfluidic chamber, along with an image of fluorescein dye solution (*right*) flowing through the same region. At far right, an intensity plot of fluorescein fluorescence shows that a linear gradient is developed and maintained over the hydrogel. (*c*) Phase-contrast and traction-force microscopy map of a neutrophil oriented within the chamber in response to a chemical gradient.