environment affects the evolution of the plaque and its cellular components. In this work, we used substrates of varying stiffness to study the behavior of macrophages, an immune cell found in plaques. We found that stiffness affected macrophage phenotype and function. For example, macrophages plated on soft gels (1-5 kPa) had only a slight increase in area over 18 hours. Macrophages on the stiff gels (280 kPa), however, had an 8-fold increase in area over the same time period. In addition to area, macrophage migration was also found to depend on stiffness. Although macrophages on both the soft and stiff substrates exhibited random motion, their speed did depend on substrate stiffness. Macrophages on the 280 kPa gel traveled at 12.5 um/hr, much faster than the average 6.6 um/hr for macrophages plated on the softer substrates. Furthermore, f-actin content in macrophages also depends on substrate stiffness. On soft substrates actin is spread uniformly throughout the cytoplasm, whereas on 280 kPa substrates, actin is organized into stress fibers. Finally, macrophages were observed to proliferate faster on stiff substrates. Cells were counted at various timepoints over 72 hours, and macrophages plated on the 280 kPa gel had a significantly smaller doubling time than those plated on soft substrates. In conclusion, these results suggest that macrophages respond in complex mechanical environments such as an atherosclerotic plaque.

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The Effect of P-Selectin Dimers on Neutrophil Rolling on Endothelium
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In inflammation, endothelial cells release adhesion molecules that mediate rolling of neutrophils. While rolling, neutrophils activate to become capable of firmly adhering to endothelial cells; after this, they migrate through the blood vessel wall into inflamed tissue. The initial phase of rolling, dominated by P-selectin-PSGL-1 bonds, is critical for preventing neutrophils from leaving the area of the inflammation prior to activating. A portion of endothelial P-selectin are expressed as dimers, and PSGL-1 are predominantly monomeric; however, monomeric P-selectin is typically used in in vitro studies and computational models of neutrophil rolling have not accounted for dimeric P-selectin. Prior to inflammation, P-selectin is stored in Weibel-Palade bodies located primarily near the boundaries between endothelial cells; secreted P-selectin mostly remains near these boundaries. It has been proposed that endothelial cells release a combination of monomeric and dimeric P-selectin, with dimeric P-selectin more stably binding neutrophils at endothelial cell boundaries, while the more diffusible monomeric P-selectin cover the center of the endothelial cell face. Here, a computational model of neutrophil rolling on endothelium is used to study the effects of distributions of monomeric and dimeric P-selectin on neutrophil rolling. Even small fractions of dimeric P-selectin greatly reduce neutrophil rolling velocity. Results are presented regarding the advantages of a mixed population of monomeric and dimeric P-selectin, the mechanics of force distribution across the neutrophil body, and how effectively neutrophils are attracted to endothelial cell boundaries.

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Three-Dimensional Traction Forces Exerted by Filopodia and Membrane Protrusions Drive Neutrophil Invasion
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The infiltration of circulating neutrophils into the vascular wall is an early step leading to inflammation and atherosclerosis, and requires transendothelial migration and invasion through the basement membrane. While much research has been dedicated to the identification of the signaling cascade involved in neutrophil recruitment, less is known about how neutrophils generate the three-dimensional (3-D) forces and shape changes required for invasion. To address this issue, differentiated HL-60 neutrophil-like cells are plated on a reconstructed basement membrane made of Matrigel and incorporated with chemoattractant fMLP. The cells subsequently adhere and invade into the 3-D Matrigel. Using 3-D Fourier traction force microscopy, we measure the evolution of cell shape and traction stresses in this 3-D invasion model. Our results show that several filopodia-like cell extensions at cell edge lead the way and exert pulling forces, which contributes to generate a large invasive protrusion that burrows into the Matrigel by generating pushing forces. We find that the total pulling force from all the filopodia structures balances the pushing force exerted by the invasive structure, revealing a coordination between morphodynamic changes and 3-D traction stresses during cell invasion. Furthermore, since the number of filopodia exceeds the number of invasive protrusions, this force balance causes the pushing forces created at the invasive site to be much higher than the pulling forces exerted by the filopodia. Thus, the 3-D traction forces exerted by filopodia play an important role in regulating protrusion dynamics of invading neutrophils in 3-D microenvironments, and are mechanistically coordinated to enhance invasion.