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Supplemental Information

Assaying How Phagocytic Success Depends on the Elasticity of a Large Target Structure

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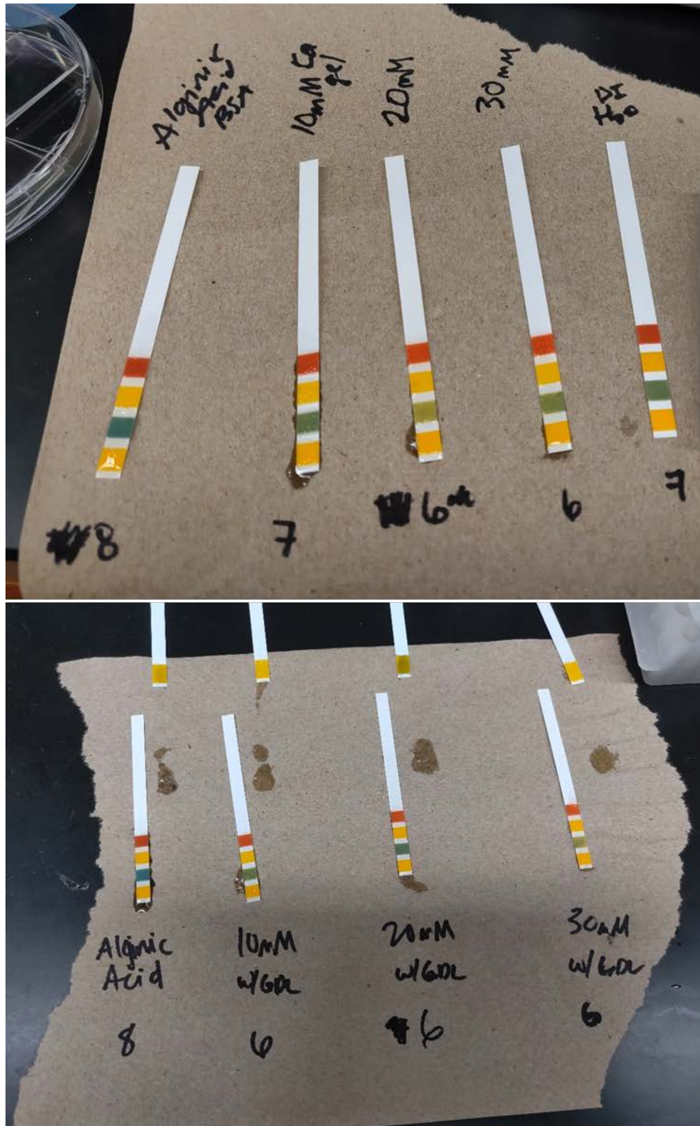

Figure S1. Images of pH test strips showing the change in pH during alginate gel fabrication. The initial alginic acid solution has a pH of 8 and upon the introduction of $CaCO_3$ and GDL the pH reduces to 6 or 7 for gels made with 10mM calcium, and a pH of 6 for gels made with 20mM or 30mM calcium.

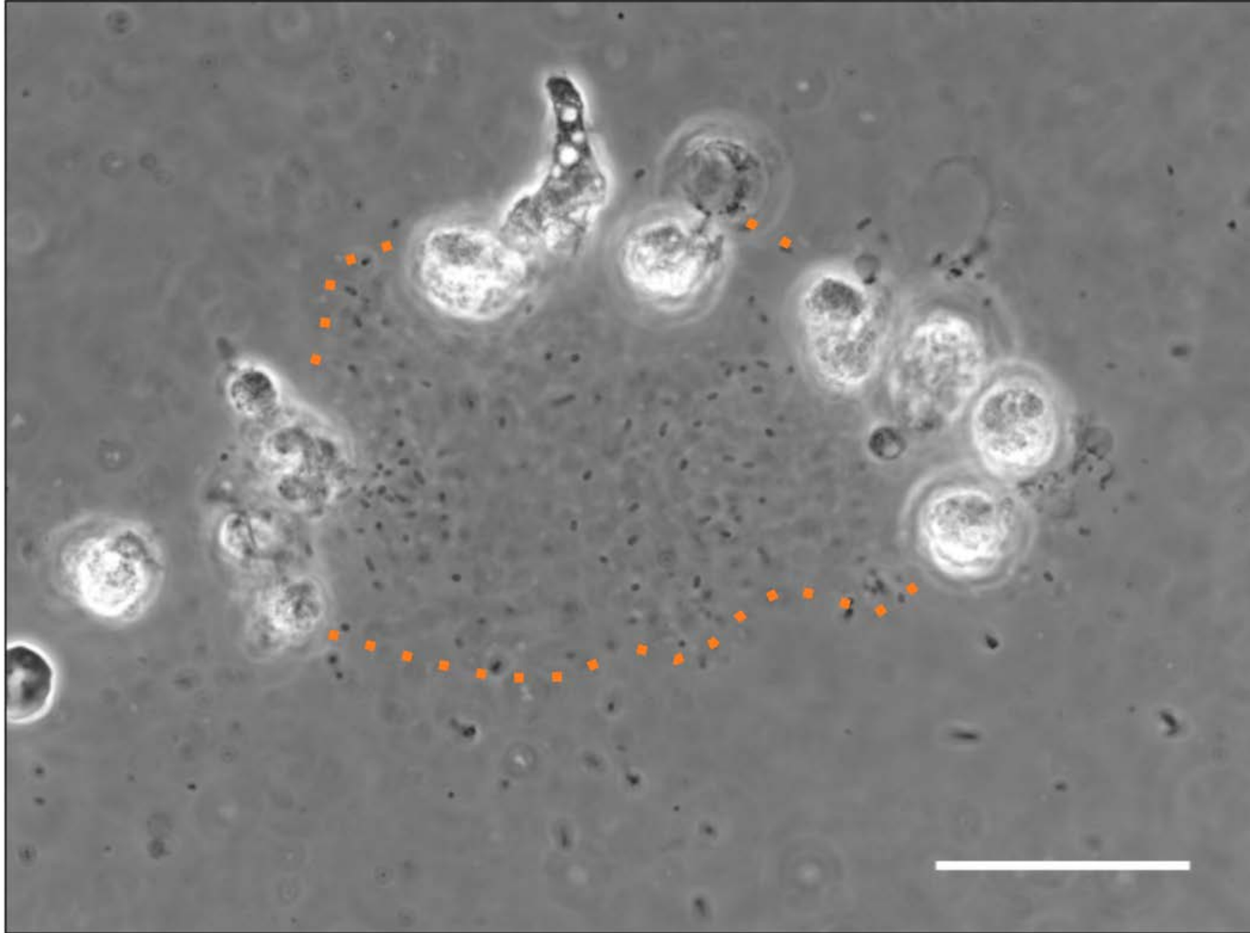

Figure S2. Still frame from neutrophil-biofilm interaction timelapse microscopy video (Supplementary Movie 1). Here the biofilm aggregate is outlined in orange for clarity. The biofilm polymer-protein matrix is not visible by phase contrast microscopy, however aggregates can be recognized as regions that are densely packed with bound bacterial cells that show neither active nor passive motility. Scalebar shown is 30 μm.

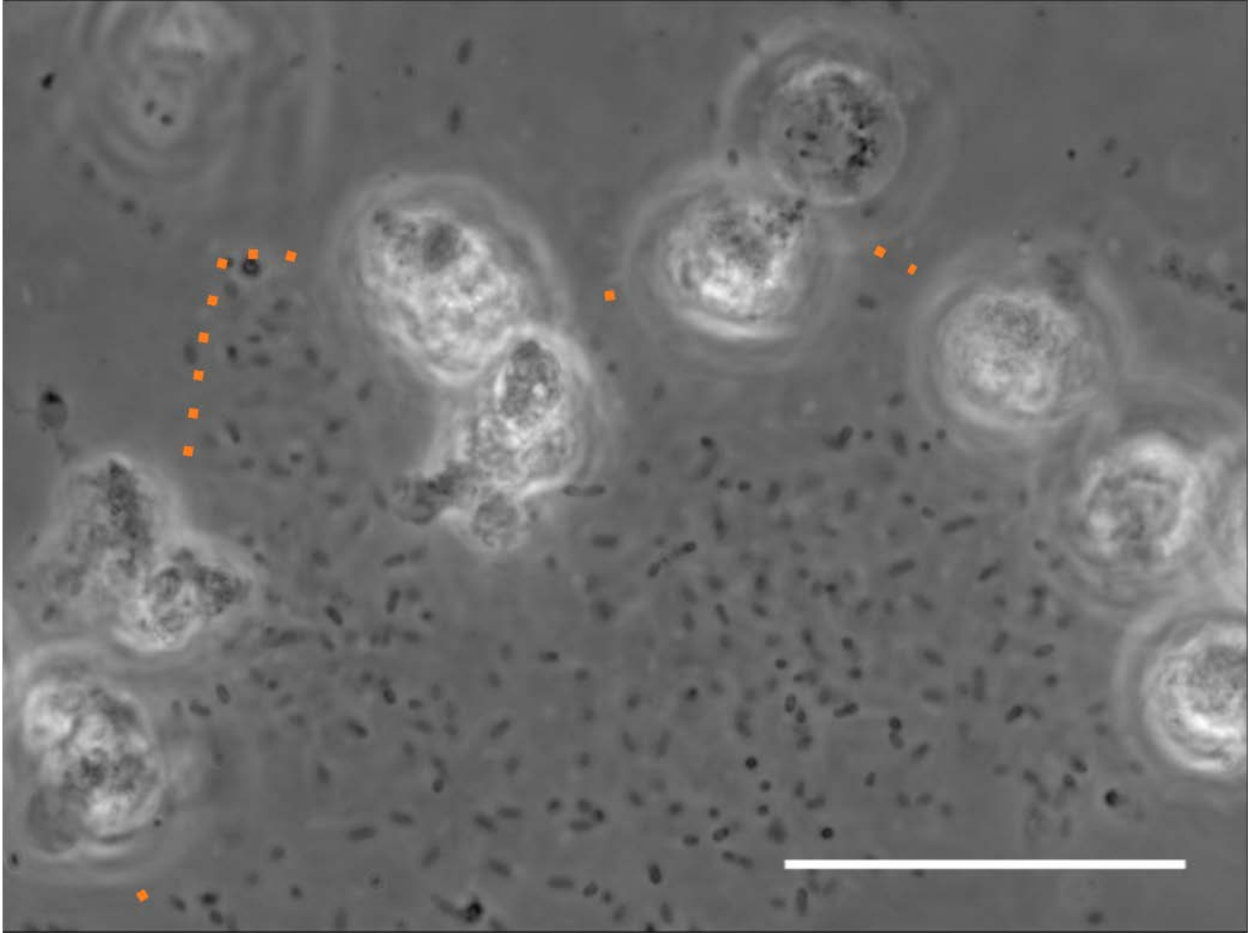

Figure S3. Still frame from neutrophil-biofilm interaction timelapse microscopy video (Supplementary Movie 2). Here the biofilm aggregate is outlined in orange for clarity. The biofilm polymer-protein matrix is not visible by phase contrast microscopy, however aggregates can be recognized as regions that are densely packed with bound bacterial cells that show neither active nor passive motility. Scalebar shown is 30 μm.

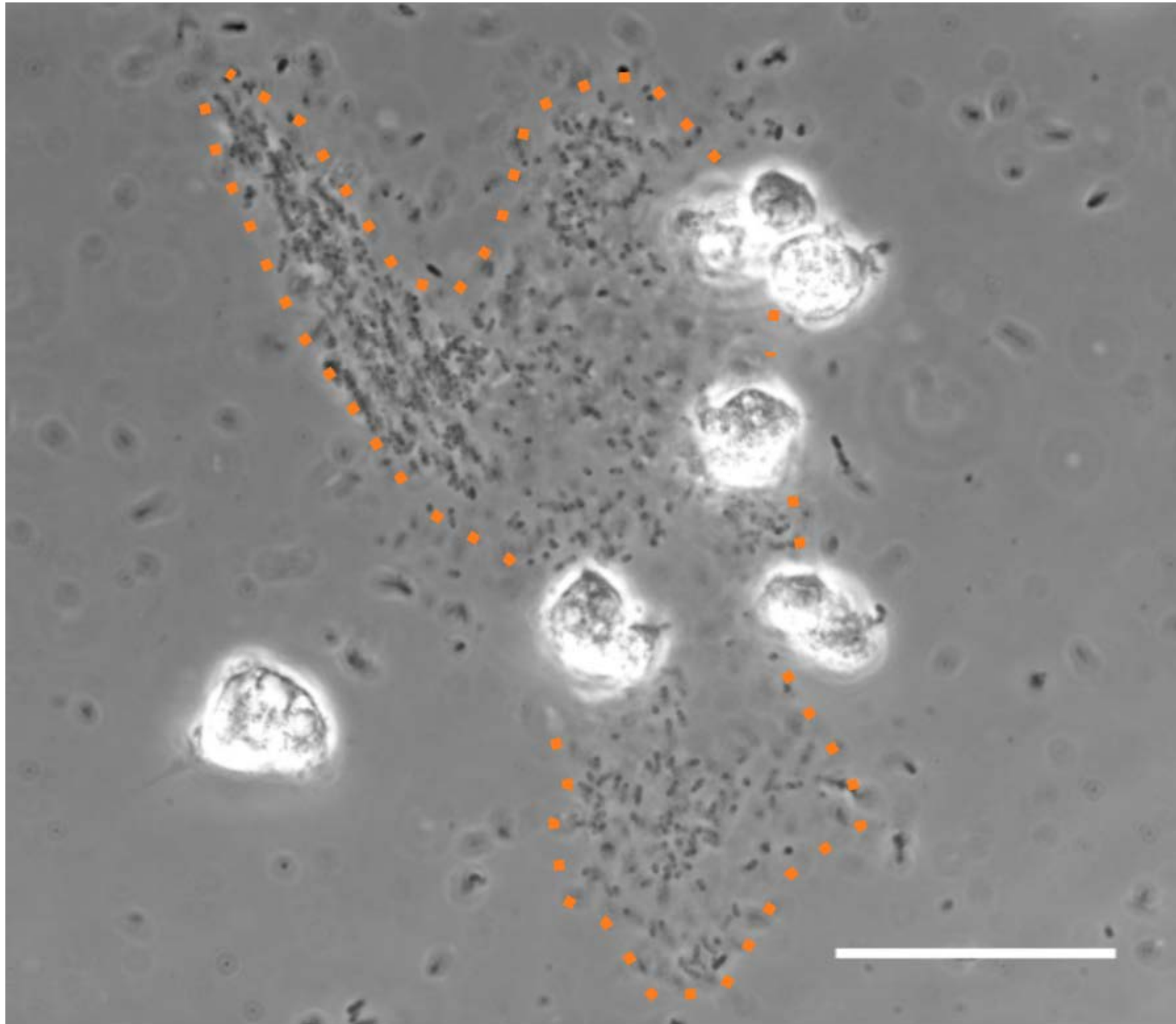

Figure S4. Still frame from neutrophil-biofilm interaction timelapse microscopy video (Supplementary Movie 3). Here the biofilm aggregate is outlined in orange for clarity. The biofilm polymer-protein matrix is not visible by phase contrast microscopy, however aggregates can be recognized as regions that are densely packed with bound bacterial cells that show neither active nor passive motility. Scalebar shown is 30 μm.

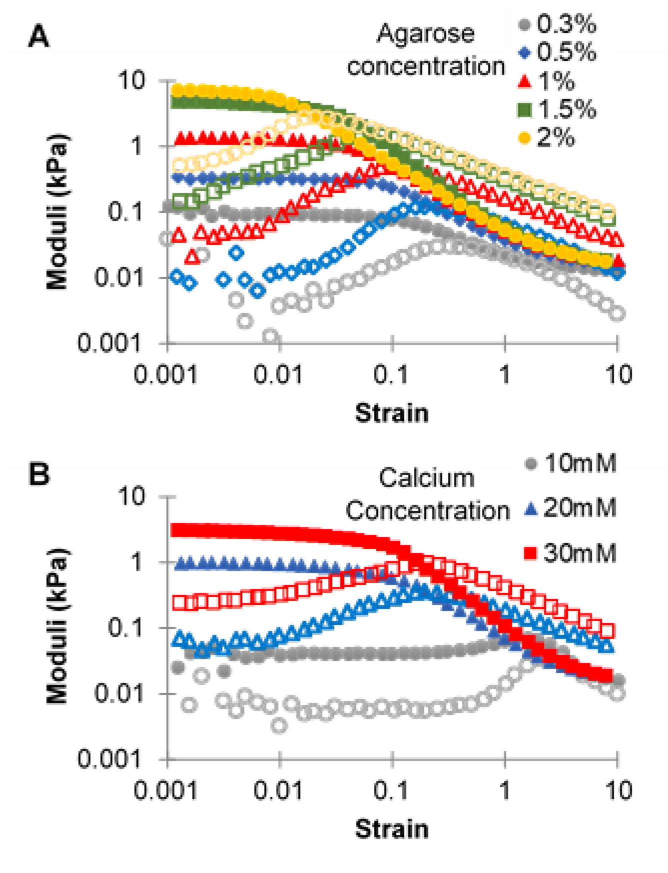

Figure S5. Representative strain sweeps for (A) agarose and (B) alginate gels, all from the same day of measurement. These gels contained fluorescent polystyrene beads and BSA at the concentrations used for engulfment tests. Frequency sweeps were done at 1% strain and strain sweeps were done at 3.14 radians/s. The elastic moduli (G') are shown with solid symbols and the viscous moduli (G'') are shown with hollow symbols of corresponding shape and color.

Supplementary Movie 1. This phase contrast micrograph movie shows neutrophils interacting with and attacking biofilm-like aggregates of bacteria at 8 times real speed. Aggregates can be recognized as the bacteria-dense regions that exclude neutrophils. Bacteria in aggregates show no active motility, such as swimming, and no passive mobility, as in Brownian motion. This is not the case for bacteria outside of aggregates, most of which are actively moving and all of which are subject to Brownian motion. Aggregated bacteria are bound together in a biofilm-like matrix of polymer and protein which is not visible under phase contrast microscopy. Scalebar shown is 30 µm.

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Supplementary Movie 4. Phase-contrast microscopy focal scan along the height of a biofilm aggregate and neutrophils (Supplementary Movie 2), showing the three-dimensional structure. Native video playback is set at 3 frames per second. Scalebar shown is 30 μm.

Supplementary Movie 5. Phase-contrast microscopy focal scan along the height of a biofilm aggregate and neutrophils (Supplementary Movie 3), showing the three-dimensional structure. Native video playback is set at 3 frames per second. Scalebar shown is 30 μm.

Supplementary Movie 6. 0.3% agarose immediately after the addition of fluorescent 200nm beads. The gel region (left) excludes the beads after initial introduction. The first frame of the movie file is transmitted light, while the second frame is a fluorescence image of the same field of view.

Supplementary Movie 7. 0.3% agarose 40 minutes after the addition of fluorescent 200nm beads. The gel region (left) continues to exclude the beads, suggesting the pore size of the gel is less than 200nm. The first frame of the movie file is transmitted light, while the second frame is a fluorescence image of the same field of view.

Supplementary Movie 8. Alginate made with 20 mM calcium immediately after the addition of fluorescent 200nm beads. The gel region is on the left of the image.

Supplementary Movie 9. Alginate made with 20 mM calcium 10 minutes after the addition of fluorescent 200nm beads. The gel region is on the left of the image and continues to exclude the beads, suggesting the pore size of the gel is less than 200nm.

Supplementary Movie 10. Alginate made with 30 mM calcium immediately after the addition of fluorescent 200nm beads. The gel region is on the left of the image.

Supplementary Movie 11. Alginate made with 30 mM calcium 10 minutes after the addition of fluorescent 200nm beads. The gel region is on the left of the image and continues to exclude the beads, suggesting the pore size of the gel is less than 200nm.

Supplementary Movie 12. 0.3% agarose immediately after the addition of dyed dextran with hydrodynamic radius 35nm. The gel already contains a great deal of dextran.

Supplementary Movie 13. Alginate made with 20 mM calcium immediately after the addition of dyed dextran with hydrodynamic radius 35nm. A small amount of dextran has entered the gel.

Supplementary Movie 14. Alginate made with 20 mM calcium 10 minutes after the addition of dyed dextran with hydrodynamic radius 35nm. The gel now contains more dextran than it did at t=0.

Supplementary Movie 15. Alginate made with 30 mM calcium immediately after the addition of dyed dextran with hydrodynamic radius 35nm. Little to no dextran has entered the gel.

Supplementary Movie 16. Alginate made with 30 mM calcium 10 minutes after the addition of dyed dextran. The amount of dextran in the gel has increased slightly or not at all since t=0.

Supplementary Movie 17. Timelapse microscopy video observing the interaction of neutrophils (on the right) with alginate gel made using 20mM of $CaCO_3$ (on the left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 450 frames were taken over the course of 450 seconds (7.5 mins). Scalebar is 60 μm.

Supplementary Movie 18. Timelapse microscopy video observing the interaction of neutrophils (on the right) with alginate gel made using 20mM of $CaCO_3$ (on the left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 551 frames were taken over the course of 551 seconds (9.18 mins). Scalebar is 60 μm.

Supplementary Movie 19. Timelapse microscopy video observing the interaction of neutrophils (on the right) with alginate gel made using 30mM of $CaCO_3$ (on the left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 450 frames were taken over the course of 450 seconds (7.5 mins). Scalebar is 60 μm.

Supplementary Movie 20. Timelapse microscopy video observing the interaction of neutrophils (on the right) with a 0.5% agarose gel. Agarose gel cannot directly be visualized by phase contrast microscopy, however polystyrene beads embedded within the gel provide visualization

of the gel region (on left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 421 frames were taken over the course of 421 seconds (7 mins). Scalebar is 60 µm.

Supplementary Movie 21. Timelapse microscopy video observing the interaction of neutrophils (on the right) with a 0.3% agarose gel. Agarose gel cannot directly be visualized by phase contrast microscopy, however polystyrene beads embedded within the gel provide visualization of the gel region (on left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 2000 frames were taken over the course of 2000 seconds (33.33 mins). Scalebar is 60 µm.

Supplementary Movie 22. Timelapse microscopy video observing the interaction of neutrophils (on the right) with a 0.3% agarose gel. Agarose gel cannot directly be visualized by phase contrast microscopy, however polystyrene beads embedded within the gel provide visualization of the gel region (on left). The neutrophils are attracted by the target and crawl along the boundary, but do not penetrate the gel. Video is displayed at 37 frames per second, and was acquired at 1 frame per minute. 2000 frames were taken over the course of 2000 seconds (33.33 mins). Scalebar is 60 µm.

Supplementary Movie 23. Confocal z-stack of 0.3% agarose gel with neutrophils on top, immediately after the addition of neutrophils to the gel. Fluorescence signal is false-colored magenta and transmitted light is false-colored blue. The neutrophils settled onto the top surface of the gel. Scalebar is 60 µm. Native video playback is set at 10 FPS.

Supplementary Movie 24. Confocal z-stack of 0.3% agarose gel with neutrophils on top, 60 minutes after the addition of neutrophils to the gel. Fluorescence signal is false-colored magenta and transmitted light is false-colored blue. Neutrophils remain localized within a similar z slice as the initial time point (Supplementary Movie 23) and do not appear to have penetrated into the gel after one hour of contact with the surface. Scalebar is 60 µm. Native video playback is set at 10 FPS.

Supplementary Movie 25. Confocal z-stack of 0.3% agarose gel with neutrophils on top, 120 minutes after the addition of neutrophils to the gel. Fluorescence signal is false colored magenta and transmitted light is false colored blue. Neutrophils remain localized within a similar z slice as the initial time point (Supplementary Movie 23) and do not appear to have penetrated into the gel after two hours of contact with the surface. Scalebar is 60 µm. Native video playback is set at 10 FPS.