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Nanopillared Surfaces Disrupt *Pseudomonas aeruginosa* Mechanoresponsive Upstream Motility

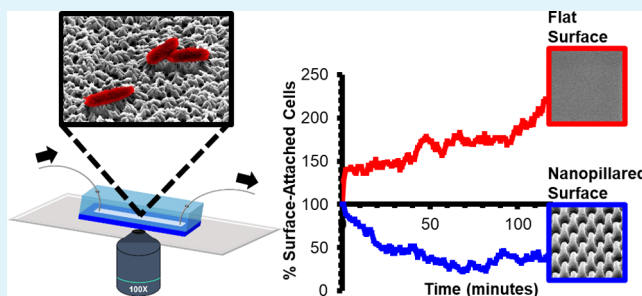
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Supporting Information

ABSTRACT: *Pseudomonas aeruginosa* is an opportunistic, multidrug-resistant, human pathogen that forms biofilms in environments with fluid flow, such as the lungs of cystic fibrosis patients, industrial pipelines, and medical devices. *P. aeruginosa* twitches upstream on surfaces by the cyclic extension and retraction of its mechanoresponsive type IV pili motility appendages. The prevention of upstream motility, host invasion, and infectious biofilm formation in fluid flow systems remains an unmet challenge. Here, we describe the design and application of scalable nanopillared surface structures fabricated using nanoimprint lithography that reduce upstream motility and colonization by *P. aeruginosa*. We used flow channels to induce shear stress typically found in catheter tubes and microscopy analysis to investigate the impact of nanopillared surfaces with different packing fractions on upstream motility trajectory, displacement, velocity, and surface attachment. We found that densely packed, subcellular nanopillared surfaces, with pillar periodicities ranging from 200 to 600 nm and widths ranging from 70 to 215 nm, inhibit the mechanoresponsive upstream motility and surface attachment. This bacteria-nanostructured surface interface effect allows us to tailor surfaces with specific nanopillared geometries for disrupting cell motility and attachment in fluid flow systems.

KEYWORDS: antibacterial, antifouling, coating, nanopillared surfaces, *Pseudomonas aeruginosa*, mechanoresponsive, type IV pili, upstream motility



INTRODUCTION

Bacteria in nature have often been found to colonize and form biofilms in environments with fluid flow such as the vasculatures of plants,¹ the lungs of cystic fibrosis patients,² and aquatic environments since their appearance in fossil records dating back to 3.25 billion years.³ Biofilm formation is problematic in industrial and health-care systems such as fuel lines in aviation,⁴ naval hulls,⁵ conduits and storage tanks for potable water,⁶ and medical devices.⁷ Biofilms consist of surface-attached cells protected by layers of secreted extracellular polymeric substances, which are key factors for cell survival in diverse environments.⁸ In health care, biofilms promote a 10- to 1000-fold decrease in the diffusion of antibacterial drugs from outside the biofilm and may alter bacterial gene expression,^{9,10} resulting in antimicrobial resistance.¹¹ Human pathogenic biofilms are responsible for 80% of clinical infections, leading to 1.7 million hospital-acquired infections and 99 000 annual deaths¹² with treatments costing 16.6 billion dollars annually in the US alone.¹³ There is an urgent need to prevent the proliferation of infectious bacteria on surfaces without the use of drugs that could lead to antimicrobial resistance.

Antimicrobial resistant, Gram-negative bacteria often persist in environments with fluid flow with the aid of mechano-responsive motility mechanisms such as those possessed by human pathogenic *Vibrio cholera*,¹⁴ *Neisseria gonorrhoeae*,¹⁵ *Escherichia coli*,¹⁶ and *Pseudomonas aeruginosa*.¹⁷ In this work, we focus on *P. aeruginosa*, a biofilm-forming, opportunistic pathogen known to cause chronic infections in immunocompetent and immunocompromised patients. *P. aeruginosa* ubiquitously contaminates medical implants and devices with fluid flow such as mechanical ventilators,¹⁸ prosthetic heart valves,¹⁹ and catheters.²⁰ *P. aeruginosa* forms biofilms through cell-to-cell communication, or quorum sensing, between surface-attached cells through regulated gene expression in response to changes in population density.²¹

Surface attachment in environments with fluid flow is facilitated in *P. aeruginosa* through the activation of its type IV pili, a mechanoresponsive motility mechanism.¹⁷ Type IV pili are flagellum-independent, filamentous appendages for motility and translocation, which were first discovered in 1961.²² These

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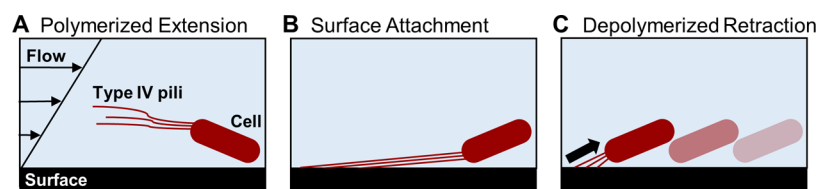


Figure 1. Schematic of type IV pili-mediated upstream motility on surfaces. *P. aeruginosa* activates mechanoresponsive motors to facilitate type IV pili cyclic sequence of (A) polymerized extension, (B) surface attachment, and (C) depolymerized retraction to generate an upward driving force.

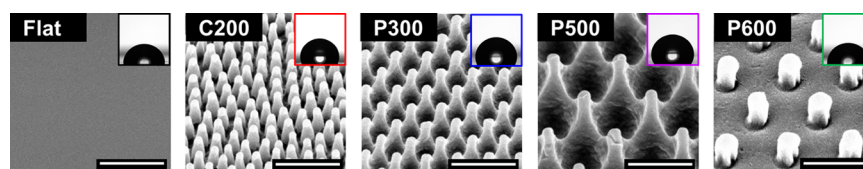


Figure 2. Scanning electron micrographs (SEMs) of PMMA surfaces taken at a 45° tilt with 3 kV, using the FEI Magellan SEM. Flat surfaces were prepared through spin coating. Nanostructured surfaces were fabricated through nanoimprint lithography (NIL) to form C200, P300, P500, and P600 surfaces. The lettering represented the negative imprinting mold originating from a cicada wing or pillared master mold. The numeric in the nomenclature represented nanometer values in center-to-center pillar periodicities per surface. Scale bars represent 500 nm. Contact angles for each surface are included as their insets and were taken using a OneAttention Theta Optical Tensiometer.

appendages mediate surface adhesion and enable *P. aeruginosa* to twitch^{14,23,24} and slingshot²⁵ upstream by the cyclic sequence of polymerized extension, surface attachment, and depolymerized retraction^{26–31} (Figure 1). These motions are facilitated by the activity of the PilT and PilU ATPase motors,³² which generate an upstream driving force and are regulated by the Chp chemosensory system.³³ Type IV pili are distributed radially at the cell poles and have been measured to be 7–20 nm in diameter and several microns in length using optical tweezers,³⁴ total internal reflection microscopy with fluorescently labeled cells on a quartz slide adhered to a silica prism,³⁵ and interferometric scattering microscopy.³⁶ Pili have been characterized as the strongest linear molecular motors to date and are able to produce 100–250 pN of shear force.^{29,37,38}

Previous studies have observed *P. aeruginosa* in flow channels with flat glass surfaces under varied flow rates and shear stresses ranging from 0.1 to 10 Pa.³² Using single and multibranch channels, previous work observed rheotaxis, colonization, and upstream migration on surfaces under varying flow rates and shear stresses.^{32,39} Along with serving as appendages for motility, type IV pili are important virulence factors during the early stages of infection^{40–42} and they mechanochemically mediate the activation of virulence when cells are surface-attached,⁴³ leading to the formation of virulent biofilms.^{17,28,40,44}

Previous work has demonstrated that surfaces with hemispherical topographies on the microscale with features of several magnitudes larger in diameter than the cellular bodies can hinder *P. aeruginosa* net motility, yet motility in relation to flow direction and surface detachment was not observed.⁴⁵ Although microtopographies have shown promise in prolonging biofilm formation by confining the cell motility in grooves between hemispheres, the use of subcellular nanotopography may, by contrast, disrupt its adhesive type IV pili mechanoresponsiveness. This would prevent *P. aeruginosa* surface adhesion from outcompeting the force of the flow. This phenomenon would inhibit both motility and surface attachment in environments where the shear stresses from fluid flow could trigger rheotactic upstream motility. The use of biomimetic, nanopillared surfaces inspired by those found on

lotus leaves (*Nelumbo nucifera*),⁴⁶ gecko feet (*Gekkonidae*),⁴⁷ and cicada wings (*Psaltoda claripennis*)^{48,49} has been promising for antibacterial medical device coatings. However, their antimicrobial properties have only been investigated in static fluid environments.⁵⁰ The effectiveness of such engineered surfaces in environments with fluid flow needs to be demonstrated.

In this work, we extend the use of bioinspired, nanopillared surfaces in fluid flow for the purpose of disrupting both upstream surface motility and attachment of *P. aeruginosa*. We demonstrate a new strategy of surface structuring a medical device material, poly(methyl methacrylate) (PMMA), without any chemical modification or addition of antibiotics. We used nanoimprint lithography (NIL), a low-cost and scalable topography engineering technique to fabricate surfaces of nanopillars with varying packing fractions. The viability of NIL has already been shown in the semiconductor industry for the manufacture of high-density circuits.^{51,52} During fluid flow, these nanopillared surfaces have been reported to possess hydrophobic properties at the nanometer length scale with minimal increased slip length, b , due to zero slip ($b = 0$) at the solid–liquid interface and perfect slip ($b = \text{infinity}$) at the liquid–vapor interfaces.^{53,54} Although such properties have been caused by nanobubbles reducing the drag in water,⁵⁵ such nanobubbles have been found to decrease over a period of an hour, resulting in zero slip.⁵⁶ Here, we found that degrees of surface hydrophobicity did not correlate with motility trends. Thus, we can assume that small changes in fluid flow insignificantly affect bacterial surface motility.

Furthermore, we analyzed the motility data of *P. aeruginosa* in relationship to the decreased surface area that the type IV pili contact due to varying packing fractions of the subcellular nanopillars on the surfaces. Herein, we present our results on quantifying the following behavior of the cells: upstream trajectory, displacement, velocity, angular trajectory, and persistent surface attachment on flat PMMA surfaces compared to those on nanopillared PMMA surfaces. These phenomena at the interface of bacteria nanotopography allow us to tailor surfaces with specific nanopillared geometries for structurally disrupting mechanoresponses, signal transduction, and motility in fluid flow. Our findings are relevant to

antibacterial, antiviral, and antibiofilm design strategies for preventing contamination in industrial and medical device applications such as catheters.

RESULTS AND DISCUSSION

We fabricated nanopillared surfaces on PMMA, an FDA-approved polymer commonly used on medical devices (Figure 2). NIL^{49,52} was used to fabricate uniform arrays of nanopillars that ranged in center-to-center pillar periodicities of 200, 300, 500, and 600 nm. The letterings for C200, P300, P500, and P600 represent the negative imprinting mold originating from a cicada wing or pillared master mold, whereas the numeric values in the nomenclature represent nanometer center-to-center pillar periodicities per surface (Table 1). For the present

Table 1. Dimensional Feature Measurements of the Nanopillared Surfaces^a

surface	period (nm)	width (nm)	height (nm)	tip packing fraction Φ	contact angle θ
C200	170	70	210	0.18	110 ± 0.8
P300	320	120	300	0.28	117 ± 0.5
P500	500	100	700	0.04	122 ± 0.6
P600	595	215	300	0.22	93 ± 0.4

^aPeriod, width, and height indicate feature measurements of individual nanopillars per surface. Tip packing fraction, Φ , indicates the ratio of the pillar tip surface area to the substrate surface area. θ indicates contact angle values. $n = 5$ for each surface. Standard errors are given for each average contact angle per surface.

case, we identified the pillar tip packing fractions, Φ , as the ratio of the pillar tip surface area to the substrate surface area in decreasing order from P300, P600, C200, to P500 (Table 1).

We next analyzed *P. aeruginosa* motility in flow cells with flat or nanopillared PMMA as the bottom surface. We fabricated the flow cells by cutting out channels in a double-sided adhesive tape and capping the channel tops using a poly(dimethylsiloxane) (PDMS) block with pre-cut inlet and outlet holes. The PDMS block placed on top of the adhesive tape on the PMMA surfaces allowed us to avoid having to use plasma bonding that may result in etching the nanopillars. The cross sections of the flow channels were 1 mm in width and 100 μm in height. These flow cells were then mounted atop an

inverted microscope (Figure 3A), and phase images were taken every minute at room temperature to create time-lapse videos (S1 and S2) of the *P. aeruginosa* motility (Figure 3B).

To demonstrate the effect of nanopillared surfaces on bacterial upstream motility, overnight *P. aeruginosa* cultures were diluted and grown to a mid-exponential phase to an optical density of 0.2 at 600 nm (OD_{600}) and seeded into each flow cell. Previous studies demonstrated that *P. aeruginosa* upstream motility was enabled by their mechanoresponsive type IV pili on glass surfaces at various flow rates ranging from 0.5 to 30 $\mu\text{L}/\text{min}$ and shear stresses ranging from 0.1 to 10 Pa.^{32,39} In this work, we applied a flow rate of 115 $\mu\text{m}/\text{min}$ using lysogeny broth (LB) media on each flow cell to induce a shear stress of ~ 1 Pa on the seeded *P. aeruginosa* cells, a value typically found in catheter tubes.⁵⁷ We constructed plots from the time-lapse videos by tracking the motion of each cell, normalizing their starting position, and quantifying their relative directions. In plots where we show the constructed trajectory, the cells that migrated from the origin 0 and extended to the negative x -axis direction indicate upstream translocation (Figure 4). The cells on the flat surfaces shifted largely in the negative direction on the x -axis compared to cells on the nanopillared surfaces. We demonstrated a correlation between surface nanostructuring and motility inhibition in the results below.

We quantified cell motility behavior on the flat and nanopillared surfaces to analyze the influence of nanopillared surface tip packing fraction, Φ , on motility inhibition. We found that 30–90% of *P. aeruginosa* cells seeded into the flow cells with nanopillared bottom surfaces did not migrate upstream compared to those on the flat surfaces. The loss of migration on the surfaces was dependent on the nanopillar packing fractions (Figure 5A). On these nanopillared surfaces, 20–80% of *P. aeruginosa* cells exhibited a lower upstream velocity compared to those on the flat surfaces. The average upstream velocity of the cells on the flat PMMA surface was ~ 1.5 $\mu\text{m}/\text{min}$, which is consistent with the previous studies on flat glass surfaces.^{32,39} The cells on P300 showed high resistance to motility followed by P600, C200, and P500 (Figure 5B). This trend correlated to the decreasing packing fraction at the top of the nanopillar arrays (Table 1). The reduction in the surface area at the pillar tips may have limited surface sensing, mechanoresponse, and attachment of the type IV pili, which resulted in the force of flow overcoming the

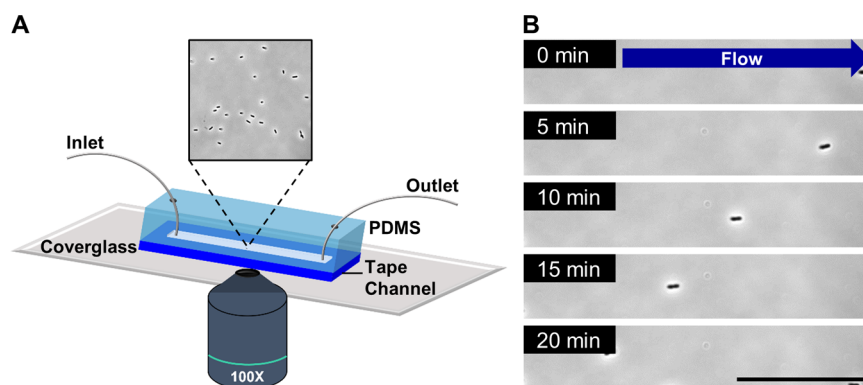


Figure 3. Continuous surface-attached cell motility imaging system. (A) Schematic of flow cell loaded with *P. aeruginosa* mounted on an inverted microscope and imaged through a 100 $\times$ oil objective. Once cells were surface-attached, only fresh lysogeny broth (LB) media flowed through the inlet at 115 $\mu\text{m}/\text{min}$. The cross sections of the channels were 1 mm wide and 100 μm tall (shear stress of ~ 1 Pa). (B) Time-lapse demonstration of *P. aeruginosa* on a flat PMMA-coated surface exhibiting upstream motility. Scale bars represent 20 μm .

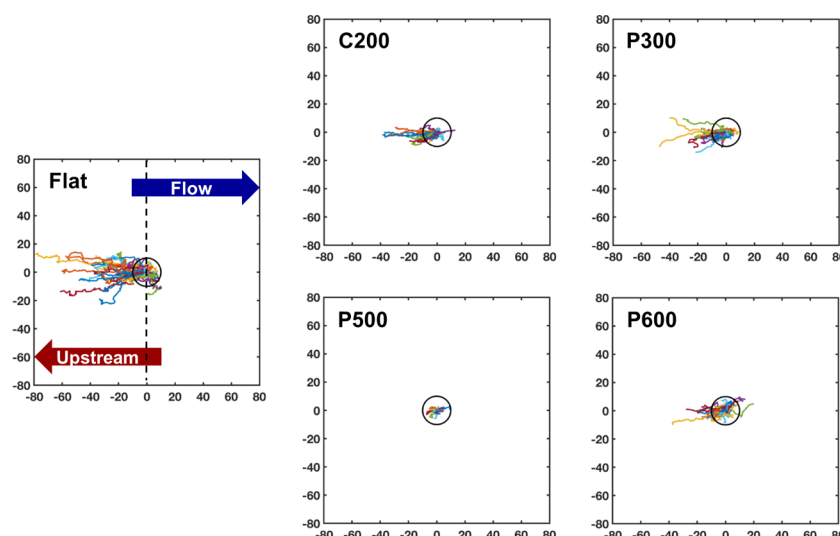


Figure 4. Single cell trajectories of *P. aeruginosa* motility on the flat and nanopillared surface structures. $n = 50$ for each surface. x -Axis and y -axis span from -80 to $80 \mu\text{m}$. Trajectories originating from 0 and extending to the $-80 \mu\text{m}$ x -direction indicated upstream motility. A ring with a radius of $10 \mu\text{m}$ is given for reference.

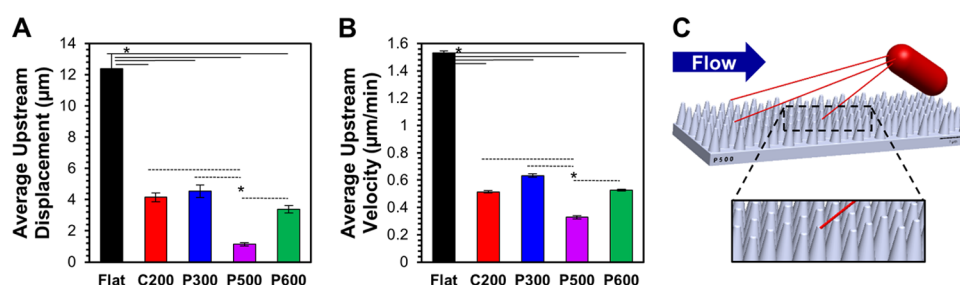


Figure 5. Nanopillared surfaces inhibited the upstream motility of *P. aeruginosa*. Data were collected from 2 h time-lapse videos using an ImageJ TrackMate plugin.⁶² $n = 50$ for each surface. Experiments were performed in triplicates of each surface. (A) Average upstream displacement of cells. (B) Average upstream velocity of cells. The decreasing upstream velocity values corresponded to the reduction in pillar tip packing fraction, Φ . Error bars represent the standard error of the mean with statistical significance assessed using Student's t test. *: $p < 0.05$. (C) Schematic rendering of *P. aeruginosa* type IV pili adhesion to the nanopillared surface structures in fluid flow.

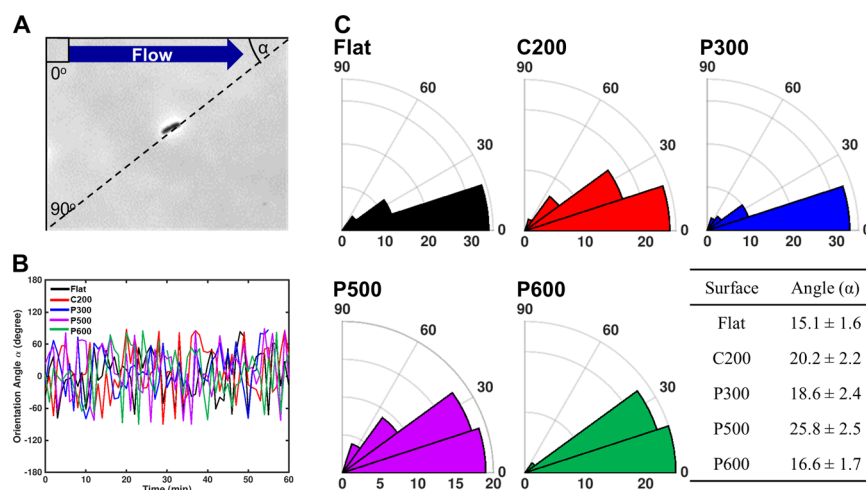


Figure 6. Angular trajectories of *P. aeruginosa* motility on the flat and nanopillared surface structures. (A) Schematic of cell orientation angle, α , measured in reference to the fluid flow direction. (B) Time lapse of angular cell trajectories was taken each minute for 1 h on all surfaces. (C) Angular trajectory histograms of cells on flat, C200, P300, P500, and P600. $n = 5$ for each surface. The larger average orientation angles on the nanopillared surfaces may indicate that the *P. aeruginosa* type IV pili explored larger areas to adhere to than on the flat surface. Standard deviations are given for each average orientation angle per surface.

force of adhesion (Figure 5C). Although there may have been an increase in slip on the nanostructured surfaces in molecular

orders of magnitude,⁵³ our results did not correlate to the degrees of apparent hydrophobicity, θ (Table 1).

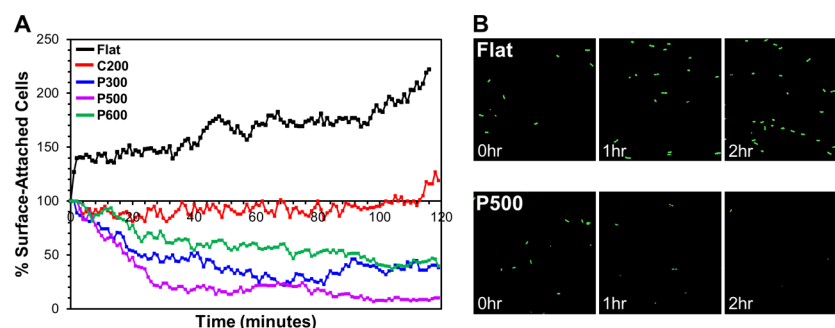


Figure 7. Nanopillared surfaces inhibit the surface attachment of *P. aeruginosa*. Data were collected from 2 h time-lapse videos using an ImageJ TrackMate plugin.⁶² (A) Percentage of surface-attached cells on the surfaces was measured in 1 frame each minute for 120 min. A decrease in cell count was observed on the nanopillared surfaces, indicating cell detachment. (B) Images of cells (green) taken at each hour on the flat and P500 surfaces to demonstrate cell division and proliferation on the flat surface and cell detachment on the nanopillared surfaces.

To further investigate the influence of reduced type IV pili surface contact area to upstream cell motility disruption, we measured the angular trajectories of the cells to monitor the degree of explored surface area compared to flow direction. Previous reports show that a zig-zag motion was observed during the upstream motion of *P. aeruginosa* on glass surfaces.^{25,32,39} In this work, the orientation angle of each cell was measured with respect to the direction of flow (Figure 6A). The motility trajectories of the cells were compared among the surfaces with orientation angle with respect to the upstream direction recorded every minute for 1 h (Figure 6B). The average orientation angle of upstream motility was collected from five individual cells on each surface attached for an hour and plotted as a histogram (Figure 6C). We found that the average orientation angles of motility increased as follows: $15.1 \pm 1.6^\circ$ for flat, $16.6 \pm 1.7^\circ$ for P600, $18.7 \pm 2.4^\circ$ for P300, $20.2 \pm 2.2^\circ$ for C200, and $25.8 \pm 2.5^\circ$ for P500. The increase in orientation angle correlated inversely with the decreasing packing fractions at the top of the nanopillar arrays (Table 1). We postulate that the larger orientation angles are due to the lack of attachable surface area at the top of the nanopillar arrays, which constrains the locations to which the type IV pili attach. Further studies imaging the type IV pili on the nanopillared surfaces in fluid flow would verify the postulation.

The shear stress from fluid flow that accompanies increased surface motility, cell proliferation, and residence time⁵⁸ in *P. aeruginosa* produces signaling precursors for infectious biofilm formation.^{17,28,40,44} Although the nanopillared surfaces inhibited upstream displacement and velocity of *P. aeruginosa*, we further measured whether the surfaces reduced surface attachment for long-term antifouling applications. Here, we recorded the percentage of cells on each surface every minute for 2 h in the same flow channels while simultaneously analyzing the cell displacements, velocities, and trajectories. The data shown in the graph display an average of triplicate surface measurements at each minute. On the flat PMMA surfaces, the cells divided and proliferated over the 2 h course. On the nanopillared surfaces, the overall population of the cells decreased due to detachment over a period of 2 h (Figure 7A). Although the increasing detachment did not directly correlate with the reduction in surface area at the top of the nanopillar arrays that the type IV pili would sense, still, these observations demonstrate that surface attachment inhibition on reduced surface areas in fluid flow played a significant role.

Although all nanopillared surfaces showed reduction in cell division and surface attachment, this effect can be most clearly observed on the P500 surface compared to that on the flat surface (Figure 7B). The study presented here provides a general technique using surface nanopatterning to avoid biofouling in fluid flow environments by systematically altering the surface using nanoimprinting. Our findings also hold significance in using nanostructured surfaces to both understand the biophysical mechanism of mechanoresponsive type IV pili in fluid flow and further investigate drug-free, antiviral, and antibiofilm coatings.

CONCLUSIONS

In summary, we have demonstrated that reducing PMMA surface contact area inhibits upstream motility and persistent attachment of *P. aeruginosa*, a virulent, biofilm-forming bacterium during fluid flow. We used NIL, a cost-effective and scalable method, to fabricate subcellular nanopillared surfaces with varying nanopillar tip packing fractions. The correlation between the decrease in upstream displacement and velocity with the decreasing PMMA nanopillar tip packing fractions provides a new direction in understanding type IV pili mechanoresponses. Additionally, we observed that cell division and proliferation occurred on the flat surfaces, whereas cell detachment and no proliferation occurred on all nanopillared surfaces. The greatest effect was observed on the P500 surface, which had the least net nanopillar tip surface area and packing fraction. Further studies on the trajectories and angular orientations of *P. aeruginosa* during the fluid flow on the flat, C200, P300, P500, and P600 surfaces demonstrated motility inhibition patterns. We have shown that in the case of *P. aeruginosa* under flow conditions, the nanostructuring targets mechanoresponsive properties of bacterial type IV pili to prevent biofilm formation. We conclude that this effect is due to the reduction in surface contact area. Together, these observations present a working model of nanopillared surfaces in fluid flow environments that disrupt motility and attachment of persistent type IV pili-possessing bacteria. These surfaces have broad applications in industrially and medically relevant systems such as mechanical ventilators, prosthetic heart valves, and catheters. The work presented here can be used as a stepping stone to investigate type IV pili mechanoresponse and regulation of surface attachment in other bacteria for antiviral and antibiofilm strategies.

■ EXPERIMENTAL METHODS

Nanopillared Surface Fabrication. Glass coverslips (24×60 mm²) (VWR, Radnor, PA) were treated with 1% aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, MO) in deionized water (DI) for 20 min to facilitate surface bonding with 120 000 MW poly(methyl methacrylate) (PMMA) (Sigma-Aldrich, Milwaukee, WI) in aqueous solution. PMMA (5% wt) in toluene was spin-coated on the treated glass coverslips at 600 rpm for 45 s. The PMMA-coated coverslips were then annealed on a hot plate at 110 °C for 5 min to evaporate residual solvent. This process was repeated three times to obtain a film thickness of 2 μ m. Each PMMA-coated coverslip was then placed in a JenOptik HX03 hot embosser (Jenoptik AG, Jena, Germany) for nanoimprint lithography (NIL). NIL molds previously described^{49,59–61} were centered on top of the PMMA-coated coverslips. A pressure of 2 MPa from the hot embosser top plate was applied to each mold while the PMMA was heated to 70 °C above its glass transition temperature (T_g) from both the top and bottom plates for 20 min. Pressure was maintained while the PMMA was cooled down to and held at room temperatures in the hot embosser for 10 min. Samples were then demolded to reveal nanoimprinted surface structures and sterilized in a fume hood under UV light for 5 min.

Nanopillared Surface Characterization. The flat, C200, P300, P500, and P600 PMMA surfaces were coated with 5 nm of iridium, using an ACE600 sputter coater (Leica Microsystems, Buffalo Grove, IL). The surfaces and their nanostructures were then characterized using an FEI Magellan 400 XHR Scanning Electron Microscope (FEI Company, Hillsboro, OR) at a 45° tilt angle with an acceleration voltage of 3 kV. Contact angle measurements were taken using a OneAttention Theta Optical Tensiometer.

Flow Cell Fabrication. The flow cell channels of 1 mm wide and 25 mm long were cut out from a 100 μ m-thick double-sided tape (3 M, Maplewood, MN). The tape with the cut channel was transferred and centered on each PMMA-coated coverslip with either flat or nanopillared surfaces, thus exposing the nanopillars. Blocks of cured poly(dimethylsiloxane) (PDMS) made from a Sylgard 184 silicone elastomer kit (1:10 cross-linker/elastomer base) (Dow Corning, Midland, MI) were cut 50 mm long, 15 mm wide, and 5 mm thick. Inlet and outlet holes, 1 mm in diameter, were pre-cut on the PDMS blocks using a biopsy hole punch. The silicone blocks were placed directly on top of the adhesive tape channels with the holes aligned above the channel ends to provide a flow cell cap. Uncured PDMS was applied on the edges of the PDMS block and cured in an oven at 65 °C for 30 min to seal the cap to the taped PMMA-coated coverslip.

Bacterial Growth Conditions and Flow Cell Loading. *P. aeruginosa* strain AFS64 (PA14 wild-type expressing GFP)⁴³ was streaked on Luria-broth/Miller (LB) (BD Biosciences, Franklin Lakes, NJ) Petri dishes and incubated for 16 h at 37 °C. Single colonies were each inoculated in 2 mL of sterilized LB media and placed on a rotating incubator for 8 h at 37 °C. The cultures for each experiment were back-diluted in LB to reach a mid-log optical density of 0.2 at 600 nm (OD₆₀₀).

A 25 cm-long polyethylene (PE) tubing (0.015 in.) was inserted into the flow channel outlet hole and connected to a Petri dish waste container. Another 25 cm-long PE tubing was inserted into the inlet hole and connected to a 1 mL syringe loaded with distilled deionized water (DDI) water with a 27G hypodermic needle. Distilled deionized water (DDI) water was then pumped at 115 μ L/min using a Legato 110 Syringe Pump (Fisher Scientific, Holliston, MA) to flush the flow cells and ensure no leakage. The syringe was then exchanged with a 1 mL syringe loaded with the bacterial cell culture and pumped at 115 μ L/min for 8 min until the syringe was empty. The cell solution was static in the channel for ~30 min to allow seeded cells to adhere to the PMMA-coated coverslips before microscopy analysis.

Bacteria Motility Microscopy. The loaded flow channels were immediately placed on an Eclipse Ti inverted research microscope (Nikon, Melville, NY) with NIS-Elements software and imaged using a 100 $\times$ oil objective at room temperature. Each PMMA-coated

surface was observed in triplicate trials. A 25 cm-long tubing was inserted into the outlet hole and connected to a waste container. A 20 mL syringe loaded with only LB media was placed on a syringe pump and connected to a 25 cm-long PE tubing inserted into the flow channel inlet hole. A flow rate of 115 μ L/min was applied to the syringe. Time-lapse movies were acquired by obtaining phase images every minute on the flat and nanopillared surfaces for 2–3 h to observe surface-attached cell motility.

Motility Analysis and Quantification. ImageJ was used to track the motility of the bacterial cells from the time-lapse videos. Data were collected from the time-lapse movies using the ImageJ TrackMate plugin.⁶² The collected data including cell positions and velocities were analyzed to generate motility analysis plots via MATLAB. Cells (50) with the longest distance traveled from each surface were included in the cell trajectory analysis and normalized to originate at (0,0) (Figure 4). The displacement and velocity of all cells were calculated and averaged to construct the average upstream displacement (Figure 5a) and average upstream velocity (Figure 5b) plots, respectively. For angular trajectories, orientation angles were calculated with respect to the flow direction (Figure 6a). The orientation angles of five cells surface-attached for longer than 1 h were tracked once per minute and displayed in the angular cell trajectories plot (Figure 6b). The absolute best-fit angle from the trajectories of cells with the longest distances traveled was calculated and displayed in the angular trajectory histograms (Figure 6c). The number of cells was quantified on each flat and nanopillared surface triplicate every minute to obtain the percentage of remaining surface-attached cells relative to the starting time point.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b22262.

P. aeruginosa upstream motility (right to left) on a flat PMMA surface under 2 h of flow (left to right); sped at 4 frames/s (ZIP)

P. aeruginosa motility on an antifouling P500 nanopillared PMMA surface under 2 h of flow (left to right); sped at 4 frames/s (ZIP)

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Author Contributions

R.R., K.P., and S.A. conceived the project. R.R. performed the nanofabrication, bacteria culture, flow experiments, and analyzed data. K.P. performed bacteria culture and flow experiments. V.K.L. performed nanofabrication and analyzed data. S.A., A.S., and A.F.Y. helped design experiments and discussed the results. R.R. and A.F.Y. wrote the manuscript, and all authors provided feedback.

Notes

The authors declare no competing financial interest.

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UC Irvine. Contact angle measurements were performed at the Lawrence Berkeley National Laboratory (LBNL) Molecular Foundry. Work at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Science, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. This research was supported by the Department of Defense (DoD) Peer-Reviewed Medical Research Program (PRMRP) award no. W81XWH-17-0355 to A.F.Y. and the National Institutes of Health (NIH) Career Transition award no. K22AI112816 to A.S.

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