A sticky bacterium can overcome various antiadhesive surfaces

Shogo Yoshimoto¹, Satoshi Ishii¹, Ayane Kawashiri¹, Taishi Matsushita², Dirk Linke³,
Stephan Göttig⁴, Volkhard A. J. Kempf⁴, Madoka Takai², Katsutoshi Hori¹*.

¹ Department of Biomolecular Engineering, Graduate School of Engineering, Nagoya University, Nagoya, Aichi 464-8603, Japan.
² Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Bunkyo-ku, Tokyo 133-8656, Japan.
³ Department of Biosciences, University of Oslo, 0316 Oslo, Norway
⁴ Institute for Medical Microbiology and Infection Control, University Hospital, Goethe University, Frankfurt 60596, Germany.

*Correspondence: Katsutoshi Hori
Tel: +81-52-789-3339
Fax: +81-52-789-3218
E-mail: khorie@chembio.nagoya-u.ac.jp
Abstract

While microorganisms have evolved to adhere and form biofilms on surfaces, many materials with antiadhesive surfaces have been developed. The Gram-negative bacterium *Acinetobacter* sp. Tol 5 exhibits high adhesiveness to various surfaces of general materials, from hydrophobic plastics to hydrophilic glasses and metals, via AtaA, an *Acinetobacter* trimeric autotransporter adhesin (TAA). However, efficient antiadhesive surfaces should prevent the adhesion of Tol 5. Here, we examined the adhesion of Tol 5 and other bacteria expressing different TAAs to antiadhesive surfaces. The results highlighted the stickiness of Tol 5 through the action of AtaA, which enabled Tol 5 cells to adhere even to antiadhesive materials, including polytetrafluoroethylene with a low surface free energy, a hydrophilic polymer brush exerting steric hindrance, and mica with an ultrasmooth surface. Single-cell force spectroscopy as an atomic force microscopy technique revealed the strong cell adhesion force of Tol 5 to these antiadhesive materials. Nevertheless, Tol 5 cells showed a weak adhesion force toward a zwitterionic 2-methacryloyloxyethyl-phosphorylcholine (MPC) polymer-coated surface. Dynamic flow cell experiments revealed that Tol 5 cells, once attached to the MPC polymer-coated surface, were exfoliated by weak shear stress. The underlying adhesive mechanism was presumed to involve exchangeable, weakly bound water molecules, suggesting that a perfect antiadhesive surface needs to possess a high free water fraction.
Main

The widespread and increasing global presence of multidrug-resistant bacteria has become a clinical problem \(^4\), and the US Centers for Disease Control and Prevention (CDC) has classified multidrug-resistant *Acinetobacter* as the top worldwide health concern \(^5\). The overuse and inappropriate prescription of antibiotics enhances the opportunity for resistant bacteria to emerge and spread \(^6\). As an alternative to antibiotics, antiadhesive (antifouling) surfaces have drawn intensive research interest because bacterial adhesion is the initial step of both infection by pathogens and the biofouling of equipment \(^1,7-9\). As a result of extensive efforts, various antiadhesive surfaces, such as fluoropolymers, polymer brushes, highly hydrophilic zwitterionic polymers, and ultrasmooth or nanopatterned surfaces, have been developed and characterized \(^10-14\). *Acinetobacter* sp. Tol 5, which is a toluene-degrading bacterium that we previously isolated from a biofiltration system, exhibits autoagglutination and high adhesiveness to solid surfaces \(^15,16\). Tol 5 cells quickly adhere to various material surfaces, from hydrophobic plastics to hydrophilic glasses and metals, independently of biofilm formation \(^15\). Atomic force microscopy (AFM) revealed that the adhesion force of Tol 5 to a sharp silicon nitride probe was near 2 nN, which was 1–2 orders of magnitude stronger than that of other highly adhesive bacteria \(^17\). This characteristic nonspecific adhesiveness of Tol 5 cells is mediated by a single protein, AtaA \(^2,18,19\), a member of the trimeric autotransporter adhesin (TAA) family \(^3\). TAAs are outer membrane proteins of Gram-negative bacteria and have been well studied as virulence factors because they enable binding to biotic molecules of mammalian host cells and, in some cases, to various abiotic surfaces \(^20,21\). Although they vary in sequence length from several hundred to several thousand amino acids, they have a common structure that includes an
N-terminal passenger domain (PSD), which is secreted onto the cell surface and is
responsible for its function, and a C-terminal transmembrane domain, which anchors the
PSD in the outer membrane. The AtaA of *Acinetobacter* Tol 5 is one of the largest
TAAs known to date. It consists of 3,630 amino acids per monomer, it shares common
structural features with other TAAs, and its N-terminal head domain is essential for
the adhesive function of AtaA. However, there have been no reports of TAA-mediated
adhesion similar to that of Tol 5 cells through the action of AtaA in terms of
nonspecificity and high stickiness for material surfaces.

Similar to the proverb known as the “shield-spear contradiction” derived from the
ancient Chinese text *Han Feizi*, which is a story about a merchant who sells spears that
can pierce anything and shields that can block anything, we do not have a good
explanation for what happens when highly adhesive Tol 5 cells encounter an
antiadhesive surface. In this study, we systematically investigated the interaction of Tol
5 and several other TAA-expressing bacterial strains with various materials, including
antiadhesive surfaces that have different repelling mechanisms.

**Adhesion of bacterial cells to various materials**

First, we compared the adhesiveness of Tol 5 and its Δ*ataA* mutant with that of
*Yersinia enterocolitica* WA-314 and *Bartonella henselae* Marseille by shaking each cell
suspension in the presence of a polyurethane support for 30 min. These Gram-negative
bacteria have also been reported to adhere to abiotic surfaces through the peritrichate
fibers of their TAAs, *YadA* and *BadA*, respectively. *YadA* is a well-studied short
TAA consisting of 422 amino acids (GenBank: CBW54734.1). *BadA* (3,973 amino
acids) (GenBank: MK993576.1) is a little larger than AtaA, and it has also been
reported to be more adhesive than other TAAs. It is worth noting that all of these TAAs
show sequence and length variations among different strains and isolates. The expression of these TAAs was confirmed by western blotting (see Supplementary Figure S1). In initial adhesion assays with a polyurethane support, Tol 5 cells expressing AtaA showed overwhelmingly strong stickiness when compared with cells expressing other TAAs (Fig. 1). Most of the Tol 5 cells adhered to the polyurethane support during shaking, and consequently, the Tol 5 cell suspension appeared clear. In contrast, the cell suspensions of Y. enterocolitica, B. henselae, and the Tol 5 ΔataA mutant remained cloudy, which indicated that many of the cells did not adhere to the polyurethane support.

Next, we quantified the adhesiveness of bacterial cells expressing TAAs to various material surfaces. Cell suspensions were incubated on polystyrene (PS), glass, stainless steel, and polytetrafluoroethylene (PTFE, known as Teflon) surfaces for only 10 min. Nonadhering cells were removed by washing with fresh medium, and the adhered cells on the material surface were quantified by crystal violet staining. In this short time, Tol 5 adhered not only to PS, glass, and stainless steel but also to PTFE, which has antiadhesive properties derived from its low surface energy (Figure 2). In contrast, the Tol 5 ΔataA mutant and Y. enterocolitica hardly adhered to any of the material surfaces. Although B. henselae showed measurable adhesiveness, the amount of adhered cells was much smaller than that of Tol 5. These results quantitatively demonstrated that Tol 5 cells exhibited remarkably higher adhesiveness to various material surfaces through the action of AtaA compared to that of bacterial cells expressing other TAAs.

Adhesion of bacterial cells to antiadhesive surfaces

To investigate whether Tol 5 cells can adhere to various other antiadhesive surfaces in addition to PTFE, we performed adhesion assays with mica, poly(oligo(ethylene glycol)
methyl ether methacrylate) (poly(mOEGMA)) brush, and 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer surfaces (Table 1). Mica is a phyllosilicate mineral of aluminum and potassium, and its cleaved surface is atomically flat. A poly(mOEGMA) brush is a neutral hydrophilic polymer brush and exerts steric repulsion. An MPC polymer is a zwitterionic hydrophilic polymer that has a high free water fraction. The surfaces of these materials have been reported to have antiadhesive properties against bacterial cells. After the incubation of bacterial cells on the antiadhesive surfaces for 10 min, it was found that Tol 5 cells adhered to the surface of PTFE but not to that of mica, the poly(mOEGMA) brush, or the MPC polymer (Fig. 3A). After incubation for 2 h, Tol 5 cells adhered to the surface of not only PTFE but also mica and the poly(mOEGMA) brush but hardly adhered to the surface of the MPC polymer (Fig. 3A). In contrast, *B. henselae* adhered to the surface of PTFE and mica but not to that of the poly(mOEGMA) brush and MPC polymer after a 2-h incubation (Fig. 3B). These results emphasized that Tol 5 cells were the only cells that adhered to the poly(mOEGMA) brush surface and showed that even sticky Tol 5 cells hardly adhered to the MPC polymer surface under these experimental conditions.

**Direct measurement of the adhesion force of bacterial cells using AFM**

In contrast to semiquantitative adhesion assays that measure the amount of cells adhering to hole plates or microwells by staining, AFM can quantify the strength of adhesion as the adhesion force in Newtons, i.e., the resistance force against peeling. We previously reported that *Acinetobacter* sp. Tol 5 exhibits an adhesion force of 2 nN to a sharp silicon nitride AFM probe, which was more than 10-fold stronger than that of the *B. henselae* strain. To quantify the strength of adhesion of Tol 5 to various materials, including antiadhesive surfaces, compared to that of the *B. henselae* strain, which
showed some adhesiveness to PTFE and mica, we measured the adhesion force of a single cell using AFM. To ensure that the adhesion force of a single cell was measured, a single colloidal probe made from PS, SiO₂, gold, poly(mOEGMA) brush-coated SiO₂, or MPC polymer-coated SiO₂ (Table 1) was pressed against the top of a single bacterial cell immobilized on a glass substrate beforehand, and the force required to pull the probe away from the cell was measured (Fig. 4A). This single-cell force spectroscopy demonstrated that Tol 5 exhibited a large adhesion force over a long distance of 500-1,000 nm (Fig. 4B-E). The median of the maximum adhesion forces of Tol 5 to PS, SiO₂, gold, and even a poly(mOEGMA) brush was 2-5 nN, which was much stronger than that of B. henselae (≤1 nN) (Fig. 4G). The adhesion work of Tol 5, which is the energy required for detachment and is shown as the peak area of the adhesion force curve, was more than 10 times larger than that of B. henselae (Fig. 4H). On the other hand, both Tol 5 and B. henselae showed little adhesion force and small adhesion work when adhered to the MPC polymer (Fig. 4F-H). These results highlighted the remarkably strong nonspecific adhesion of Tol 5 to PS, SiO₂, gold, and even a poly(mOEGMA) brush, except for the MPC polymer, demonstrating its remarkable antiadhesive property.

**Behavior of Tol 5 cells on the MPC polymer surface under flow**

To investigate the behavior of Tol 5 cells on the MPC polymer surface in the presence of shear forces, we observed Tol 5 cells on the surface by using a flow cell system with a square glass tube (Fig. 5A) 29. The glass tube with or without the MPC polymer coating was filled with a Tol 5 cell suspension and incubated for 10 min. Then, the cell suspension was replaced with fresh BS-N buffer (34.5 mM Na₂HPO₄, 14.7 mM KH₂PO₄, 15.5 mM K₂SO₄, pH 7.2) by flowing slowly at 1 cm/min for rinsing, and the
fluid velocity was increased stepwise, as shown in Figure 5B, while observing the inner surface of the bottom of the glass tube under a microscope. Unexpectedly, Tol 5 cells adhered to the MPC polymer-coated glass as much as to the bare (noncoated) glass under static conditions and remained adhered after rinsing at 1 cm/min (Fig. 5C). When the fluid velocity was increased to 2 cm/min, a small fraction of previously adhered cell clumps started to move and slip on the surface (see Supplementary Movie S1), but many cells still resisted detachment after 10 min of flow (Fig. 5C, 2 cm/min). At a high fluid velocity of 5 cm/min or more, the Tol 5 cells firmly adhered to the bare glass, whereas the cells attached to the MPC polymer were exfoliated, rolled, and washed off from the surface by shear stress (≥5.94 mN/m$^2$) (Fig. 5C, ≥5 cm/min; see Supplementary Movie S1).

Discussion

To date, various antiadhesive materials have been developed on the basis of different repelling mechanisms. Fluoropolymers, including PTFE with a low surface free energy, are widely used in not only cookware but also labware and medical equipment. Polymer brushes with a high grafting density have been especially studied as powerful antiadhesive surfaces for cell adhesion based on their hydrophilicity and steric repulsion. The finding that Tol 5 is able to adhere to these antiadhesive materials shows its remarkably wide range of adhesion preferences.

Although BadA is similar to AtaA in size and its fibrous molecules peritrichously cover the bacterial cells, the adhesiveness of $B. henselae$ to material surfaces was lower than that of Tol 5, and in contrast to Tol 5, $B. henselae$ did not adhere to the poly(mOEGMA) brush (Fig. 3, 4). Their difference in adhesiveness demonstrated the functional diversity of the TAA family as a result of protein evolution; $B. henselae$ is a
zoonotic pathogen and has therefore adapted to mammalian surfaces\(^\text{24}\), and Tol 5 has
adapted to environmental and abiotic surfaces. Our study demonstrates the importance
of using a variety of bacteria in the evaluation of antiadhesive surfaces.

Dynamic adhesion experiments using a flow cell system revealed that Tol 5 cells even
adhered to an MPC-polymer-coated surface, but their interaction was so weak that the
cells could be exfoliated by a weak shear stress (Fig. 5). In the static adhesion assays
using a microwell (Fig. 3), the Tol 5 cells must have been detached by the washing step.
In addition, the exfoliated and rolling cell clumps seemed to incorporate and remove
cell clumps that were still adhered owing to the autoagglutinating property of Tol 5 cells
\(^\text{29}\), resulting in the self-cleaning of the surface coated with the MPC polymer (see
Supplementary Movie S1). This suggested that the autoagglutinating property, which
generally promotes adhesion and microcolony formation\(^\text{32}\), negatively affects adhesion
to the MPC polymer. The high potential of MPC polymers to inhibit adhesion, which
can prevent even the adhesion of highly sticky Tol 5, as an antiadhesive material was
thus reaffirmed. MPC is a methacrylate monomer with a phosphorylcholine (PC) group,
which is a hydrophilic polar head group also present on phospholipids comprising
eukaryotic cell membranes\(^\text{33}\). The underlying inhibitory mechanism for the adhesion of
proteins and cells to MPC polymers is based on their interactions with water molecules:
while there are many free water molecules present\(^\text{27}\), only a few bound water molecules
are captured by the PC groups of the MPC polymers\(^\text{33-35}\). Tol 5 cells could adhere to the
surfaces of the poly(mOEGMA) brush and mica but could only interact very weakly
with the surface coated with MPC polymers, despite similar levels of hydrophilicity of
these materials, as shown by the static contact angles of air in water (Table S2). This
suggested that exchangeable, weakly bound water molecules contribute to the
interaction between AtaA and the material surfaces.

Analogous to antibiotic resistance acquisition, bacteria can adapt to newly developed antiadhesive surfaces to enable adhesion to surfaces. It is thus difficult to develop an ultimate material that repels any bacteria in any environment. However, using the highly adhesive strain Tol 5 gives us an efficient model system for the development of a potentially perfect antiadhesive surface; our work suggests that this surface needs to possess a high free water fraction.
Methods

Materials

A polyurethane foam support (1 cm$^3$ cube; CFH-30) was obtained from Inoac Corporation (Aichi, Japan). Polystyrene plates (PS2035-1), glass plates (FF-001), stainless steel plates (SUS430 grade; EA441WA-21), PTFE plates (J1-537-01), mica disks (V-1 grade), and square glass tubes (VitroTubes, 8100) were purchased from Hikari Co., Ltd. (Osaka, Japan), Matsunami Glass Ind., Ltd. (Osaka, Japan), ESCO Co., Ltd. (Osaka, Japan), AS ONE Corp. (Osaka, Japan), TED PELLA, Inc. (CA, USA), and Vitrocom (Mountain Lakes, NJ), respectively. A poly(mOEGMA) brush surface was prepared on glass plates as described previously. The MPC polymer-coated surface for the adhesion assays was prepared as described below. A glass plate was dipped into 2% MPC polymer solution (Lipidure-CM5206, a copolymer of MPC and butyl methacrylate; NOF Corp., Tokyo, Japan) dissolved in ethanol and shaken at 70 rpm for 3 min. After shaking, the glass plate was rinsed in pure water and dried at 60 °C for 3 h. The MPC polymer-coated glass tube was prepared as described below using the flow cell system. A solution of 2% MPC polymer dissolved in ethanol was flushed through a square glass tube (50 mm in length and 1 mm in every internal dimension) at 10 cm/min for 3 min, and then pure water was flushed through at 50 cm/min for 2 min. After passing air through at 50 cm/min for 2 min, the glass tube was removed from the flow cell system and dried at 60 °C for 3 h.

AFM colloidal probes modified with silica (CP-PNPL-SIO-A), polystyrene (CP-PNPL-PS-A) and gold (CP-PNPL-Au-A) colloids of 2 μm in diameter were purchased from NanoAndMore (Wetzlar, Germany). A poly(mOEGMA) brush-modified colloidal probe was prepared by grafting the poly(mOEGMA) brush onto the silica-colloidal
probe as described previously. An MPC polymer-coated colloidal probe was prepared by immersing the silica-colloidal probe in 0.2% MPC polymer solution and incubated for 1 h at room temperature. After incubation, the MPC polymer-coated colloidal probe was rinsed in pure water.

Microwells used in the adhesion assays with various material surfaces were constructed in our lab as follows: Four sheets of vinyl electrical tape (ASKUL Corporation, Tokyo, Japan) were stacked, and holes with a diameter of 6 mm were punched into the stacked tape. The punched tape was placed on a material plate as shown in Supplementary Figure S2.

Prior to use, glass was washed with piranha solution (H₂SO₄:30% H₂O₂ = 3:1) followed by rinsing with pure water, mica was cleaved with scotch tape, the MPC polymer-coated surface was rinsed with pure water, and the other surfaces were rinsed with pure ethanol.

The static contact angles (SCAs) of air bubbles in water on each material surface were measured with a contact angle meter (CA-W, Kyowa Interface Science Co., Tokyo, Japan) at room temperature and are listed in Supplementary Table S2. Material substrates were immersed in water, and 10 μL of air bubbles were placed on the substrates.

**Bacterial strains and culture conditions**

The bacterial strains used in this study are listed in Supplementary Table S1. These bacterial strains were grown as previously described. *Acinetobacter* strains were grown in Luria-Bertani (LB) medium at 28 °C for 8 h. An overnight culture of *Y. enterocolitica* strains grown in LB medium at 28 °C was used to inoculate the LB
medium at a 1:100 dilution, and the medium was incubated at 37°C for 6 h to induce
yadA expression \(^{37}\). *B. henselae* strains were grown for 5 days on Columbia agar
supplemented with 5% sheep blood at 37 °C in a humidified atmosphere with 5% CO\(_2\).
The expression of trimeric autotransporter adhesins in each strain was confirmed by
western blotting using anti-Ata\(_{699-1014}\) antiserum, anti-BadA antibodies \(^{38}\), or anti-YadA
antibodies (sc-22472; Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

**Polyurethane foam support adhesion assays**

The polyurethane foam support adhesion assays were performed as previously described
\(^{39}\), with slight modifications. Bacterial cells were suspended in BS-N buffer, and the
optical density of the cell suspension at 660 nm (OD\(_{660}\)) was adjusted to 1.0. Four pieces
of the polyurethane foam support were placed into 20 mL of the cell suspension and
shaken at 115 rpm at 28 °C. After a 30-min incubation with shaking, the transparency of
the cell suspension was observed and photographed by a digital camera.

**Microwell adhesion assay**

Bacterial cells were suspended in BS-N buffer, and the OD\(_{660}\) was adjusted to 0.5. The
cell suspensions (50 μL each) were placed into a microwell with the materials and
incubated at 28 °C for 10 min. When the antiadhesive surfaces were used, the
incubation time was extended to 30 min and 2 h. The cell suspensions were removed
after incubation using a pipet, and the wells were washed for 10 s by shaking in BS-N
buffer at 70 rpm. Cells adhering to the well were stained with 50 μL of 0.1% crystal
violet solution for 15 min and washed for 10 s by shaking in BS-N buffer at 70 rpm.
Finally, the stain was eluted with 200 μL of 70% ethanol, and the absorbance of the
elution at 590 nm ($A_{590}$) was measured with a microplate reader (ARVO X3; PerkinElmer, Inc., MA, USA).

**Single-cell force spectroscopy**

Bacterial cells were immobilized on a glass bottom dish (FluoroDish, FD5040-100; World Precision Ins., Sarasota, FL, USA) as previously described. Single-cell force spectroscopy was performed using a NanoWizard 3 BioScience AFM system (JPK Ins., Berlin, Germany) in contact mode at room temperature in BS-N buffer. For measurement, colloidal probes with a diameter of 2 µm and spring constants of 0.06-0.09 N/m were used. The spring constants of the cantilevers were measured using the thermal noise method. The parameters used in the measurement were as follows: Z-length: 3 µm; applied force: 0.2 nN; speed: 1 µm/s; cell surface dwell time: 0.1 s; and sample rate: 5000 Hz.

**Flow cell system**

A rectangular flow cell system, which we have previously reported, was used with slight modifications: a syringe pump (Legato 200; KD Scientific, Holliston, MA) was directly connected to a square glass tube 50 mm in length and 1 mm in every internal dimension without using a three-way stopcock valve. Tol 5 cells were suspended in BS-N buffer at an OD$_{660}$ of 0.2, and the suspension was subjected to sonication to break up the cell clumps. The glass tube with or without an MPC polymer coating was filled with the cell suspension and incubated for 10 min at room temperature. The suspension was replaced with fresh BS-N buffer by flowing slowly at 1 cm/min for 35 min, and the fluid velocity was increased stepwise. Live images of the
behavior of Tol 5 cells during their adhesion and detachment to/from the inner surface of the glass tube were recorded under a digital microscope (VHX-200; Keyence, Osaka, Japan). Wall shear stress ($\tau$) was calculated using Equation (1), as previously described:

$$\tau = \frac{8\mu U}{D}$$

Equation (1)

where $\mu$, $U$, and $D$ are the fluid viscosity, fluid velocity, and diameter of the glass tube, respectively. The equivalent diameter ($De$) was used for the $D$ value in Equation (1), which was calculated according to Equation (2):

$$De = \frac{4A}{P}$$

Equation (2)

where $A$ and $P$ are the sectional area and wetted perimeter, respectively.

Acknowledgments

We thank Kai Iio and Eriko Matsui for their technical assistance. This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant Numbers JP17H01345, JP18K14062, JP21H05227), by the Research Council of Norway (grant 331752) to DL, and by the Deutsche Forschungsgemeinschaft (DFG research group 2251) to SG and VAJK.

Author contributions

K.H. designed the study, and S.Y., A.K., and K.H. wrote the paper. S.Y. and A.K. conducted the adhesion assays. S.I. and S.Y. conducted the AFM analysis. A.K., T.M., and M.D. prepared the poly(mOEGMA) brush surface and measured the static contact angles of air bubbles in water. S.I. and A.K. conducted the cultivation and protein
analysis of bacterial cells with help from D.L, S.G., and V.A.J.K. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Data and materials availability
The data within this paper are available from the corresponding author upon reasonable request.

References


Muller, N. F. *et al.* Trimeric autotransporter adhesin-dependent adherence of *Bartonella henselae, Bartonella quintana,* and *Yersinia enterocolitica* to matrix components and endothelial cells under static and dynamic flow conditions. *Infect Immun* 79, 2544-2553 (2011).


Koiwai, K., Hartmann, M. D., Linke, D., Lupas, A. N. & Hori, K. Structural basis for toughness and flexibility in the C-terminal passenger domain of an


Fig. 1. Adhesion of bacterial cells to a polyurethane surface. Each panel shows the bacterial cell suspension after shaking for 30 min with a polyurethane foam support.
Fig. 2. Adhesion of bacterial cells to various materials. Adhesion of *Acinetobacter* sp. Tol 5, Tol 5 Δ*ataA*, *B. henselae* (*B. h.*), and *Y. enterocolitica* (*Y. e.*) to polystyrene, glass, stainless steel, and PTFE was assessed by microwell adhesion assays after 10 min of incubation. Data are expressed as the means ± SEMs (n=3). Significant differences are indicated by an asterisk (Student’s t test, p<0.05). The upper photographs show the adhered cells on the material surfaces after staining.
Fig. 3. Adhesion of bacterial cells to antiadhesive surfaces. Adhesion of Tol 5 (A) and B. henselae (B) to PTFE, mica, a poly(mOEGMA) brush on glass and MPC polymer-coated glass was assessed by microwell adhesion assays. Data are expressed as the means ± SEMs (n=3). The upper photographs show the adhered cells on the material surfaces after incubation for 2 h.
**Fig. 4.** Single-cell force spectroscopy using colloidal probes. (A) Schematic illustration of the measurement. (B-F) Typical force curves of bacterial cell adhesion to colloidal probes made of various materials: polystyrene (B), SiO$_2$ (C), gold (D), a poly(mOEGMA) brush (E), and an MPC polymer (F). (G, H) Box and whisker plot of the adhesion force (G) and adhesion work (H) of bacterial cells adhered to various colloidal probes. The boxes represent the data from the 25th to the 75th percentile, and the whiskers extend to the 10th/90th percentile. The horizontal lines in the box represent the median values.
Fig. 5. Observation of the behavior of Tol 5 cells adhered to the MPC polymer surface beforehand. (A) Schematic representation of the flow cell system used in this study. (B) Transition of the fluid velocity. The fluid velocity was increased stepwise every 5 min. The black arrowheads indicate the time at which snapshots of the inner surface at the bottom of the glass tubes were captured. (C) Snapshots captured as described in (B).
Table 1. Surface materials used in this study.

<table>
<thead>
<tr>
<th>Property</th>
<th>Microwell material</th>
<th>AFM probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>Polystyrene</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>Hydrophilic</td>
<td>Glass</td>
<td>SiO₂</td>
</tr>
<tr>
<td>Metal</td>
<td>Stainless steel</td>
<td>Gold</td>
</tr>
<tr>
<td>Low surface energy</td>
<td>PTFE/Teflon</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atomically flat</td>
<td>Mica</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Strong hydrophilic/nonionic, steric repulsion</td>
<td>poly(mOEGMA) brush-coated glass</td>
<td>poly(mOEGMA) brush-coated SiO₂</td>
</tr>
<tr>
<td>Strong hydrophilic/high free water fraction/zwitterionic</td>
<td>MPC polymer-coated glass</td>
<td>MPC polymer-coated SiO₂</td>
</tr>
</tbody>
</table>

<sup>a</sup> PTFE and mica were not able to be used as AFM probes.