Supporting Information for:

Tuning Bacterial Attachment and Detachment via the Thickness and Dispersity of a pH-Responsive Polymer Brush

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Table S1. Static water contact angles for *S. epidermidis* on two lawns prepared from independent cultures. Surface energy was calculated by the method of Wu.[1]

	Water [°]	Diiodomethane [°]	Ethylene Glycol [°]	Surface Energy [mN/m]
Sample 1	48 ± 4	63 ± 2	58 ± 2	48
Sample 2	47 ± 4	60 ± 2	55 ± 2	49

Table S2. Zeta potential for *S. epidermidis* obtained from the independent cultures, and measured at pH 4 and pH 9. Zeta potential changes slightly on changing the pH of solution and is reproducible between independent cultures.

	Zeta Potential at pH 4 [mV]	Zeta Potential at pH 9 [mV]
Sample 1	-22 ± 2	-29 ± 1
Sample 2	-21 ± 2	-30 ± 1

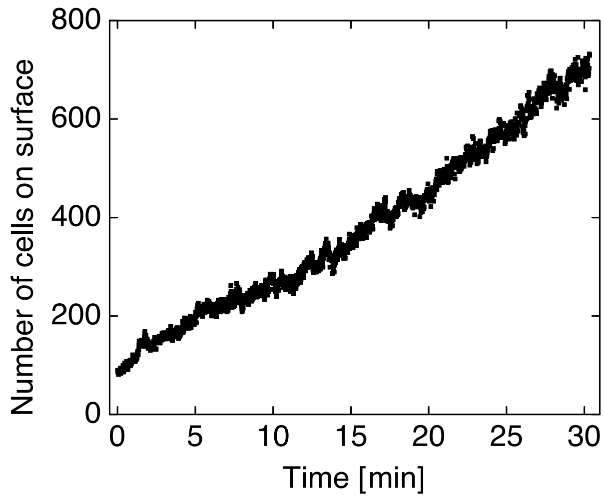
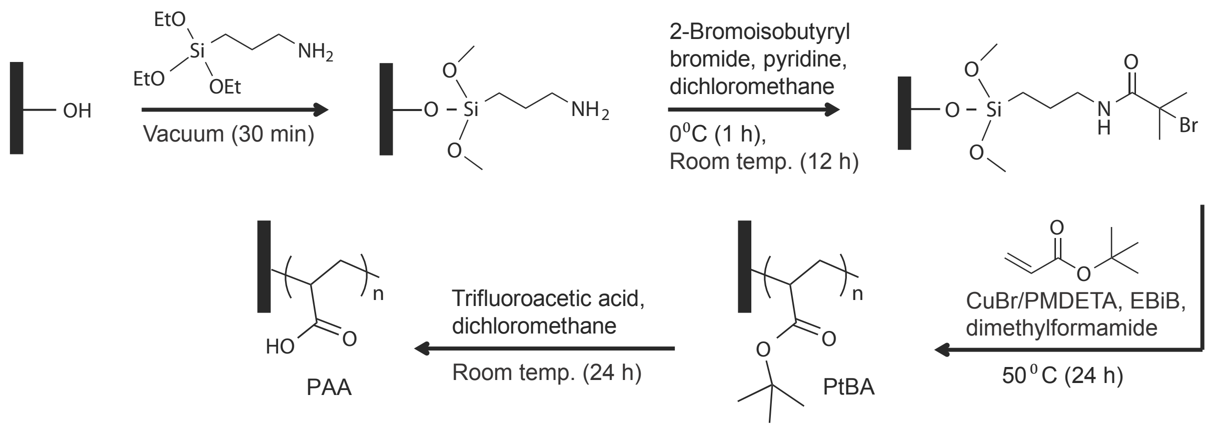


Figure S1. Number of *S. epidermidis cells* deposited on a glass substrate as a function of time from a solution of optical density OD = 0.40, measured using confocal microscopy.



Scheme S1. Synthesis of PAA brushes.

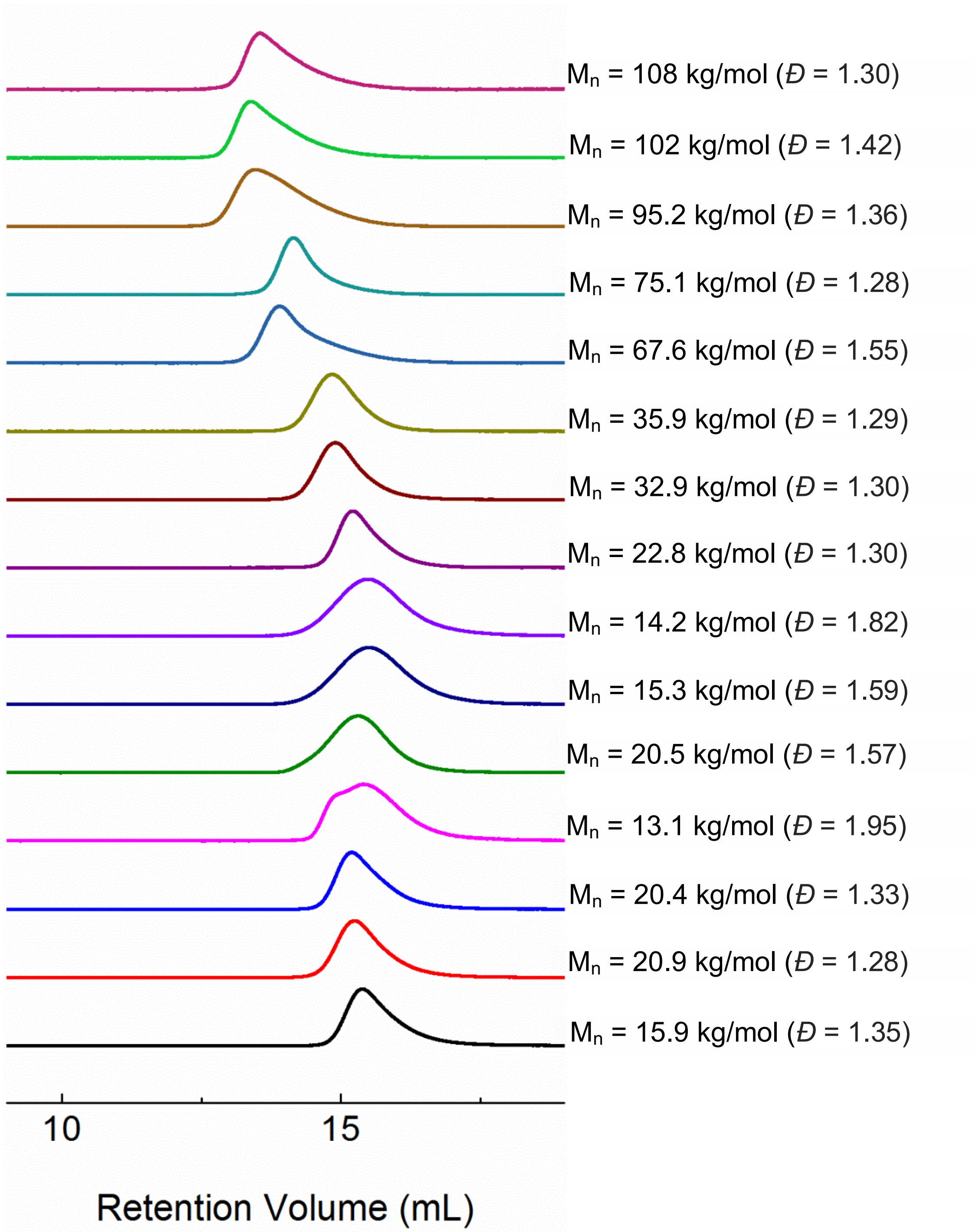


Figure S2. GPC refractometer data for PtBA polymerized in solution, concurrent with the synthesis of PtBA brushes on silicon substrates. Molecular weight and dispersity for each polymer are given in Table S3.

Table S3. Average as-synthesized dry thickness (with error bar) of each PtBA and PAA brush reported in the main text, corresponding molecular weight and dispersity of the PtBA that was polymerized in solution simultaneously during the PtBA brush synthesis, and reduction in brush thickness upon hydrolysis to PAA. Thickness was measured by ellipsometer and instrument has an error of 1 nm. Standard deviation obtained from thickness measurements at different location on the surface was less than 1 nm.

Average PtBA Brush Thickness (nm)	**M_n (kg/mol) of Solution PtBA (*Đ*)**	**Average PAA Brush Thickness (nm)**	**Reduction in PtBA Brush Thickness Upon Hydrolysis to PAA**
11 ± 1	15.9 (1.35)	5 ± 1	54 %
9 ± 1	20.9 (1.28)	4 ± 1	55 %
8 ± 1	20.4 (1.33)	4 ± 1	50 %
9 ± 1	13.1 (1.95)	4 ± 1	55 %
18 ± 1	20.5 (1.57)	8 ± 1	55 %
18 ± 1	15.3 (1.59)	8 ± 1	55 %
19 ± 1	14.2 (1.82)	9 ± 1	53 %
21 ± 1	26.2 (1.16)	10 ± 1	52 %
22 ± 1	22.8 (1.30)	10 ± 1	54 %
28 ± 1	32.9 (1.30)	13 ± 1	53 %
30 ± 1	35.9 (1.29)	14 ± 1	53 %
33 ± 1	67.6 (1.55)	15 ± 1	54 %
42 ± 1	75.1 (1.28)	18 ± 1	57 %
48 ± 1	95.2 (1.36)	21 ± 1	56 %
52 ± 1	102 (1.42)	22 ± 1	58 %
63 ± 1	108 (1.30)	28 ± 1	55 %

Interaction of *S. epidermidis* bacteria with solution PAA

To assess the strength of the interaction of bacteria with PAA, we measured the size of S. epidermidis bacteria that were exposed to solutions of poly(acrylic acid) (PAA) of $M_v \approx 450{,}000$ Da (Sigma-Aldrich) using dynamic light scattering (DLS). DLS data were collected using an ALV goniometer equipped with a He-Ne laser (wavelength λ = 632.8 nm) and an ALV-5000/EPP Multiple tau Digital Correlator (ALV-GmbH, Langen, Germany). Samples were loaded into glass culture tubes for DLS experiments. We collected the light scattered at a fixed angle θ and a temperature of 25°C for 60 s and repeated this measurement five times at a scattering angle of θ = 90°, corresponding to a wavevector $q = \left(\frac{4\pi n}{\lambda}\right)\sin(\theta/2)$ = 18.7 μm^{-1}, where n = 1.332 is the refractive index of water. From the scattered intensity as a function of time, the normalized intensity-intensity correlation function $g_2(q,t) = \langle I(t_0+t)\,I(t_0)\rangle / \langle I(t_0)\rangle^2$ was calculated at lag times t ranging from 0.1 μs to 16 s. To extract the hydrodynamic radius, we fitted the correlation functions to a cumulant fitting form,[6]

$$g^{(2)} - 1 = B + \beta \exp\left(-2\bar{\Gamma}t\right)\left(1 + \frac{\mu_2}{2}t^2\right)^2$$

where B accounts for noise and β is a correction factor of order 1. From the measured correlation time $\tau = (\bar{\Gamma})^{-1}$, we used the Stokes-Einstein relationship to calculate the hydrodynamic diameter $d = k_B T\tau q^2/3\pi\eta$, where η is the viscosity of the solution determined at each PAA concentration using bulk rheology. At pH 4, the solution viscosities for PAA concentrations of 0, 0.005, and 0.2 mg mL^{-1} were determined to be 0.86, 0.89, and 0.98 mPa·s, respectively; at pH 9, the solution viscosities for 0, 0.005, and 0.2 mg mL^{-1} were determined to be 0.86, 0.96, and 1.27 mPa·s, respectively. We also calculated the polydispersity, is $\mu_2/\bar{\Gamma}^2$.

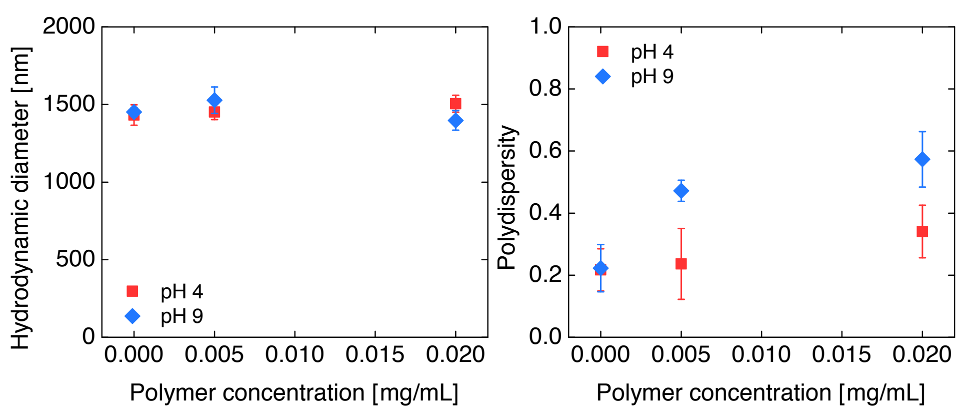


Figure S3. Hydrodynamic diameter (left) and polydispersity (right) of *S. epidermidis* bacteria exposed to poly(acrylic acid) (PAA) in solutions adjusted to pH 4 (squares) or pH 9 (diamonds), extracted from dynamic light scattering measurements. Error bars represent the 95% confidence intervals from the averages of five correlation functions per sample.

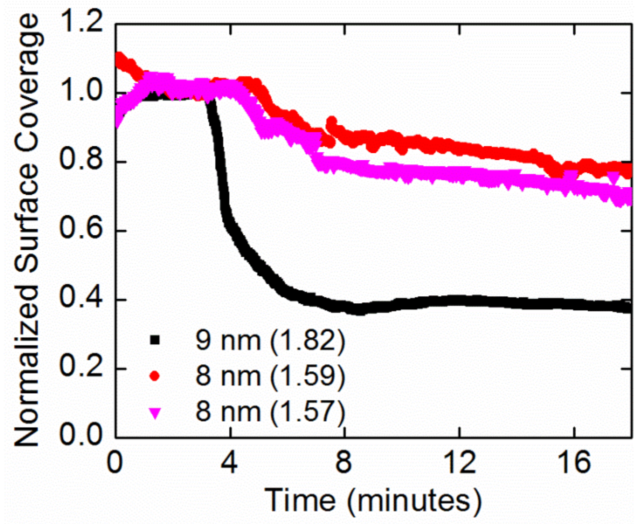


Figure S4. Normalized surface coverage as a function of time after start of pH 9 flow for PAA brushes with varying dispersity. Increase in dispersity leads to greater reduction in surface coverage.

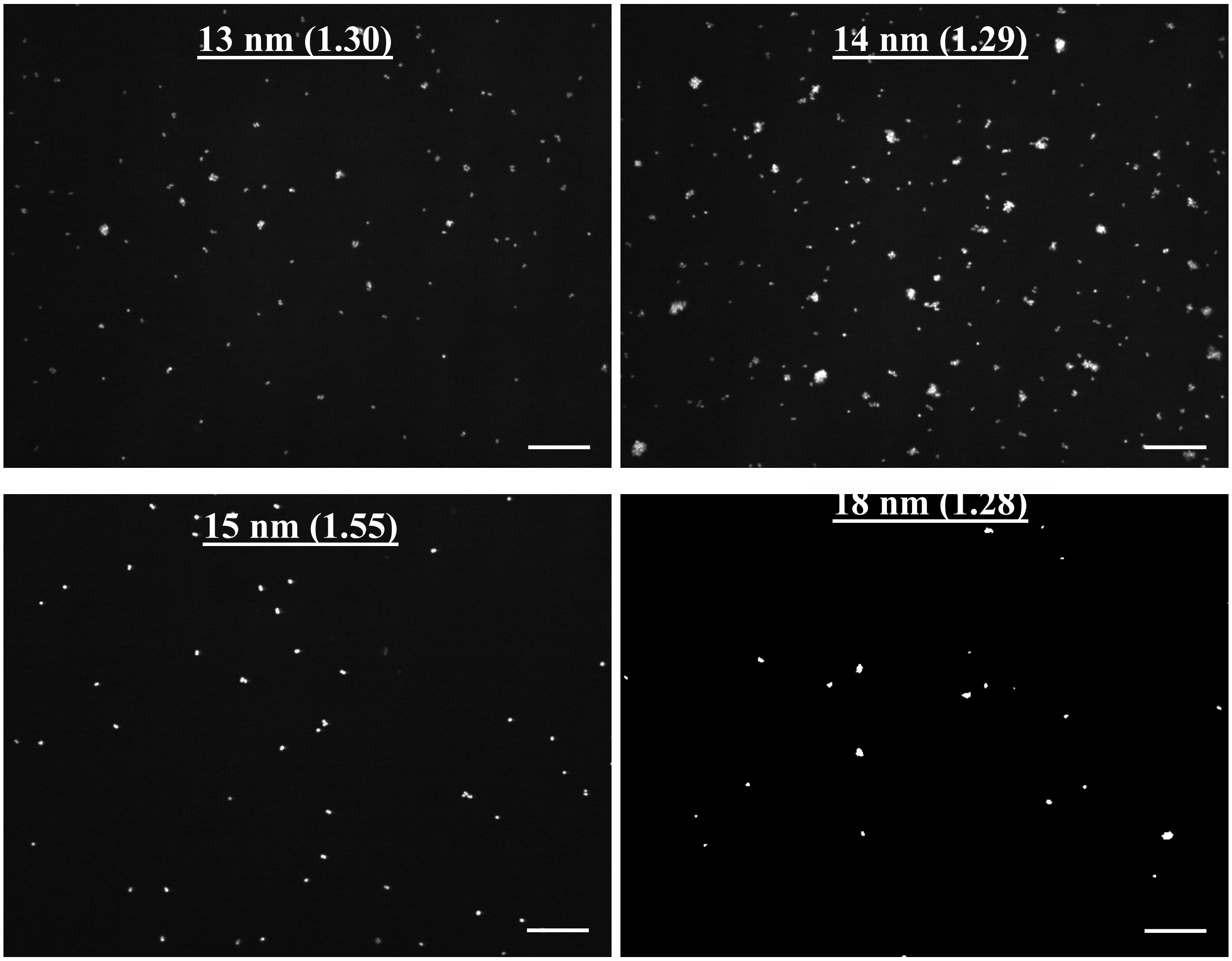


Figure S5. Micrographs of the PAA surfaces exhibiting very low bacterial adhesion after the pH4 flow. Each micrograph shows the PAA brush thickness (dispersity) used for the experiment. Scale bar signifies 40 μm.

Expression for electrostatic interaction energy between PAA brush and bacteria

Charge density of PAA brushes: $\sigma = 0.36 \times (1e) \times N$
(0.36 is grafting density and N is degree of polymerization)

Electric field due to charged PAA brushes: $E = \sigma/2\varepsilon$ (ref [2])
(ε is the permittivity constant)

Charge of the bacteria: $Q = 4\pi\varepsilon\zeta R(1+\kappa R)$ (ref [3])
(ζ is the zeta potential, R is the radius of a bacterium, and κ is the inverse Debye length)

Free energy of electrostatic interactions: $w = -\int F\cdot dr = -\int QE\cdot dr$

- ⇨ $w = 2\pi\zeta R(1+\kappa R) \times 0.36 \times (1e) \times N \times d$
- ⇨ d is the distance between brush and bacteria
- ⇨ $d \le d_{max}$

- ⇨ $d_{max} = N \times (0.36)^{-1/3} \times (f/(1-f))^{1/3} \times ([H^+] + IS)^{1/3}$ (ref [4])
  (f is the degree of dissociation, IS is the ionic strength)

Therefore, $w \propto N^2$, so that increasing the molecular weight (and brush thickness) leads to greater repulsion between PAA brushes and bacteria.

Estimate of the gravitational force of a sedimenting bacterium on brushes

First, we measured the gravitational flux by counting the number of bacteria that approached the surface in a quiescent solution over time (following Ref. [5]). The measurement (Fig. S1) yielded a flux $k = 0.0006$ cells min^{-1} μm^{-2}. Using the argument in Chen et al.[5], the flux k is (to first order) proportional to the terminal sedimentation velocity v_s,

$$k = v_s C_0$$

where $C_0 = 4 \times 10^8$ cells mL^{-1} is the cell concentration in the free volume (measured through plate-counting and through direct counting of the number of bacteria in a 3-D volume using confocal microscopy). The terminal sedimentation velocity is proportional to the density difference between bacteria and water,

$$v_s = \frac{g(\rho_p - \rho)d_p^2}{18\mu}$$

where d_p is the diameter of a bacterium (estimated as a sphere, $d_p = 1$ μm), μ = 0.89 mPa·s is the viscosity of water, and g is the gravitational coefficient. Finally, combining these two expressions allows us to estimate the magnitude of the gravitational force exerted by a bacterium on a brush as

$$F = \frac{4}{3}\pi\left(\frac{d_p}{2}\right)^3(\rho_p - \rho)g = 3\frac{\pi\mu d_p k}{C_0}$$

This expression yields 0.2 fN as the upper bound on the force exerted by a bacterium on the brushes.

References

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