Supporting Information

Self-Assembled Poly(ethylene glycol)-co-Acrylic Acid Microgels Inhibit Bacterial Colonization of Synthetic Surfaces

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S1. Bulk Gel Swelling Properties

Figure S1 describes the effects on swelling of changes in gel composition, solvent used during synthesis, and precursor concentration in the solvent prior to polymerization. We used bulk gels with dry weights of about 0.2 g to determine the pH-dependent swelling properties. Each data point represents the average of measurements on three nominally identical gels. Error bars correspond to the standard deviation about the average. Fig. S1A shows the swell ratio as a function of pH for pure PEG gels synthesized as 20 wt% solutions in either DCM or in a 50% H_2O – 50% EtOH solution. PEGDA 575 is not fully soluble in pure water. In both cases, due to the absence of charge, the swell ratio of the pure PEG gel shows no systematic dependence on pH. Adding 10 vol% acrylic acid slightly decreases the swell ratio at lower pH relative to pure PEG (fig. S1B). In addition to covalent crosslinks introduced during the free-radical acrylate polymerization, at lower pH an additional set of pH-dependent crosslinks is introduced by hydrogen bonding between the AA and PEG.[1] These would reduce the effective mesh size of the gel and reduce the extent to which the gels can swell relative to pure PEG gels. At higher pH values, however, the swell ratio increases significantly. The transition occurs at pH values between 6 and 7 consistent with results summarized by Choi and Rubner [2] on the pH-dependent degree of ionization of poly(acrylic acid). They found that the pH at which 50% of the acid groups deprotonate is 6.5. In this state, the negatively charged carboxyl groups will repel each other as well as the PEG ether oxygen, thus swelling the gel network and increasing its capacity to accumulate both bound and free water.

Figure 1C includes the pH-dependent swell ratios determined from gels made from 7% precursor concentrations of PEG-0.10AA. At this lower concentration, PEGDA 575 is soluble in pure water, and we include water as a third solvent. The swell ratios for gels made in all three solvents are substantially higher when gels are made from 7% solutions than from 20% solutions. This is due to the fact that the lower precursor concentration increases the probability of PEGDA self-reaction during polymerization thus increasing the ratio of loops to crosslinks. Second, the effect of increasing pH is substantially more pronounced, though the transition to increased swelling still appears between pH values of 6 and 7. While the nominal composition of the gels is no different, the increased mesh size of the 7% gels presumably reduces steric constraints so the greater electrostatic repulsion can increase that mesh size even more.

S2. Antimicrobial Susceptibility Tests

The MIC and MBC of L5 and vancomycin were determined using a broth microdilution assay according to the CLSI MO7-A8 criteria.[3] Two types of *S. epidermidis* strains were tested (NJ 9709 and ATCC 35984). Both strains are vancomycin susceptible, and vancomycin (Fluka Analytical), a broad-spectrum antibiotic, was used as positive control. Briefly, working solutions of antimicrobials were prepared in 96-well plates at final concentrations ranging from 512 to 0.0625 μg/ml in CAMHB. Each plate chamber was inoculated with the test organisms at a final concentration of $5*10^5$ CFU/ml and incubated for 24 hours at 37 ºC. We also tested the vancomycin susceptibility of NJ 9709 at $2.5*10^7$ CFU/ml, which is the inoculum concentration used to challenge our gel-modified surfaces. All tests were repeated three times. Table S1 summarizes the results of these measurements.

Table S1 – Minimum Inhibitory (MIC) and Minimum Bactericidal (MBC) Concentrations of L5 cationic peptide and vancomycin.

Antimicrobial	MIC (⌠g/mL), *S. epidermidis*			MBC (⌠g/mL), *S. epidermidis*		
	NJ 9709 $5*10^5$ CFU/mL	ATCC 35984 $5*10^5$ CFU/mL	NJ 9709 $2.5*10^7$ CFU/mL	NJ 9709 $5*10^5$ CFU/mL	ATCC 35984 $5*10^5$ CFU/mL	NJ 9709 $2.5*10^7$ CFU/mL
L5	1	2	2	2	4	8
Vancomycin	2	2	4	4	4	8

S3. L5 Peptide Retention after Soaking in PBS

We used FITC-labeled L5 to study the L5 retention in medium. FITC-L5 loaded microgel-modified surfaces were immersed in PBS (0.15 M salt) for 3 days. The specimens were then washed with DI water, dried in air, and imaged using confocal microscopy. As a control, another sample was prepared and keep dry in the dark for the same three-day period. All images were taken using the same microscope excitation and emission parameters. Typical results are presented in fig. S2. The fluorescent intensity profiles were analyzed along the red line in each image. These show that there is no obvious decrease in FITC-L5 concentration due to aging in PBS indicating that the FITC-L5 is not released by elution. We do observe a decrease in fluorescent intensity when the pH is lowered to 2 and then returned to 7.4 (data not shown). These findings are consistent with those reported previously by Pavlukhina et al.[4] showing that L5 is retained in poly(acrylic acid) [PAA] thin-film hydrogels under physiological conditions and is released upon lowering of the pH. Note, however, that the FITC label introduces the possibility of additional hydrophobic interactions with the PEG-AA hydrogels that may be absent with unlabeled L5.

Supporting Information References:

1. Sukhishvili, S.; Granick, S., Layered, erasable polymer multilayers formed by hydrogen-bonded sequential self-assembly. *Macromolecules* **2002,** 35, 301-310.
2. Choi, J.; Rubner, M. F., Influence of the degree of ionization on weak polyelectrolyte multilayer assembly. *Macromolecules* **2005,** 38, 116-124.
3. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. CLSI document M7-A8. In Clinical and Laboratory Standards Institute: 2009.
4. Pavlukhina, S.; Lu, Y.; Patimetha, A.; Libera, M.; Sukhishvili, S., Polymer Multilayers with pH-Triggered Release of Antibacterial Agents. *Biomacromolecules* **2010,** 11, (12), 3448-3456.

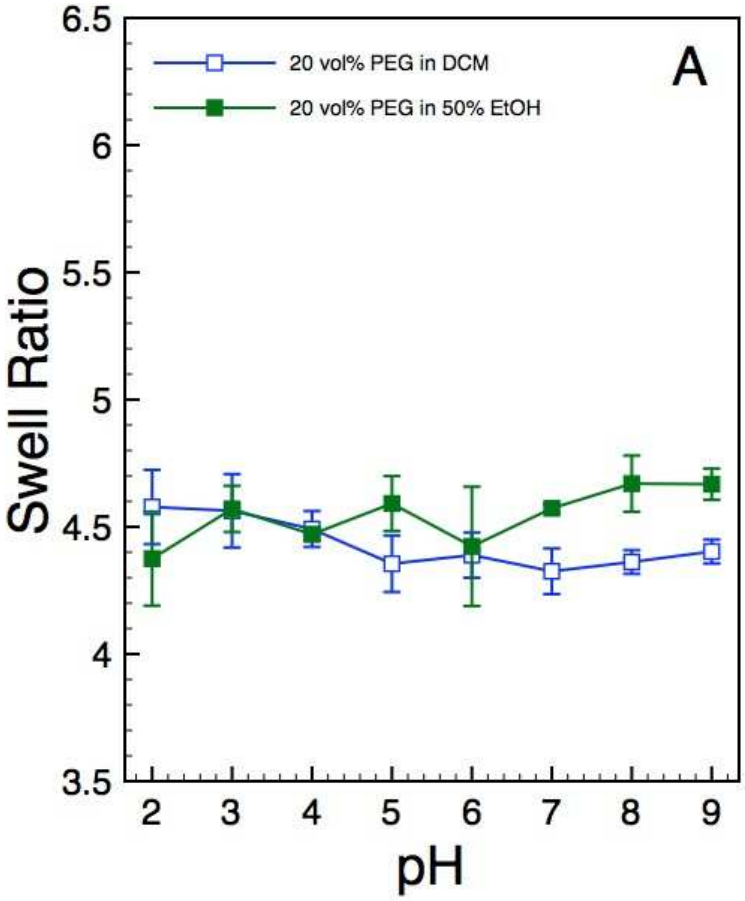

Fig. S1A - the swell ratio as a function of pH for pure bulk PEG gels synthesized as 20 wt% solutions in either DCM or in a 50% H_2O – 50% EtOH solution.

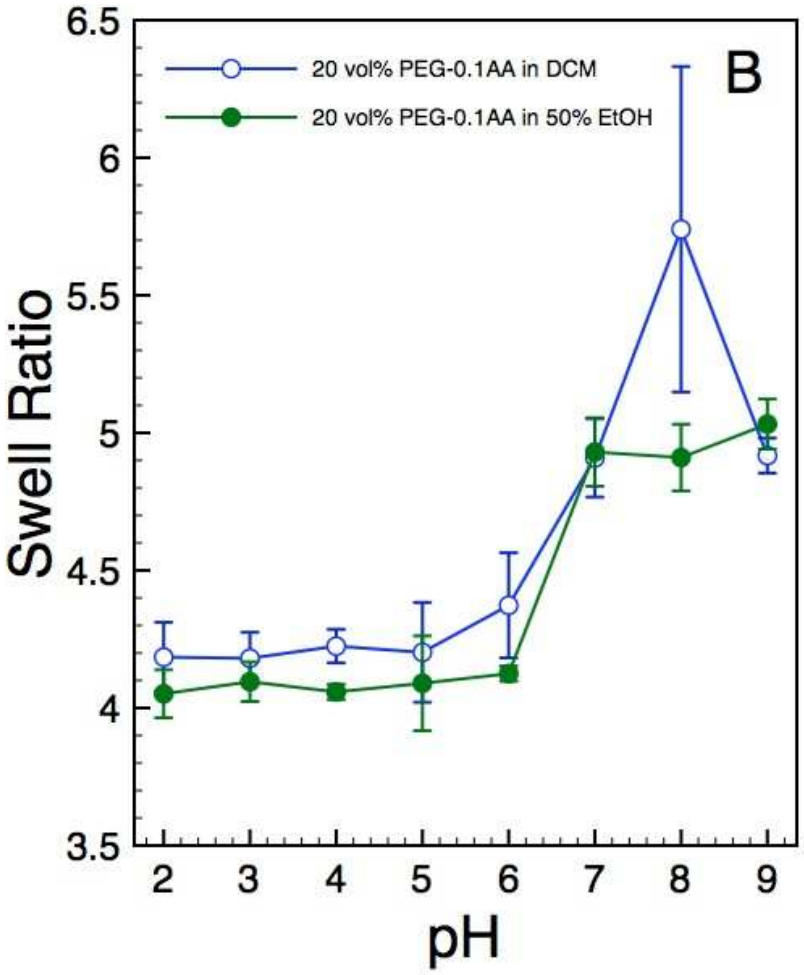

Fig. S1B - the swell ratio as a function of pH for bulk PEG-0.1 AA gels synthesized as 20 wt% solutions in either DCM or in a 50% H_2O – 50% EtOH solution.

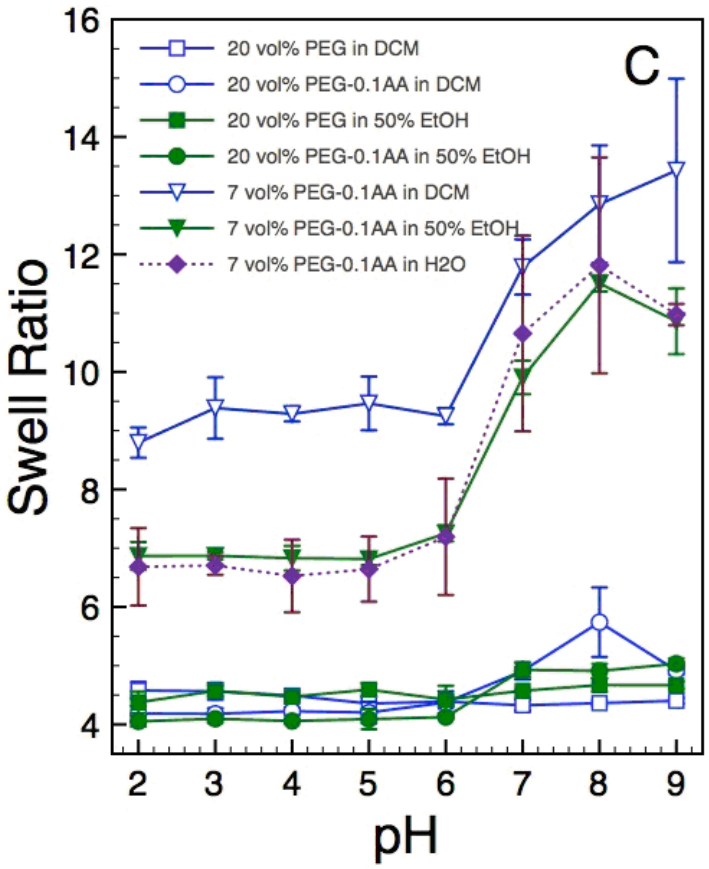

Fig. S1C – The pH-dependent swell ratios of bulk gels of pure PEG and PEG-0.1AA synthesized in various solvents and at two different dilutions.

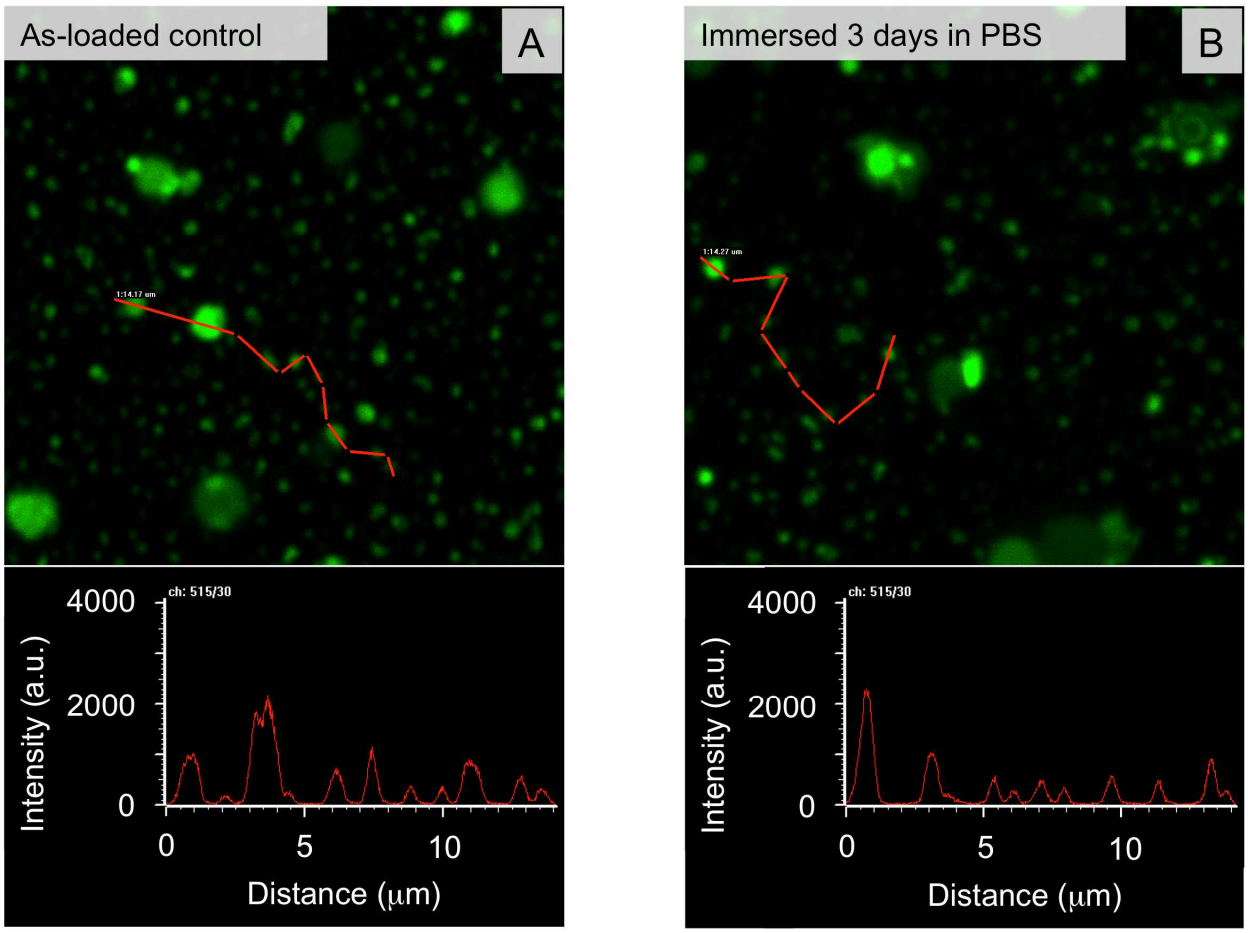

Fig. S2 – Confocal image (top) and fluorescent intensity profile (bottom) from FITC-labeled L5-loaded, microgel-modified silicon surfaces immediately after FITC-L5 loading (A) and after soaking in PBS at pH 7.4 and 0.15M NaCl concentration (B). In both cases, the intensity profile follows the red line to sample a number of different microgels.