Supporting Information

Bacterial Adhesion is Effected by the Thickness and Stiffness of Poly(ethylene glycol) Hydrogels

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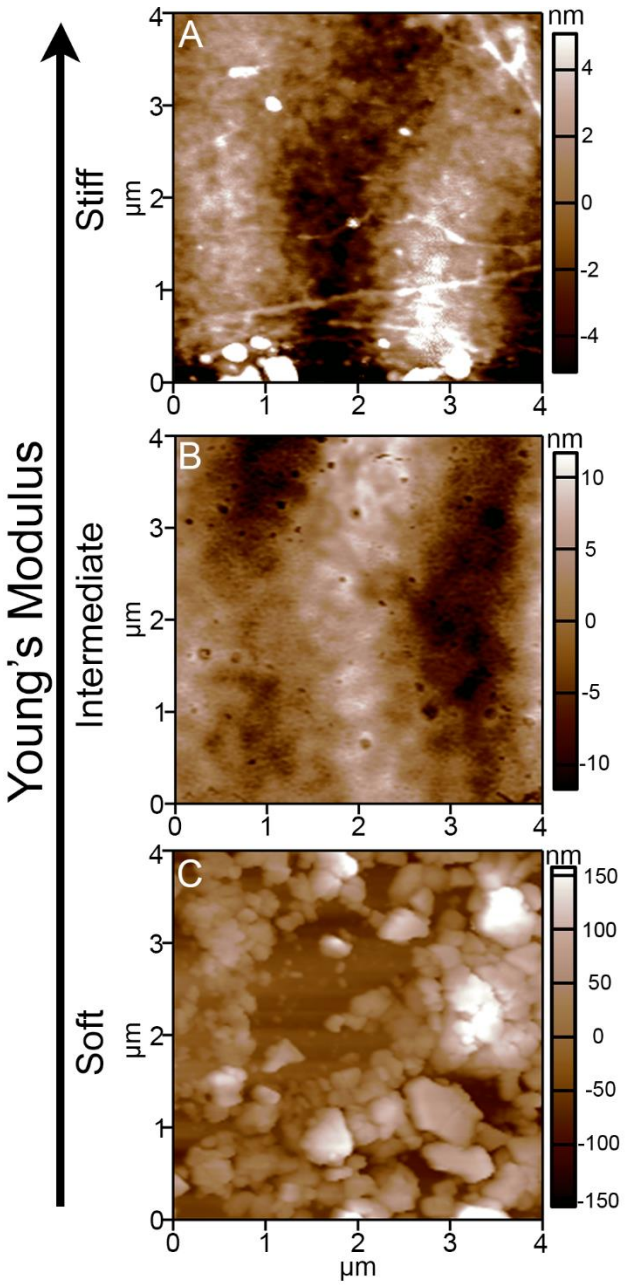

Figure S1. To demonstrate the need to acquire measurements on hydrated hydrogels, provided are representative AFM surface topography images of (A) soft, (B) intermediate, and (C) stiff PEG hydrogels that were dried at room temperature. Hydrogels were 150 µm thick. A z-scale is provided alongside each image.

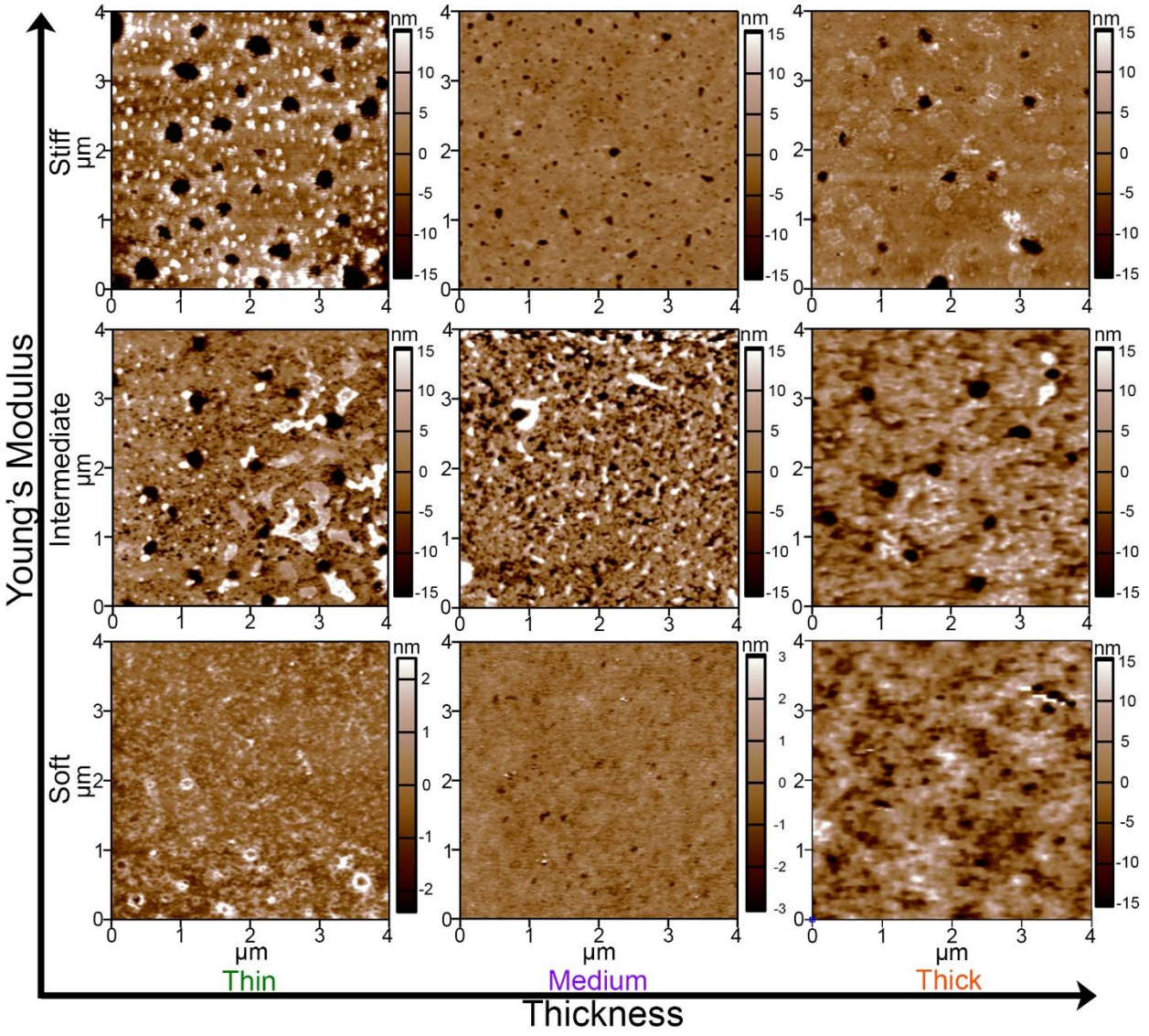

Figure S2. Representative AFM surface topography images of hydrated PEG hydrogels. Displayed is a grid of the hydrogels organized by Young's modulus and thickness. A z-scale is provided alongside each image.

Table S1. Surface roughness values for the PEG hydrogels, including, route mean square roughness (R_q), average roughness (R_a), skewness (R_{shw}), kurtosis (R_{kur}), minimum roughness (R_{min}), and maximum roughness (R_{max}). Standard deviation is provided.

Hydrogel		R_q (nm)	R_a (nm)	R_{skw} (nm)	R_{kur} (nm)	R_{max} (nm)	R_{min} (nm)
Stiff	Thin	11.03±2.0	8.01±1.63	-0.75±1.10	3.53±4.25	37.99±5.78	-78.29±31.18
	Medium	3.71±3.29	2.67±2.41	-0.82±1.14	5.88±4.81	18.01±12.64	-27.74±23.88
	Thick	12.15±1.01	8.16±0.30	2.19±0.32	10.20±2.46	105.58±20.495	-49.34±42.80
Int	Thin	7.27±0.50	4.89±0.58	-0.46±0.52	7.84±2.21	50.76±4.8	-58.67±0.97
	Medium	11.98±2.60	8.25±1.86	-0.18±1.07	5.12±1.08	55.90±15.99	-66.38±11.47
	Thick	17.43±1.00	12.03±0.53	2.22±0.21	7.45±1.20	166.91±36.85	-33.74±2.41
Soft	Thin	0.71±0.09	0.51±0.06	2.05±0.49	15.34±9.85	12.11±6.36	-4.07±0.46
	Medium	10.40±0.66	7.29±0.34	0.56±0.08	3.63±1.49	102.59±28.05	-62.08±9.05
	Thick	9.36±3.18	7.141±2.5	0.54±0.48	4.39±4.33	75.70±14.23	-48.76±19.81

†Roughness values were determined as the average of 4 μm^2 subsections of topographical scans.

Table S2. Properties of PEG hydrogels, including Young's modulus and mesh size (ξ). Standard error is provided.

Hydrogel	Young's Modulus (kPa)				ξ (nm)^
	Atomic Force Microscopy†			Rheology‡	
	Thin	Medium	Thick	Bulk gel	Bulk gel
Stiff	950±90	1000±90	1100±90	3900±700	1.0±0.1
Int	420±40	280±20	470±20	330±120	1.9±0.04
Soft	27±10	20±11	32±4.0	29±8	2.7±0.3

†Measured using AFM nanoindentation. ‡Measured using oscillatory shear rheology.
^Theoretical mesh size (ξ) was determined using a modified version of the Flory theory[1] using a method previously reported[2] and **Equation S1**:

$$\xi = \upsilon_{2,s}^{-1/3}(\bar{r}^2)^{1/2} \qquad \textbf{Equation S1}$$

Where $\upsilon_{2,s}$is the swollen volume fraction of polymer and $(\bar{r}^2)^{1/2}$ is the average end-to-end distance of the crosslinked PEG.

Reference:

(1) Canal, T.; Peppas, N. Correlation between Mesh Size and Equilibrium Degree of Swelling of Polymeric Networks *J. Biomed. Mater. Res.* **1989**, 23, 1183-1193.
(2) Kolewe, K.; Peyton, S. R.; Schiffman, J. D. Fewer Bacteria Adhere to Softer Hydrogels. *ACS Appl. Mater. Interfaces* **2015**, *7*, 19562–19569.

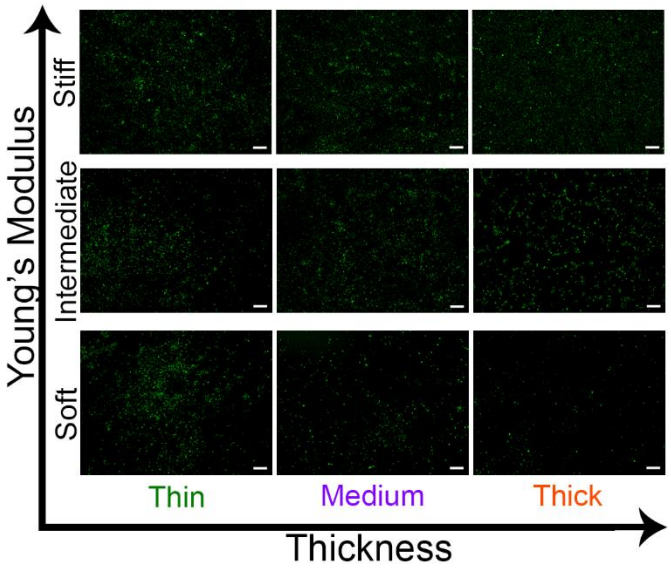

Figure S3. Representative fluorescent micrographs of *S. aureus* adhesion after a 24 h incubation period on thin (15 µm), medium (40 µm), and thick (150 µm) PEG hydrogels that were soft (30 kPa), intermediate (400 kPa), and stiff (1000 kPa). A 100 µm scale bar is displayed.

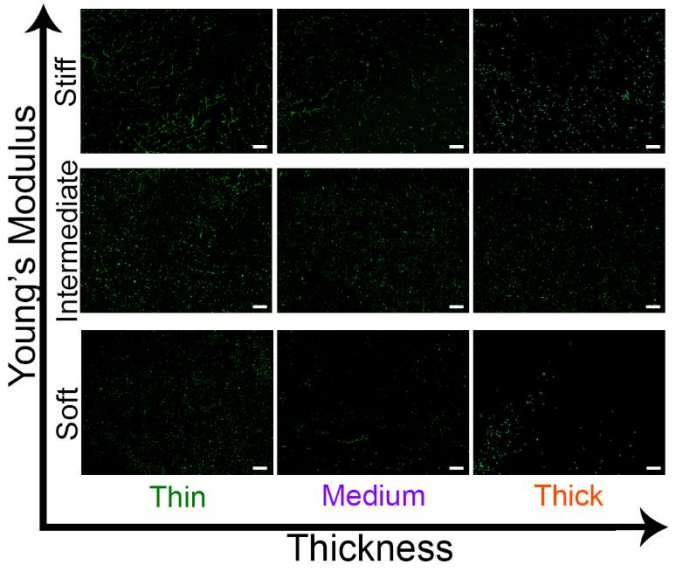

Figure S4. Representative fluorescent micrographs of *E. coli* adhesion after a 24 h incubation period on thin (15 µm), medium (40 µm), and thick (150 µm) PEG hydrogels that were soft (30 kPa), intermediate (400 kPa), and stiff (1000 kPa). A 100 µm scale bar is displayed.