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Research Article

Stiffness and Oligomer Content Affect the Initial Adhesion of *Staphylococcus aureus* to Polydimethylsiloxane Gels

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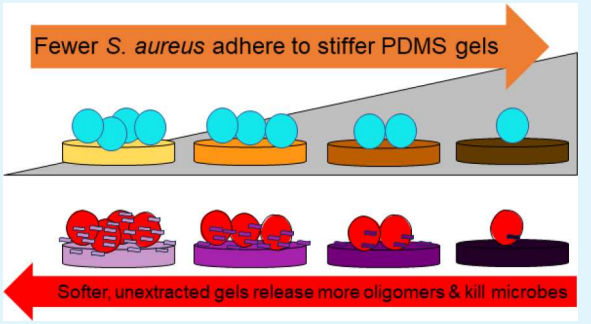
ABSTRACT: The growing prevalence of methicillin-resistant *Staphylococcus aureus* (*S. aureus*) infections necessitates a greater understanding of their initial adhesion to medically relevant surfaces. In this study, the influence of the mechanical properties and oligomer content of polydimethylsiloxane (PDMS) gels on the initial attachment of Gram-positive *S. aureus* was explored. Small-amplitude oscillatory shear rheological measurements were conducted to verify that by altering the base to curing (B:C) ratio of the commonly used Sylgard 184 silicone elastomer kit (B:C ratios of 60:1, 40:1, 10:1, and 5:1), PDMS gels could be synthesized with Young's moduli across four distinct regimes: ultrasoft (15 kPa), soft (30 kPa), standard (400 kPa), and stiff (1500 kPa). These as-prepared gels (unextracted) were compared to gels prepared from the same B/C ratios that underwent Soxhlet extraction to remove any unreacted oligomers. While the Young's moduli of unextracted and extracted PDMS gels prepared from the same B:C ratio were statistically equivalent, the associated adhesion failure energy statistically decreased for the ultrasoft gels after extraction (from 25 to 8 J/mm^2). The interactions of these eight well-characterized gels with bacteria were tested by using *S. aureus* SH1000, a commonly studied laboratory strain, as well as *S. aureus* ATCC 12600, which was isolated from a human lung infection. Increased *S. aureus* inactivation occurred only when the bacteria were incubated directly on top of the unextracted gels prepared at high B:C ratios (40:1 and 60:1), whereas none of the extracted gels (no unreacted oligomers) had significant levels of inactivated bacteria. *S. aureus* adhered the least to the stiffest extracted PDMS gels (no unreacted oligomers) and the most to soft, unextracted PDMS gels (with ∼17% unreacted oligomers). These findings suggest that both unreacted oligomers and Young's moduli are important material factors to consider when exploring the attachment behavior of Gram-positive *S. aureus* to hydrophobic elastomer gels.



KEYWORDS: *antifouling, biomaterial, gel, polydimethylsiloxane, Staphylococcus aureus*

INTRODUCTION

In clinical settings, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common antibiotic-resistant bacteria, and it is also responsible for most *S. aureus* infections. MRSA is a leading source of hospital-acquired infections (HAIs) that are commonly associated with incidences of ventilator-associated pneumonia, catheter-associated urinary tract (CAUTI), and central line-associated bloodstream infections (CLABSI).[1] Medical research and improvements to hospital sanitation protocols have focused on creating specific guidelines aimed at reducing infections from antibiotic-resistant bacteria.[2−5] While these guidelines have reduced the incidence of MRSA infections by 45−68%, CDC data from 2015 to 2020 reported that the incidence of MRSA infections has stagnated at ∼300,000 cases per year, suggesting a constant presence of this bacteria.[3−6] From 2017 to 2020, this stagnation continued, with the overall incidence ranging from ∼280,000 to 320,000 cases per year. Additionally, cases of MRSA specifically originating in hospital settings during this time-frame steadily increased to ∼50,000 cases per year. This statistic has likely been further aggravated by the COVID-19 pandemic at the onset of 2020. A 2022 preliminary report from the CDC indicates that the overall incidence of MRSA infections and CLABSIs had regressed to pre-2015 levels, which is alarming since the previous 5 years of progress in lowering the overall incidence of MRSA was undone in just one year. This startling regression indicates that the current antibacterial techniques, combined with an increased awareness of sanitation protocols, are insufficient for preventing and treating MRSA infections. Thus, the development of alternative strategies that prevent or at least delay HAIs are needed.[6]

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Current HAI prevention strategies rely on commercial antibiotics to kill the microorganisms, and repeated antibiotic usage accelerates the development of antibiotic-resistant strains like MRSA.[7] An alternative approach is to design biomaterials that innately delay the onset of HAIs, thus providing clinicians with more time to identify and treat them, using fewer antibiotics in the process. One emerging method has focused on understanding how the bulk mechanical properties of biomaterials influence microbial attachment. Numerous previous studies have explored how Young's modulus of elastic hydrophilic hydrogels impacts bacterial adhesion. While our group has investigated poly(ethylene glycol) dimethacrylate (PEG) and agar hydrogels,[8−11] a wide range of hydrogel chemistries, such as agarose, polyvinylpyrrolidones, poly(acrylic acid), and polyacrylamide, have also been evaluated.[12−15] In most cases, the elastic hydrogel's properties impacted microbial attachment, but a universal trend was not revealed. For example, more *Escherichia coli* and *S. aureus* adhered to stiffer PEG hydrogels,[8,9] more *S. aureus* adhered to stiffer polyacrylamide hydrogels, and more *Pseudoalteromonas sp.* adhered to stiffer agarose hydrogels. Conversely, the attachment of *Bacillus sp.* to agarose hydrogels was reported to be unaffected by agarose stiffness.[12] While hydrophilic biomaterials have been well explored, fewer studies have focused on hydrophobic biomaterials, which are also relevant in many biomedical devices and clinical applications.[16−18]

One commonly used hydrophobic biomaterial chemistry is polydimethylsiloxane (PDMS), due to its ease of manufacturing into devices such as catheters, implant coatings, and biosensors.[19−25] These PDMS biomaterials are substrates on which HAIs develop, as previously discussed. PDMS gels are typically manufactured using a commercially available kit produced by DOW known as Sylgard 184, which contains a base and a curing agent. The manufacturer recommends that gels are prepared by vigorously mixing a mass ratio of 10 base to 1 curing agent, as this ratio provides the best cross-linking, stability, and biocompatibility, as well as other desirable mechanical properties.[26]

Previously, researchers have studied the interactions between microorganisms and PDMS gels prepared according to the manufacturer's recommendations, as well as gels prepared using 5:1, 20:1, and 40:1 base-to-curing agent ratios (B:C ratios), wherein greater amounts of base lead to softer gels. More Gram-negative bacteria, including *E. coli* RP437 and *Pseudomonas aeruginosa* PAO1, attached to and formed biofilms the fastest on softer PDMS gels (40:1) in comparison to the stiffer gels (5:1).[16] As reported by Song et al.,[16] mostly antibiotic-resistant persister cells attached to stiff PDMS (5:1), while the bacteria that attached to softer PDMS gels (40:1) were more susceptible to antibiotics. An additional study from the same group demonstrated that when the *oprF* gene in *P. aeruginosa* PAO1 was neutralized, the attachment, growth, and size of cells present were equivalent for all PDMS gels tested.[17] Other work that focused on mutations to genes responsible for motility, flagella synthesis, and fimbriae synthesis for *E. coli* RP437 revealed that the bacteria used the *motB* gene to avoid attachment to stiffer PDMS.[18] Similarly, more *E. coli* BW25113, *Staphylococcus epidermidis* ATCC 155, and *P. aeruginosa* DSM 1117 attached to softer PDMS (40:1), but *S. aureus* DSM 20231 (also known as ATCC 12600) did not respond to stiffness as much as the other bacteria.[27,28] In summary, of the five reports that have studied the attachment of microorganisms to PDMS gels, interestingly, all of the reported Gram-negative bacterial strains and the Gram-positive *S. epidermidis* strain were impacted by stiffness. However, Gram-positive *S. aureus* DSM 20231 remained mostly unimpacted. Here, we explore whether clinical *S. aureus* strains are impacted by PDMS stiffness.

Importantly, studies have also noted that PDMS fabrication can lead to the presence of unreacted oligomer concentrations in the as-prepared PDMS gels.[24,27,29−32] When PDMS undergoes free radical polymerization, the radicals on the active chain in the base can react with other radicals of active chains, leading to less cross-linking in the bulk gel and uncrosslinked oligomers.[24,29,33,34] This is especially apparent in gels with larger ratios of B:C, such as 40:1 and 60:1. Uncrosslinked oligomers are a concern because they can diffuse out of the PDMS gels over time and lead to negative biological effects, such as transport into mammalian cell membranes, protein transport interference, and cytotoxicity.[19,29,30,32,35,36] The presence of uncrosslinked oligomers also impacts PDMS material properties, as greater uncrosslinked oligomer content increases the associated adhesion force and lowers the deformation resistance of PDMS gels.[37,38] Despite the breadth of studies demonstrating the biological and mechanical impacts of uncrosslinked oligomers, to the best of our knowledge, their effects on the bacterial adhesion to PDMS have only been assessed in one study that came online as we were submitting this manuscript. Sun et al.[39] tested the proliferation of *S. aureus* ATCC 25293 after exposure to the as-prepared 10:1 PDMS gels, as well as those that underwent a hexane extraction process. Using plate counting assays, they reported that more *S. aureus* bacterial colony-forming units grew after exposure to the extracted PDMS gels than to the unextracted PDMS gels; they also conducted a broader study with *E. coli* BL21 (DE3) and reported the same trend.

For the first time, we investigated the attachment of *S. aureus* SH1000 and *S. aureus* ATCC 12600 to PDMS by assessing the impacts of both PDMS gel stiffness and the presence of uncrosslinked oligomers in PDMS gels, see Figure 1. Our

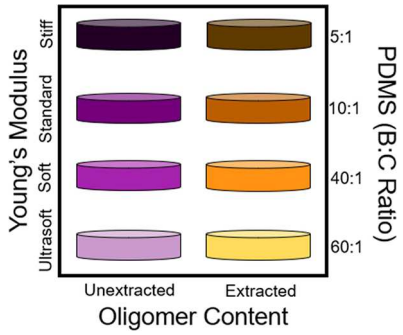

Figure 1. Schematic of the eight PDMS gels tested in this study. Throughout the results section, we will refer to a PDMS sample by its oligomer content and stiffness (bottom and left axes, respectively). The B:C ratio used to fabricate PDMS gels with different stiffnesses is noted on the right axis.

PDMS gels were manufactured at four different B:C ratios, including a softer 60:1 B:C ratio not yet tested in literature. The impact of extracting the uncrosslinked oligomer content on gel material properties and bacterial interactions for each of the four B:C ratios was determined. *S. aureus* SH1000 was selected because it is a commonly studied laboratory Gram-positive strain, whereas *S. aureus* ATCC 12600 was selected to

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serve as a clinically relevant strain that was isolated from a human lung infection. By studying a clinically relevant strain's adhesion to PDMS biomaterials, we suggest that the results may provide guidance for the design of commercially available PDMS-based medical devices.

MATERIALS AND METHODS

Materials and Chemicals. All compounds were used as received, unless otherwise noted. Sylgard 184 silicone elastomer base and curing agent were purchased from Ellsworth Adhesives (Germantown, WI). M9 minimal salts, D-(+)-glucose, magnesium sulfate, calcium chloride, chloramphenicol (BioReagent grade), glycerol, tryptic soy broth (TSB), propidium iodide (PI), and 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO). ACS-certified toluene was purchased from Fisher Scientific (Hampton, NH). Deionized (DI) water was obtained from a Barnstead Nanopure Infinity water purification system (Thermo Fisher Scientific, Waltham, MA).

Fabrication of Unextracted and Extracted PDMS Gels. To make PDMS gels, various Sylgard 184 silicone elastomer B:C mass ratios (B:C ratios of 60:1, 40:1, 10:1, and 5:1) were vigorously mixed together for 15 min at 20 °C and then poured into square, sterile polystyrene Petri dishes (100 × 100 mm, Thermo Fisher Scientific, Waltham, MA), which were degassed under vacuum to remove air bubbles.[26] The volumes of uncured PDMS added to the Petri dishes were controlled to ensure a final gel thickness of 0.5 cm. PDMS gels used for bacterial experiments were cleaned square and Fisher Scientific glass microscope slides (22 × 22 mm) were placed on the bottom of the Petri dishes prior to adding uncured PDMS. All glass was cleaned by submersion in acetone at 20 °C for 15 min, rinsed with sterile DI water three times, dried at 100 °C for 1 h, and treated with UV/ozone (UV/Ozone ProCleanerTM, Bioforce Nanosciences, Ames, IA) for 15 min. All PDMS gels were cured on a hot plate at 60 °C for 16 h and then cut into 22 mm square samples using a razor blade that was sterilized with ethanol. Throughout this manuscript, PDMS gels prepared at B:C ratios of 60:1, 40:1, 10:1, and 5:1 were used as-prepared and will be referred to as unextracted ultrasoft, soft, standard, and stiff, respectively.

To remove any uncrosslinked oligomers, a Soxhlet extraction procedure using toluene was employed.[24,40−44] Unextracted PDMS gels, freestanding or mounted on glass slides, were submerged in toluene for 96 h at 20 °C to ensure that equilibrium swelling was reached. Next, the swollen gels were removed from the toluene and placed in an oven at 40 °C for 48 h to evaporate any residual toluene. Throughout this paper, PDMS gels that have undergone the toluene extraction process will be referred to as extracted ultrasoft, soft, standard, and stiff, respectively. Prior to their use in microbial assays, all PDMS gels (unextracted and extracted) were sterilized via submersion in 70% ethanol for 15 min, rinsed with sterile DI water, and dried in a sterile biohood for 16 h at 20 °C.

Characterization of Unextracted and Extracted PDMS Gels. The chemical compositions of the PDMS gels were determined using a PerkinElmer Spectrum 100 Fourier transform infrared spectrometer (FTIR, Waltham, MA). All spectra were recorded from 4000 to 500 cm^{-1} by the accumulation of 32 scans and with a resolution of 4 cm^{-1}. Scans were performed in duplicate on three replicates for unextracted and extracted gels of each stiffness.

To quantify the concentration of uncrosslinked oligomers (ω_{sol}) present within the unextracted PDMS gels, eq 1[46] was used:

$$\omega_{sol}\ (\%) = \frac{m_0 - m_{dry}}{m_0} \times 100 \tag{1}$$

where the mass of the unextracted PDMS gels is m_0 and the mass of the same gel after toluene submersion and fully drying the gel is m_{dry}. The equilibrium degree of swelling (Q) was calculated using eq 2[45]:

$$Q = \frac{1}{\Phi} = \frac{\frac{m_{dry}}{\rho_P} + \frac{m_{sw} - m_{dry}}{\rho_s}}{\frac{m_{dry}}{\rho_P}} \tag{2}$$

where m_{sw} is the mass of the swollen gel, m_{dry} is the mass of the dry gel, ρ_P is the density of PDMS and ρ_s is the density of the solvent. The cross-link density (n) of the PDMS gel was calculated via the Flory−Rehner equation, eq 3[46]:

$$-\left[\ln\left(1 - \Phi\right) + \Phi + \chi \times \Phi^2\right] = V_1 \times n \times \left(\Phi^{\frac{1}{3}} - \frac{\Phi}{2}\right) \tag{3}$$

where Φ is the volume fraction of the polymer in the swollen mass, V_1 is the molar volume of the solvent, and χ is the Flory−Huggins solvent-polymer interaction term, calculated using eq S1.

Contact angle measurements were acquired using an in-house-constructed apparatus that was equipped with a Nikon D5100 digital camera with a 60 mm lens and 68 mm extension tube (Nikon, Melville, NY).[47] At least 15 measurements of 5 μL drops of DI water were acquired for each sample, with at least three samples per sample type. The images were analyzed using *ImageJ* software (National Institutes of Health, Bethesda, MD).[48]

Small-amplitude oscillatory shear (SAOS) measurements were acquired using a Kinexus Pro rheometer (Malvern Instruments, U.K.) equipped with plate−plate geometry with a diameter of 20 mm and a gap of 0.5 cm. Circular PDMS gels (25 mm diameter ×0.5 cm height) were loaded into the rheometer and trimmed to size using a razor blade. A strain amplitude sweep was performed at a strain of 0.1% to ensure that experiments were conducted within the linear viscoelastic region. Oscillation frequency sweeps were conducted using a constant strain of 0.5% over an angular frequency domain of 1.0 to 300 rad/s at 20 °C. Dynamic strain-sweep measurements to determine the linear viscoelastic range were at a fixed angular frequency of 10 rad/s and a strain of 0.01 to 500%. Complex moduli (G), mesh size (ξ), and Young's moduli (E) were determined using the linear region of a constant angular frequency of 10 rad/s and a strain of 0.5% using eqs 4−6.[49,50]

$$G = \sqrt{G'^2 + G''^2} \tag{4}$$

$$\xi = \sqrt[3]{\frac{6RT}{\pi N_a G}} \tag{5}$$

$$E = 2G(1 + \upsilon) \tag{6}$$

where G' is the elastic modulus, G'' is the loss modulus, R is the gas constant, T is the absolute temperature, υ is the Poisson's ratio, and N_a is Avogadro's number.

The adhesion failure energy (J/m^2) of the PDMS gels was also quantified by using the Kinexus Pro Rheometer. After the probe (20 mm) contacted the sample, it was pressed at a constant normal force (F_N) of 10 N for 5 s. Then, the probe was removed from the sample at a rate of 5 mm/s until it reached a 25 mm gap distance. Adhesion failure energy was calculated as the area under the removal force ($-F_N$) versus the gap distance curve.[51−53] Measurements were taken on three replicates for each gel stiffness.

Bacterial Assays Using Unextracted and Extracted PDMS Gels. The Gram-positive strains used include *S. aureus* SH1000 (*S. aureus* SH1000) with the high efficiency pCM29 sGFP plasmid[54] containing a chloramphenicol antibiotic resistance gene (a generous donation from Dr. Alexander Horswill, University of Colorado Anschutz Medical Campus) and *S. aureus* ATCC 12600. *S. aureus* SH1000 (inoculated with 10 μg/mL chloramphenicol) and *S. aureus* ATCC 12600 (no antibiotic) were cultured overnight in TSB (with chloramphenicol present at 10 μg/mL for SH1000) at 37 °C to a concentration of 10^8 cells/mL, washed twice, and resuspended in M9 medium before use.

To assess *S. aureus* inactivation, experiments were conducted by placing square unextracted and extracted PDMS gels (22 × 22 mm) in separate wells of 6-well polystyrene plates (Fisher Scientific) and run in parallel to internal controls (cleaned square glass coverslips in

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triplicate, 22 × 22 mm, Fisher Scientific, Hampton, NH). The samples were submerged in 5 mL of M9 medium containing 10^8 cells/mL of *S. aureus* SH1000 and 10 μg/mL of chloramphenicol and incubated without shaking at 37 °C for 2 h. Bacteria were stained using PI (60 μM, excitation/emission at 535 nm/617 nm), incubated for 15 min,[55] and rinsed gently with M9 medium before acquiring 15 random images per sample using a Zeiss Microscope Axio Imager A2M (20x objective). GFP expressing *S. aureus* SH1000 was considered viable, while PI-stained cells were considered inactivated. *ImageJ*[48] was used to quantify the viable and dead cells, and the percentage of viable bacteria was determined using eq 7.

$$\text{viable } S.\ aureus\ (\%) = \frac{\text{viable } S.\ aureus}{\text{viable } S.\ aureus + \text{inactivated } S.\ aureus} \times 100 \quad (7)$$

Bacterial attachment onto the unextracted and extracted PDMS gels was assayed using our previously described procedure.[8,9] The PDMS square gels and glass controls were placed in separate wells of 6-well polystyrene plates before being submerged in 5 mL of M9 medium containing 10^8 cells of either *S. aureus* SH1000 (inoculated with 10 μg/mL of chloramphenicol) or *S. aureus* ATCC 12600 (no antibiotic) and incubated without shaking at 37 °C for 24 h. To visualize *S. aureus* ATCC 12600, cells were stained with DAPI (10 μM, excitation/emission at 358/461 nm) and allowed to incubate for 5 min.[56] Samples were rinsed and imaged as previously described. *ImageJ* was used to calculate the bacteria colony area coverage over the total acquired 5,504,455 μm^2 area. All bacterial experiments were run in triplicate, and three experiments were conducted per sample type. Throughout the results, statistical significance was determined using a two-tailed, unpaired student's *t*-test using Microsoft Excel.

RESULTS AND DISCUSSION

Characteristics of Unextracted PDMS Gels. By systematically controlling the B:C ratio used during gel manufacturing, PDMS gels with differing mechanical properties were successfully produced. As shown in Figure 1, the as-prepared gels will be referred to as unextracted because they contain uncrosslinked oligomers. It is well known that the as-prepared PDMS gels contain unreacted oligomers because the active polymer chain reacts with itself during cross-linking. This self-reaction occurs more frequently at larger B:C ratios, where fewer curing agents are available for the active chain to react with. The uncrosslinked oligomers increase gel tackiness, and therefore, it is important to investigate if their presence influences bacterial adhesion, especially for high B:C ratio gels.

As calculated via eq 1, the uncrosslinked oligomer content in the unextracted gels was ∼46, 33, 11, and 1% for the ultrasoft, soft, standard, and stiff gels (Figure 2 and Table 1). While the ultrasoft is being examined for the first time by our group, our findings align with previous studies, where the ethanol[27] and hexane[39] extraction processes determined the oligomer content to be 24 and 38% for soft gels (40:1), 8 and 3% for standard gels (10:1), and 5 and 3% for stiff gels (5:1).

The low percentage of free oligomers in stiff and standard gels compared to the soft and ultrasoft gels is consistent with predicted PDMS polymerization behavior and also correlates to differences in equilibrium swelling degree and cross-link density, as calculated using eqs 2 and 3. Ultrasoft and soft gels have statistically greater swelling ratios than standard and stiff gels, ∼10 and 6 versus ∼2 and 2, respectively, indicating a less structured polymer network able to facilitate greater solvent absorption. This is consistent with the lower cross-link density for ultrasoft and soft gels as compared to standard and stiff gels, where the lower number of cross-links leads to a more spacious and flexible polymer matrix that can contain uncrosslinked oligomers without disturbing the overall polymer structure.[57]

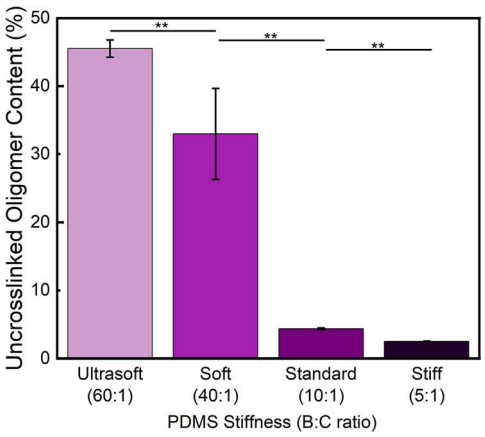

Figure 2. Uncrosslinked oligomer content of unextracted PDMS gels decreased with decreasing base to curing agent ratio (B:C). Error bars denote the standard deviation. Two asterisks (**) denote that the values are significantly different at the 0.01 level.

Material Characterization of Unextracted and Extracted PDMS Gels. A toluene extraction procedure was employed on the as-prepared gels to extract any uncrosslinked oligomers; these PDMS gels do not contain any free oligomers and are referred to as extracted. The FTIR spectra was consistent for unextracted and extracted gels; see Figure S1. Additionally, contact angle measurements for each PDMS gel were ∼120°, thereby confirming that each gel was hydrophobic regardless of its stiffness, as expected.

Rheological analysis of the unextracted and extracted PDMS gels was conducted to characterize the mechanical properties of the gels. As seen in Figures S2 and S3, the storage modulus (G'), i.e., the energy stored by the gel when it is deformed by an external force, was greater than the loss modulus (G''), i.e., the energy lost to internal friction during that deformation process, for gels made from all B:C ratios. Additionally, as the B:C ratio increased, both G' and G'' decreased. These findings are consistent with previous reports that have conducted rheology on PDMS gels.[58] As shown in Figure S4A,B, the tan δ, a measure of elastic behavior against viscous behavior of a material by comparing loss and storage moduli, was slightly greater than 0 for all unextracted and extracted PDMS gels. This indicates that, in all cases, PDMS gels are more elastic than viscous. After obtaining storage and loss moduli from rheological analysis of the unextracted and extracted PDMS gels, eq 4 was used to calculate their complex moduli, as seen in Figure S4C,D. Complex moduli were then used in eq 6 to calculate the Young's modulus for unextracted and extracted PDMS gels.

By altering the B:C ratio during gel fabrication, we successfully produced PDMS gels with different Young's moduli, displayed in Figure 3 and Table 2. Our unextracted gels had Young's moduli of 15 ± 4, 30 ± 12, 400 ± 80, and 1500 ± 180 kPa for the gels prepared at B:C ratios of 60:1, 40:1, 10:1, and 5:1. Unextracted PDMS gels prepared at each B:C ratio had a statistically equivalent Young's moduli to their extracted counterpart; this is because the mechanical properties of PDMS gels are dominated by the polymer fraction as opposed to the unreacted components.

Our findings are consistent with the work of Valentin et al.,[58] which used rheological analysis to obtain Young's moduli

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Table 1. Properties of the Unextracted PDMS Gels

sample	swelling ratio[a]	uncrosslinked oligomer, ω_{sol}, (%)[b]	equilibrium degree of swelling, Q[c]	cross-link density, n (mol/mL)[d]	contact angle (deg)
ultrasoft	9.53 ± 0.8	45.52 ± 1.29	19.38 ± 1.5	$3.7 \times 10^{-6} \pm 5 \times 10^{-7}$	120 ± 3
soft	6.38 ± 0.4	32.97 ± 6.70	10.64 ± 1.7	$1.2 \times 10^{-5} \pm 4 \times 10^{-6}$	121 ± 4
standard	1.99 ± 0.04	10.64 ± 1.71	2.12 ± 0.01	$3.4 \times 10^{-4} \pm 3 \times 10^{-6}$	118 ± 4
stiff	1.91 ± 0.004	$1.20 \pm 3.6 \times 10^{-6}$	2.13 ± 0.003	$3.6 \times 10^{-4} \pm 1 \times 10^{-6}$	122 ± 3

[a]Calculated using eq S2. [b]Calculated using eq 1. [c]Calculated using eq 2. [d]Calculated using eq 3.

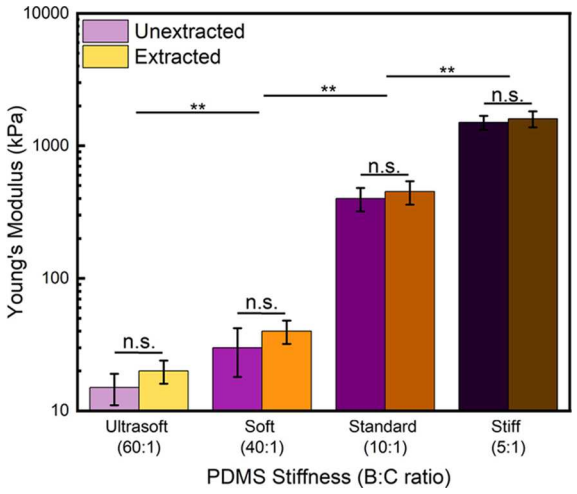

Figure 3. Young's moduli of the unextracted and extracted PDMS gels. Standard deviation is provided as error bars in the graph. Two asterisks (**) denote that the values are significantly different at the 0.01 level, whereas n.s. indicates that the samples are not statistically different. No significant differences due to extraction (gels prepared from the same B:C ratio) were found. To simplify the plot, the intergroup bars (different B:C ratios) are displayed via the same bar; gels prepared from different B:C ratios (comparing unextracted gel to unextracted gel and extracted gel to extracted gel) were both significantly different at the 0.01 level.

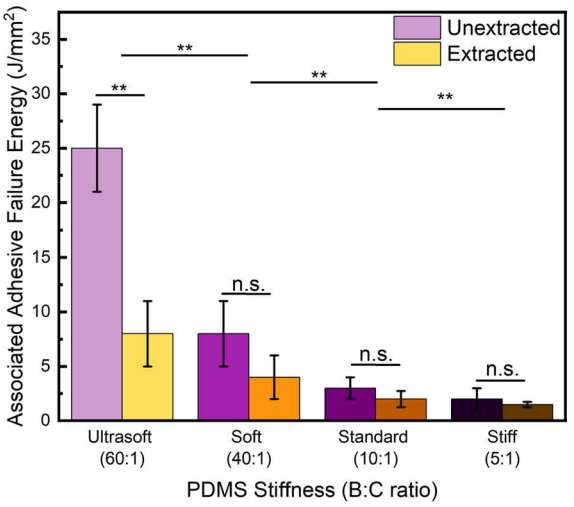

Figure 4. Adhesive failure energy of the unextracted and extracted PDMS gels. Standard deviation is provided as error bars in the graph. Two asterisks (**) denote that the values are significantly different at the 0.01 level, whereas n.s. indicates that the samples are not statistically different. Significant differences due to extraction (gels prepared from the same B:C ratio) are displayed. To simplify the plot, the intergroup bars (different B:C ratios) are displayed via the same bar; gels prepared from different B:C ratios (comparing unextracted gel to unextracted gel and extracted gel to extracted gel) were both significantly different at the 0.01 level.

values of 21 ± 1 and 565 ± 20 kPa for B:C ratios of 40:1 and 10:1, respectively. We note that previous PDMS gels used in bacterial adhesion studies, including B:C ratios of 5:1, 10:1, and 40:1, were characterized using atomic force microscopy (AFM) or dynamic mechanical analysis,[16,28] which makes a direct comparison impossible. However, trends reported in these papers mirror our data, where the Young's moduli for standard and stiff gels are 1−2 orders of magnitude greater than the Young's moduli for soft gels.

While there was no statistical difference between the Young's moduli of the unextracted and extracted gels prepared from the same ratio, the presence or absence of uncrosslinked oligomers could alter the tackiness of the PDMS gel. Typically, the greater the adhesive failure energy (i.e., tackiness) associated with a surface, the greater the force needed to remove objects adhered to that surface. Figure 4 demonstrates that higher concentrations of uncrosslinked oligomers in the unextracted PDMS gels increased the adhesive failure energy associated with those surfaces. The unextracted ultrasoft and soft PDMS gels had significantly greater adhesive failure energy than standard and stiff samples, 25 and 8 J/mm^2 compared to 3 and 2 J/mm^2, respectively. This difference indicates that a greater free oligomer content positively correlates with increased adhesive failure energy and gel tackiness.

The same trend was present for the extracted PDMS gels, but the overall associated energy values were lower. The decrease in adhesive failure energy of the PDMS gels as a result of oligomer removal was most notable for the samples with the lowest Young's modulus; the ultrasoft unextracted and extracted gels had adhesive energy failure values of 25 and 8 J/mm^2, respectively. The data indicate that uncrosslinked oligomer content impacts the tackiness of ultrasoft PDMS gels.

Table 2. Young's Modulus and Adhesive Failure Energy of Unextracted and Extracted PDMS Gels

		unextracted PDMS		extracted PDMS	
sample	B:C ratio	Young's modulus (kPa)	adhesive failure energy (J/mm^2)	Young's modulus (kPa)	adhesive failure energy (J/mm^2)
ultrasoft	60:1	15 ± 4	25 ± 4	20 ± 4	8 ± 3
soft	40:1	30 ± 12	8 ± 3	40 ± 8	4 ± 2
standard	10:1	400 ± 80	3 ± 1	450 ± 90	2 ± 0.75
stiff	5:1	1500 ± 180	2 ± 1	1600 ± 220	1.5 ± 0.25

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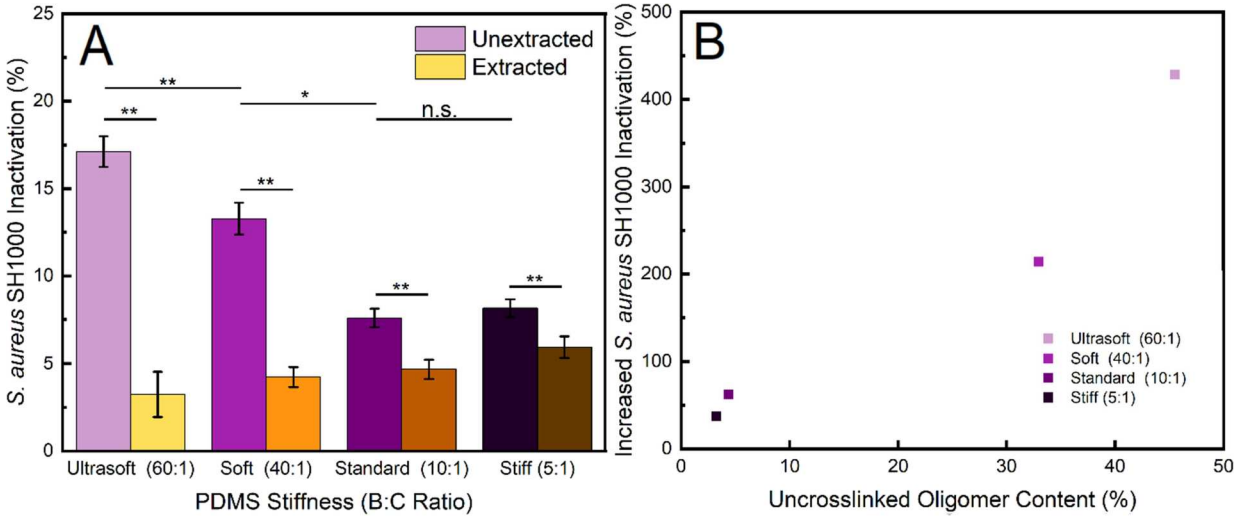

Figure 5. (a) *S. aureus* SH1000 inactivation after a 2 h incubation on top of the unextracted and extracted PDMS gels. Standard error is provided. One asterisk (*) and two asterisks (**) indicate that the values are significantly different at 0.05 and 0.01 levels, while n.s. indicates no statistical significance. There was no statistical difference in *S. aureus* inactivation by any of the extracted PDMS gels; not shown to simplify this plot. Significant differences due to extraction (gels prepared from the same B:C ratio), as well as the significant differences between unextracted gels prepared from different B:C ratios are provided. (b) Increased *S. aureus* SH1000 inactivation by unextracted PDMS gels (versus their extracted counterpart) is plotted as a function of the uncrosslinked oligomer content in the unextracted gels. The uncrosslinked oligomer content was calculated using eq 1, whereas the increased *S. aureus* SH1000 inactivation was calculated using eq S3.

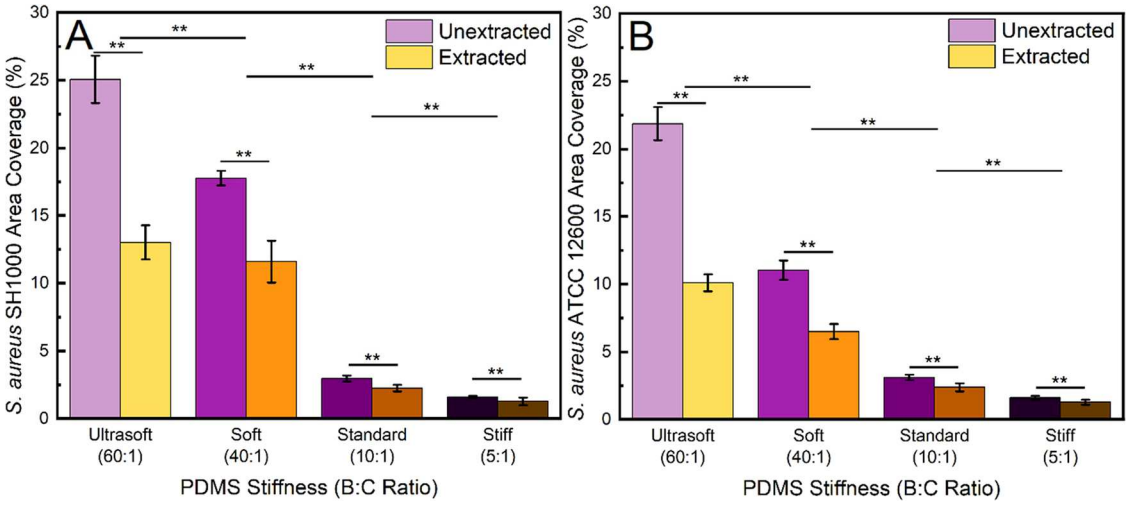

Figure 6. Adhesion of (a) *S. aureus* SH1000 and (b) *S. aureus* ATCC12600 to unextracted and extracted PDMS gels after a 24 h incubation period. Standard error is provided. Two asterisks (**) indicate that the values are significantly different at 0.01 level. To simplify the plots, bars comparing gels prepared from different B:C ratios (extracted gels to extracted gels) were omitted from Figure 6 but they are provided in Figure S5. Significant differences due to extraction (gels prepared from the same B:C ratio), as well as the significant differences between unextracted gels prepared from different B:C ratios are provided.

Inactivation of *S. aureus* after Incubation on Unextracted and Extracted PDMS Gels. Next, we aimed to investigate the impact that uncrosslinked oligomers and gel mechanics have on microbes. First, inactivation assays were conducted to determine whether there were any differences in cell viability that could be attributed to the free oligomers. Figure 5A displays *S. aureus* SH1000 inactivation after a 2 h incubation of the microbes on top of the unextracted and extracted PDMS. The ultrasoft unextracted PDMS gels inactivated the most bacteria, with a 17.13% inactivation, whereas the stiff unextracted PDMS gels exhibited only 8.15% inactivation.

All extracted gels exhibited no statistical difference in the *S. aureus* inactivation. For example, the ultrasoft extracted PDMS gels demonstrated a 3.24% inactivation, which was statistically equivalent to the inactivation caused by our internal glass control (3.8% inactivation, data not plotted). This indicates that PDMS gels without uncrosslinked oligomers have equivalent levels of inactivation to our cleaned and sterilized glass control.

Figure 5B shows the relationship between the uncrosslinked oligomer content and bacterial cell inactivation as calculated using eq S3. While the bacterial inactivation was statistically different between all unextracted and extracted PDMS gels prepared at the same B:C ratio, we highlight the increase that occurred at the higher B to C ratios. The *S. aureus* SH1000 inactivation on ultrasoft and soft gels increased by 428 and 214% versus the extracted gels with the same Young's modulus.

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While the standard and stiff unextracted also had increased inactivation, at 62.37 and 37.37%, respectively, these values were lower than those of the gels with more free oligomers. Thus, changes in *S. aureus* viability are likely linked to the presence and/or diffusion of uncrosslinked oligomers from the unextracted gels. For unextracted gels with greater uncrosslinked oligomer content, we may consider if the increased tackiness of the gel might lead to increased amounts of dead cells, since these cells might be harder to rinse off. While this is a possibility, it would likely apply only to bacteria that first adhered to the surface and then died. Current literature on *S. aureus* surface sensing suggests that *S. aureus* interactions with surfaces are protein-mediated and that the potential microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), such as the ClfA protein, are responsible for *S. aureus* adhesion to fibrinogen.[59] For dead *S. aureus* cells, these protein MSCRAMMs would be inactive, and therefore, the dead cells would be unlikely to be able to sense the oligomer content of the PDMS gel and adhere.

Microbial Adhesion to Unextracted and Extracted PDMS Gels. Microbial attachment assays were used to assess the adhesion of *S. aureus* strains to unextracted and extracted PDMS gels. For both unextracted and extracted PDMS, an inverse correlation between gel stiffness and microbial area coverage occurred: more *S. aureus* adhered to softer PDMS gels as seen in Figure 6. When the adhesion of *S. aureus* SH1000, a common laboratory strain (Figure 6A), was explored on the unextracted PDMS gels, it was determined that the ultrasoft had 25.07%, soft had 17.79%, standard had 2.98%, and stiff had 1.62% area coverage. The ultrasoft gels had 93.5% more *S. aureus* SH1000 area coverage than the stiff, unextracted PDMS gels. With regard to *S. aureus* ATCC 12600, which was isolated from a human lung *S. aureus* infection (Figure 6B), similar area coverage (to SH1000) was observed (21.87, 11.03, 3.12, and 1.62%) on ultrasoft, soft, standard, and stiff unextracted gels. Again, the ultrasoft unextracted gels had 92.6% more *S. aureus* ATCC 12600 area coverage than the stiff gels.

As Young's moduli of the extracted PDMS gels decreased, more *S. aureus* adhered. *S. aureus* SH1000 adhered to extracted PDMS gels, with area coverages of 13.01, 11.59, 2.26, and 1.29% for the ultrasoft, soft, standard, and stiff samples, respectively. Similarly, the *S. aureus* ATCC 12600 area coverage on extracted PDMS gels was 10.09, 6.51, 2.39, and 1.29% on ultrasoft, soft, standard, and stiff gels, respectively. When directly comparing the ultrasoft to the stiff extracted gels, there was 91.1 and 87.2% more *S. aureus* SH1000 and ATCC 12600, respectively. *S. aureus* exhibits greater adhesion to softer PDMS, regardless of the extraction status.

Discussion on the Adhesion of Microorganisms to PDMS Gels. As presented in the last section, the microbial attachment was greater on unextracted gels than on extracted gels. Figure 7 plots this increase, as calculated via eq S4, as a function of the uncrosslinked oligomer content in the unextracted PDMS gel. As discussed in Figure 5, there was an increase in the level of bacterial inactivation for bacteria incubated on top of the softer, unextracted gels. While *S. aureus* SH1000 was visualized using GFP, a visualization of the *S. aureus* ATCC 12600 was achieved by staining all cells using DAPI stain. This, along with other biological reasons, might account for the difference in the increased adhesion observed by these two strains on the soft and ultrasoft gels. However, differing cell visualization methods do not account for the overall trend of why more cells of both strains attach to softer PDMS gels than to stiffer PDMS gels.

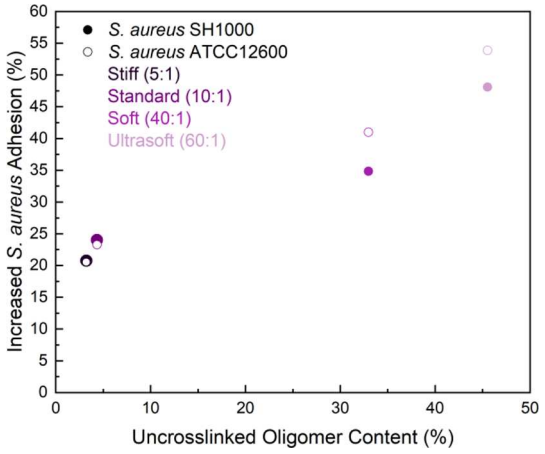

Figure 7. Increased *S. aureus* adhesion observed on unextracted PDMS gels (versus their extracted counterpart) is plotted as a function of the uncrosslinked oligomer content in the unextracted gels. Closed circles represent *S. aureus* SH1000, open circles represent *S. aureus* ATCC12600, and the color represents the PDMS gel, where dark to light purple represents the stiffest to softest gels. The uncrosslinked oligomer content was calculated using eq 1, whereas increased *S. aureus* adhesion was calculated using eq S4.

We can again consider differences that may be due to the adhesive failure energies discussed in Figure 4, which indicates that free (uncrosslinked) oligomers contributed to the greater tackiness of the gels. One hypothesis is that the removal of these uncrosslinked oligomers would lead to reduced bacterial area coverage. However, even with the free oligomers removed, *S. aureus* adheres more to softer gels, indicating that a combination of the material's stiffness and adhesive failure energy causes changes in bacterial adhesion. We note that this study focused only on the material properties and not the biological mechanism(s) that might be influencing bioadhesion.

Previously, Straub et al.[28] reported that *S. aureus* DSM 20231 adhered similarly to gels with Young's moduli ranging from 60 to 4520 kPa (measured using AFM nanoindentation) prepared from B:C ratios of 5:1, 10:1, 20:1, and 40:1. Recently, Sun et al.,[39] reported that more *S. aureus* ATCC 25923 (a strain commonly used for quality control tests for antibiotics) grew after exposure to extracted 10:1 PDMS gels more than unextracted PDMS gels. Here, we report that after 24 h of incubation directly on PDMS gels, statistically more bacteria attached to unextracted PDMS gels (B:C ratios of 5:1, 10:1, 40:1, and 60:1) than to extracted PDMS gels. These manuscripts all report different findings, possibly due to the different bacteria and microbiology analysis techniques used. Sun et al.[39] used a plate-counting process that removed bacterial cells from the PDMS with a saline solution prior to a serial dilution and redepositing the cells onto an agar plate for growth overnight. In contrast, we used fluorescence microscopy, which, after a light rinse to remove loosely adhered bacteria, immediately imaged and quantified the bacteria grown directly on the PDMS gels. Despite differences in methodologies, we are excited for laboratories across the globe to conduct more related experiments that will yield a unified story in the future.

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CONCLUSIONS

In this work, we have fabricated and characterized unextracted and extracted PDMS gels that span a wide range of Young's moduli (50−1500 kPa), including an ultrasoft gel, which has not previously been explored in literature. The PDMS gels' degree of swelling, uncrosslinked oligomer content, and crosslink density were distinct at each B:C ratio. To remove uncrosslinked oligomers, a toluene extraction process was used; the amount of uncrosslinked oligomers was quantified, and their role in bacterial inactivation and adhesion was assessed. Increased *S. aureus* inactivation occurred only for the unextracted gels prepared at high B:C ratios, whereas none of the extracted gels (no unreacted oligomers) had significant levels of bacteria inactivation. The adhesion of *S. aureus* ATCC12600 and *S. aureus* SH1000 decreased with an increase in PDMS gel stiffness, meaning that more bacteria adhered to softer PDMS gels. Furthermore, across both bacterial strains, unextracted PDMS gels had greater bacterial adhesion compared to extracted PDMS gels of the same B:C ratio. After a 24 h incubation period, soft PDMS gels demonstrated ∼90% greater bacterial adhesion than the stiff PDMS gels. Comparing the free oligomer content present in the PDMS gels to bacterial adhesion suggests that the increased presence of uncrosslinked oligomers leads to increased bacterial adhesion. Our findings suggest that material stiffness impacts *S. aureus* adhesion to PDMS gels, as more bacteria adhere to softer PDMS gels irrespective of the presence of uncrosslinked oligomers. At the same time, all unextracted PDMS gels statistically had more bacteria adhered than their extracted counterpart gel. These findings suggest that *S. aureus* is sensitive to the mechanical and material properties of hydrophobic gels and that oligomer content plays a role in both bacterial cell adhesion and inactivation; we are excited for the future research that unveils the biological mechanism(s) that likely also play important roles in the phenomena.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.3c11349.

FTIR, angular frequency sweeps, tan δ, complex moduli plots, *S. aureus* adhesion to PDMS gels, and additional equations (PDF)

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Notes

The authors declare no competing financial interest.

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