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ABSTRACT

Liquid-infused polymers are recognized for their ability to repel foulants, making them promising for biomedical applications including catheter-associated urinary tract infections (CAUTIs). However, the impact of the quantity of free liquid layer covering the surface on protein and bacterial adhesion is not well understood. Here, we explore how the amount of free silicone liquid layer in infused silicone catheter materials influences the adhesion of bacteria and proteins relevant to CAUTIs. To alter the quantity of the free liquid layer, we either physically removed excess liquid from fully infused catheter materials or partially infused them. We then evaluated the impact on bacterial and host protein adhesion. Physical removal of the free liquid layer from the fully infused samples reduced the height of the liquid layer from 60 μm to below detection limits and silicone liquid loss into the environment by approximately 64% compared to controls, without significantly increasing the deposition of protein fibrinogen or the adhesion of the common uropathogen *Enterococcus faecalis*. Partially infused samples showed even greater reductions in liquid loss: samples infused to 70%–80% of their maximum capacity exhibited about an 85% decrease in liquid loss compared to fully infused controls. Notably, samples with more than 70% infusion did not show significant increases in fibrinogen or *E. faecalis* adhesion. These findings suggest that adjusting the levels of the free liquid layer in infused polymers can influence protein and bacterial adhesion on their surfaces. Moreover, removing the free liquid layer can effectively reduce liquid loss from these polymers while maintaining their functionality.

I. INTRODUCTION

Placing medical instruments within the human body presents numerous challenges due to the inevitable exposure of medical materials to various contaminants such as bodily fluids, proteins, and mucus. This exposure can lead to contamination and colonization of pathogens. For example, after the insertion of a urinary catheter, fibrinogen, a sticky protein released by the human host would coat the urinary catheter surface, which provides binding sites for pathogen colonization and induces the occurrence of catheter-associated urinary tract infection (CAUTI).[1–3]

Liquid-infused polymers are a promising solution for medical instruments and devices due to their documented antifouling properties.[4–8] In liquid-infused polymers, the infusing liquid penetrates the polymer matrix, forming a free-flowing liquid layer on the polymer surface upon saturation. As long as the infusing liquid

has a higher affinity for the solid material than contaminants, it can effectively repel them.[4,9] Multiple excellent reviews have been published describing in detail the physics and chemistry behind liquid-infused surfaces in general, and liquid-infused polymers specifically; the reader is to refer to these works for more in-depth information.[4,10–13] However, on a fundamental level, an infused polymer system can be made with any liquid-polymer pair in which (1) the liquid infuses into the solid polymer and (2) the liquid remains immiscible with the surrounding fluid. Following these rules, medical-grade silicones can be effectively infused with silicone liquid to create a highly stable liquid-infused polymer system.[1,14]

For individuals considering catheterization, the presence of issues such as allergies and comfort levels pose significant concerns. Silicone is a nonallergenic material which stands out as an excellent choice for those with latex allergies. In contrast to latex, silicone is not only nonallergenic but also biocompatible. Studies have demonstrated that silicone does not adversely affect the viability and metabolic activity of human cells.[15] Furthermore, the United States Centers for Disease Control and Prevention recommends silicone as a preferred catheter material for individuals prone to frequent obstructions. In comparison to latex, silicone exhibits higher resistance to kinking, enhancing both patient comfort and catheter efficiency.[16,17] This combination of nonallergenicity, biocompatibility, and resistance to obstructions or kinking makes silicone a compelling option for individuals seeking a reliable and comfortable catheterization experience.[17,18] Silicones are also easily infused with medical-grade silicone liquids to create liquid-infused polymers,[8,14,19] making them well suited to act as next-generation infection-resistant materials for catheters. Infused silicones have shown promising results in both *in vitro* and *in vivo* settings, effectively reducing adhesion by a range of medically relevant proteins and microorganisms.[4,10,14,19–22]

Recently, an *in vivo* study by Andersen *et al.* has shown that liquid-infused catheter materials can reduce the adhesion of fibrinogen, a host protein that facilitates the attachment and proliferation of microorganisms, in a murine model of CAUTI.[1] This work demonstrated that preventing the adhesion of fibrinogen decreased subsequent attachment by six of the most prevalent pathogens in CAUTI, as well as significantly reducing bladder colonization and their dissemination to other organs. In urinary catheters, the intermittent flow of urine over the surface of the catheter is very likely to result in the loss of free surface silicone liquid from liquid-infused surfaces.[23] At the same time, repeated contact of the exterior surface of the catheter with urethral and bladder tissue is likely to physically remove the free liquid layer present there. The success of liquid-infused polymers in repelling proteins and bacteria in this study is, therefore, intriguing as it suggests that even with the removal of some level of the liquid layer, the material remains functional. Moreover, the understanding of how the free liquid layer on liquid-infused polymers impacts protein and bacterial adhesion is crucial for their use as medical devices since liquid loss into the human body is not a desired outcome for these applications.[24–26]

In this work, we explore the effect of the removal of the free liquid layer from the surface of infused silicone materials on protein and bacterial adhesion relevant to CAUTI. We show that liquid layer removal significantly decreases silicone liquid lost into the environment without disrupting protein- and bacterial-resistance as long as a certain volume of silicone liquid (~80%) remains in the system. These results will aid the potential future applications of liquid-infused materials as urinary catheter materials or other medical devices and enhance our understanding of the interaction between the liquid layer and protein and pathogen adhesion.

II. EXPERIMENT

A. Infused silicone material preparation

Silicone tubing (8060-0030, NalgeneTM 50 Platinum-cured Silicone Tubing, Thermo Scientific, USA) was cut into 2 cm sections, before being fully submerged in 20 cSt silicone liquid (DMS-T12, Polydimethylsiloxane, Trimethylsiloxy terminated, 20 cSt, Gelest, USA). 20 cSt silicone liquid was chosen due to its use in recent *in vivo* CAUTI models.[1] Any air bubbles in the tube lumen were removed to ensure uniform infusion. For samples with a free liquid layer (LL) and without a free liquid layer (ØLL), the tube sections were removed from silicone liquid after 5 days and excess liquid was allowed to flow out of the tube by holding it vertically for at least 1 mi or until all the excess liquid had dripped off the sample. These materials were then used as the LL samples; for the ØLL samples, the sections were gently blotted on both the exterior and interior surfaces using an absorbent cellulose wipe (Kimwipe, Kimberly-Clark Corp., USA) to absorb the free liquid. For partially infused samples, the sections were removed at defined points in the infusion process then weighed to determine the infusion percentage. All infused samples were allowed to rest for at least 24 h before further testing to permit equilibration of silicone liquid within the polymer.

B. Confocal imaging of infused samples with and without liquid layer

A mixture of 850 μg of BDP FL alkyne laser dye (D14B0, Lumiprobe, USA), 10 ml of dichloromethane, and 100 g of silicone elastomer base (Dow SILGARDTM 184 Clear, Dow, USA) were combined in a planetary centrifugal mixer (ARE-310, Thinky, USA) at 2000 rpm for 1 min, followed by an additional mixing at 2200 rpm for 1 min. The mixture was left in a desiccator overnight to remove any trapped gases. Next, 10 g of curing agent (Dow SILGARDTM 184 Clear, Dow, USA; in a 10:1 ratio) was added to the resulting solution, which was mixed again in the centrifugal mixer using the same settings. Aliquots of 0.9 ml were then transferred into the square depressions ($2.0 \times 2.0 \times 0.5$ cm^3) of a mold master and were degassed for 2 h and cured overnight at 70 °C.

To prepare the dyed silicone liquid for infusion, approximately 9 mg of pyrromethene (05971, Pyrromethene 597-8C9, Exciton, USA) was added to every 100 ml of silicone liquid and thoroughly mixed. The solution was filtered through a 0.45 μm filter to remove any particulates. The silicone squares were fully submerged in the infusion liquid for over 96 h. LL samples were placed vertically to drain off excess liquid. ØLL samples were placed vertically to allow the excess infusion solution to drain off and then gently dabbed on an absorbent cellulose wipe (Kimwipe, Kimberly-Clark Corp., USA). Finally, the samples were imaged using a Leica Stellaris

confocal microscope equipped with a white light laser, using an HC PL APO CS2 20×/0.75 mm objective lens. False coloring was added to the image with Clip Studio Paint (Clip Studio Paint, Japan).

C. Sliding angle and droplet speed test

For sliding angle tests, silicone tubing sections were placed on a tilt stage at 0°. A digital angle gauge (AccuMASTER 2 in 1 Magnetic Digital Level and Angle Finder, Calculated Industries) was affixed to the tilt stage to measure any changes in the angle. Using a pipette, a 20 μl water droplet was deposited into the lumen of the silicone tubing. The tilt stage was then gradually inclined until the droplet began to move off the tubing. The minimum angle at which the droplet began to move was then recorded. For droplet speed tests, the tilt stage was set at 30° and a digital camera (EOS Rebel T5 Digital SLR Camera; Canon; USA) was used to record the sliding of the droplet down the lumen. A frame-by-frame analysis was then used to determine the time the droplet took to travel from one end to the other.

D. Free liquid removal of infused silicone samples

The dyed silicone liquid was prepared following the previously defined procedure. A standard curve of the dyed silicone liquid in 18.2 MΩ cm water (Millipore Milli-Q Direct 8 Water Purification System; 18.2 MΩ cm) was created by adding a fixed percentage of dyed silicone liquid in 10 ml of 18.2 MΩ cm water (Fig. S1 in the supplementary material). Afterward, 1 ml of toluene (108–883, Toluene anhydrous, Alfa Aesar, USA) was pipetted into the 18.2 MΩ cm water, and the mixture was manually shaken for 10 s and left to settle for at least a minute to separate into upper and bottom layers. The top layer was then carefully extracted and placed in a glass cuvette for absorbance measurement via a spectrophotometer (840–277 000, GENESYS™ 30 Visible Spectrophotometer, Thermo Fisher, USA). The absorbance of the samples was measured at 2 nm intervals within the 350–650 nm range. A minimum of 20 samples per standard solution were used in the generation of the standard curve, adhering to the published recommended number for establishing the limit of detection.[27] The limit of detection, determined following the guidelines set forth by the Clinical and Laboratory Standards Institute, was calculated to be 0.0012%.

The assessment of free liquid removal from silicone materials infused with dyed silicone liquid followed a consistent procedure. Instead of dispensing a fixed volume of dyed silicone liquid into 18.2 MΩ cm water, we immersed the samples infused with dyed silicone liquid, prepared using the aforementioned infusion method, into 10 ml of 18.2 MΩ cm water, then completely removed the samples from the water. This immersion-withdrawal cycle was repeated ten times. Subsequently, the absorbance readings were compared against a standard curve to quantify the amount of liquid extracted.

E. Immunolabeling of fibrinogen and *E. faecalis*

Adhesion testing with human fibrinogen (Fb) free from plasminogen, von Willebrand factor (Enzyme Research Laboratory #FB3), and *Enterobacter faecalis* (ATCC 47077) were conducted using the previously described methods.[1,28] Briefly, after overnight incubation with Fb in phosphate-buffered saline (PBS; 150 μg/ml) or *E. faecalis* in human urine (supplemented with 20 mg/ml BSA), the silicone samples were fixed using 10% neutralized formalin, followed by blocking and staining steps. For Fb, goat anti-Fb primary antibody (Sigma) was used in staining at a dilution of 1:1000, followed by Donkey anti-Goat IRD800 antibody (Invitrogen; 1:5000). For *E. faecalis*, rabbit primary antibody was used followed by Donkey anti-Rabbit IRD680 antibody (Invitrogen; 1:5000). After overnight drying at 4 °C, the silicone catheter materials were imaged using an Odyssey Imaging System (LI-COR Biosciences) to visualize and quantify the infrared signal. The controls were established using the same method, except that they were incubated in sterile PBS instead of Fb or *E. faecalis*. The intensities for each catheter piece were normalized against a negative control and then expressed relative to the pieces coated with Fg, which was assigned a value of 100%.

F. Statistical analysis

The statistical significance of the experimental results was evaluated using GraphPad Prism, version 7.03 (GraphPad Software, San Diego, CA). The data underwent an initial assessment to determine their adherence to Gaussian distribution. Based on the outcomes of these assessments, appropriate statistical tests were chosen. When the data exhibited Gaussian distribution, a one-way ANOVA was employed. Conversely, when the data did not conform to Gaussian distribution, the Kruskal–Wallis test was used. Significance levels on the graphs are denoted as follows: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.0001$, and $^{****}p \leq 0.00001$.

III. RESULTS

A. Removal of the free liquid layer does not significantly affect surface slipperiness

To better understand the role of the free liquid layer in a protein and bacterial adhesion system, e.g., those found in infused urinary catheters,[1] we first fabricated infused silicone tubes by immersing them in 20 centistoke (cSt) trimethoxy-terminated silicone liquid until complete saturation was achieved, indicated by a plateau in weight gain.[1,29] Samples were then removed from the liquid and all excess liquid was allowed to drain from the surface: these samples were considered to have an intact free liquid layer (LL). A subset of catheter samples was then subjected to removal of the free liquid layer (ØLL) via absorption of the liquid from both interior and exterior surfaces by light contact with a cellulosic wipe [Fig. 1(a)]. In previous reports, the free liquid layer was removed via rinsing with water,[30–32] which could lead to syneresis, defined as free silicone liquid molecules migrating to the surface of the material.[30,33] Our treatment here was intended to deplete the surface layer to the point where it would not substantially increase via syneresis over the duration of the experiments (<48 h). This ensures that the material being tested remains stable throughout the experiment and is not subjected to significant changes in the liquid layer during the experimental process.

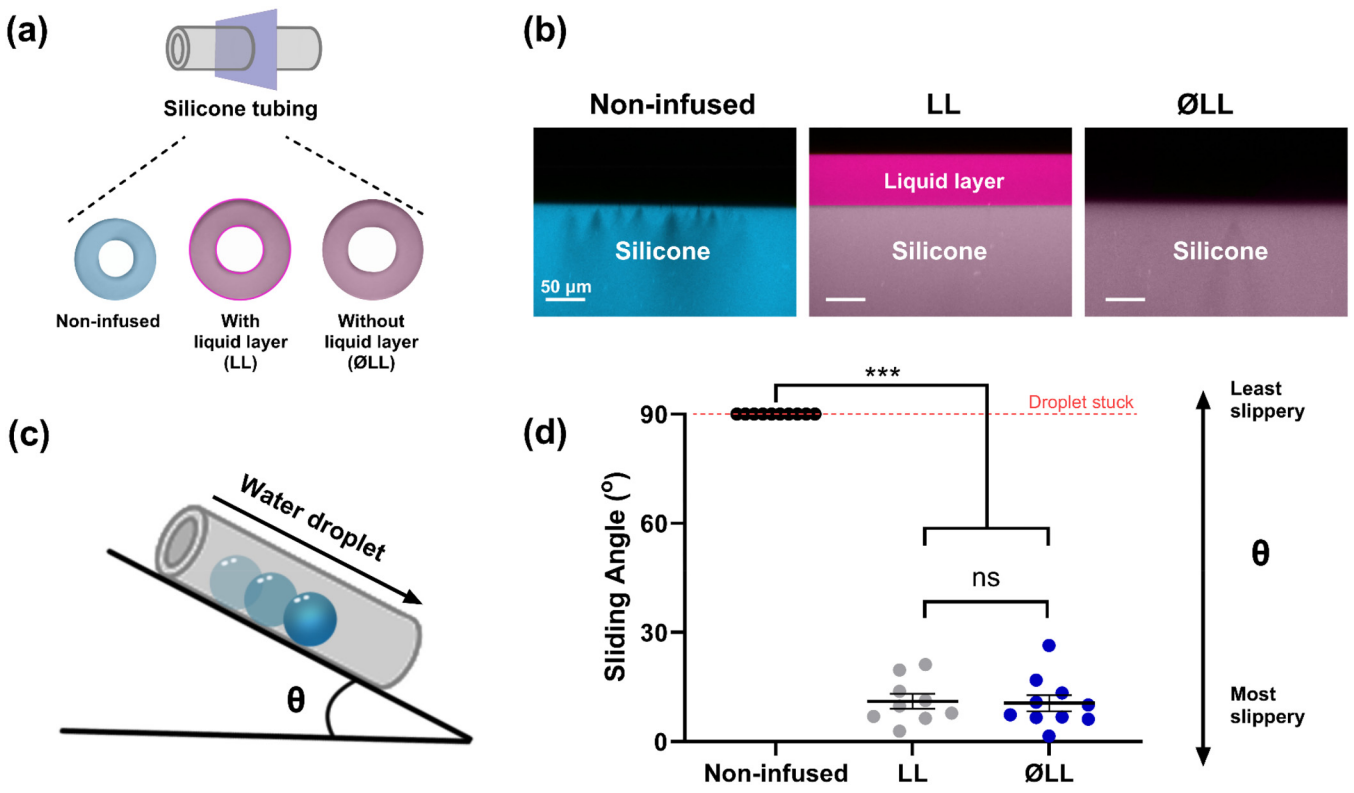

FIG. 1. Removal of free liquid on fully infused silicone tubes. (a) Schematic of silicone tubes fabricated with or without a liquid layer (LL; ØLL). (b) Confocal image analysis of noninfused silicone tubes; silicone tubes with and without a liquid layer. All scale bars are 50 μm. (c) Schematic of the sliding angle test to characterize a slippery surface. (d) Sliding angle test of noninfused silicone tubes, and silicone tubes with (LL) or without a liquid layer (ØLL). In all graphs presented, the error bars represent the standard error of the mean. Statistical significance between the groups was evaluated using the ANOVA test. *** = $P < 0.0005$ and ns = not significant.

The results of removing the free liquid layer from the surface were visualized using confocal microscopy with different fluorescent dyes for solid silicone and liquid silicone [Fig. 1(b)].[8,31] The images of the LL samples showed a layer of ~60 μm in thickness, in agreement with previous reports on similar systems.[8] In contrast, the ØLL samples showed a marked reduction of the free liquid layer to a value below what could be observed using this technique (≤500 nm), confirming the successful removal of nearly all free liquid at the sample surface.

To initially explore the effect of free liquid removal on surface properties, we performed sliding angle tests [Fig. 1(c)] where the critical angle at which a water droplet starts to slide down the sample. For liquid-infused surfaces, the sliding angle serves as an indicator of the fluid repellency of the surface and, therefore, the likelihood of resisting adhesion.[10,27–30] We observed no difference in sliding angle between the LL and ØLL samples [Fig. 1(d), $P > 0.9999$]. Measurements of the droplet speed,[34] which provide an indication of surface uniformity, yielded similar results [Fig. S2(a) in the supplementary material]. Based on the previously published studies, it is generally expected that samples with low sliding angles and high sliding rates would result in the most effective antifouling surfaces.[8,22,29] With these findings, we conclude that infused silicone tubes without a liquid layer exhibit a comparable antifouling surface to the silicone tubes with a liquid layer.

B. Exploration of foulant repelling ability of silicone catheter materials without liquid overlayer

Previous investigations of liquid-infused silicone have suggested that a thin, stable free liquid layer is critical to successful fouling resistance, as it acts as a physical barrier and reduces the force required to release attached fouling organisms.[35,36] To assess if the removal of the free liquid layer in our system impacted the ability of foulants relevant to CAUTI to adhere to the surface, we incubated both the LL and ØLL samples with the host protein fibrinogen (Fb) and the bacterium *Enterococcus faecalis*.

The results showed that both the LL and ØLL samples effectively resisted Fb [Fig. 2(a)] and *E. faecalis* [Fig. 2(b)] adhesion, showing significantly less surface attachment compared to controls (Fb: $P < 0.0001$ and $P = 0.0001$, respectively; *E. faecalis*: $P < 0.0001$ and $P = 0.009$, respectively). Moreover, the results showed no significant difference in either Fb or *E. faecalis* adhesion between LL and

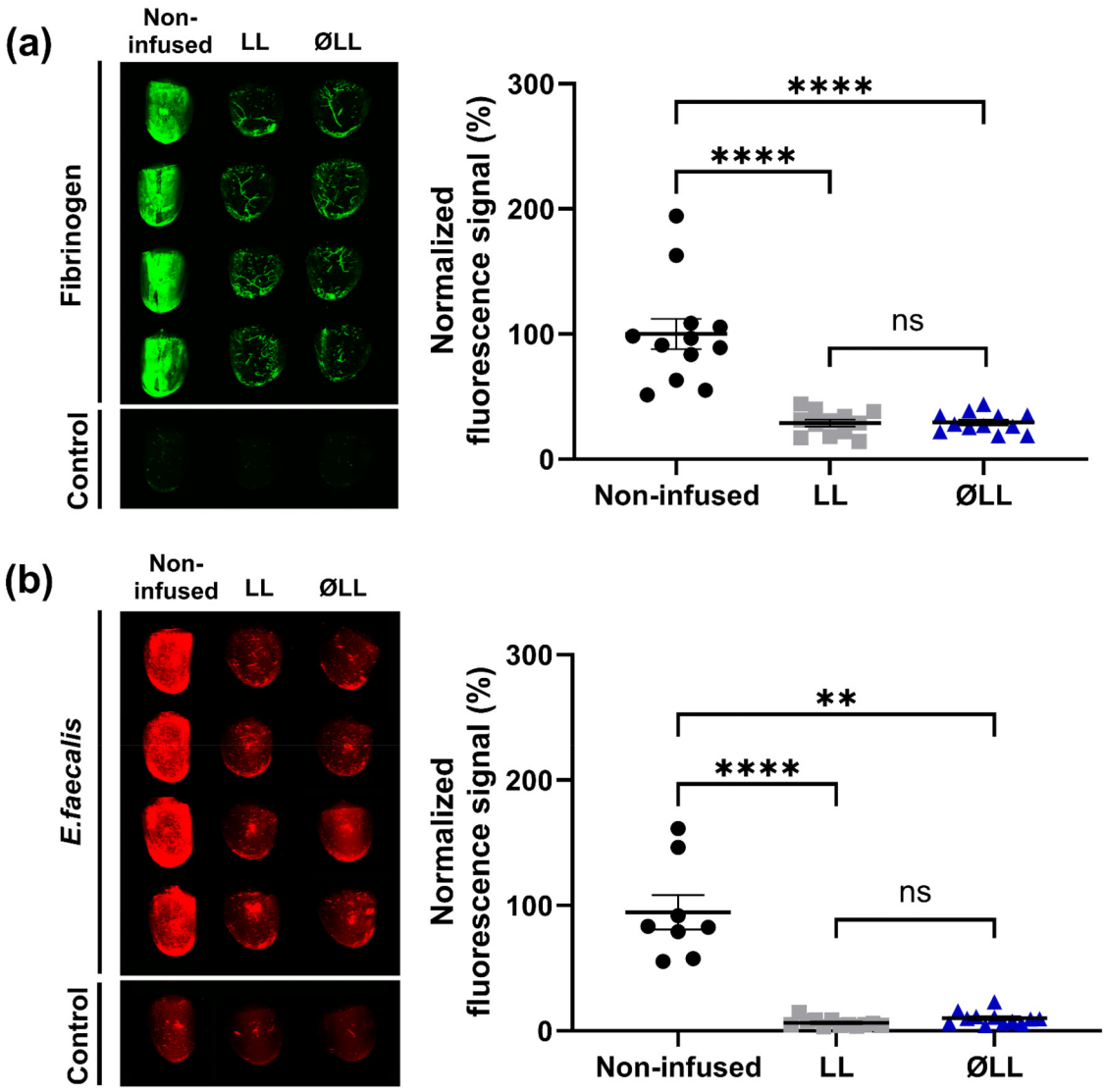

FIG. 2. Comparison of (a) fibrinogen or (b) *E. faecalis* adhesion levels on tubes that are noninfused, with (LL), or without (ØLL) a liquid layer. The images on the left show the visualization of the samples while the plots on the right show the fluorescence quantification. At least three replicates with n = 4–5, each were conducted. In all graphs presented, the error bars represent the standard error of the mean. Statistical significance between the groups was assessed using ANOVA, with **** = $P < 0.0001$; ** = $P < 0.005$; and ns = not significant.

ØLL samples ($P > 0.9999$ and $P = 0.25$, respectively), suggesting that removing the free liquid overlayer does not have a significant impact on the material's ability to resist fouling in terms of Fb and pathogen adhesion.

C. Reducing the amount of liquid infused into silicone tubes

Research has demonstrated that infused silicone polymers can undergo a process called syneresis, in which unbound oligomers or silicone liquid molecules slowly migrate to the surface of the material, reforming a free liquid layer which is then available to be lost into the environment.[30,36,37] However, this can be minimized by reducing the amount of free silicone liquid in the system.[32] Infused materials exhibit a progressive mass increase and swelling during the infusion process until they reach a state of saturation, referred to as full infusion.[29] The degree of swelling (Q) in the silicone tubes resulting from the absorption of a solvent such as silicone liquid can be quantitatively described as $Q = \frac{W_s}{W_d}$, where W_s represents the mass of the silicone tube after infusion and W_d represents

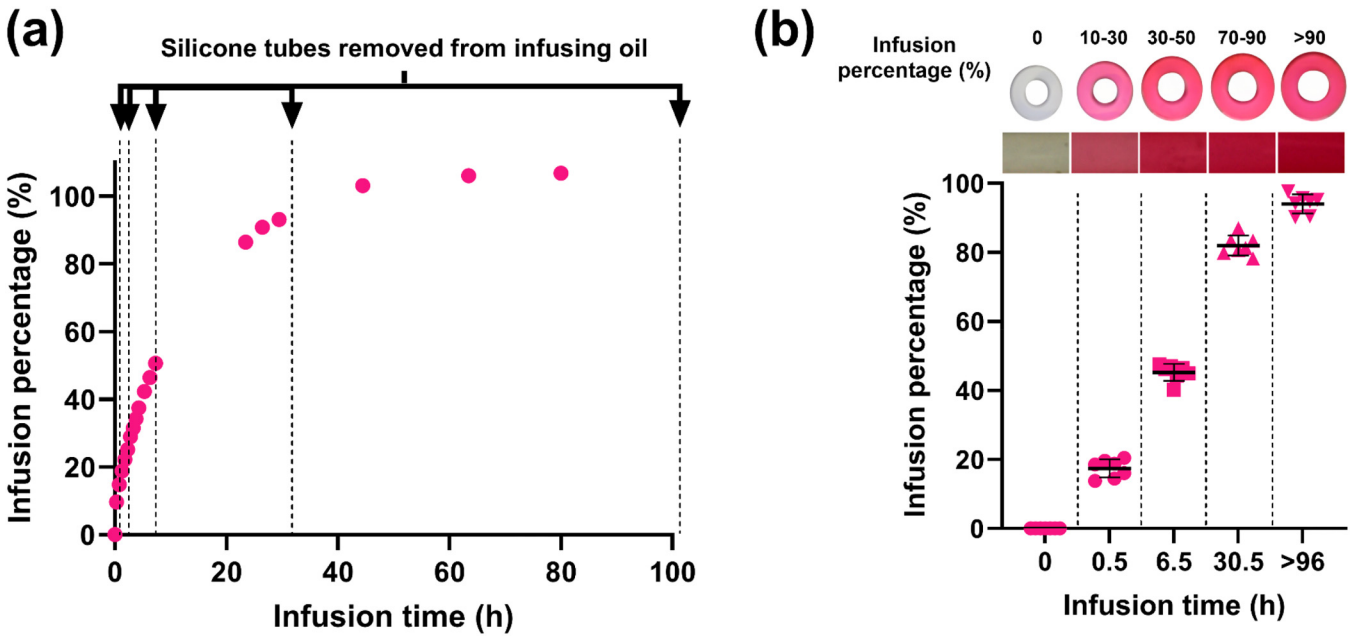

FIG. 3. (a) Various levels of infusion percentage (%) vs infusion time. The arrows denote the timepoints when silicone tubes were removed from infusing silicone liquid. (b) (Top) images of sample cross sections in which the infusing liquid was dyed for the visualization of distribution throughout the material. Close-up images of the color are shown in the boxes underneath. (Bottom) silicone tubes infused for 0.5; 6.5; 30.5; and >96 h and their resulting infusion percentages.

the mass of the noninfused silicone tube.[32] The infusion percentage (I%) can then be calculated through the following formula:

$$I\% = \frac{Q - 1}{Q_{max} - 1} \times 100\% ,$$

where Q_{max} represents the maximum degree of swelling when the silicone tube is fully infused. The Q_{max} value for silicone tubes used in our experiments infused with 20 cSt silicone liquid is experimentally determined to be ~1.95 (±0.02).

In our system, partial infusion was achieved by removing the silicone tubes at defined time points during the infusion process, as shown in Fig. 3(a), followed by absorbing excess liquid from the surface to prevent further infusion. The result [Fig. 3(b)] was a series of well-defined groups of samples with increasingly less free liquid distributed throughout the matrix. Specifically, samples infused for 0.5 h resulted in 17.4 (±0.99)% of infusion, those infused for 6.5 h achieved 45.25 (±0.94)% of infusion, and samples infused for 30.5 h reached 81.98 (±1.08)% of infusion. Samples were considered fully infused if they were removed from the liquid bath at >96 h and had an infusion percentage value of >90.0%.

D. Functionality of partially infused silicone tubes is comparable to fully infused material

Partially infused silicone tubes were tested for sliding angle to initially explore their surface properties. The results of the sliding angle test (Fig. 4) showed that as the infusion percentage increases, (i.e., a higher amount of free silicone liquid is present in the system), the sliding angle gradually decreases. Samples infused to 30%–50% already showed a significant difference in sliding angle compared to noninfused controls (P = 0.03). Although the samples

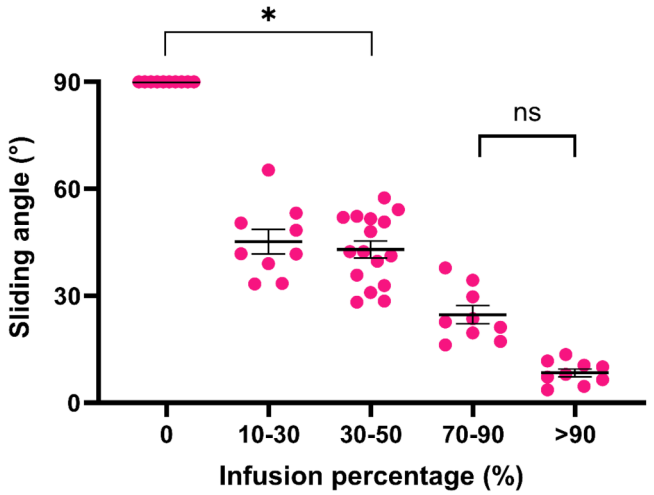

FIG. 4. Sliding angle of a water droplet on silicone tubes with varying infusion percentage (%). Statistical significance between the groups was assessed using ANOVA; * = P < 0.05 and ns = not significant.

infused to 70%–90% showed somewhat higher values than the fully infused samples [24.71 (±2.54) compared to 8.41 (±1.20), respectively], the difference was found to be nonsignificant ($P > 0.9999$). In the droplet speed test, a similar outcome was observed [Fig. S2(b) in the supplementary material].

E. Significant reduction in protein and bacteria adhesion is achievable without full infusion of silicone tubes

To assess the effectiveness of partially infused silicone tubes in repelling fouling agents, we again incubated the samples with either Fb or *E. faecalis* followed by immunolabeling to assess the levels of adhesion. The results indicate that, as the infusion level increased, the adhesion levels of both Fb and *E. faecalis* gradually decreased. Notably, the first significant difference was observed between non-infused silicone tubes and those infused to 70%–90% ($P < 0.0001$ for both Fb and *E. faecalis*). In addition, the silicone tubes infused to 70%–90% did not show a significant difference compared to the fully infused silicone tubes ($P = 0.19$ for Fb; $P > 0.9999$ for *E. faecalis*).

F. Removal of the free liquid layer and partially infused silicone tubes significantly reduces liquid loss into the environment

To use liquid-infused materials in medical devices effectively, it is crucial to minimize the leakage of infused silicone liquid into the environment, as silicone liquid escaping from the infused material can potentially trigger immune responses.[24–26,38] To compare the quantity of liquid that could be lost into the environment in LL vs ØLL samples, silicone tubes infused with dyed silicone liquid underwent repeated passage through an air-water interface to strip away the liquid layer. The liquid extracted from the infused silicone tubes into water was extracted into toluene and, subsequently, measured for concentration using a spectrophotometer and quantified via a standard curve (Fig. S1 in the supplementary material). The LL samples were found to lose 0.05 (±0.01) μl of silicone liquid/mm of sample length, while the ØLL samples lost significantly less, 0.02 (±0.006) μl of liquid/mm [$P = 0.038$; Fig. 5(a)].

To investigate the liquid loss levels of partially infused silicone tubes, the samples were again repeatedly exposed to an air-water interface to strip away the liquid layer. The results are shown in Fig. 6(b). The fully infused samples were found to lose 0.025 (±0.004) μl of liquid, while samples at 70%–90% infusion lost significantly less at 0.004 (±0.0004) μl ($P < 0.0001$). Samples at 30%–50% infusion lost 0.004 (±0.0013) μl of liquid, which was not significantly different from samples at 70%–90% infusion ($P > 0.9999$). Neither samples at 30%–50% nor 70%–90% infusion were found to be significantly different from noninfused controls ($P = 0.50$ and 0.52, respectively), unlike the fully infused samples, which were significantly higher ($P < 0.0001$).

G. Discussion

Current understanding of the mechanism of antifouling action of liquid-infused surfaces relies most heavily on the presence of a free and continuous liquid layer.[36] It is thought that such a layer presents a physical barrier to contaminants and can deceive the mechano-sensing mechanism of fouling organisms, preventing the initiation of their adhesive behavior.[5,6,8,35] Furthermore, the presence of the free liquid overlayer is thought to contribute to increased slipperiness on the infused surface, reducing the energy required for detaching fouling organisms and facilitating their release.[39] The results indicating no significant difference in slippery properties and antifouling functionality between LL and ØLL infused silicone materials are, therefore, surprising.

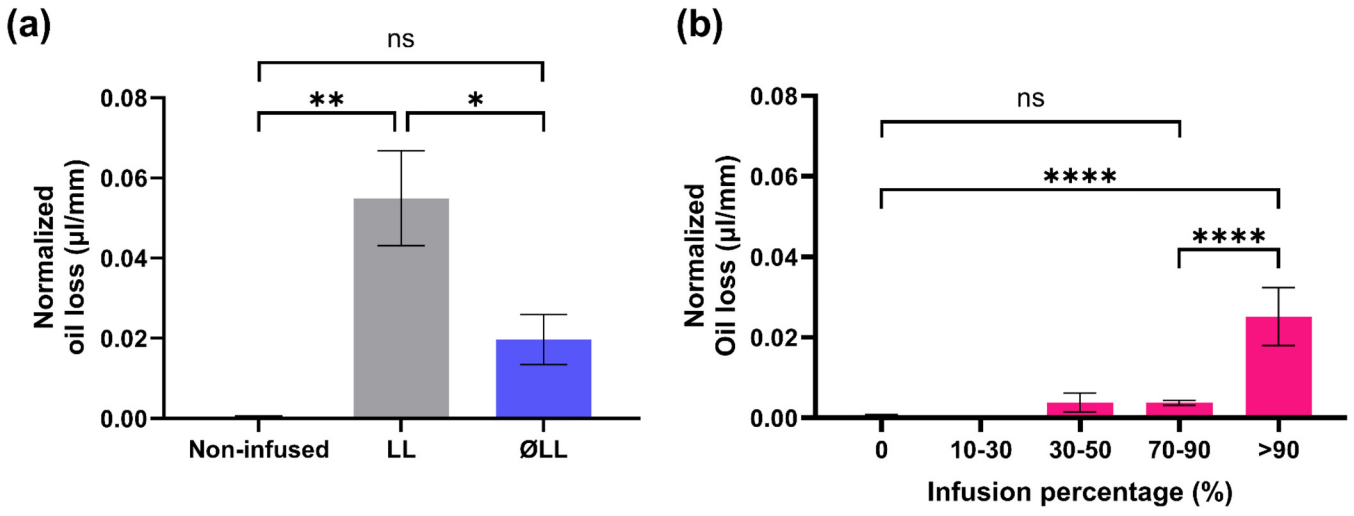

FIG. 5. Amount of liquid loss per millimeter of sample length for (a) LL and ØLL silicone tubes and (b) partially infused silicone tubes. Statistical significance between the groups was assessed using ANOVA. **** = $P < 0.0001$; * = $P < 0.05$; and ns = not significant.

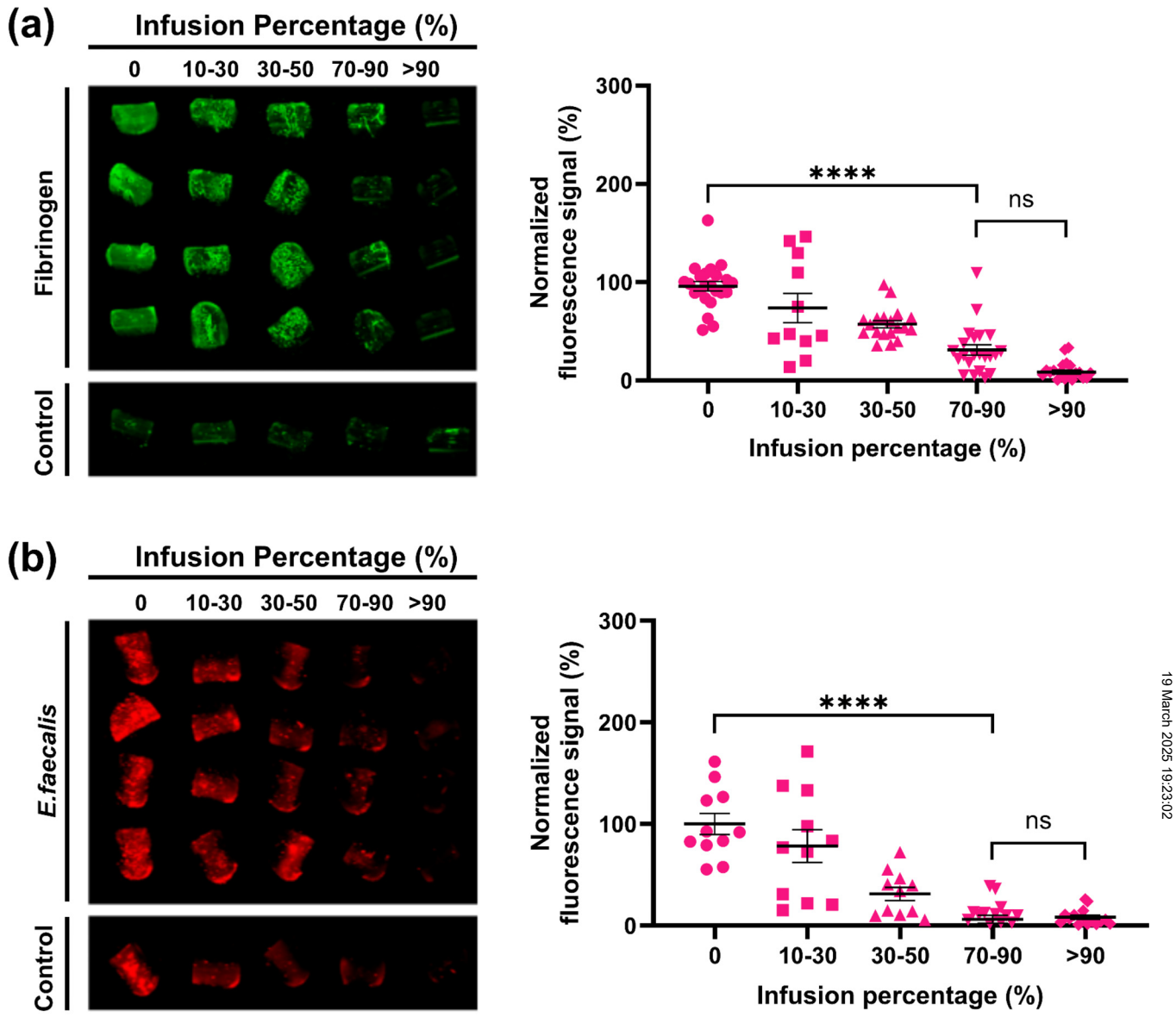

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FIG. 6. Immunolabeling of fibrinogen and *E. faecalis* on partially infused silicone tubes. (a) Fb or (b) *E. faecalis* adhesion levels on silicone tubes infused into various infusion percentages (%). The images on the left show the visualization of the samples while the plots on the right show the fluorescence quantification. At least three replicates with n = 4–5 each were conducted. In all graphs presented, the error bars represent the standard error of the mean. Statistical significance between groups was assessed using ANOVA. **** = $P < 0.0001$; and ns = not significant.

Several possibilities could explain this phenomenon: it is possible that the presence of a free and continuous liquid layer is still necessary for optimal functionality, and that ØLL still contains a liquid layer, albeit significantly thinner than LL. Since the characterization of the liquid layer in ØLL was conducted under a confocal microscope, which has a resolution limitation of 500 nm,[40,41] any liquid layer below 500 nm thickness would not be visible. It is, therefore, possible that a continuous liquid layer below 500 nm thickness would still be present. However, it is unlikely that this is the case as adhesion tests on partially infused catheters also showed a decrease in adhesion, even at infusion percentages as low as 30%–50% (albeit not significant compared to controls).

According to the literature, the lower concentration of excess liquid in partially infused materials reduces the amount of free liquid available to accumulate at the surface.[32] This suggests that the decrease in adhesion may not be entirely due to the presence of a free continuous liquid layer. In agreement with our findings, Kolle *et al.*[36] calculated that a ~20% loss of free liquid from a liquid-infused silicone bulk would result in a system in which a free liquid layer would no longer be able to reform at the surface. Our results suggest that for medical applications, silicone infused to ~80% of its maximum infusion value may contain just enough free liquid to create a dynamic interface that resists adhesion by proteins and microorganisms, while not enough to be easily lost into the environment.

Previous studies have suggested that in silicone liquid-infused silicone materials, bacterial flagella, such as those found in *E. coli* and *P. aeruginosa*, interact with the liquid layer.[8] It has been demonstrated that biofilm-forming signals are triggered when bacterial flagella come into contact with a solid material.[42] The interaction between bacterial flagella and the liquid layer may enable some degree of flagellar movement, thereby reducing the likelihood of initiating such signals and, consequently, lowering adhesion. Recent RNA sequencing research has also revealed that the introduction of silicone liquid to silicone solids leads to an upregulation of ten distinct genes in *P. aeruginosa* while simultaneously downregulating a gene that may impede initial adhesion, although the precise mechanism behind this phenomenon remains unknown.[43] Furthermore, factors such as the stiffness of the material might also impact the adhesion levels of bacteria. Research on noninfused dry silicone materials with varying degrees of stiffness has revealed that *E. coli* and *P. aeruginosa* attach more readily to softer silicone surfaces than to harder ones.[44] As it has been observed that stiffness levels correspond to the extent of infusion,[32] the alteration in the stiffness level may also be a contributing factor affecting bacterial adhesion on infused materials. These studies suggest that the adhesion of bacteria on infused materials involves numerous complex interactions and is influenced by various factors.

In fact, the understanding that liquid-infused silicone surfaces are more nuanced and dynamic than previously thought has been growing. Lavielle *et al.*[30] reported that when the free liquid layer was removed from the fully infused samples via washing with water, it would spontaneously regenerate over the following 360 h, increasing linearly from ~50 nm to ~1 μm. Cai *et al.*[31] demonstrated that free silicone liquid could spontaneously separate from the silicone solid at the edge of a water droplet. Wong *et al.*[37] also showed that free molecules within the silicone solid could migrate to the surface in response to the presence of a water drop, but, importantly, also showed that they could return to the bulk after the droplet was removed. These studies point to infused silicone surfaces that are able to dynamically respond to changes in conditions, which might also explain our results.

Silicone materials and silicone liquids are widely used in clinical settings, including heart valves, breast implants, retinal tamponades, and syringe barrel lubricants[24,45–48] due to their versatility and relative biocompatibility. Recent findings have shown that when free silicone liquid is infused into silicone catheter materials, there is a remarkable decrease in both Fb and pathogen adhesion levels, both *in vivo* and *in vitro*.[1] This reduction in adhesion levels holds promise in effectively reducing the development of CAUTI without the need for antibiotics[1–3,49]—a critical need given the rapid increase in resistant microorganisms.[50,51]

However, the potential for free silicone liquid to separate from the surface is a critical concern that must be addressed to increase the safety of these materials. For example, previous studies on ocular tamponades and connective tissues have suggested adverse effects of silicone liquid leakage, including heightened inflammatory cell response and antibody production.[25,26,38,52] It is believed that the immune response is linked to the formation of protein aggregates around silicone liquid droplets. *In vivo* studies have also indicated elevated concentrations of antidrug antibodies in the presence of silicone liquid-protein complexes.[25,26,38]

The potential for loss of the free liquid layer from the liquid-infused system is well documented.[19,39,53,54] Under water, an infused surface can maintain a low sliding angle under both turbulent flow and laminar flow conditions for a little over an hour before experiencing a significant loss of the free liquid layer.[55] However, it is well documented that when a surface with a free liquid layer is exposed to an air/water interface, the amount of liquid lost undergoes a substantial increase of one to two orders of magnitude, compared to being placed directly in water under flow.[19] In addition, it is well understood that physical contact of a solid with a free liquid layer can also easily result in layer disruption and removal.[36] Given these facts, it is most likely that the use of liquid-infused materials for medical purposes, such as liquid-infused urinary catheters,[1] are being frequently subjected to conditions which can disrupt and/or remove any free liquid layer present on the catheter surface. Temperature changes are known to affect silicones and may also be playing a role.[56]

We found that the removal of the free liquid layer in liquid-infused silicone materials resulted in a significant decrease in the amount of liquid that could be lost into the environment, suggesting that intentional removal of the free liquid layer may be an option to increase the safety of liquid-infused materials for medical use. We further found that we could decrease the amount of liquid that could be removed from the surface by only partially infusing the catheter samples with silicone liquid. Due to the lower concentration of excess liquid in the system, the amount of free liquid available to accumulate at the surface is reduced.[32] We found that at ~80% of infusion, the amount of free silicone liquid that could be removed from the surface was significantly decreased compared to fully infused controls, and there was no statistically significant difference between Fb and *E. faecalis* adhesion at this level. This is in agreement with a previous work, which showed a significant reduction in *P. aeruginosa* adhesion even at infusion levels of 30% of their maximum.[37]

Finally, although many investigations of liquid-infused silicones have examined their ability to repel bacterial adhesion,[6,8,14,19,20,34,37,39,43,57] notably fewer have examined protein deposition.[1,35,58] However, recent work has begun to reveal not only the critical role that proteins play in infection,[2,3,49] but also the ability of liquid-infused silicones to robustly resist their deposition.[1] Here, we further show that protein deposition on liquid-infused surfaces can be modulated by adjusting the quantity of silicone liquid embedded in the polymer network. The ability to precisely modulate surface protein levels could potentially open new

doors in a variety of fields, particularly, materials engineering. In addition, the mechanism by which liquid-infused surfaces reduce protein deposition will be an important area of investigation going forward and may involve multiple interacting factors, including masking of microscale defects and neutralization of surface charges.[37,57,59–62]

IV. SUMMARY AND CONCLUSIONS

The understanding of how a free liquid layer impacts the antifouling functionality of liquid-infused materials is critical for the effective design of liquid-infused medical devices. Here, we removed a bulk of the free liquid layer from the silicone tubes by absorbing the liquid, resulting in a decrease in thickness of the liquid layer from ~60 μm to below detection, and in a significant decrease in the amount of free silicone liquid that can be lost into the environment. Importantly, we find that removal of the liquid layer in this way does not prevent the surface from resisting deposition of Fb and adhesion of *E. faecalis*, a host protein and a uropathogen that have been shown to play a significant role in CAUTI. Our results suggest that a minimal to no continuous free liquid layer may be required to be effective in medical applications such as device-associated infection prevention. Further investigation using silicone catheter materials only partially infused with silicone liquid revealed that at ~80% infusion, infused silicones retained their ability to resist deposition and adhesion by Fb and *E. faecalis*, respectively, while the amount of free liquid that could be removed from the surface was minimized. Both protein and bacterial adhesion were found to increase inversely with infusion levels below 80% suggesting that it may be possible to tune protein deposition and microbial adhesion using this method. Together, our results suggest potential benefits of incorporating the removal of the free liquid layer into the fabrication process of liquid-infused materials as a method of preserving antifouling properties while also reducing the loss of excess liquid into the host.

SUPPLEMENTARY MATERIAL

See the supplementary material for droplet speed test results and standard curve for silicone liquid-pyrromethene mixture in toluene.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Ethics Approval

Ethics approval is not required.

Author Contributions

ChunKi Fong: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **Marissa Jeme Andersen:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (equal); Writing – review & editing (supporting). **Emma Kunesh:** Data curation (equal); Formal analysis (equal); Investigation (equal); Writing – review & editing (supporting). **Evan Leonard:** Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – review & editing (supporting). **Donovan Durand:** Data curation (supporting); Investigation (supporting); Writing – review & editing (supporting). **Rachel Coombs:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting). **Ana Lidia Flores-Mireles:** Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – review & editing (equal). **Caitlin Howell:** Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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