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Making Silicone Rubber Highly Resistant to Bacterial Attachment Using Thiol-ene Grafting

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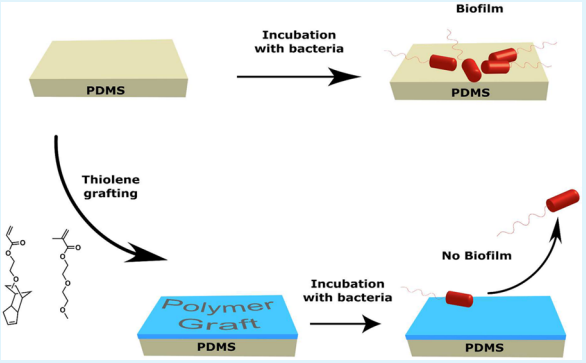
Supporting Information

ABSTRACT: Biomedical devices are indispensable in modern medicine yet offer surfaces that promote bacterial attachment and biofilm formation, resulting in acute and chronic healthcare-associated infections. We have developed a simple method to graft acrylates to silicone rubber, polydimethylsiloxane (PDMS), a commonly used device material that is often colonized by bacteria. We demonstrate a novel method whereby nontoxic bacteria attachment-resistant polymers can be readily grafted from and grafted to the surface using thiol-ene chemistry, substantially reducing bacterial colonization. With use of this approach, bacterial biofilm coverage can be reduced by 99% compared with standard PDMS in an in vitro assay. This grafting approach offers significant advantages over commonly used physisorbed coatings, especially in areas of high shear or mechanical stress. Furthermore, the approach is versatile such that the grafted material properties can be tailored for the desired final application.



KEYWORDS: *biomaterials, PDMS, silicone, bacteria, polymers, Pseudomonas, catheter*

INTRODUCTION

Biomedical devices are essential for the treatment and management of diseases in the twenty-first century. Many materials that have been chosen to make these devices have been chosen or adapted for their structural or physical attributes.[1] However, these materials often induce a biological response that is counter to their intended function, for example, stents that induce blood clotting[2] and catheters that promote urinary tract infections.[3] Biomedical device-associated infections account for a quarter of healthcare infections and are often promoted by surfaces that support bacterial attachment and subsequent biofilm formation.[4] In the age of widespread multiantibiotic resistance, new technologies are required to reduce the initial bacterial attachment to the device to prevent infection and minimize dependence on antibiotics. As the physical attributes of many devices are often optimal for their given application, much research directed toward reducing biofilm formation has been directed toward modification or adaptation of current device technology. For example, through modification via poly(ethylene glycol),[5,6] topography,[7,8] zwitterions,[9−11] increased microbicidal activity,[12,13] or a combination of these[14] a reduction in bacterial attachment has been observed.[15] Recently, a novel class of nontoxic bacterial antiadhesive materials have emerged that significantly reduce bacterial attachment and biofilm formation both in vitro and in an in vivo foreign body mouse infection model.[16−18] These are polyacrylates, with monomers characterized by their weakly amphiphilic nature[16] that reduce colonization of surfaces, and since they prevent biofilm formation rather than killing bacteria, minimize the selective pressure on bacteria to evolve antibiotic resistance. An example of one such monomer is ethylene glycol dicyclopentenenyl ether acrylate (EGdPEA). However, this monomer forms hard glassy polymers and so must be copolymerized with a monomer such as di(ethylene glycol) methyl ether methacrylate (DEGMA) to improve the mechanical properties without adversely affecting antibacterial function.[19]

Polymer coating of medical devices using noncovalent adsorption is a technique commonly employed to modify surfaces;[20] however, despite its advantages such as simplicity and batch production of polymers for coating, the efficacy of the product is often limited by the gradual attrition of the polymer from the device surface, especially in environments of high-shear stress.[21] Covalent attachment provides a more durable approach to modifying the surface properties of medical devices;[6,22,23] however, chemical modification of polydimethylsiloxane (PDMS) can prove challenging as the surface is relatively chemically inert. Consequently, the material is subjected to irradiation or etching to generate surface active groups.[24] Alternatively, the surface is modified to introduce chemical functionalities which may initiate polymerization such as bromoester formation necessary for atom transfer radical

Received: August 31, 2016
Accepted: October 24, 2016
Published: October 24, 2016

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

polymerization (ATRP).[21] However, ATRP has limitations; for example, it can be sensitive to air,[25] technically difficult to carry out, and utilizes potentially cytotoxic copper salts.[26,27] Recently, residual Si−H groups at the surface of PDMS were used to covalently graft a vinyl-terminated polymer that resulted in that resulted in a 91% reduction in bacterial attachment.[28] Although a significant improvement, the limited surface density of the grafted polymer chains resulted in more bacterial attachment compared with applying a noncovalent attachment-resistant coating.[17,18] When grafting polymers onto a surface, it is often difficult to obtain sufficient surface coverage. This limitation is largely attributable to steric hindrance at the material surface after initial chains have been successfully grafted.

Thiol-ene polymerizations are emerging as a biocompatible process for modifying material surfaces. The chemistry allows polymerization of a number of different vinyl-based monomers containing the ene functionality $H_2C{=}CHR$, for example, methacrylate, acrylate, norbornene, vinyl ether, and vinyl silazane.[29] For surface modification this is often done through the deposition of self-assembled monolayers and subsequent radical initiation.[30] The polymerization mechanism involves the formation of both radicals on the surface and in the bulk solution and so polymers may form in either location. For this reason, the process can neither be described purely as "grafting to" nor "grafting from". This methodology offers a number of advantages over the conventional grafting-to approach whereby preformed polymers are attached to a surface using reactive end groups and adhesive functionalities. When following a classical grafting-to approach, polymer surface coverage can be limited by steric hindrance, especially with longer polymer chains. Polymer chains initially grafted to the surface of interest block the active sites, preventing further grafting.[31] Thiol-ene polymerizations have been demonstrated to be useful for generating peptide-functionalized hydrogels to promote cell attachment,[32,33] and microfluidics.[34]

Recently, it has been demonstrated that (3-mercaptopropyl)-trimethoxysilane (MTS) can be incorporated into PDMS surfaces, generating chemically attached thiol functionalities available for surface polymerization.[35] In this study, nontoxic polymeric materials have been covalently attached to medical-grade silicone using thiol-ene chemistry to generate novel, hybrid biomaterials. The methodology offers an advance over dip coating for durability and is sufficiently versatile to allow the grafting of acrylate-based monomers of varying functionalities. The materials have been tested in vitro with bacteria and shown to reduce bacterial attachment by a factor of ∼100.

MATERIALS

PDMS tubing and sheets were obtained from Appleton Woods Laboratory Equipment and Consumables and manufactured by Sterilin. (3-Mercaptopropyl)trimethoxysilane (MTS), EGdPEA, DEGMA, potassium hydroxide (KOH), 2,2-dimethoxy-2-phenyl-acetophenone (DMPA), and azobis(isobutyronitrile) (AIBN) were obtained from Sigma-Aldrich and used without further purification. Methanol, dichloromethane (DCM), and toluene were obtained from Fischer Scientific. Dithiothreitol (DTT) was obtained from Fluorochem Ltd.

METHODS

Preparation of Thiol-Functionalized Silicone. Silicone catheters and sheets were modified according to a procedure previously published in the literature.[35] Briefly, (3-mercaptopropyl)-trimethoxysilane (10% v/v) and KOH (1% w/v) were dissolved in methanol. PDMS catheters and sheets were sectioned into areas 2 × 0.5 cm and placed into the reaction solution. The vessel was sealed and sonicated at 50 °C for 6 h. The modified silicone was then washed by sonication twice with fresh methanol for 5 min. For the final washing step the silicone was stirred in DCM for 3 h and repeated three times. The PDMS was removed from the DCM and dried briefly under vacuum at 1.6 mbar before storing at 5 °C in methanol. Before use the methanol was removed and thiols were recovered to the surface by use of DCM.[35] Oxidized thiols were reduced for samples stored longer than 3 months from initial production using 100 mM DTT and phosphate buffer (pH 8) for 30 min at room temperature.

Time of Flight-Secondary Ion Mass Spectroscopy (ToF-SIMS). ToF-SIMS measurements were conducted using a ToF-SIMS IV (IONTOF GmbH) instrument operated using a 25 keV Bi_3^+ primary ion source exhibiting a pulsed target current of >0.3 pA. Samples were scanned at a pixel density of 512 pixels/mm, with 8 shots per pixel over a given area. An ion dose of 2.45×10^{11} ions/cm^2 was applied to each sample area, ensuring static conditions were maintained throughout. Negative secondary ion spectra were collected (mass resolution of >7000 at $m/z = 29$), over an acquisition period of 15 scans. Owing to the nonconductive nature of the samples, charge compensation was applied in the form of a low-energy (20 eV) electron flood gun.

X-ray Photoelectron Spectroscopy (XPS). Samples were analyzed using the Kratos AXIS ULTRA with a monochromated Al Kα X-ray source (1486.6 eV) operated at 10 mA emission current and 12 kV anode potential (120 W). A charge neutralizer filament was used to prevent surface charging. Hybrid-slot mode was used measuring a sample area of approximately 0.5 mm^2. Approximately 0.5 cm sections of the samples were mounted on a standard Kratos sample bar with double-sided tape with the external wall analyzed. The analysis chamber pressure was better than 5×10^{-9} mbar. Two areas per sample were analyzed. A wide scan at low resolution (1400−5 eV binding energy range, pass energy 80 eV, step 0.5 eV, sweep time 20 min) and a short high-sensitivity scan over the S 2p energy region (175−155 eV binding energy, pass energy 80 eV and step 0.5 eV, sweep time 10 min). These were used to estimate the total atomic percentage of the detected elements. High-resolution spectra at pass energy of 20 eV with steps of 0.1 eV and sweep times of 10 min each were also acquired for photoelectron peaks from the detected elements, O, C, and Si, and these were used to model the chemical composition. The high-resolution spectra were charge-corrected to the CH_3 peak in the silicone to 285.0 eV, CasaXPS (version 2.3.18dev1.0x).

Fourier Transform Infrared Spectroscopy (FTIR). Infrared spectra were acquired using the Agilent Cary 630 FTIR Spectrometer (Agilent Technologies) with a-Sampler ATR accessory (diamond crystal, single-bounce beam path, 45° incident angle, 32 scans, 1 cm^{-1} resolution. Absorbance was recorded between 650 and 4000 cm^{-1}.

Surface "Monomer Grafting from" Polymerizations. A polymerization mixture was prepared containing 1.88 mL (8.19 mmol) of EGdPEA, 0.53 mL (2.85 mmol) of DEGMA, and 24 mg (0.094 mmol) of DMPA. This mixture was homogenized by vortexing for 30 s. After this time, sections of PDMS were placed into a 24-well polypropylene tissue culture plate. The plate was placed into a glovebox and sealed. The chamber was degassed with argon until O_2 < 0.2%. After this period 5 μL of the mixture was added to both the thiol-modified and nonmodified PDMS. The plate was placed on a reflective surface and irradiated with ultraviolet (UV) light for 60 min. For thermal polymerizations 3 mg (0.73 mmol) of AIBN and 5 mL of toluene were added instead of DMPA. The vessel was sealed, degassed and heated to 70 °C. When the reactions were complete, both PDMS sample types were washed by mixing with toluene for 30 min (2 × 25 mL), DCM (2 × 25 mL), methanol (2 × 25 mL), and water (2 × 25 mL) before drying under vacuum for 60 min at 1.6 mbar.

Cobalt-Mediated Polymerization for the "Polymer Grafting to" Approach. A vessel was prepared containing 75.20 mL (328.58 mmol) of EGdPEA, 20.80 mL (112.72 mmol) of DEGMA, 120.50 mg of AIBN, 50 mg of bis[(difluoroboryl)diphenylglyoximato]cobalt(II) (CoPhBF), and 200 mL of toluene. The contents were degassed for 30 min before the temperature was raised to 80 °C. The reaction was

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

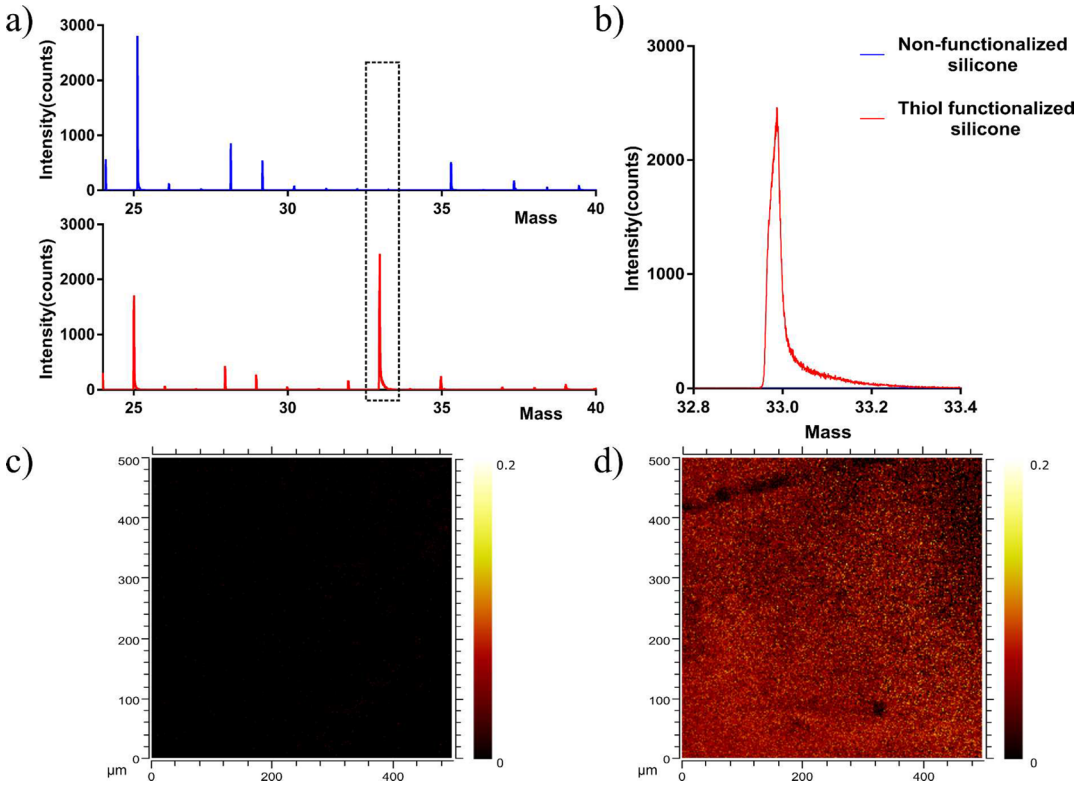


Figure 1. (a) ToF-SIMS spectra of the unmodified and thiol-modified medical-grade silicone with SH^- peak highlighted in the dashed box, (b) zoomed region in ToF-SIMS spectra for SH^-. Normalized ToF-SIMS image of (c) unmodified and (d) modified PDMS tubing showing presence and distribution of SH^- after washing samples with DCM and methanol.

followed for 26 h. The polymers were obtained by precipitation three times into hexane and dried under vacuum at 1.6 mbar before storing at −20 °C.

Grafting to of Polymer Produced by Cobalt-Mediated Polymerization to PDMS. Polymer was dissolved in toluene at concentrations of 10%, 20%, and 30% w/v and DMPA was then added at a concentration of 1% w/v. Five microliters of these solutions were pipetted onto the PDMS sheets in an inert nitrogen atmosphere before exposing to UV light for 60 min. After this time the sheets were washed for 30 min in DCM (2 × 20 mL), methanol (2 × 20 mL), and water (2 × 20 mL) before drying under vacuum for 60 min at 1.6 mbar before analysis.

Scanning Electron Microscopy (SEM). SEM was carried out on a Jeol 6060LV variable pressure scanning electron microscope (Jeol UK Ltd.). Before insertion into the chamber, samples were cut in half through the coated areas, mounted onto carbon discs, and then coated in gold for 120 s using a Leica EM SCD005 Sputter Coater. Images were taken at 40×, 250×, 370×, or 650×. Polymer thickness was calculated using SMile View using three measurements across the polymer coating.

Statistical Analysis. Statistical analysis of the silicone signal obtained from ToF-SIMS of PDMS and grafted polymer was carried out using an unpaired *t* test in Prism Version 7.01.

Biofilm Studies. The ability of the grafted PDMS to resist bacterial attachment and subsequent biofilm formation was tested according to a method previously reported in the literature.[17,18] Red fluorescence emitting *Pseudomonas aeruginosa* PA01-N mCherry and *Proteus mirabilis* DZM226637 dsRed (Nottingham collection) were streaked onto LB agar and grown overnight at 37 °C. After this the plates were stored at 5 °C for no longer than 1 month. A primary culture was produced by selecting a single homogeneous colony and adding this to LB medium (10 mL). This tube was placed in an incubator at 37 °C rotating at 200 rpm. The next day the bacteria were pelleted by centrifugation at 9500 rpm for 5 min and the supernatant was removed. The bacteria were resuspended in RPMI 1640 and pelleted again to remove the LB medium. Polymer-grafted PDMS was UV-sterilized in a 50 mL falcon tube for 20 min using a desktop UV sterilization unit. RPMI 1640 was added to the polymer-grafted PDMS followed by the bacterial suspension to obtain a final OD_{600} of 0.01. The mixture was placed in an incubator at 37 °C rotating at 60 rpm for 3 days. After this time, the catheters were washed with sterile phosphate buffered saline (2 × 10 mL) and sterile deionized water (1 × 10 mL) for 5 minutes at 100 rpm each before being dried and placed on glass slides for confocal microscopy.

Statistical Analysis. Statistical analysis of biofilm coverage was carried out using a two-way ANOVA in Prism Version 6.05. Images for analysis were imported into ImageJ 1.49k, converted into an 8-bit format. This image was threshold-adjusted and the fluorescence quantified as a percentage of the region of analysis. Means and standard deviations were calculated in Excel for each sample type and the mean of the total sample set was used to calculate the two-way ANOVA.

RESULTS AND DISCUSSION

Modification of PDMS. Recently, a base-catalyzed method to introduce thiols to the surface of PDMS objects has been published using PDMS elastomer discs.[35] We adapted this technique and applied it to commercial PDMS tubing and PDMS sheets. The polymer is commonly found in biomedical uses where it is used for breathing, feeding, and drainage tubes. The use of sheets provided a convenient flat surface for manipulation and analysis. In the base-catalyzed process, OH^- nucleophilically cleaved the siloxane bonds on both the silicone and the alkoxysilane bond on (3-mercaptopropyl)-trimethoxysilane, which was followed by metathesis to allow incorporation of the (3-mercaptopropyl)trimethoxysilane onto the surface. The addition of (3-mercaptopropyl)-trimethoxysilane at the surface was confirmed by ToF-SIMS.

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

The key thiol (SH^-) ion at m/z 32.9893 from the (3-mercaptopropyl)trimethoxysilane was identified on the PDMS surface (Figure 1).

The intensity of the ion SH^- was distributed over the 500 × 500 μm area analyzed (Figure 1), suggesting the thiol functionality was dispersed across the PDMS tubing with the exception of areas interpreted to be topography or a contaminant particle. This is important for further modifications where a homogeneous and continuous coating is desired to prevent bacterial attachment. After visual inspection no effects were observed upon the elasticity or transparency of the PDMS after modification.

XPS is a surface-sensitive technique probing the top ~10 nm of a material; therefore, it is sensitive to surface modification of the silicone. The thiol-modified PDMS material was analyzed by XPS to examine the inclusion of sulfur into MTS onto the polymer (Figure S1 of the Supporting Information). The data is summarized in Table 1.

Table 1. Quantification of the Survey/Wide Energy Range Spectra and S 2p High-Sensitivity Spectra Using Peak Areas, Kratos Transmission Function, and Kratos Relative Sensitivity Factor Library To Estimate Detected Elemental Atomic Percentage

sample	C 1s (%)	F 1s (%)	O 1s (%)	S 2p (%)	Si 2p (%)
PDMS	53.1	0.2	24.3	0.00	22.5
PDMS	53.9	0.6	22.4	0.00	23.1
MTS modified	57.0	0.0	21.4	0.03	21.5
MTS modified	57.4	0.1	21.5	0.05	20.9

XPS of the bare silicone surface found a composition very close to that expected for a simple silicone structure (namely, 25 at. % silicon, 25 at. % oxygen, and 50 at. % carbon). There was a slight enrichment in carbon which we attribute to surface contamination. A sulfur peak equivalent to 0.05 at. % was observed after MTS treatment, suggesting that the MTS was successfully grafted to the PDMS. These data supported the information from the ToF-SIMS that the grafting of MTS to the silicone surface was successful.

Monomer Grafting Polymerizations on PDMS (Grafting from). Thiol-ene polymerization reactions were utilized to further modify the silicone surface. The aim was to introduce nontoxic polymers that have been demonstrated to resist bacterial attachment.[17,18] Polymers containing EGdPEA and DEGMA were grown from the silicone surface, initiated by the surface thiols. This monomer combination was chosen for its broad resistance to bacterial attachment and its favorable mechanical properties.[19] Thiyl radical ($S^\bullet$) initiation was achieved through either thermal decomposition of AIBN or with UV light irradiation with DMPA (Scheme 1).[36] It was noted that for these reactions UV initiation allowed faster and more complete polymerization, whereas using thermal processes allowed for gradual addition of polymer to the PDMS.

After the grafting from polymerization, the PDMS was extensively washed using increasingly hydrophilic solvents of toluene, dichloromethane, methanol, and water followed by vacuum drying. This was done to remove noncovalently bound polymers formed in the bulk solution, reaction by-products, and also solvents that would be toxic to the bacteria. After washing, the covalently attached polymers were detected by infrared spectroscopy through the increasing intensity of carbonyl stretching at ~1716 cm^{-1} (Figure 2b).

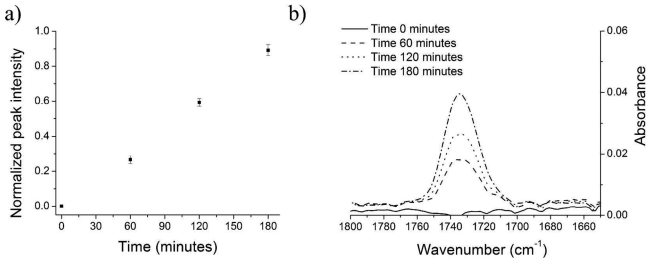
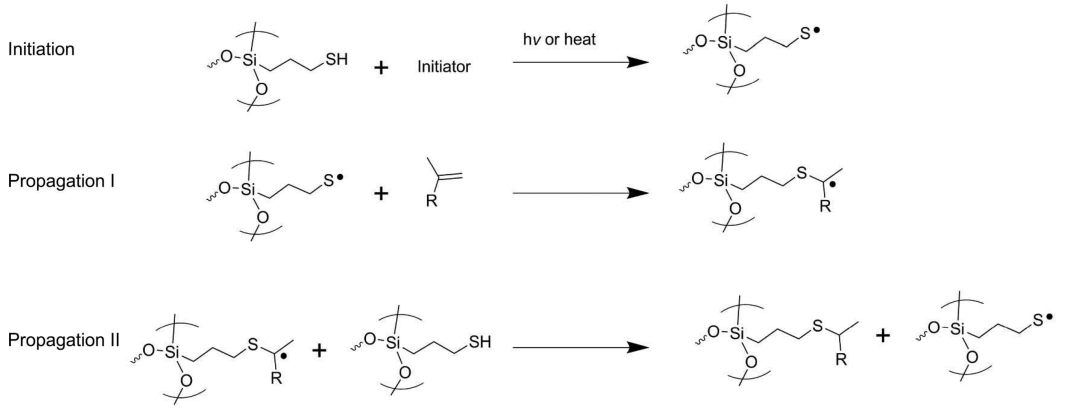


Figure 2. (a) Grafting from polymerization kinetics of EGdPEA and DEGMA on thiol-modified PDMS as determined using ATR FTIR at carbonyl peak integration 1760−1700 cm^{-1}; (b) FTIR peaks of PDMS over time between 1800 and 1650 cm^{-1}.

To determine whether the polymers were growing off or grafting to the silicone surface, a kinetic time course experiment was carried out. PDMS was polymerized with EGdPEA using heat-activated free-radical polymerization in four reaction tubes and each polymerization was stopped at 1 h internals. Over the time course the quantity of polymer attached to the surface, as determined by ATR FTIR, increased (Figure 2). However, over time the length of polymer chains formed in and sampled from the solution did not increase as measured by GPC (M_n 60−80 kDa). This suggests that the chain growth occurred more slowly at the surface relative to the bulk, perhaps due to steric

Scheme 1. Reaction Mechanism of Thiol-ene Surface-Initiated Polymerization



DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

limitations or that the number of polymer chains attached to the PDMS increased as a function of time.

The addition of polymer to the catheter proceeded at a linear rate ($R^2 = 0.999$) over the 180 min time course, indicating zero-order kinetics.

The carbonyl stretch peak integrations achieved for using thermal polymerization were lower than those obtained using UV polymerization. For example, after 60 min of thermal polymerization a carbonyl peak integration of 0.27 (AU) was observed, compared to 15.65 (AU) obtained using UV initiation.

By SEM analysis, no polymer layer could be imaged on the thermally grafted from surfaces; however, a carbonyl signal was clearly observed by FTIR, indicating a coating was present. Unfortunately, XPS analysis (Figures S1c and S2) was unable to calculate coating thickness because of siloxane oligomer migration through the coating as noted in the subsequent ToF-SIMS analysis. This means that there was no unique element to locate the position of the PDMS substrate.

These results differed from the UV-grafted from surfaces where the polymer layer thickness after washing was readily apparent and measured to be 34 ± 1.05 μm as determined by SEM (Figure 3).

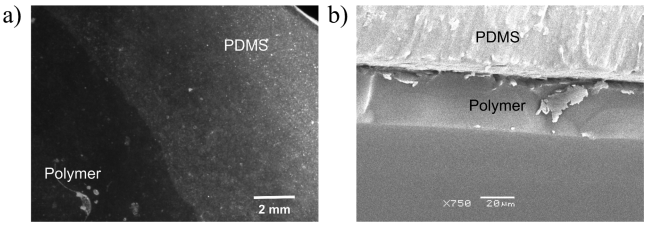


Figure 3. (a) Micrograph image of UV grafted from PDMS sheet with polymer=grafted area bottom left and ungrafted area top right. (b) SEM image of polymer PDMS boundary after cutting through grafted area.

The coating thickness exceeded that of a single monolayer as the polymerization process occurred with the monomers deposited on the PDMS surface and those monomers in proximity to the surface covalently attached to the surface and anchored the polymer to the PDMS.

Grafting Preformed Polymer to PDMS. Grafting polymers to PDMS allowed the materials that were to be grafted to be better characterized before grafting and surface termination reactions to be minimized as compared to the grafting from approaches. A copolymer was synthesized from EGdPEA and DEGMA, and subsequently grafted to the surface (Figure 4a).

The cobalt-mediated polymerization method was utilized for several reasons. The reduced- polymerization kinetics compared with standard free-radical polymerization minimized cross-linking through the pendant group of monomer EGdPEA. It also preserved the terminal vinyls at the ω-chain ends[37] for α-methyl-substituted monomers,[38] thus ensuring that the polymer double bonds could participate in further reactions.[39]

The polymerization rate followed linear, pseudo first-order kinetics as seen in Figure 4b. This indicated that the degree of control obtained during the polymerization was acceptable and that retention of terminal vinyl functionality was likely. This was crucial for the polymer to engage in thiol-ene polymer grafting to the modified silicone surfaces.

At the conclusion of the reaction the polymer had a molecular weight (M_n) of 13.7 kDa and a polydispersity (M_w/M_n) index (PDI) of 3.9. This value was high given the degree of control seen observed over the rate. The cause for this large PDI was likely to be as a result of cross-linking reaction occurring on the EGdPEA pendant group.

As shown for grafting monomers from PDMS the presence of vinyl groups was essential to participate in the thiol-ene grafting to process according to Scheme 1. Concentrations of 10%, 20%, and 30% w/v in toluene were tested with the polymer for thiol-modified and nonmodified PDMS. The inclusion of non-thiol-modified silicone was done to verify the thiol-ene process.

By FTIR no carbonyl peak was detected on the non-thiol-modified silicone, suggesting no polymer had been successfully grafted. However, polymers grafted onto the thiol-modified surface could be readily detected by FTIR, resulting in strong carbonyl stretching (Figure 4c). Little difference between each concentration applied to the thiol modified PDMS was detectable by infrared. Therefore, the thickness of the grafted polymers was determined using scanning electron microscopy (SEM). The SEM results showed that the thickness of the polymer coating grafted onto the PDMS increased from 56 to 141 μm with increasing polymer concentration (Figure 5c). This thickness was determined by cutting vertically through the sample and measuring the thickness of the polymer layer attached to the PDMS.

The increase in grafted to polymer thickness with polymer concentration suggested that the increased concentration of terminal vinyl groups had a positive impact upon the quantity of polymer that could be grafted. Additionally, the polymer may be able to participate in intermolecular attachment to increase chain length more frequently at higher concentrations.

A visible presence of polymer on the PDMS surface was apparent after the successive washing steps (Figure 5b,d). ToF-SIMS of these grafted polymers revealed an absence of signal for cobalt ions (Co^+ at m/z 58.9327) (Figure S4), indicating that any anti-attachment activity would be solely attributed to the action of the polymer rather than residual transition-metal ions from the catalyst used in the polymerization process. When the same washing process was carried out using non-thiol-modified PDMS, the polymer was washed off (Figure 5a). This observation was supported by the FTIR data (Figure 4c) that showed the complete absence of polymer. These data demonstrate the importance of the surface thiol for successful, covalent polymer grafting.

The sample was later analyzed by ToF-SIMS after 1 day and 6 months to investigate if siloxane oligomers were migrating over the surface. We found that the grafted polymer after 1 day had a coating of silicone as detected from the mass fragment $Si(CH_3)_3^+$ at m/z 73.0665 (Figure S3). This signal intensity was significantly less ($p < 0.0001$) on the polymer-grafted samples compared to that on bare silicone (normalized ion intensity 0.178 and 0.3367, respectively). Over the following 6 month period this signal changed only marginally. We interpret this as the presence of siloxane oligomers which have reached an equilibrium level at a very early time point. It is notable that these do not significantly inhibit the performance of the coatings.

Bacterial Attachment Resistance of Polymer-Modified PDMS. The ability of the polymer-grafted PDMS to resist bacterial attachment and colonization was explored using a 72 h biofilm assay in a manner analogous to that previously reported

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

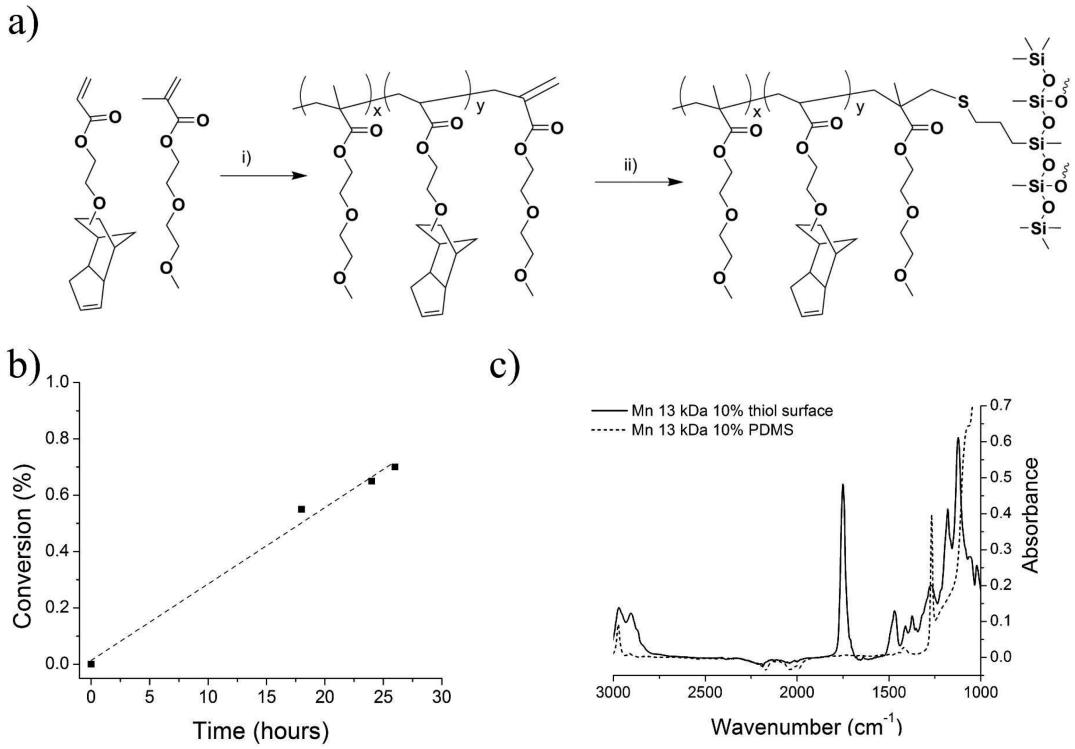
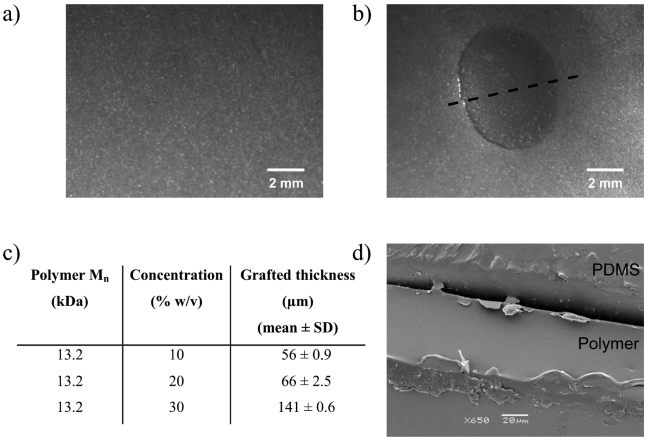


Figure 4. (a) Schematic overview for synthesis of polymer-grafted PDMS. (i) Cobalt-mediated polymerization of EGdPEA (329 mmol) and DEGMA (113 mmol) at 80 °C in toluene (200 mL) with AIBN (0.7 mmol) and cobalt (CoPhBF) (50 mg). (ii) Polymer grafting to PDMS by dissolving polymer (10%, 20%, and 30% w/v) and 2,2-dimethoxy-2-phenylacetophenone (1% w/v) in toluene (10 μL) and exposing to UV for 60 min under argon. x = 1, y = 3. (b) Polymer conversion over time as determined by ^{1}HNMR. (c) FTIR absorbance spectra of polymer grafted to samples onto PDMS sheets either functionalized or nonfunctionalized with thiols.



Polymer M_n (kDa)	Concentration (% w/v)	Grafted thickness (μm) (mean ± SD)
13.2	10	56 ± 0.9
13.2	20	66 ± 2.5
13.2	30	141 ± 0.6

Figure 5. (a) Image of grafting-to area on non-thiol-modified PDMS sheet. (b) Image of grafted area on thiol-modified PDMS sheet. (c) Grafted polymer thickness layer as determined by SEM as a function of applied polymer concentration. (d) Scanning electron microscope image at 650× magnification of polymer Mn 13.7 kDa (bottom) onto thiol-modified PDMS sheet (top) applied at a concentration of 10%.

in the literature.[17,18] The growth medium used was RPMI-1640 as this is a defined medium that is nutritionally deficient such that it induces a stress response in bacteria, inducing biofilm formation. *Pseudomonas aeruginosa* and *Proteus mirabilis* were genetically modified to express the fluorescent proteins mCherry and dsRed, respectively. In this way, a simple yet quantifiable measure of bacterial attachment to the silicone surface was possible without the need for staining.

The two bacterial species were chosen as they frequently colonize biomedical materials and are common clinical pathogens.[40] The PDMS was only partially coated such that the nongrafted surface could serve as an internal control.

To analyze the resistance to bacterial attachment and biofilm formation using preformed polymer, a sample with a molecular weight of 13 kDa at a grafting to concentration of 10% was used as this produced a coating efficiently for the quantity of material used and had a similar thickness to the coating obtained via the monomeric grafting from approach. The biofilm coverage on the PDMS was significantly reduced from 10 to 15% on the thiol-modified surface to less than 1% on both polymer surfaces for both *P. aeruginosa* and *Pr. mirabilis*, respectively (Figure 6).

This reduction of bacterial attachment is similar to that obtained when using a physisorbed coating applied non-covalently onto PDMS yet has the advantage of being covalently attached to the PDMS surface.[17] The technique that appeared to be the most effective was that of the grafting-to approach. However, upon carrying out a two-way ANOVA on the results using a Sidak's multiple comparison test, the differences between the approaches were not statistically significant.

The pretreatment to induce the surface thiols also had a beneficial effect upon bacterial attachment resistance. However, the final modification, whereby the functional polymers were covalently attached, reduced coverage to less than 1% using either approach. This beneficial effect upon bacterial attachment was present despite the detection of siloxane oligomer egressed over the grafted polymer surface which can commonly occur.[41] These versatile approaches were thus able to prevent attachment of two important pathogens known to colonize

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787

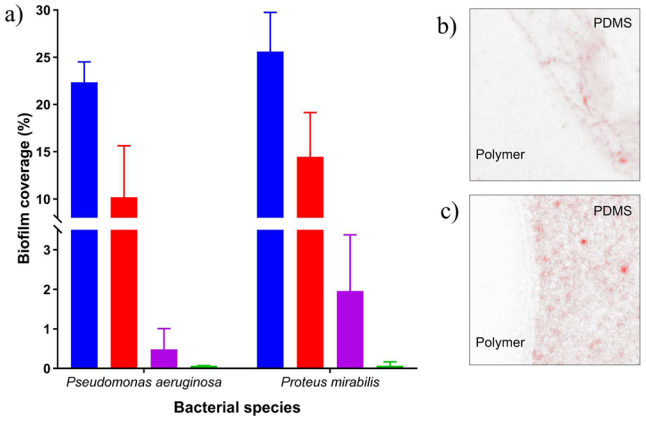


Figure 6. (a) Percentage of *P. aeruginosa* and *Pr. mirabilis* biofilm coverage on sheets of silicone (blue), thiol-modified silicone (red), grafting from approach using monomers EGdPEA and DEGMA (purple), and grafting to using preformed co-polymers of EGdPEA and DEGMA (green). (b) Boundary between monomer-grafted surface (left) and silicone (right). (c) Boundary between polymer-grafted surface (left) and silicone (right). Total size of each micrograph 625 μm^2.

PDMS surfaces. Additionally, the coatings were tolerant to a number of solvents including toluene, dichloromethane, methanol, and water, suggesting that this covalent coating was highly durable in challenging environments.

CONCLUSION

We have demonstrated a novel and facile method to modify medical-grade polydimethylsiloxane with commercially available (3-mercaptopropyl)trimethoxysilane to generate nontoxic polymer surfaces that are highly resistant to bacterial biofilm formation by both the grafting-from and grafting-to approach. With use of this method, bacterial coverage can be reduced by 99%. The reaction may be carried out using radicals generated through heat or more efficiently by UV irradiation. The final properties of the material can be tailored through judicious monomer selection and so may have a variety of applications or target organisms. Covalent attachment of polymers to the PDMS surface can enable the coating to be sufficiently durable to resist various solvents, hydrolytic degradation in service, and mechanical stresses for long-term device use.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b10986.

Further information on XPS and ToF-SIMS results of modified PDMS surfaces (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by the Wellcome Trust (grant numbers 103882, 103884); and the Engineering and Physical Sciences Research Council (grant number EP/K005139/1).

Notes

The authors declare no competing financial interest.

Data availability: All relevant data are available from the University of Nottingham Research Data Management Repository, under the DOI: http://dx.doi.org/10.17639/nott.66.

ACKNOWLEDGMENTS

Thanks to Christine Grainger-Boultby, B.A., FRMS, RSci, MIST, Nanoscale and Microscale Research Centre, University of Nottingham, for her expertise in SEM imaging of the samples. Dr. Emily F. Smith at the Nanoscale and Microscale Research Centre (NMRC), University of Nottingham, for acquiring the XPS spectra and data interpretation.

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on November 2, 2016. A data availability statement was added to the paper, and the corrected version was reposted on November 4, 2016.

DOI: 10.1021/acsami.6b10986
ACS Appl. Mater. Interfaces 2016, 8, 30780−30787