Surface Chemistry

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Facile and Efficient Control of Bioadhesion on Poly(dimethylsiloxane) by Using a Biomimetic Approach**

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Poly(dimethoxysiloxane) (PDMS) elastomers have found many applications in microfluidic and in biomedical devices, ranging from intraocular lenses to catheters,[1] but their hydrophobicity is prone to generate uncontrolled and deleterious bioadhesion. Surfaces of implanted biomedical devices promote protein and cell adhesion that can mediate a variety of adverse reactions, such as inflammation, thrombosis, occlusion, bacterial infection, or fibrosis.[2] To circumvent this problem, a variety of strategies have been developed to modify the surface properties of PDMS by physical modifications, such as exposure to oxygen plasma,[3] corona discharge,[4] ultraviolet light/ozone,[5] physical adsorption of charged surfactants,[6] and polyelectrolyte multilayers,[7] as well as entangling amphiphilic copolymers[8] or by covalent modifications, usually by polymers, requiring activation of the surface[9] or the doping of PDMS matrices by reactive molecules.[10,11] However, important limitations, such as the modifications of mechanical, optical, or gas permeability properties of silicones, the use of organic solvents or potentially toxic reactants, and, to some extent, the requirement of multistep strategies, have hampered the development of antiadhesive coatings on PDMS, especially in the field of biomedical devices. Simple, reproducible, and cost-effective methods to create long lasting antiadhesive PDMS are therefore still needed.

Herein we describe the design and the preparation of an antiadhesive nanofilm onto commercial PDMS by mimicking the glycocalyx, the external region of cell membrane exhibiting highly glycosilated molecules. Our general approach takes advantage of residual SiH groups in silicone elastomers, after the curing step, to graft, in water and in one single step, a methylated polysaccharide (methylcellulose) bearing vinyl groups (Figure 1a).

PDMS material is known to be chemically inert; thereby, it does not possess an appropriate chemical group to tether a passivating polymer, requiring an activation step, usually by plasma oxidation, to create reactive moieties. In fact, the only reactive groups on PDMS chains are the vinyl and the hydrosilane groups (SiH) that ensure the cross-linking of the silicone elastomers by a platinum-catalyzed hydrosilylation reaction. Although both chemical groups are initially present at the uncured state, we wondered about their presence at the end of the curing step that is supposed to consume them. To answer this question, SiH functions were titrated by infrared spectroscopy during the curing step at 120 °C (Figure 1b). Two widely used silicone elastomers were tested: SYLGARD 184 (Dow Corning) and the medical grade MED-4765 (NuSil) for long-term implantation. The SiH titration curves are quite similar for both and exhibit a dramatic decrease of SiH functions within the first minutes of the curing step to reach a plateau with 25 to 35% of the initial SiH that are still present at the end of the curing process. Residual SiH functions in silicone matrices are, moreover, extremely stable when exposed to air and do not suffer of prolonged exposition to water (Supporting Information). We hypothesized that these

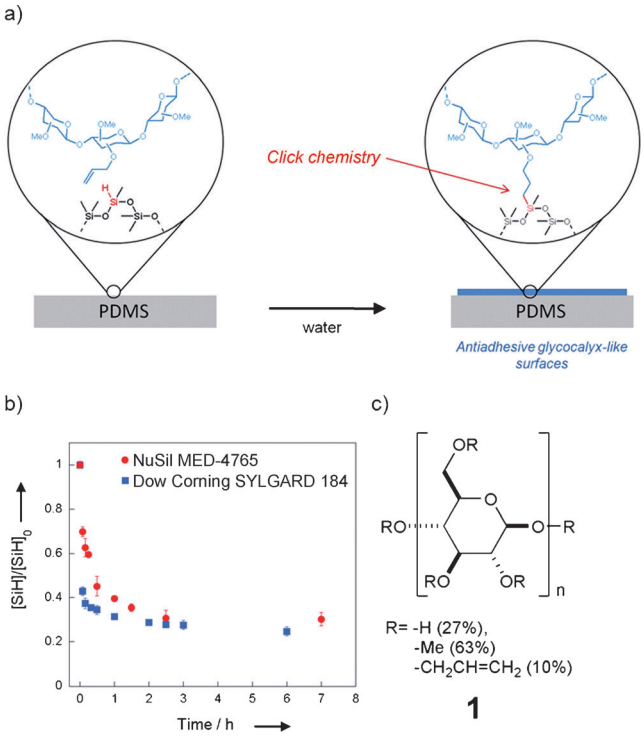

Figure 1. General principle of the biomimetic design of antiadhesive PDMS surfaces. a) The polysaccharide grafting step. b) Consumption of SiH groups during the curing step at 120 °C for silicone elastomers made with SYLGARD 184 or MED-4765. c) Vinyl-modified methylcellulose derivative **1** used to create antiadhesive glycocalyx-like PDMS surfaces.

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hydrosilane groups would therefore constitute an excellent chemical group to develop a click reaction based on hydrosilylation with vinylated polymers.

Several antiadhesive polymer backbones were considered for grafting on PDMS surfaces. Polyethyleneglycols (PEGs) are the most widely used passivating antiadhesive polymers. However, simple linear PEGs offer only two extremities for chemical grafting and furthermore their susceptibility to thermal and oxidative degradation limits strongly their use.[12,13] Mimicking the non-adhesive properties of a glycocalyx provides a potential solution to undesirable bioadhesion on implantable devices.[14] Methylcellulose (MeCe) was chosen for this purpose. Indeed MeCe possesses four molecular characteristics essential to inhibit protein adsorption: MeCe is hydrophilic, it contains hydrogen bond acceptors, it contains very few hydrogen bond donors, and its overall electrical charge is neutral.[15,16] Furthermore, MeCe resists to biodegradation in vivo and is thermally stable. It is noteworthy that MeCe has been used for several biomedical applications, such as a blood expender, as intraocular lenses, or as food additives, without any safety concerns.

A significant challenge in engineering a glycocalyx-like surface on biomaterials is to reach a high surface density of polysaccharides. To achieve this requirement, commercial MeCe (8000 cps grade, ca. 90 000 g mol^{-1}) was alkylated with allyl bromide to afford **1**, which bears several vinyl groups along its backbone (Figure 1c). The derivative **1** in water (1 mg mL^{-1}) reacted readily with both PDMS surfaces (MED-4765 and SYLGARD 184) at 65 °C during 2 h with, or more surprisingly, without the addition of a platinum catalyst, leading to a more wettable surface characterized by $\theta_{adv} \approx 90°$ and $\theta_{rec} \approx 35°$ contact angle as opposed to $\theta_{adv} \approx 110°$ and $\theta_{rec} \approx 75°$ for the untreated PDMS. The same results were obtained with silicones at different stage of curing (from uncured to fully cured).

These surfaces were characterized by ToF-SIMS (time-of-flight secondary-ion mass spectrometry) in positive mode (Figure 2). As expected, the characteristic peaks of siloxane polymer dominate the spectrum (Si^+, $SiC_3H_9^+$, $Si_2OC_5H_{15}^+$, $Si_3O_3C_5H_{15}^+$, $Si_3O_2C_7H_{21}^+$). Similarly, ToF-SIMS analysis reveals strong fragments from the MeCe backbone, such as $C_5H_9O^+$, $C_5H_7O_2^+$, and $C_5H_9O_2^+$, populating the spectrum in the mid-mass range (m/z: 80–150) with a whole series of $C_aH_bO_c^+$ detectable secondary ions. Analysis of the bare PDMS substrate references shows the absence of major positive ToF-SIMS peaks associated with the MeCe domains. Moreover, AFM analysis indicates that the coating is homogeneous and corresponds to the deposition of one monolayer with an average thickness of 16 nm.

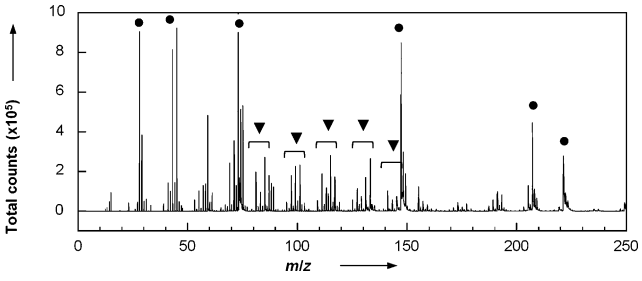


Figure 2. ToF-SIMS spectrum in positive mode of Sylgard 184 grafted with **1**, assigned with fragment ions of PDMS (●) and **1** (▼).

When the reaction was performed at 4 °C with **1** or the PDMS surfaces were incubated with the unmodified methylcellulose at 65 °C, no surface modification was observed. These results rule out nonspecific adsorption of polysaccharides and confirm the covalent grafting of **1** by hydrosilylation. We hypothesized that the Pt catalyst trapped in PDMS elastomers at the end of the curing step would catalyze the surface modification. This assumption was validated by the inhibition of the grafting when the reaction between the PDMS surface and **1** was poisoned by dithiotreitol (1 mg mL^{-1}).

Thus the intrinsic SiH groups contained in commercial silicone elastomers constitute a chemical function of choice to graft molecules by the hydrosilylation reaction. This one-step reaction, performed in water, fulfills the criteria of a click reaction.[17] These findings are contrary to the knowledge built over the decades about the chemical inertness of PDMS elastomers and highlight the importance of considering the residual chemical functions after the curing step for surface modification in material sciences.

To investigate the biotechnological and the biomedical applications of these glycocalyx-like surfaces, modified and unmodified surfaces were incubated with plasmatic proteins labeled with fluorescein isothiocyanate (FITC), such as bovine serum albumin and fibrinogen. Compared to control PDMS surfaces, PDMS surfaces grafted with **1** were able to reduce strongly the adsorption of both proteins below the detection limit (Figure 3a). In the case of fibrinogen, the addition of thrombin generates the formation of highly

a)

Sample	Protein adsorption[a] (ng/cm^2)	
	Fibrinogen	Albumin
NuSil MED-4765	400±120	645±114
NuSil MED-4765 grafted with **1**	<5[b]	<5[b]

[a] Adsorption of FITC labelled proteins [b] Detection limit

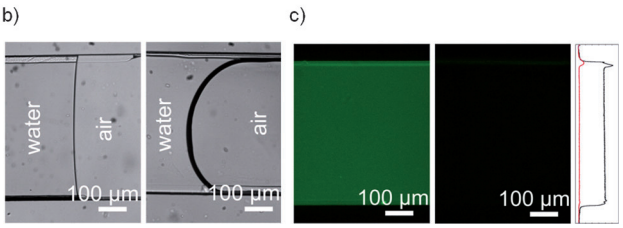
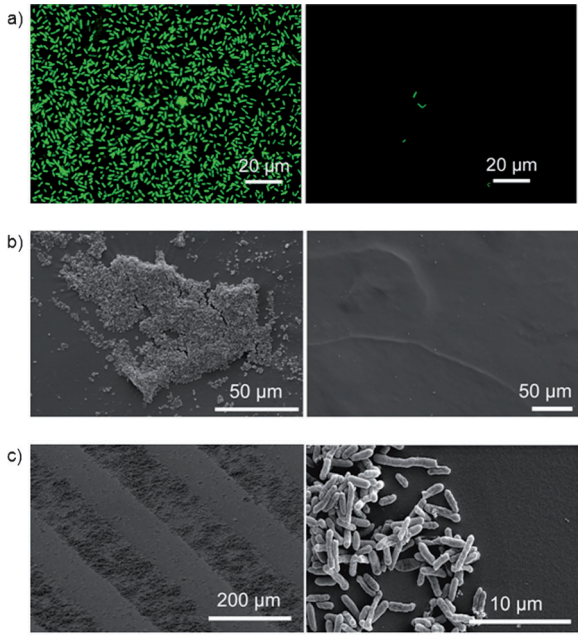


Figure 3. a) FITC-labeled plasmatic-protein adsorption on modified and unmodified medical-grade silicone elastomers. b) Wettability of PDMS microchannel (left) and PDMS microchannel modified with **1** (right); the microfluidic channel walls were coated by flowing **1** (1 mg mL^{-1} in water) through the channels for 2 h at a rate of 2 µL min^{-1}, 70 °C. c) Adhesion of FITC-labeled fibrinogen in uncoated (left) and coated (right) microchannels (500 µm wide). Fluorescence intensity profiles were recorded in untreated PDMS (black) and PDMS modified with **1** (red). Coated and uncoated channel were incubated with a FITC-labeled fibrinogen (1 mg mL^{-1}, 10 min), washed with a phosphate buffer saline (PBS; pH 7.2), and then filled with a sodium bicarbonate solution for imaging.

branched polymeric fibrin on the hydrophobic PDMS surface, leading to a surface-induced clot or fouling of biomedical devices (Supporting Information).[18] In contrast, polymerization of fibrinogen was impaired at the MeCe-grafted surface and no adsorption of fibrin was observed.

The use of PDMS in microfluidic devices is particularly prone to generate uncontrolled nonspecific bioadhesion on surfaces, as the surface-to-volume ratio is much greater than for macroscale systems. In this context, we have demonstrated that our glycocalyx-like surfaces were able to greatly enhance the wettability of microchannels and to lower considerably the adsorption of FITC-labeled fibrinogen (Figure 3b,c, respectively). Thus our biomimetic nanoconstructs are an ideal tool to control bioadhesion in microfluidic devices, as the PDMS surface modification, performed in water, is straightforward and does not require organic solvents, which are usually not compatible with PDMS.[19]



d)

Bacterial strain	Adhered bacteria (cell/cm^2)	
	MED-4765	MED-4765 grafted with **1**
E. coli MG1655	1.5×10^7	7.2×10^4
S. epidermidis ATCC 35984	5.1×10^7	4.6×10^5
P. aeruginosa ATCC 15442	2.6×10^6	2.8×10^4
S. aureus ATCC 6538	4.0×10^7	1.8×10^5

Figure 4. a) Fluorescence images of adhered *E. coli* MG 1655 stained with SYTO 9, on untreated MED-4765 PDMS (left) and on MED-4765 PDMS-grafted **1** (right) after an overnight incubation in LB medium and a washing step with PBS. b) SEM images biofilm forming *S. epidermidis* ATCC 35984, on control MED-4765 PDMS (left) and on MED-4765 PDMS grafted with **1** (right) after an overnight incubation in LB medium and a washing step with PBS. c) SEM images of biofilm (*E. coli* MG1655 F+) grown on patterned surfaces (100 µm width stripes of PDMS grafted with **1** alternating with bare PDMS stripes). Biofilm formation is only observed on bare PDMS stripes. d) Cell density of adhered bacterial matter on PDMS grafted with **1** and on uncoated PDMS.

Furthermore, we hypothesized that the MeCe-grafted PDMS surfaces would prevent bacterial adhesion. Indeed, the resulting surfaces were also found to reduce dramatically the adhesion of different bacterial strains: (Figure 4a,c). The ability to prevent bacterial adhesion is correlated with the molecular weight of the MeCe derivatives grafted on PDMS, and optimal antiadhesive properties were obtained with molecular weights higher than 50000 g mol^{-1} (ca. 600 cps grade MeCe), ensuring a good steric repulsion.[20] In the case of bacterial strains forming biofilms, these surfaces were particularly efficient in suppressing bacterial biofilm (Figure 4b,d). This remarkable characteristic was also observed in microfluidic devices under flow conditions (Figure 5a). Indeed, the injection of an initial bacterial innoculum followed by a continuous flow of Luria Bertani (LB) medium leads to significant biofilm formation on control PDMS surfaces, whereas no biofilm was detected for glycocalyx-like nanofilm on PDMS surfaces (Figure 4a).

Similarly, the evaluation of antiadhesive properties of the generated surfaces was extended to mammalian cells. Figure 5b shows a patterned culture of Madin Darby Canine Kidney (MDCK) cells on a PDMS surface exhibiting adhesive patterns (bare PDMS, 600 µm diameter disks) surrounded by MeCe. All together, the glycocalyx-like PDMS surfaces demonstrate effective suppression of non-specific protein adsorption as well as cell adhesion, highlighting the usefulness of this approach on implantable devices to control bioadhesion.

The MeCe-grafted surfaces are extremely stable and can be stored in air for a long period of time (at least 2 years) without any loss of the antiadhesive properties. This observation suggests that **1** cross-links the PDMS chains at the surface, suppressing its hydrophobic recovery.

In summary, antiadhesive biomimetic glycocalyx-like nanofilms on PDMS have been developed through the

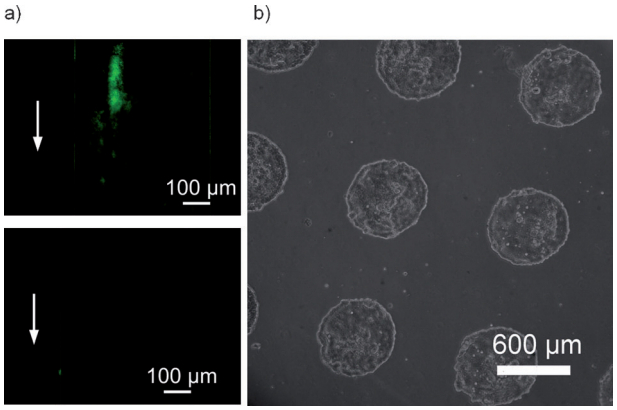


Figure 5. a) Fluorescence microscopy images of biofilms grown in microchannels (500 µm width) made in PDMS (SYLGARD 184). Unmodified microchannel (top) and microchannel grafted with **1** (lower) were filled with a bacterial suspension (*E. coli* MG1655 F+, 10^4 bacteria mL^{-1}), and after 1 hour were flowed overnight with LB medium (50 µL min^{-1}). Then they were washed with a PBS solution, revealing biofilm formations in the untreated microchannel. The arrows indicate the flow direction. b) MDCK cell adhesion grown onto circular patterns (600 µm diameter) of bare PDMS surrounded by area of PDMS grafted with **1**.

combination of the hydrosilylation click reaction and a methylated polysaccharide derivative (methylcellulose). The robust and efficient nature of the chemistry, taking advantage of free SiH groups remaining in commercial silicone elastomers, allows the generation of these bioinspired nanoassemblies at the PDMS surface in one step and in water. This integrated strategy was found to produce surfaces highly resistant to bioadhesion and demonstrates an advance in the state-of-the-art. We thus anticipate this simple strategy will be developed not only in microtechnology (microfluidic, micropatterning) but also in the biomedical field to produce medical devices exhibiting extremely low interactions with surroundings tissues.

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