Silanization of Silicone Elastomer with Zwitterionic Surface Modifier for Robust Biocompatibility

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Abstract—**Biofouling generally causes adverse effects to health, such as thrombosis, infection, and pathogenic calcification. Hydrophilic and charge-balanced surface is proved to provide an energy barrier serving as a strong repulsive force against the nonspecific absorption. Unfortunately, the state-of-art technology for silicone modification cannot provide a stable and effective coating for the long-term applications under complex conditions. In this study, we aim to modify the silicone surfaces with a zwitterionic surface ligand to resist nonspecific adsorption of protein, lipid and bacteria. We synthesized a silanized surface ligand conjugated with a head residue of zwitterionic sulfobetaine (SBSi) that bears positively charged quaternary amine and negatively charged sulfonate groups. The bacterial adhesion tests and protein fouling test revealed the excellent antifouling properties of modified silicones. For the real-world application, we modified commercially available silicone hydrogel contact lenses with developed zwitterionic ligands and showed their capability of anti-bacterial adhesion and anti protein fouling. In summary, the strategy of surface engineering in this work can be applied to not only contact lenses but other silicone-based medical devices in facile and effective fashion.**

Keywords— **Biocompatibility, anti-fouling, zwitterionic materials, contact lenses.**

I. Introduction

Silicone is widely used biomaterial in medical devices and catheters due to its non-sensitizing and non-irritating properties. As the silicone material exhibits hydrophobic characteristic, the non-specific adsorption of proteins, cells or bacteria occurs frequently and generally induces serious pathogenic problems to constrain their exploitation [1, 2]. A hydrophilic surface enables to strongly interact with water molecules to form a tightly bound water layer, which provides an energy barrier against the protein absorption. Based on that, the facile and widely used method to reduce protein fouling of silicones is to make the surface hydrophilic by oxidizing the surface using oxygen plasma or UV-ozone [3], or an oxidative wet chemical method [4]. These strategies can generate hydroxyl groups directly onto the silicone surface to increase surface hydrophilicity, and hence reduce nonspecific protein fouling events. However, silicone chains reconstruct and reorganize almost surface coatings [5, 6]. Therefore, these modifications often last only a few hours or sometimes a matter of minutes. This study aims to modify the surfaces of silicone materials with zwitterionic silane build block to increase surface wetting and effectively reduce bio-fouling under a physiological condition. We synthesized 3-(Dimethyl(3-(trimethoxysilyl)-propyl)ammonio)propane-1-sulfonate (sulfobetaine silane, SBSi) which bears positive charged quaternary amine and negative charged sulfonate groups [7]. We applied SBSi onto the silicone surfaces to develop a super hydrophilic and charge-balanced antifouling biointerface (Fig. 1). The fouling resistance of the modified PDMS elastomer was verified by exposing to bacterial, protein, and lipid solutions. The results revealed its excellent capability to repel the foulant adsorption, even after 30-day storage in ambient. The cytotoxicity test for SBSi was carried out by MTT assay. In addition, the modification strategy was applied to silicone hydrogel contact lenses to verify the applicability to the commercial products.

II. Results and Discussion

A. Surface Characterization and Long-Term Stability

The elemental composition of the SBSi-tailored PDMS was determined using XPS. XPS signatures originating from Si, C, O, N, and S atoms within the sample were measurable. The [N]/[S] atomic ratios derived from their XPS spectral area ratio were 0.94, which means approximately

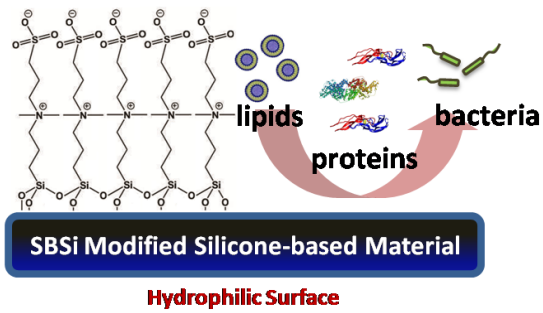

Fig. 1 Surface modification of PDMS with SBSi for antifouling properties.

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Table 1 Atomic percentage at listed binding energy (eV) of SBSi modified PDMS surface as determined by XPS

Atom	Si	C	O	N	S
Atomic percentage (%)	13.87	3.17	52.48	2.97	3.17

equal quantity for both elements (Table 1). As a result, the N and S spectra clearly indicate the presence of SB zwitterions on the PDMS sample. The surface energy was tested by static contact angle measurements. As shown in Fig. 2, the contact angles of the O_2-plasma treated PDMS significantly increased with time. This hydrophobic recovery was observed in previous research, attributed to the thermodynamically favorable rotation of the oxidized groups and the diffusion of low-molecular-weight chains from the bulk.[8-10] On the contrary, the SBSi-modified PDMS retained the hydrophilic property for more than 400 h. The slight increase in the contact angle should be the result of the disappearance of the unreacted oxidized groups on PDMS.

B. Resistance to Bacterial Adsorption

The fouling properties of SBSi-modified PDMS were verified by challenging with common biomaterial-associated pathogens, *P. aeruginosa* and *S. epidermidis,* and observed the adherent bacteria under a fluorescent microscope (Fig. 3). In order to compare the effectiveness of the SBSi adlayer, PDMS was partially modified with SBSi and incubated in bacterial solutions under a physiological condition. As shown in Fig. 3a and b, the SBSi-modified areas obviously have lower bacterial densities than bare ones. Moreover, Gram-positive *S. epidermidis* exhibited weaker adsorption capability than Gram-negative *P. aeruginosa.* We further quantified the adhered bacteria by using software ImageJ and determined the long-term fouling resistance of modified substrates. Fig. 3c indicates that O_2-plasma treated PDMS enables reducing adsorption of *S. epidermidis*, but exhibits a limited capability to *P. aeruginosa.* However, after 30-day

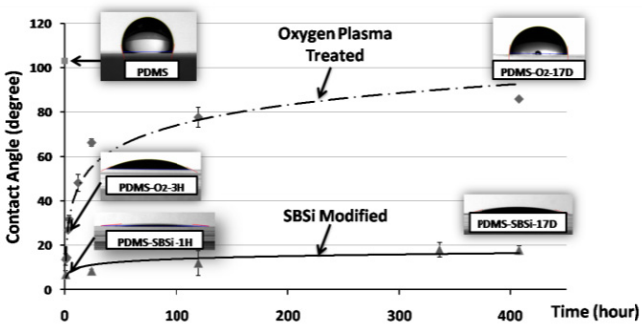

Fig. 2. Contact angle changes with time for SBSi-modified and O2-plasma treated PDMS samples.

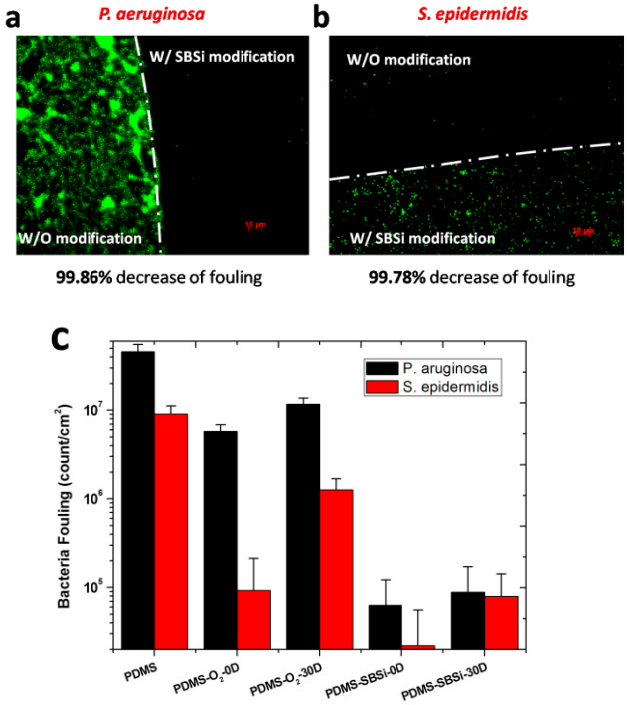

Fig. 3 P. aeruginosa (a) and S. epidermidis (b) adsorption on partially modified PDMS. The quantitative results of bacterial adsorption on PDMS samples with various treatments and aging time (c).

storage, the oxidized PDMS had reduced fouling resistance to both bacteria, which should be due to the hydrophobic recovery. [8-10] For the SBSi-modified PDMS, the reduction rate for *P. aeruginosa* and *S. epidermidis* were 99.86 % and 99.78 %, respectively, by using freshly prepared samples (PDMS-SBSi-0D). For the aged sample (PDMS-SBSi-30D), its capability to resist *P. aeruginosa* adsorption was comparable with the freshly prepared one, while slightly loss of resistance to *S. epidermidis.* Nevertheless, the overall fouling reduction rates of SBSi-modified PDMS reached over 90 % relative to bare samples.

The feasibility of SBSi for modification of commercial products was demonstrated with silicone hydrogel contact lenses (Fig. 4). Contact lenses become daily consumable of silicone-based medical device, on which the accumulation of bacteria and proteins from tear can cause protein denaturation, bacterial infection and conjunctivitis.[11] In this work, the surfaces of the contact lenses were tailored with SBSi as the procedure for PDMS modification described above. The contact lenses were brought to contact with the *P. aeruginosa* solution. As one can see, the number of adherent bacteria on SBSi-modified contact lens is much less than on unmodified one. This result reveals the potential of SBSi for industrial implementation.

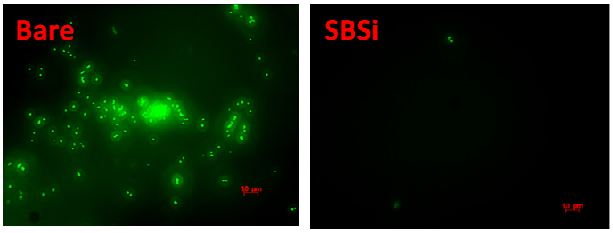

Fig. 4 SBSi modification for silicone hydrogel contact lenses.

C. *Resistance to Protein and Lipid Fouling*

BSA, mucin, lysozyme and lipids were used for investigating the antifouling properties of SBSi-PDMS by ELISA and fluorescence scanner. In the Fig. 5, the fouling levels on bare, oxidized and SBSi-modified PDMS samples were present. BSA is a standard protein that has been widely investigated in the field of biomaterials and has also been reported to adsorb on hydrophobic surfaces [12]. Many of reports have used BSA as a target to examine nonfouling property [13, 14]. Moreover, albumin is the most abundant protein in blood circulation, and easily to fouling on any medical devices which once contacted to blood to cause further biological interaction. Reduction of the albumin fouling level highlighted the potential of SBSi modified PDMS application in short/long-term blood contacting implants. The reduction rates of SBSi-modified PDMS were 98 % for BSA relative to unmodified sample (Fig. 5a). Fig. 5c and d show PDMS-SBSi resist 97 % and 49 % of mucin and lysozyme fouling level, respectively, relative to unmodified sample. Clearly, resistance of SBSi to biomolecules was shown, reflecting the function of superhydrophilic and

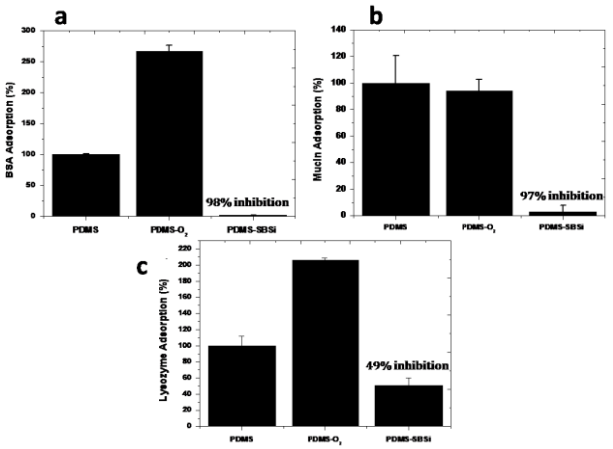

Fig. 5 ELISA measurements for (a) BSA, (b) mucin and (c) lysozyme on PDMS samples.

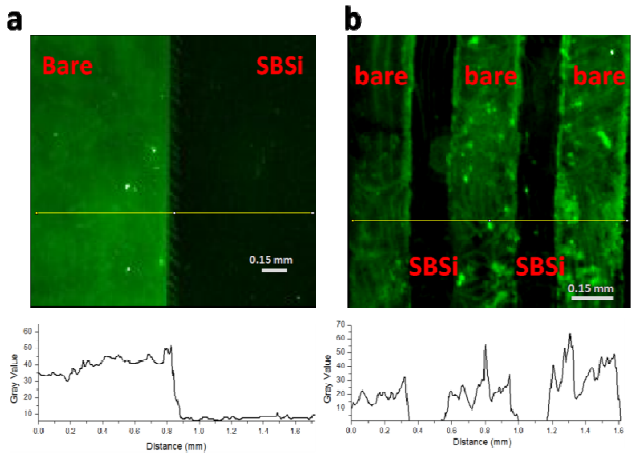

Fig. 6 Adsorption of SRB-encapsulated liposomes on SBSi-patterned PDMS.

charge-balanced properties of SB moieties. Interestingly, the fouling levels on O_2-plasma treated PDMS were higher than unmodified ones. This observation can be explained by increased surface roughness after irradiation, offering larger areas for adsorption [15]. The hydrophobic silicone elastomer is lipophilic, facilitating absorption of lipids and other nonpolar agents. Early study indicated that the silicone-containing heart valves failed in vivo due to significant dimensional swelling by absorption of lipids from blood.[16] Therefore, the adsorption of lipid on substrates was investigated by observing the fluorescence signals from SRB-encapsulated liposome (Fig. 6). The patterned surfaces were prepared by elastomeric stencils and microchannels as removable masks for the selective modification with SBSi as the methods present in previous publication.[17] The unprotected areas were oxidized by O_2-plasma irradiation, followed by contacting with the SBSi solution. After adsorption of SRB-encapsulated liposomes, the fluorescence images and the intensity profiles were recorded using fluorescence microscope. The clear edges in profiles between bare and SBSi-modified surfaces indicate that the liposomes namely adsorbed on the unmodified areas for the samples patterned by both methods.

D. *Cytotoxicity of SBSi*

The concern of the cytotoxicity of SBSi may be arisen before introduction it to the in vivo test. SBSi and its precursors, 1,3-propanesultone and DMASi, were dissolved in the culture medium at concentrations ranging from 0.1 to 20 mM. After 16-h incubation, the viability of cells was determined by MTT assay. As shown in Fig. 7, the cytotoxicity of 1,3-propanesultone is very high, which is classified as a toxic and carcinogenic agent. On the contrary, the SBSi and

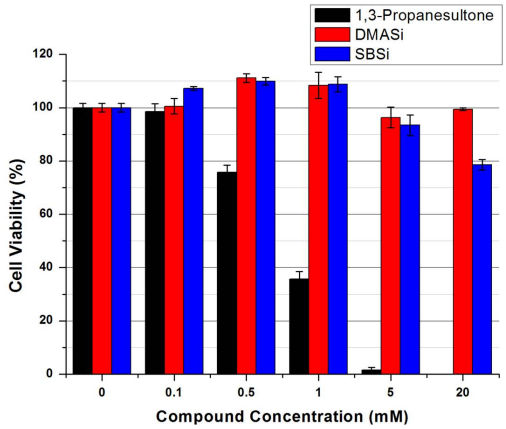

Fig. 7 MTT assay for the viability of cells after the incubation with compounds of SBSi, DMASi, 1,3-propanesultone.

DMASi have almost no cytotoxicity at a concentration below 5 mM. At a concentration of 25 mM, SBSi exhibits low cytotoxicity. However, as a thin layer coating, the concentration of SBSi in solution should be extremely low even if the leaching occurred. Consequently, the SBSi can be regarded as a very low cytotoxic and biocompatible material for potential uses in vivo.

III. Conclusion

In this study, a surface coating strategy for modification of silicones based on zwitterionic sulfobetain silane (SBSi) for robust biocompatibility was proposed. The coating was characterized using x-ray photoelectron spectroscopy and contact angle goniometer. The long-term stability of SBSi-modified PDMS was demonstrated by high surface wetting and excellent capability to resist bacterial adsorption, which should be results of suppressing the rotation of PDMS backbones and transport of low-molar-mass molecules by network crosslinking and superhydrophilicity of SBSi adlayers. Biomolecules, including bovine serum albumin, mucin, lysozyme and lipid, were effective repelled from the silicone by the SBSi coatings, rendering improved biocompatibility of silicone materials. The experiment for the cytotoxicity indicated that SBSi has very low cytotoxicity. Moreover, the applicability of SBSi for modification of commercial products was demonstrated by applying it on silicone hydrogel contact lenses. The improvement in the biocompatibility and stability of silicone-based medical devices with SBSi modification enables a wide range of applications, particularly implants for in vivo uses.

Acknowledgment

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