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Facile surface modification of silicone rubber with zwitterionic polymers for improving blood compatibility

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ABSTRACT

A facile approach to modify silicone rubber (SR) membrane for improving the blood compatibility was investigated. The hydrophobic SR surface was firstly activated by air plasma, after which an initiator was immobilized on the activated surface for atom transfer radical polymerization (ATRP). Three zwitterionic polymers were then grafted from SR membrane via surface-initiated atom transfer radical polymerization (SI-ATRP). The surface composition, wettability, and morphology of the membranes before and after modification were characterized by X-ray photoelectron spectroscopy (XPS), static water contact angle (WCA) measurement, and atomic force microscopy (AFM). Results showed that zwitterionic polymers were successfully grafted from SR surfaces, which remarkably improved the wettability of the SR surface. The blood compatibility of the membranes was evaluated by protein adsorption and platelet adhesion tests in vitro. As observed, all the zwitterionic polymer modified surfaces have improved resistance to nonspecific protein adsorption and have excellent resistance to platelet adhesion, showing significantly improved blood compatibility. This work should inspire many creative uses of SR based materials for biomedical applications such as vessel, catheter, and microfluidics.

1. Introduction

Due to good mechanical properties, softness, gas permeability, and low cost, silicone rubber is particularly attractive for biomedical applications [1–7]. However, the hydrophobic surface, possessing strong interaction with proteins, could lead to negative host response and biological reactions [8–10], which restricts its applications practically in blood related fields [11].

In order to improve the surface properties and control the subsequent biological interaction, much attention has been paid to the surface modification of SR with functional organic materials [12–14]. Poly (ethylene glycol) (PEG) is one of the materials that is most frequently used in this regard [15]. It is prevalently considered that PEG chains could bind water through hydrogen bond, leading to a "barrier" in addition to the steric repulsion around the PEG chains [15–17], therefore, the modification of SR with PEG chains could not only improve the wettability, but also enhance the ability to resist the adsorption of proteins and the adhesion of cells [18–21]. However, PEG is widely known to be prone to oxidation in an in vivo environment [22].

Zwitterionic materials have attracted much attention and have been shown to be among the most effective polymers in improving biocompatibility and anti-biofouling [23–29]. It is believed that zwitterionic surfaces could form a hydration layer via electrostatic interaction in addition to hydrogen bond, therefore, it could bind a significant amount of water [30], leading to a strong repulsive force to protein at specific separation distances or making the protein contact with the surface in a reverse manner without a significant conformation change [31]. More important, zwitterionic surfaces have less interaction with biomacromolecules and thus could maintain the "normal conformation" of biomacromolecules [32,33].

During the past two decades, numerous attempts have been developed to construct these bio-functional materials on the SR. Bae et al. prepared a blood compatible PDMS-polyurethane block polymer, on which PEG grafts were bonded via the reaction between reactive hydrogen and isocyanate groups [20]. Ishihara et al. tethered an ABA-type block polymer containing phospholipids on PDMS through a swelling–deswelling method for biocompatible improvement [34]. Our group fabricated two zwitterionic polymers (phosphobetaine and carboxybetaine) onto SR surface through an ozone-induced random radical polymerization grafting for improving the blood compatibility [35,36]. In the recent years, SI-ATRP is a method developed for constructing functional polymers with uniform chains, tailorable length, and relative high grafting density on substrates [37–39]. Taking advantage of ATRP, zwitterions have been directly grafted from metallic and inorganic substrates [40–42], and synthesized as

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block coating materials for biomedical application [43]. However, there is very little literature reporting on direct grafting of polymers from SR membranes through ATRP.

Unlike hydrophilic substrates such as cellulose, the surface of SR is too inert to covalently bonding ATRP initiators, a process of surface activation is required. Ozone [36,44], UV [45–47], chemical oxidation [48,49], and plasma [50,51] are the methods that frequently used for the surface activation of polymers. Among these methods, plasma has been shown to be an effective way with minimum damage to the substrates. As reported, the $-OSi(CH_3)_2O-$ groups can be converted to $-O_nSi(OH)_{4-n}-$ groups under oxygen or air plasma treatment [52–54], offering active silanol group for further surface reactions.

In the present study, based on the plasma treatment and SI-ATRP, an approach to fabricate zwitterionic polymers onto SR surface for improving the blood compatibility was investigated. The surface composition, wettability, and surface morphology of the SR substrates were systematically characterized by XPS, WCA, and AFM, respectively. The blood compatibility of the SR substrates that modified and unmodified was evaluated by nonspecific protein adsorption test and platelet adhesion test in vitro.

2. Materials and methods

2.1. Materials

The silicone rubber membranes were purchased from Shanghai Rubber Institute, and were cut into circle pieces (d = 11 mm) and Soxhlet extraction with methanol for 24 h before use. 2-Bromoisobutyryl bromide (BIBB, 97%), and 2-dimethylaminopryridine (DMAP, 97%) were purchased from Alfa Aesar. Copper (I) bromide (CuBr, 98.5%) was purchased from Sinopharm Chemical Reagent Co., Ltd and purified before use. 2, 2′-bipyridine (BPY, 99.5%), triethylamine (TEA, 99%), chloroform ($CHCl_3$, AR) and tetrahydrofuran (THF, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. [2-(methacryloyloxyethyl) ethyl]-dimethyl-(3-sulfopropyl)-ammonium (DMMSA, 97%) was purchased from Sigma-Aldrich. 2-methacryloyloxyethyl phosphorylcholine (MPC, 98%) was purchased from Joy-Nature Technology Institute, Nanjing, China. Sodium dodecyl sulfate (SDS, 99.9%) was purchased from Nanjing Sunshine Biotechnology Co., Ltd. (3-aminopropyl) triethoxysilane (APTES, 98%) was purchased from Zhangjiagang Guotai-Huarong New Chemical Materials Co., Ltd. Fresh whole blood and fresh platelet rich plasma (PRP) were provided by Blood Center of Jiangsu Red Cross. Phosphate buffered saline (PBS, 0.02 M phosphate, 0.15 M sodium chloride, pH 7.4) was purchased from Boster Biotechnology Co., Ltd. A Micro-BCA protein assay reagent kit (#23235) was purchased from Pierce Chemical (Rockford, IL, USA).

2.2. Preparation of DMVSA monomer

In a round-bottom flask, *N*-(4-vinylbenzyl)-*N*, *N*-dimethyl amine (40 mmol) was dissolved in 40 mL dry chloroform under stirring. Then, 1, 3-propanesulfone solution (44 mmol in 20 mL dry chloroform) was added drop-wise into the flask in 1 h. The reaction was conducted at 30 °C for 12 h. After filtration and recrystallized in ethanol, a white powder product was obtained (Yield 60%). ^{1}H NMR (300 MHz, D_2O): δ 7.50 (q, 4H, aromatic), 6.75 (q, 1H, ═CH), 5.85 (d, 1H, cis, ═CH_2), 5.30 (d, 1H, trans, ═CH_2), 4.42 (s, 2H, −CH_2), 3.36 (m, 2H, CH_2−N), 2.97 (s, 6H, −CH_3), 2.88 (t, 2H, −CH_2), 2.23 (m, 2H, −CH_2−).

2.3. Preparation of 2-bromo-2-methyl-N-(3-(triethoxysily) propyl) propanamide (BTPAm)

To prepare the surface-attachable initiator BTPAm, APTES (8 mmol), TEA (10 mmol) and toluene (20 mL) were charged to a dry round-bottom flask and stirred at 0 °C. 2-Bromoisobutyryl bromide solution (8 mmol in 10 mL of dry toluene) was added drop-wise into the flask in 1 h. The reaction was performed for 3 h at 0 °C and additional 21 h at room temperature. After filtration and evaporated at reduced pressure, a colorless oil-like liquid was obtained (85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.58 (t, 2H, $SiCH_2$), 1.17 (t, 9H, $\underline{C}H_3CH_2OSi$), 1.58 (m, 2H, CH_2), 1.82 (q, 6H, CH_3C), 3.15 (t, 2H, $\underline{C}H_2NH$), 3.7 (t, 6H, $CH_3\underline{C}H_2OSi$), 6.85 (s, 1H, NH).

2.4. Immobilization of BTPAm on SR membrane

A two-step procedure was performed in the immobilization of initiator onto SR membrane (Scheme 1).

The initiator of BTPAm (0.4 mL) was firstly hydrolysed in a mixed solution of methanol (2 mL) and DI water (17.6 mL). The mixture was stirred at 40 °C until the cloudy solution turned transparent, indicating the hydrolysis was completed. During the hydrolysis process, the SR membranes (Soxhlet extraction with methanol for 24 h) were treated with an air-plasma (Harrick air-plasma cleaner, U.S.A.) for 2 min, and then kept in air for 5 min before immersed in initiator solution. After 10 min incubation in the initiator solution, the substrates were rinsed with copious methanol, cured at 75 °C for 10 min in a vacuum oven, and sonicated with methanol (3 × 10 min). The air plasma activated SR membranes and initiator immobilized SR membranes here are referring to SR-O and SR-Br, respectively.

2.5. SI-ATRP from SR membranes

For the SI-ATRP from SR membranes, six SR-Br sheets and CuBr (71 mg, 0.5 m mol) were placed in a dry flask, after which the flask was pumped and back-filled with nitrogen for five times. Then 20 mL of degassed solution (methanol and DI water in 1:1 volume ratio) containing zwitterionic monomer (4.0 m mol) and BPY (156 mg, 1.0 m mol) was transferred into the flask under nitrogen protection and polymerized for 1 h at room temperature. After the polymerization, the substrates were thoroughly sonicated in DI water-PBS-DI water (each for 10 min), and finally dried under vacuum. The obtained membranes here are referring to SR-X, where X is the abbreviation of corresponding zwitterionic monomer.

2.6. Surface and interfacial characterization

The X-ray photoelectron spectroscopy (XPS) measurements were obtained on an ESCALAB 250 (Thermo Scientific, USA), using an Al Kα radiation source ($h\nu$ = 1486.6 eV) and operating at 150 W. The take-off angle of the photoelectron and the X-ray beam spot were kept at 90° and 500 μm, respectively. All the binding energies were referenced to the C_{1s} hydrocarbon peak at 285.0 eV.

Water contact angles were measured and calculated in static mode on a DataPhysics Instrument (OCA-30, Germany) at ambient temperature. One drop of water (3 μL) was put on the surface of the substrate with an automatic piston syringe and photographed. Three spots were measured for each sample, and triplicate specimens were measured for each group. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparison.

The surface morphology of the substrates was observed by a NanoScope IIIa scanning probe microscope (Digital Instruments, Inc., U.S.A.). A piece of properly sized dry substrate was placed on the surface of a clean metal wafer (fixed by silicone tape) and observed in tapping mode with a scanning rate of 1 Hz. The root-mean-square (rms) roughness was determined over a 2 × 2 μm^2 area using the Nanoscope software.

2.7. Protein adsorption test

To measure protein adsorption of the SR substrates, the total protein adsorption was measured using the Micro-BCA method. SR