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Preparation of bactericidal cationic PDMS surfaces using a facile and efficient approach

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ABSTRACT

Two cationic polymers containing vinyl and quaternary ammonium groups were successfully grafted on silicone surfaces containing Si—H groups. The reaction was carried out by straightforward hydrosilylation reaction in water without using exogenous catalyst and without doping the surface with Si—H groups. The Si—H content was varied by changing the curing time or the ratio between silicone components. The surfaces obtained exhibited quaternary ammonium densities ranging from 1.8×10^{14} to 2.8×10^{15} charge/cm^2 and higher polarity and hydrophilicity in comparison with non grafted surfaces. Bactericidal effects of these surfaces against *Escherichia coli* and *Staphylococcus epidermidis* were evidenced. This study have evidenced also the fouling of the poly(quaternary ammonium) (PQA) surfaces by contact with plasmatic proteins probably limiting their use in some biomedical field (e.g. implantable medical devices).

1. Introduction

Antimicrobial coatings are used in various application fields such as medical products, packaging materials and filters for air-conditioning systems. To obtain antimicrobial properties the substrate is usually impregnated with an antibacterial agent (e.g. silver derivatives, rifampicine...). This strategy requires the use of large quantities of the antimicrobial agent, which is, in addition, gradually released to the environment. Such materials also reduce the probability of generating microbial resistance. Therefore, to avoid this phenomenon, antimicrobial agents must be covalently grafted on material surfaces.

The wet-chemical surface modification using classical organic reactions is the most efficient and controlled tool to modulate the surface properties, in particular when the grafting of active polymers is concerned. Indeed, when grafted, polymers offer numerous advantages comparing to small molecules, i.e. high surface coverage and stability as well as high bioactivity.

Two approaches are commonly used to afford antibacterial properties to a material surface. The first one consists in reducing bacteria attachment by increasing the surface hydrophilicity through grafting of polar and hydrophilic polymers such as polysaccharides [1–7] or PEG [8–12]. The second strategy consists in grafting of biocidal groups onto the surface. Actually, several recent studies have reported the design of biocidal surfaces by covalent grafting of bioactive polymers onto glass [13–17], polymer materials [18–25], paper, [17] and metal [26]. Usually, the bioactive polymer involved cationic groups, such as alkyl pyridinium or quaternary ammonium.

Poly(dimethylsiloxane) (PDMS) elastomers are materials of choice for a wide range of applications such as microfluidic systems, [27] micro-electromechanical systems (MEMS), [28] cellular study [29]. Silicone polymers have also many attributes that make them excellent materials for biomedical and drug delivery applications. For example, silicone polymers have been used as ophthalmic (lens) and blood-contacting biomaterial. However, their use in these applications and their future evolution as biomaterials is limited by their extremely high surface hydrophobicity, which results in the adsorption of significant quantities of proteins from the surrounding biological environment causing thrombosis and adhesion of pathogens like bacteria.

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One of the strategies developed to avoid these problems is the covalent grafting of bioactive polymers onto silicone surfaces. Direct chemical coupling is stable but is difficult to achieve because PDMS is known to be chemically inert. Typically, the first step is to apply plasma treatment to PDMS surface to produce functional anchoring groups such as hydroxyls. Those functional groups render the surface hydrophilic and allow chemical coupling [30]. However, plasma treatment is not sustainable because of the hydrophobicity recovery of treated PDMS, implying that chemical coupling can be performed only few minutes after this treatment [31]. Plasma also has the tendency to damage PDMS [32]. Furthermore, the use of plasmas is only applicable to planar surfaces because of its limited penetration depth, particularly with tubes.

Recently chemical strategies pathways were developed to functionalize PDMS surfaces [1,9–12], particularly in the biomedical field, in order to improve their biocompatibility [9–11]. The silicone surfaces were functionalized during a simple two step procedure via hydrosilylation reaction between small molecules or PEG bearing one alkene group and Si—H bond from the surface [9–11]. The Si—H groups were generated on the surfaces by acid-catalyzed insertion of polymethylhydroxysiloxane (surface doping). Alternatively, residual Si—H groups remaining after silicone curing by hydrosilylation were recently used to graft copolymers onto PDMS surfaces, which constitute a promising pathway of PDMS surface treatment [1,11,12].

Herein, we describe the chemical grafting, in one step, of high molecular weight cationic polymers bearing vinyl and ammonium groups onto PDMS surfaces, taking advantage of remaining Si—H groups from partially to fully cured silicone elastomers. The process involved hydrosilylation in aqueous medium. Neither exogenous catalyst nor surface doping was employed. A widely used and available silicone (Sylgard® 184) as well as a medical grade silicone (NuSil® MED-4750) for long-term implantation (greater than 30 days, e.g. catheters), were tested as models. In order to vary the polymer grafting density, the surface density of residual Si—H was varied by different methods. Finally, the PQA surface properties as well as their stability after contact with plasmatic proteins were investigated.

2. Experimental

2.1. Materials

Poly(vinyl benzyl chloride) (PVBC, weight average molecular weight: $\overline{M_w} = 100,000$ g/mol, dispersity: (Đ) = 1.82) and Poly(diallyldimethylammonium chloride) (PDADMAC, 20 wt% in water, $\overline{M_w} = 400,000–500,000$ g/mol), 3-buten-1-amine, hexadecyltrimethyl ammonium chloride, fibrinogen from porcine plasma and bovine serum albumine (BSA), were purchased from Sigma-Aldrich (France). *N*,*N*-dimethylbutylamine was supplied by Alfa Aesar (France). *Escherichia coli* (MG1655) and *Staphilococcus epidermidis* (ATCC 35984) were purchased from Pasteur Institute (France). Sylgard® 184 was purchased from Neyco (Paris, France). It was supplied as a two-part kit of liquid components with a curing agent (part *A*) and a base (part *B*). The base and the curing agent are usually mixed in an *A*/*B* weight ratio of 1:10. Then, the mixture is cured at 70 °C for 24 h. Biomedical grade silicone NuSil®MED-4750 was from Nusil Technology (Carpinteria, USA). It was supplied as a solid mixture of two components *A* and *B*. These components are thoroughly mixed using a cooled two-roll mill. The *A*/*B* weight ratios used were of 1:1 and 2:1. These mixtures were used at the cured (at 120 °C for 1 h) and uncured states for the chemical grafting. All other reagents and solvents were commercially available as pure grade and were used as purchased from Sigma-Aldrich (France). Ultra-pure water was used in all the experiments.

2.2. Synthesis of Polymer **1** Fig. 1

120 μL (1.3×10^{-3} mol) of 3-buten-1-amine were added to a stirred solution of 2 g of PVBC in 50 mL of THF at room temperature. The reaction was allowed to stir for 24 h at 50 °C. Then, 8.2 mL (5.75×10^{-2} mol) of *N*,*N*-dimethylbutylamine was added to the previous solution. 30 mL of ethanol was added to the solution after 1 h of stirring because of the polymer precipitation. The reaction was allowed to stir for 24 h at 50 °C. After, the solution was concentrated and the resulting product was dissolved in 50 mL of ethanol. Then, 8.2 mL (5.75×10^{-2} mol) of *N*,*N*-dimethylbutylamine was added to this ethanol solution in order to complete the conversion of the chloride groups into quaternary ammonium. After stirring for 24 h at 50 °C, the obtained random copolymer (Polymer **1**) was precipitated on diethyl ether, dried under vacuum, dissolved in water and lyophilized (yield: 83%). *Characterizations:* ^{1}H NMR (D_2O, 300 MHz) δ/ppm: 7.02 and 6.47 (m, 4*H*, $CH{=}C\underline{H}$); 4.16 (m, 2*H*, $—Ar—C\underline{H}_2—N^+$); 2.70 (m, 8*H*, $C\underline{H}_3—N^+$ et $—CH_2—C\underline{H}_2—N^+$); 1.3–2 (m, 3*H*, $—C\underline{H}_2—C\underline{H}—Ar$); 0.9–1.3 (m, 4*H*, $—C\underline{H}_2—C\underline{H}_2—CH_3$); 0.74 (m, 3*H*, $—CH_2—C\underline{H}_3$). FTIR-ATR (diamond): $\nu_{O–H(water)} = 3374\ cm^{-1}$; $\nu_{=C–H} = 3023\ cm^{-1}$; $n_{CH_2,CH_3} = 2990 - 2770\ cm^{-1}$; $\nu_{C=C(arylandbutene)} = 1631\ cm^{-1}$; $\delta_{CH_2,CH_3} = 1482 - 1380\ cm^{-1}$; $\delta_{=C–H} = 866–810\ cm^{-1}$; complete disappearance of $\nu_{Ar–Cl(PVBC)} = 1263\ cm^{-1}$. UV (water): ε (λ_{max}) (mol/L/cm): 476 (264 nm), 6828 (219 nm), 54,186 (187 nm).

2.3. Chemical grafting of cationic polymers onto silicone surfaces

Samples were prepared by immersion of the silicone elastomers in an aqueous polymer solution (1 g/L and 1 cm of depth) followed by an evaporation of water at 70 °C over night or at 120 °C during 1 h. Then, samples were intensively washed with water and immersed in 10 successive water baths during 3 days in order to remove the non covalent attached polymer on the surfaces.

2.4. Measurements

^{1}H NMR spectra were recorded on a Bruker Avance (300 MHz) Spectrometer. IR spectra were recorded on a FTIR (Nicolet Magna 550), equipped with a diamond ATR device (Attenuated Total Reflection). Spectra were obtained from 32 scans in the 4000 to 500 cm^{-1} range. UV–vis spectra were recorded on a Perkin-Elmer Lambda 800. Static contact angle measurements with water were performed on modified and unmodified silicone surfaces using Digidrop Goniometer (GBX, France). The static contact angles were measured at the equilibrium time. At least five measurements on different points were performed to calculate the mean contact angle and its standard deviation. Fluorescence spectroscopy was performed using Fluoromax spectrofluorimeter (France). Surface fluorescence analyses were performed using a commercial epifluorescence upright microscope (DMR, Leica microsystems) equipped with 63× and 40× water immersion objectives. The images were recorded with a color CCD camera (CoolSnap, RS Photometrics) and analyzed with a computer imaging system allowing fluorescent intensities measurements. Surface quaternary ammonium densities were quantified according to a method described by Tiller et al. [13]. Briefly, modified and unmodified PDMS samples (7×7 mm^2) were immersed in a solution of fluorescein sodium salt (1% in distilled water) for 10 min. Due to their negative charges, the fluorescent probe bind strongly to the cationic sites and the unreacted molecules can then be removed by exhaustive washing with distilled water. The bounded fluorescein molecules were then exchanged by immersing the modified samples in 2 mL of an aqueous solution of hexadecyltrimethyl ammonium (0.5%), and sonicated for 45 min. After adding 0.3 mL of saturated $NaHCO_3$ solution (pH = 8.3), the absorbance of the resulting solution was