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Solvent-free functionalization of silicone rubber and efficacy of PAAm brushes grafted from an amino-PPX layer against bacterial adhesion

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ABSTRACT

Silicone rubber is a frequently employed biomaterial that is prone to bacterial adhesion and biofilm formation. In this study, the surface of silicone rubber was solvent-free functionalized by chemical vapor deposition (CVD) of poly(*o*-amino-*p*-xylylene-*co*-*p*-xylylene (amino-PPX). Subsequently, the amino groups of the amino-PPX layer were used to introduce the initiator from a vapor phase for atom transfer radical polymerization of acrylamide to form polyacrylamide (PAAm) brushes. The modification steps were verified by means of X-ray photoelectron spectroscopy and attenuated total reflection–Fourier transform infrared spectroscopy. Adhesion of *Staphylococcus aureus* ATCC 12600 and *Escherichia coli* 3.14 to an amino-PPX–PAAm brush coating in a parallel plate flow chamber was strongly reduced with respect to non-coated silicone rubber – by 93% and 99%, respectively. For *E. coli* 3.14, this reduction is larger than that obtained for solvent functionalization of γ-aminopropyltriethoxysilane–PAAm brushes due to the higher density of amino groups introduced by the CVD of amino-PPX.

1. Introduction

Silicone rubber is one of the few synthetic materials that have found their way into the biomedical field for use as implants or devices. Microbial adhesion to silicone rubber implant or device surfaces may cause severe deterioration of properties and functioning [1], sometimes with lethal infections as a result. Therefore different approaches have been taken to modify silicone rubber surfaces in order to discourage microbial adhesion. Silicone rubber has been hydrophilized using plasma etching [2,3], CO_2-pulsed laser beam irradiation [4] and plasma immersion ion implantation [5,6]. Coatings based on biosurfactants and colloidal palladium/tin salt mixtures have also been applied in order to improve the silicone rubber resistance to microbial adhesion [7,8].

Densely packed polyacrylamide (PAAm) brushes grown from silicone rubber surfaces have hitherto demonstrated the largest reductions in microbial adhesion to silicone rubber and it has been demonstrated for a number of different strains and species that adhesion was reduced up to a factor of hundred [9]. Surface grafting of polymers resulted in stable end-tethered polymer chains and, depending on the number of grafted chains per nm^2, molar mass and interaction with water, isolated polymer coils can be obtained with more or less random coil conformations (mushroom regime) or stretched coils with greatly extended average chain lengths (brush regime) [10–12]. The latter situation is interesting as a biomaterial coating, because the brush constitutes a steric barrier, preventing protein adsorption and microbial adhesion [13,14].

Atom transfer radical polymerization (ATRP) is a very suitable technique for the growth of polymer brushes from surfaces because it tolerates the use of many functional groups, it is easy to apply and results in highly uniform polymer chains with a good control over the brush thickness and density [15]. Using ATRP, PAAm brushes were grown from the surface of silicone rubber [9], but the needed surface modification steps require aggressive solvents, such as toluene, dichloromethane and dimethylformamide. Those solvents are usually not recommended for the surface modification of silicone rubber because they cause swelling of the rubber and cracking by solvent penetration into the rubber. Upon implantation, the solvent can diffuse toward the implant surface and cause inflammatory tissue reactions. Therefore, it would be advantageous for the sustainability of an implant or device to be able to functionalize silicone rubber surfaces by a procedure that involves fewer modification steps and avoids the use of solvents.

It has been reported before that substituted poly-*p*-xylylenes are biocompatible and may offer a direct attachment point for polymeric drug release systems, like poly(N-isopropylacrylamide) (NIPAAm)-*co*-poly(acrylic acid) [16], or anticoagulant proteins such as r-hirudin [17]. Chemical vapor deposition (CVD) allows

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direct conversion of monomer into a polymer film on metals and polymers without using solvents, catalysts or initiators [16–19] with good control of the film thickness and homogeneity. Poly(*o*-amino-*p*-xylylene-*co*-*p*-xylylene) (amino-PPX) has been polymerized onto a poly(vinylidene fluoride) surface and further used to graft fibronectin in order to induce the attachment of human osteoblasts [20]. Silicone rubber was also coated with a deposited amino-PPX layer in order to prepare elastomeric stamps [21].

This study aims to investigate whether PAAm brushes can be grafted from silicone rubber without using aggressive solvents and fewer reaction steps. Thus the grafting procedure was done by using CVD of an amino-PPX film. Secondly, amino-PPX–PAAm brush coatings on silicone rubber were evaluated for their ability to withstand bacterial adhesion.

2. Materials and methods

2.1. Materials

Silicone rubber sheets with a thickness of 0.5 mm were obtained from Medin, Groningen, The Netherlands and cut into 2.5 cm × 2.5 cm samples. RBS35 neutral concentrated detergent was purchased from Brunschwig Chemie BV, Amsterdam, The Netherlands and diluted to a solution of 2% (v/v). 4-(Chloromethyl)benzoyl chloride (CMBC), acrylamide (AAm), 2,2-dipyridyl and copper(I) chloride were purchased from Aldrich. All solvents were reagent grade and used without further purification.

2.2. Surface preparation and modification

2.2.1. Surface preparation

Silicone rubber samples were first cleaned by sonication in 2% RBS35 in demineralized water and subsequently thoroughly rinsed with demineralized water and sterilized with 70% ethanol for 10 min. Finally, silicone rubber samples were dried under vacuum and used further for the modification process.

2.2.2. Aminofunctionalization

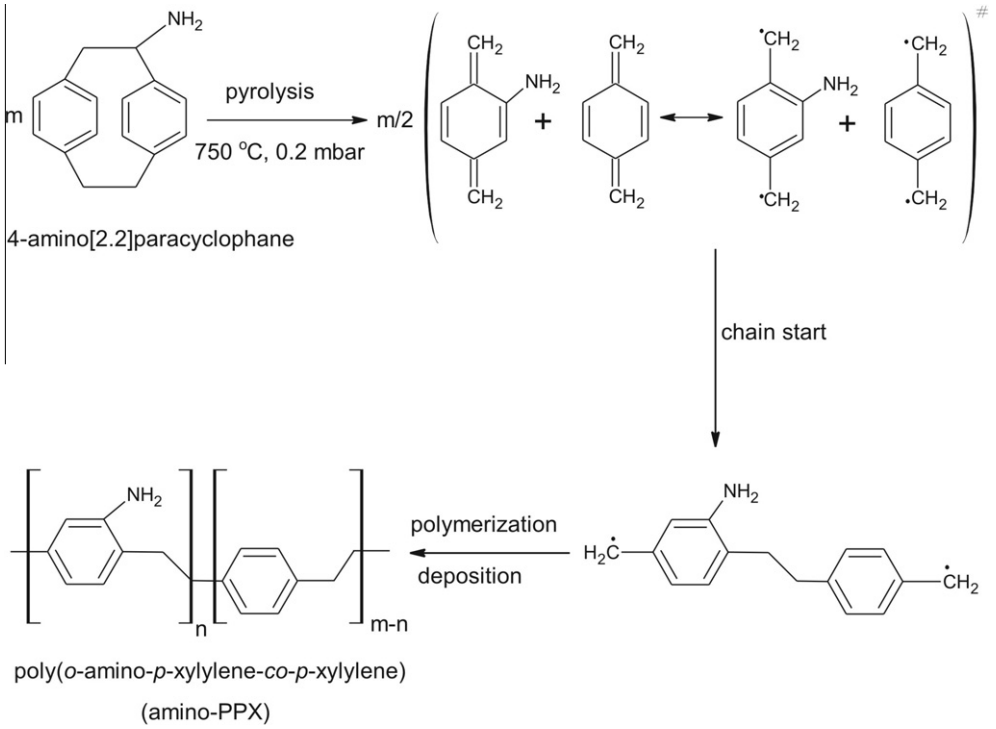
Amino-PPX deposition was performed using CVD. CVD was carried out in a home-built installation at DWI RWTH AAchen, Germany. The monomer 4-amino[2.2]paracyclophane was synthesized from [2.2] paracyclophane, as described previously [22]. The monomer was first vaporized at 200 °C and then pyrolyzed for 30 min at 750 °C at a pressure of 20 Pa and a monomer stream flow of 15 sccm (standard cubic centimeters per minute). Finally it was polymerized and condensed at 7 °C on the silicone rubber surface as amino-PPX (Scheme 1), which is the coupling layer for the PAAm brush.

2.2.3. ATRP initiator attachment

Initiator attachment was done in the presence of CMBC vapor instead of using dichloromethane solvent, as reported previously [9], in order to prevent the rubber from swelling and cracking. Silicone rubbers coated with an amino-PPX layer were placed with the modified side down in a special device to keep them 1.5 cm above the bottom of the flask, containing 0.2 g of CMBC. A vacuum was applied for 5 min to the flask containing both CMBC and silicone rubber surfaces coated with amino-PPX layer. Ultimately, the flask was placed in an oven at 50 °C and kept for different time intervals to study the conversion of amino groups into chlorine groups with X-ray photoelectron spectroscopy (XPS). The functionalized surfaces were washed in ethanol under sonication to remove the non-grafted material and dried under vacuum.

2.2.4. PAAm grafting

Finally, after the initiator attachment, the atom transfer radical polymerization (ATRP) of acrylamide took place in water as a solvent in order to graft the PAAm brush [9]. After polymerization, the silicone rubber samples were removed from the solution and washed with deionized water for 48 h in a Soxhlet to remove any unreacted monomer, catalyst and non-grafted material. Finally, the surfaces were dried under vacuum.



Scheme 1. Schematic presentation of 4-amino[2.2]paracyclophane polymerization.

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