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Effect of surface proteins on *Staphylococcus Epidermidis* adhesion and colonization on silicone

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Abstract

Shunt infections are one of the most serious complications in shunt implant surgery. Previous studies have suggested that cerebrospinal fluid (CSF) proteins could affect bacterial adhesion and subsequent shunt infection. A systematic study using immobilized protein on the surface of silane-modified silicone was conducted to determine how these modifications influenced *Staphylococcus epidermidis* adhesion and colonization. A comparison was also made with silicone having physically adsorbed protein. A colony-counting adhesion assay and scanning electron microscopy (SEM) were used to provide quantitative analysis of bacterial adhesion and semi-quantitative analysis of bacterial colonization, respectively. In order to determine the appropriate silanization process for effective protein immobilization, the effect of bovine serum albumin (BSA) immobilized on *n*-3-(trimethoxysilyl)propyl-ethylenediamine (AEAPS)/silicone, aminopropyltriethoxysilane (APTMS)/silicone, 3-(glycidyloxypropyl)trimethoxysilane (GPTMS)/silicone, and octadecyltrichlorosilane (OTS)/silicone on bacterial adhesion was investigated. Upon identifying that OTS is the most effective silane, different types of proteins, including: BSA, human serum albumin (HSA), γ-globulin, and fibrinogen were immobilized on OTS/silicone by a photo-immobilization method. Immobilized protein on modified silicone surfaces was found to be stable in saline for 30 days, while physically adsorbed protein showed instability within hours as determined by contact angle measurements and X-ray photoelectron spectroscopy (XPS). For HSA/OTS/silicone, BSA/OTS/silicone, γ-globulin/OTS/silicone, fibrinogen/OTS/silicon, and physically absorbed BSA on silicone, the contact angles were 78.5°, 80.7°, 78.9°, 81.3°, and 96.5°; and the amount of nitrogen content was found to be 4.6%, 5.0%, 5.6%, 7.2%, and 3.2%, respectively. All protein immobilized on OTS/silicone surfaces significantly reduced bacterial adhesion by around 75% compared to untreated silicone, while physically adsorbed BSA on silicone reduced by only 29.4%, as determined by colony-counting adhesion assay. However, there was no significant difference on bacterial adhesion among the different types of proteins immobilized on OTS/silicone. Minimizing bacterial adhesion and colonization can be attributed to the increased concentration of $-NH_2$ group, and stability and more hydrophilic nature of the protein/OTS/silicone surfaces.

Keywords: Bacterial adhesion; Colonization; Immobilized protein; *Staphylococcus epidermidis*; Silicone

1. Introduction

Catheter infection is the one of the most frequent complications of ventricular shunt implantation for the treatment of hydrocephalus, with a reported frequency varying from 7 to 30% [1]. Eighty percent of shunt infections occur within 6 months of surgery [2]. Thus, there is an increasing effort to develop new shunt materials to prevent shunt infection by minimizing the incidence of initial bacterial adhesion.

Many patients treated with a cerebrospinal fluid (CSF) shunt have raised CSF protein concentrations [3]. In particular, premature infant patients have a higher incidence of shunt infection [4,5]. Moreover, previous studies have found that plasma proteins, CSF proteins, and other proteins rapidly coat implanted devices and possibly affected bacterial adherence to the shunt catheter [6,7]. However, there have been conflicting reports regarding the influence of protein on bacterial adherence, with some indicating that protein adsorption enhanced bacterial adhesion [3,8], while other studies have shown that pre-incubating

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surfaces with plasma, albumin, or CSF, reduced the adherence of staphylococci to a wide variety of polymers [7,9–11]. It should be noted that in the latter studies the proteins were physically adsorbed on the surface. To the best of our knowledge, no study has been made on the effect of "immobilized" (covalently bonded) protein to silicone surfaces on bacterial adhesion. Immobilization of protein through covalent bonds should be much more stable compared to physically adsorbed protein [12]. Therefore, with immobilization, the effects of different proteins on bacterial adhesion and colonization can be evaluated without the concern of dynamic protein adsorption and desorption processes.

Immobilization of protein onto a solid surface can be achieved by various surface modification techniques [13–17]. In order to achieve covalent bonding between a biomolecule and a polymer surface, active functional groups must exist in the outermost layer of the polymer surfaces for chemical bonds with bio-molecules. These functional groups include amino ($–NH_2$), carboxyl (–COOH), and hydroxyl (–OH) groups [14]. Recent studies have shown that deposition of a self-assembled monolayer (SAM) or multilayer of organosilane is promising for protein immobilization [13,18–20]. Organosilane coatings on solid surfaces can be formed in solution and by vapor deposition techniques [21,22]. The stability of various SAMs and multilayers formed by organosilanes on silicon has been reported [23]. However, the stability of protein on organosilanes coated on silicone has not been explored.

Bovine serum albumin (BSA), human serum albumin (HSA), γ-globulin (Glo), and fibrinogen (Fg) were used in this study. BSA (molecular weight, 66.4 kDa; 59 lysine residues (59 amino groups) [24]) is an acidic protein with a comparatively low molecular weight. HSA (molecular weight, 66.2 kDa; 58 lysine residues [25]) accounts for 56–76% of total protein in CSF [26,27]. It is the predominant adsorbed protein on hydrocephalus shunt catheters [28]. γ-globulin (molecular weight, 155–160 kDa [29]; 75 lysine residues [30]), accounts for 7–12% of total protein concentration in CSF [26,27]. Fibrinogen (molecular weight, 340 kDa [31]; 39 lysine residues on the chain [32]), is a glycoprotein found in the blood which has been found to be quickly adsorbed on polyethylene, silicone rubber, polystyrene, and glass [33].

The objectives of this study were to perform a systematic investigation to (1) evaluate different silanization processes for protein immobilization; (2) compare the degree of bacteria adhesion and colonization on physically adsorbed protein and immobilized protein; and (3) gain a better understanding of the relationship between bacterial adhesion and colonization and the nature of modified surfaces.

2. Materials and methods

2.1. Materials

Silastic silicone sheets (thickness: 0.4 mm) were obtained from Specialty Manufacturing, Inc. (Saginaw, MI) and were cut into uniform 21 mm diameter disks. OTS (97.5%, $CH_3(CH_2)_{17}SiCl_3$) was purxased from United Chemical Technologies (Bristol, PA). AEAPS (97%, $NH_2(CH_2)_2NH(CH_2)_3Si(OCH_3)_3$), GPTMS (98%, $CH_2OCHCH_2OCH_2CH_2CH_2Si(OCH_3)_3$), APTMS (97%, $NH_2(CH_2)_3Si(OCH_3)_3$), 1-3-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (98% water-soluble carbodiimide, WSC), glutaraldehyde (Glu, 50 wt.%, $OCH(CH_2)_3CHO$), BSA, HSA, γ-globulin from human blood, fibrinogen from human plasma, and phosphate-buffered saline (PBS, pH 7.4) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). 4-azidobenzoic acid was obtained from TCI (Portland, OR). Ethyl alcohol (200 proof, absolute, dehydrated) was purchased from Pharmco Products Inc. (Brookfield, CT). Sodium chloride (NaCl), dioxane and toluene (anhydrous) were obtained from Fisher Scientific (Pittsburgh, PA). Deionized water (DI water) with resistivity of 18 MΩ cm was obtained with a Barnstead Nanopure Systems (Dubuque, IA). All chemicals were used without further purification.

2.2. Immobilization method

2.2.1. Surface modification of silicone with silanes

The silastic disks were cleaned by immersion in anhydrous ethyl alcohol in an ultrasonic cleaner (Branson 2200, Danbury, CT) for 5 min, and dried with nitrogen. The samples were then treated by oxygen plasma cleaner (PDC-3XG, Harrick Scientific Corporation, Ossining, NY) for 5 min at the high power level. Subsequently, different silanes (APTMS, GPTMS, AEAPS, and OTS) were coated on the plasmatreated silicone samples. For AEAPS- and OTS-coated samples, the disks were exposed together with a glass cup filled with 1 mL AEAPS or OTS in a sealed chamber (3 L) at 10^{-3} Torr and at room temperature for 4 h. The samples remained in the sealed chamber with AEAPS or OTS at 10^{-2} Torr for an additional 12 h. Only the exposed surface was modified with an AEAPS or OTS SAM layer.

To obtain a GPTMS coated surface, the plasma treated silicone was put in 1% (wt./wt.) GPTMS/toluene (anhydrous) solution for 3 h at 65 °C, followed by rinsing four times at room temperature with toluene; while for an APTMS multilayer, the plasma treated silicone was put in 1% APTMS/dioxane (wt./wt.) solution in the presence of 0.2% water at 65 °C for 1 h, followed by washing four times at room temperature with dioxane.

2.2.2. Immobilization of BSA on AEAPS or APTMS modified silicone

BSA/Glu/AEAPS and BSA/Glu/APTMS were prepared according to the procedures of Liu et al. [13]. The AEAPS and APTMS on silicone samples were first immersed in 5% (v/v) glutaraldehyde solution with 0.01 M PBS (pH 7.4) for 6 h at room temperature, and then washed with DI water, followed by immersing in 15 mL of 3 mg/mL BSA in PBS (pH 5.0) at 30 °C for 4 h. The samples were then rinsed with DI water.

2.2.3. Immobilization of BSA on GPTMS modified silicone

BSA/GPTMS was prepared according to Yang et al.'s approach [19]. The GPTMS on silicone samples were immersed in 15 mL of 1 mg/mL BSA in PBS at 4 °C for 12 h. The samples were then rinsed with DI water.