

Influence of polymerized siloxanes on growth of nosocomial aerobic bacteria

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Abstract. Health care associated infections are the fourth leading cause of disease in industrialized countries, and the most common complication affecting hospitalized patients. Recently a polymerized siloxane coating substrate was suggested as a promising candidate for the coating of materials which are aimed to be used in cardiovascular tissue engineering. To reduce the risk of tissue remodeling failure after implantation of such engineered tissue implants, coatings substrates for tissue engineering scaffolds might be helpful to lower the risk of bacterial attachment and growth during and after implantation.

In the presented study a coating based on polymerized siloxanes was tested with respect to these properties. Tests were performed with aerobic nosocomial bacteria (*Escherichia coli* and *Staphylococcus epidermidis*) and an exemplary selection of materials (stainless steel, polycarbonate, soda-lime glass). The siloxane coating was able to significantly reduce bacterial adherence, and it is supposed that this effect is mainly a result of the high hydrophobicity of the coating substrate.

Keywords: Biomaterial, siloxanes, coating, bacterial growth, biofilm

1. Introduction

Recently a substrate based on polymerized siloxanes was suggested as a promising candidate for the coating of materials which are aimed to be used in cardiovascular tissue engineering approaches. The siloxanes become electrostatically bond on biomaterials creating a hydrophobic surface [1]. Surface hydrophobicity is well known as a key factor to govern bacterial cell attachment. It was shown that coating of exemplary biomaterials like silicone with an aqueous emulsion of polydimethylsiloxane (PDMS) did not negatively impact cell morphology and cell layer formation [1]. Cell layer confluence was even higher after cell growth with extracts of the coated materials in contrast to cell growth in extracts of the non-coated samples. Additionally the PDMS coating resulted in a decreased LDH

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release, and in the literature it is described that silica conditioned media significantly induce proliferation of fibroblasts from rats (RAT-2 cells) [2] and hamsters [3] and also from other cell types (e.g. osteoblasts [4, 5]).

However, before PDMS-based coating substrates can be used for therapeutic approaches it has to be guaranteed, that the coating does not promote bacterial growth. The latter can cause destruction of the engineered tissue and engineered tissue failure respectively. Main factors influencing bacterial reactivity and bacterial growth on siloxane surfaces are the local charge distribution on the surface [6], and the surface hydrophobicity [7, 8]. In the absence of nearby isomorphous cation substitutions that create negative charge a siloxane surface functions only as a mild charge donor [9]. The predominant effect of the siloxane coating comes therefore from the hydrophobicity, which makes the surface less resistant to bacterial adhesion than hydrophilic surfaces [10, 11].

The presented study aimed to evaluate the potential of the polymerized siloxane coating to influence bacterial growth. Tests were performed with exemplary nosocomial gram-negative and gram-positive bacteria (*Escherichia coli*, *Staphylococcus epidermidis*), and an exemplary selection of materials (stainless steel, polycarbonate, soda-lime glass).

2. Experimental

2.1. Bacteria

Tests were performed with an *S. epidermidis* strain provided by ATCC (strain 12228). Additionally an *E. coli* strain was used that was isolated from the feces of a conventional housed C57Bl/6N mouse. Strain identification was done with an automated system (Vitek 2 System, Biomérieux). To exclude tests with *E. coli* parovars (EAEC, EHEC, EPEC, EIEC, ETEC) real-time multiplex PCR was performed (R-Biopharm). *S. epidermidis* was used as a representative for nosocomial aerobic gram-positive bacteria, and *E. coli* served as representative for nosocomial aerobic gram-negative bacteria. Both bacterial strains were initially grown on glucose nutrient agar plates (2 vol-% agar, SIFIN) at 36°C, and then transferred into glucose nutrient broth to establish suspension cultures (36°C, 100 rpm). Bacterial growth was controlled by measuring optical density at 590 nm. For test purposes the exact bacterial concentration was calculated by plating the suspended bacteria on glucose nutrient agar plates and subsequent counting of the colony forming units (cfu).

After 1 hour of incubation of the samples in the bacterial suspension at 36°C under permanent mild shaking at 100 rpm samples were washed three times in sterile physiological saline solution to get rid of the non- or loosely adherent bacteria. Thereafter samples were transferred into sterile physiological saline solution, and bacteria were detached by ultrasonic treatment (1 min, room temperature, frequency: 60 Hz, non-modulated). Detached bacteria were quantified by spread-plate method as described above.

2.2. Materials

Tests were performed using discs (diameter 2.6 cm, thickness 0.2 cm) with a centered fixation hole (diameter 0.5 cm). The discs were made of stainless steel, polycarbonate or soda-lime glass, respectively. All discs were cleaned before usage. The cleaning procedure started with incubating all discs for 12 hrs in 100% ethanol (Sigma Aldrich), and a subsequent treatment for 20 min in an ultrasonic bath (35 kHz, Elmasonic One) filled with ethanol. Thereafter the discs were rinsed three times with ddH₂O (sterile, endotoxin-free), dried at 37°C for 2 hrs, coated with the PDMS substrate, and sterilized using autoclaving (121°C, 20 min).

The discs made of soda lime glass were initially rinsed in acetone, then for 12 hrs in 2% HCl, and finally once with ddH₂O, before the cleaning procedure was started.

2.3. Coating substrate

The coating substrate consisted of an aqueous emulsion of polydimethylsiloxane (PERMANON), which was used with and without supplementation of 0.5 vol-% of a quaternary ammonium compound (Advisal 400, ENVISAL, Reppenstedt, Germany). The constitution of the PDMS coating substrate is described in more details elsewhere [1].

2.4. Coating

Dip coating was used to apply the polymerized siloxanes onto the materials. Therefore the materials were dipped into an aqueous emulsion of 2.0 vol-% PDMS three times for 10 min each (overall coating time 30 min) at $22 \pm 2^\circ\text{C}$.

2.5. Counting of adherent bacteria

Detached bacteria were serially diluted with physiological saline solution and plated with T-spatula on glucose nutrient agar plates. Plates were incubated overnight at 36°C . CFU were counted manually and values converted to original concentration.

2.6. Scanning electron microscopic (SEM) examination

The samples were washed two times in 0.1 M cacodylate buffer at 21°C , three times in physiological saline solution, transferred into a modified Karnovsky solution (7.5% glutaraldehyde, 3% paraformaldehyde, buffered at pH 7.4) for 24 hours at 7°C , and afterwards postfixed in 1% osmium tetroxide mixed with 0.2 M cacodylate buffer. The postfixed samples were washed twice with buffer, dehydrated in an ascending series of ethanol (starting with 30%, and finally into two changes of absolute ethanol) and then covered twice with hexamethyldisilazane (HMDS, Roth) for 15 minutes. After drying overnight under the fume hood to remove HMDS, the samples were sputter-coated with gold-palladium for 2 min (approx. thickness 2 nm) and examined using a FEI Quanta 200 ESEM at an accelerating voltage of 15 kV.

2.7. Statistics

Data were reported as mean value \pm standard deviation for continuous variables, and were analysed by Student's *t*-test (paired). A *p* value of less than 0.05 was considered significant.

3. Results

The number of adherent bacteria was highest on PDMS polycarbonate and lowest on PDMS coated glass ($p < 0.05$, Fig. 1), and on all coated materials *S. epidermidis* was present in significantly higher numbers than *E. coli*.

However, the PDMS coating proved to be able to lower the adherence of *E. coli* and *S. epidermidis*. The number of adherent *E. coli* (see Fig. 2) and *S. epidermidis* on PDMS coated stainless steel, polycarbonate, and glass was reduced in comparison to the number of adherent bacteria on the non-

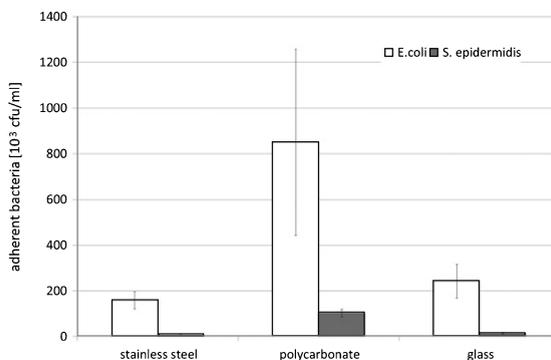


Fig. 1. Bacterial adherence on stainless steel, polycarbonate, and glass after coating with 2 vol-% PDMS, and subsequent incubation in a bacterial suspension (0.2 OD_{590 nm} in glucose nutrient broth) for 1 hr; number of adherent bacteria expressed in as 10³ cfu/ml; means and standard deviation, $n = 10$.

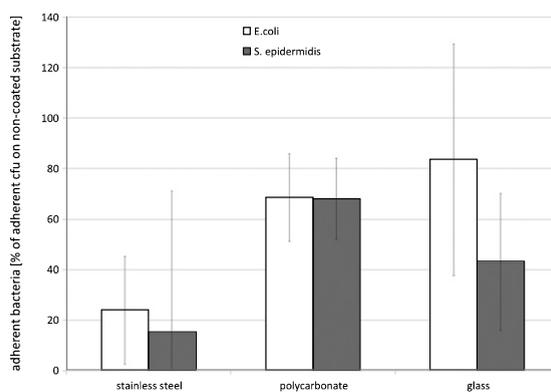


Fig. 2. Bacterial adherence on stainless steel, polycarbonate, and glass after coating with 2 vol-% PDMS, and subsequent incubation in a bacterial suspension (0.2 OD_{590 nm} in glucose nutrient broth) for 1 hr; number of adherent bacteria expressed in percentage of adherent cfu on non-coated glass; means and standard deviation, $n = 10$.

coated materials ($p < 0.05$), with PDMS coating of stainless steel being most effective for adherence reduction of both bacterial strains (see Fig. 3).

Exemplarily tests with glass coated with the polysiloxane emulsion after supplementation with a quaternary ammonium compound revealed comparable results to the tests with the non-supplemented coating substrate. After 1 hr of incubation the number of adherent *E. coli* was not significantly different between glasses which were coated with PDMS and disinfectant and with non-supplemented PDMS (see Fig. 4).

4. Discussion

Most bacteria are able to adhere to material surfaces and to form complex and heterogeneous microbial communities and biofilms [12]. Significant efforts are currently being directed to prevention of bacterial adhesion and subsequent formation of biofilms. Many surface modification techniques have been used to investigate inhibition of bacterial adhesion and biofilm formation. Among these methods chemical coating strategies are promising approaches for bacterial adherence reduction [13–16]. The presented study was aimed to test the effect of a PDMS coating substrate on bacterial adherence, and

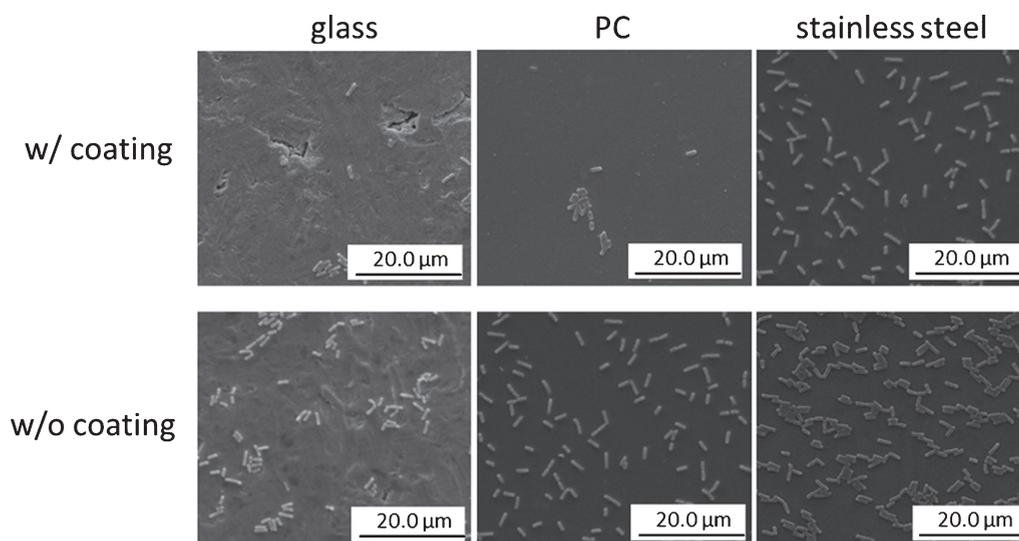


Fig. 3. Bacterial adherence on glass, polycarbonate (PC), and stainless steel after coating with 2 vol-% PDMS (w/siloxanes), and subsequent incubation in a *E. coli*-suspension ($0.2 \text{ OD}_{590 \text{ nm}}$ in glucose nutrient broth) for 1 hr, scanning electron microscopy.

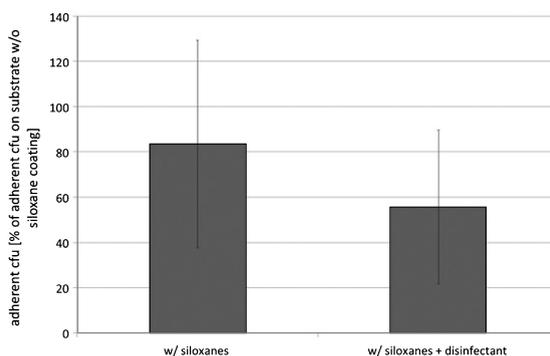


Fig. 4. *E. coli*-adherence after incubation of glass coated with 2 vol-% PDMS (w/siloxanes), or with 2 vol-% PDMS plus 0.5-vol% disinfectant (Advisal 400, ENVISAL; w/siloxanes+disinfectant) in a *E. coli*-suspension ($0.2 \text{ OD}_{590 \text{ nm}}$ in glucose nutrient broth) for 1 hr; number of adherent bacteria expressed in percentage of adherent cfu on non-coated glass; means and standard deviation, $n = 10$.

proved this substrate to be able to reduce adherence of defined aerobic nosocomial bacterial strains (*E. coli*, *S. epidermidis*).

Bacterial adhesion is generally described as a twostep process. Initially the bacteria became reversible bonded to the material surface based on physico-chemical forces. Thereafter the contact becomes irreversible due to hydrophobic interactions and interactions between functional groups on bacterial cells and material surfaces [13]. It has been reported that physico-chemical properties, e.g. surface potential, roughness and hydrophobicity, affect the rate of the initial bacterial adhesion [17–20]. Hydrophilic materials are described to be more resistant to bacterial adhesion than hydrophobic materials. For instance in a study of Fletcher and Loeb [23] the adherence of *Pseudomonas sp.* to hydrophobic plastics with low surface charges was pronounced in comparison to hydrophilic metals with a positive or neutral surface charge, and glass which is characterized by a hydrophilic and negatively charged surface.

Beside of the hydrophobicity of the material surface also the hydrophobicity of the bacterial cell wall determines bacterial adhesion [21–24]. The hydrophobicity of bacteria varies according to bacterial species and is influenced by growth medium, bacteria age, and bacterial surface structure. In the presented study the bacterial were exposed to the materials in a liquid, and were therefore expected to be tested with a hydrophilic cell wall. The polysiloxane coating established a hydrophobic surface on the tested materials [1], and resulted in a change of the metal and glass surface from hydrophilic to hydrophobic. Due to this change of the surface hydrophobicity the number of *E. coli* and *S. epidermidis* that became adherent to these materials might have been reduced after surface coating.

5. Conclusion

The hydrophilic siloxane coating was able to lower bacterial adherence of aerobic nosocomial bacteria (*E. coli*, *S. epidermidis*) on stainless steel, polycarbonate, and soda-lime glass. Bacterial adherence on the polysiloxane coating alone was not different to adherence after supplementation with 0.5 vol-% of a quaternary ammonium compound.

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