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Influence of polymerized siloxane coating on growth and biofilm formation of aerobic grown nosocomial bacteria

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Abstract. A recent study showed that polymerized siloxane (PDMS) coating of biomaterials like polycarbonate, stainless steel or glass results in a hydrophobic and positively charged surface, which is known to be advantageous for cell adhesion. However, when *Escherichia coli* and *Staphylococcus epidermidis* were cultured on PDMS coated materials for one hour, this resulted in a significant decrease of bacterial adherence compared to non-coated materials. The study therefore aimed at investigating antimicrobial effects of PDMS. We used nosocomial aerobic grown bacteria *S. epidermidis, Pseudomonas aeruginosa, E. coli* and the biofilm formation model organism *Bacillus subtilis*, which were exposed to PDMS in a planktonic culture assay and additionally while biofilm formation. PDMS had a significant impact on the growth of these bacteria in both culture assays. In planktonic culture, PDMS exposition resulted in a decreased growth of all bacteria tested, which was strongly species specific. Biofilm culture in contrast caused an increased growth of *E. coli* and *P. aeruginosa*, while growth of *S. epidermidis* and *B. subtilis* was decreased. However, these results are based on tests of single species biofilms. Previous to practical application it is necessary to confirm these results by tests in which different bacterial species are able to interact like in natural biofilms resulting in modified bacterial growth behavior and toxin resistance.

Keywords: Siloxanes, biofilm, toxicity, coating, biomaterials, bacterial growth

1. Introduction

Health care-associated infections (HAI's), or infections acquired in health-care settings are the most frequent adverse event in health-care delivery worldwide. In developed countries 7% of hospitalized patients at any given time, will acquire at least one HAI. In developing countries, case frequency increases onto 10% in hospitalized patients [1]. The main group of HAI's are those related to the use of invasive devices. 50–70% of the HAI's reported by the Centers for Disease Control in U.S. are caused by biofilm formation on indwelling medical devices [2, 3]. For this reason, the development of biomaterials and material coating substrates that prevent the formation of biofilms is of great importance to lower the risk of bacterial attachment and growth during and after implantation of a medical device. Recently an aqueous emulsion of polydimethylsiloxane (PDMS), which is a widely

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used silicone oil, was used as a coating substrate on stainless steel, polycarbonate and soda-lime glass. This coating proved to be non-cytotoxic to eukaryotic cells (fibroblasts), but significantly reduced adherence of aerobic nosocomial bacteria (*Escherichia coli* and *Staphylococcus epidermidis*) [4]. However, PDMS provides a hydrophobic surface, which is described to be less resistant to bacterial adhesion than hydrophilic surfaces [5, 6]. For this reason, a study was conducted to clarify whether PDMS can reduce the growth of nosocomial bacteria in a planktonic culture assay. Additionally biofilm growth was studied due to the different behavior of bacteria in planktonic culture and in biofilms [7].

2. Materials and methods

2.1. Bacteria

The study was performed using Gram-positive bacteria (*Staphylococcus epidermidis* [ATCC strain-no. 12228], *Bacillus subtilis* subsp. *spizizenii* [ATCC strain-no. 6630]), and Gram-negative bacteria (*Pseudomonas aeruginosa* [ATCC strain-no. 27853; LGC, Germany], *Escherichia coli*-strain ECM1 which was isolated from C57Bl/6N mouse feces and had proved to not contribute to any diarrheagenic pathovar [4]).

Bacteria were initially cultured on sheep blood agar plates (OXOID, Germany), and after 24 hrs at 36°C transferred to glucose nutrient agar plates (2 vol-%, Sifin diagnostics, Germany), where they were grown for 18 hrs at 36°C before further usage.

2.2. Coating substrate

For biofilm assay, we coated polystyrene 96-well microplates with an aqueous emulsion of PDMS (Permanon, Germany). The composition of the PDMS coating substrate is described in more detail elsewhere [1]. For application of the polymerized siloxane, each well was completely filled with an aqueous emulsion of 2.0 vol-% PDMS. After 10 min at 22 ± 2°C the PDMS-solution was exhausted. This coating procedure was repeated three times, resulting in an overall coating time of 30 min.

2.3. Biofilm culture assay

This test was performed with single species biofilms using a semi-quantitative biofilm assay as described elsewhere [8, 9]. In brief, one colony of *E. coli,* one colony of *B. subtilis*, two colonies of *P. aeruginosa*, or three colonies of *S. epidermidis* were inoculated into 50 ml glucose nutrient broth (Sifin diagnostics, Germany) each. Cultures were incubated for 18 hrs at 36°C under continuous shaking (100 rpm). 5 µl aliquots of each bacterial culture were transferred into eight wells of a polystyrene-based 96-well microplate. All wells were pre-filled with 200 µl DMEM high glucose medium (Sigma Aldrich, Germany). After 18 hrs of biofilm formation at 36°C, bacteria were stained with 0.5% crystal violet (Merck, Germany). Subsequently, cultures were washed three times with H_2O_{dd}, followed by destaining with 70% ethanol. Supernatants were transferred to a fresh, uncoated 96-well microplate. Photometrical measurement at 540 nm was performed for quantification of crystal violet.

2.4. Planktonic culture assay

5.6 ml of a PDMS in water emulsion in different concentrations (0.2, 2.0, 5.0 and 7.0 vol-%) were added to 74.4 ml glucose nutrient broth to a final volume of 80 ml.

Planktonic growth under the influence of different PDMS concentrations was tested three times separately and in triplicates for each bacterial species. One to three colonies of each bacterium were inoculated into PDMS-supplemented glucose nutrient broth (one colony of *E. coli*, two colonies of *P. aeruginosa,* and three colonies of *S. epidermidis*; depending on individual, species-related different colony sizes). Bacterial growth was quantified using photometric measurement of the optical density (OD) at 590 nm every 30 min for 300 min (550 microplate reader, Bio-Rad, Germany). We excluded *B. subtilis* from this test due to its strong autoaggregation, which would have resulted in non-assessable measurement inaccuracies.

Because of initial OD reduction in the PDMS-broth mixtures they were pre-incubated for 18 hrs at 36°C under continuous shaking (100 rpm) before inoculation with the bacteria to generate a constant OD.

2.5. Statistics

The results were evaluated with the statistics software OriginPro 2016 Version 93G (OriginLab Corporation, Northampton, MA, USA). The significance level was set at 0.05 for all tests performed. The subdivision of the significance levels was defined in * with $p \leq 0.05$, ** with $p \leq 0.01$ and *** with $p \leq 0.001$. Normal distribution was proved by Kolmogorov-Smirnow test. Normally distributed samples could be further investigated with student *t*-test. Otherwise Welch test was used. For comparison of growth curves, Bonferroni correction was used.

3. Results

3.1. Effects of PDMS on bacterial growth in planktonic cultures

In planktonic cultures with and without PDMS, the growth curves showed different phases of growth. *E. coli* almost reached the end of exponential growth or log phase in the examination time period of 300 min. For all bacteria tested, initial growth was retarded due to lag phase, followed by exponential growth. *S. epidermidis* and especially *P. aeruginosa* had a slower growth rate than *E. coli*. After 300 min growth *P. aeruginosa* was still in the early exponential phase, while *S. epidermidis* has onward progressed in this phase. None of the bacteria reached the stationary phase. This allowed us to investigate the influence of PDMS on growing cultures and a comparison of effects during growth in a statistical end point analysis.

The impact of PDMS on the growth rate differed between the bacterial species. A dose-dependent effect of PDMS could be shown on *E. coli* and *S. epidermidis* as well as for 0.2 to 5 vol-% PDMS on *P. aeruginosa*. Most commonly, higher concentrations inhibited growth stronger than lower concentrations, except for *P. aeruginosa* with 7 vol-% PDMS, which reduced bacterial growth less than 5 vol-%.

A 2 vol-% PDMS supplementation to liquid growth medium significantly reduced the growth rate of *E. coli*, *P. aeruginosa* and *S. epidermidis* ($p < 0.001$; see Fig. 1). Due to the reduced growth of every tested bacterial strain by the used siloxane concentration, we defined 2 vol-% as a sufficient and sensible effective concentration for further tests. After 300 min of incubation in presence of 2 vol-% PDMS the OD of the *P. aeruginosa* culture was reduced by 89.8% compared to the OD without PDMS ($p < 0.001$). *S. epidermidis* showed an OD impairment and growth reduction of 71.5% ($p < 0.001$) and *E. coli* exhibited an impairment of 35.0% ($p < 0.001$). In general, growth of *P. aeruginosa* was reduced the most, closely followed by *S. epidermidis* and followed by distance to both by *E. coli*.

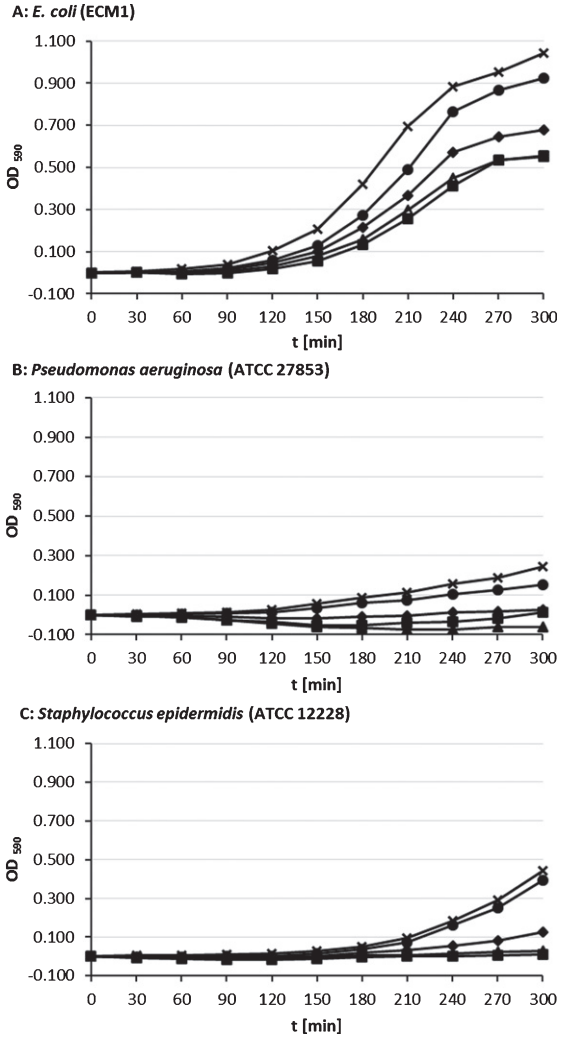

Fig. 1. Growth of planktonic bacteria cultures in glucose nutrient broth with 0 vol-% (x), 0.2 vol-% (●), 2 vol-% (♦), 5 vol-% (▲) and 7 vol-% (■) PDMS supplementation; means of three individual tests with triple measurements, each.

With 7 vol-% supplementation of siloxane maximum growth reduction for *S. epidermidis* (97.4 %; $p < 0.001$) and *E. coli* (45.7%; $p < 0.001$). For *P. aeruginosa* we found a maximum growth reduction with 5 vol-% supplemented PDMS (125 %; $p < 0.001$), whereas bacterial growth with 7 vol-% was less reduced (94.7 %; $p < 0.001$).

We calculated negative values for optical density for *P. aeruginosa* and *S. epidermidis* cultures with 2, 5, and 7 vol-% PDMS, because of blanking against OD of equivalent pre-incubated broth-PDMS mixtures.

3.2. Effects of PDMS on bacterial single species biofilm formation and growth

Even without PDMS supplementation cell mass production of the used bacteria differed strongly. While for planktonic cultures the growth rate was the main determining factor, other biofilm-specific factors became important in this assay. Without PDMS exposition *S. epidermidis* showed the strongest biofilm growth within the time period of testing (18 hrs) followed by *P. aeruginosa*. Intensity of PDMS-

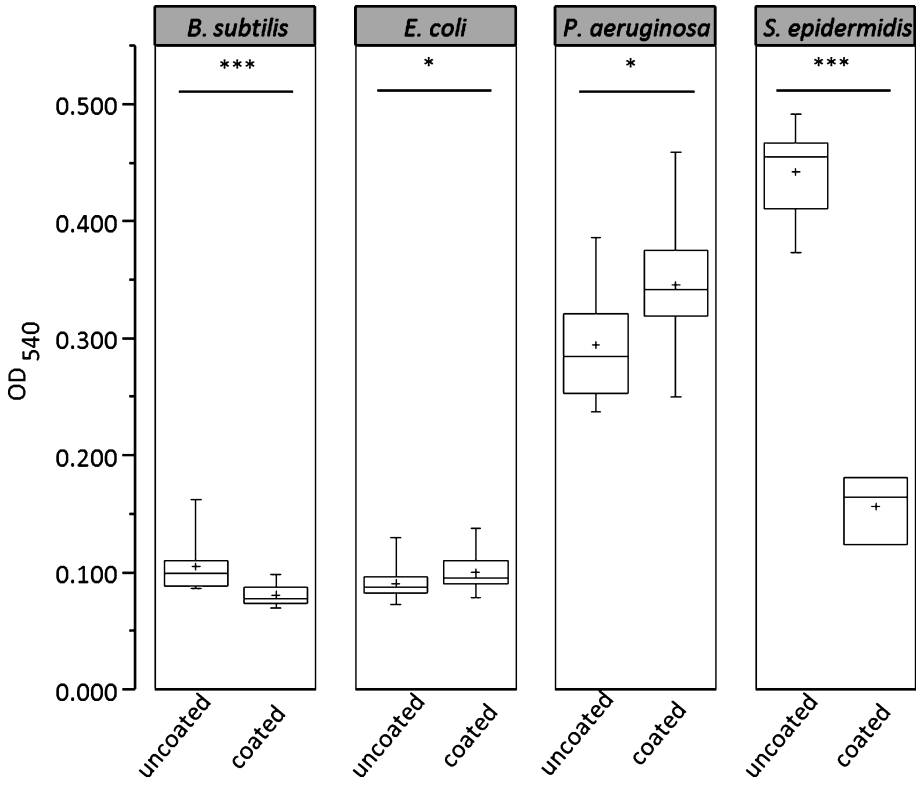

Fig. 2. Growth of *B. subtilis* subsp. *spizizenii*, *E. coli*, *P. aeruginosa* and *S. epidermidis* after 18 hrs of biofilm culture on PDMS-coated/uncoated polystyrene, $n = 8$.

free biofilm growth of *E. coli* and *B. subtilis* was similar and much weaker compared to *S. epidermidis* and *P. aeruginosa* (see Fig. 2).

With PDMS exposition, biofilm growth of *S. epidermidis* and *P. aeruginosa* was again stronger compared to *E. coli* and *B. subtilis*. The impact of PDMS on biofilm production was significant and strongly dependent on the used bacteria. *E. coli* and *P. aeruginosa* showed increased biofilm formation on PDMS coated polystyrene, while *S. epidermidis* and *B. subtilis* in contrast showed significantly reduced biofilm formation. The bacterial cell mass in biofilms formed by *S. epidermidis* and *B. subtilis* was significant reduced by 64.7% (*S. epidermidis*, $p < 0.001$) and 23.1% (*B. subtilis*, $p < 0.001$) on the PDMS-coated surface, whereas cell mass in biofilms of *P. aeruginosa* and *E. coli* was 17.5% (*P. aeruginosa*, $p = 0.011$) and 10.1% (*E. coli*, $p = 0.033$) higher than without surface coating.

4. Discussion

Surface coating for bacterial adherence and growth reduction [3, 10–12] is a promising approach to address the problem of health care-associated infections (HAI's) which are related to invasive medical devices. A coating based on polymerized siloxanes and establishing a hydrophobic and positively charged surface already proved to reduce the number of adherent aerobic nosocomial bacteria on stainless steel, polycarbonate, and soda-lime glass [4]. The study showed that PDMS also affects the growth rate of aerobic nosocomial bacteria, which were grown planktonically, or in biofilms. This confirms results of a study from Yan and Li [13] which exposed *S. epidermidis* and *P. aeruginosa* to silicon oil on blood agar and found PDMS to be antimicrobial. Comparable results were found with

anaerobic bacterial strains (*Propionibacterium acnes, Peptostreptococcus* spp., *Bacteroides fragilis, Fusobacterium* spp., and *Clostridium tertium*) [14]. However, in our study different effects of PDMS on bacterial growth in the different culture assays were obvious.

Less intensive growth in tested planktonic bacteria strengthens the growth inhibition due to PDMS. The inhibitory effect of PDMS on bacterial growth seems to be strongly depended on growth rate. *P. aeruginosa* growth rate is typically slow and we could investigate the strongest inhibitory effect in planktonic cultures onto this bacterial culture. In contrast, growth of *E. coli* with (a typically) shorter generation time compared to the other bacteria used in this study could not be influenced that intensely.

In planktonic culture with addition of PDMS in different concentrations, even the weakest tested concentration of 0.2% PDMS led to a significant growth reduction of *E. coli* and *P. aeruginosa.* Starting from 2 vol-% PDMS growth of *S. epidermidis* was also significantly inhibited. Effect strength increased with increasing PDMS concentration, implying a dose dependent effect. An exception was *P. aeruginosa* incubated with 7 vol-% PDMS, because maximum effect for *P. aeruginosa* was found at 5 vol-% PDMS supplementation.

We calculated negative OD values for some initial points of time in PDMS supplemented cultures of *P. aeruginosa* and *S. epidermidis*. This was due to blanking against pre-incubated PDMS-broth mixtures of the same PDMS concentrations. Negative values are based on three additive factors: Slow growing cultures, cytotoxic effects, and putative changing of refraction index due to PDMS and bacterial cell interactions. Temporarily decline of growth curves can be an indicator for loss of bacterial cell integrity. PDMS seems to induce cell death due to cytotoxic effects, delays reproduction and reduces maximum growth rate respectively, as it was shown for silver nanoparticles in *E. coli* [15]. For *P. aeruginosa* a cytotoxic effect based on silver that diffused from coating to bacteria was presumed [16].

Biofilms and planktonic cultures of *P. aeruginosa* differ in gene expression and therefore metabolism like other bacteria, too [17]. Due to optimal growth and reproduction conditions for single cells, metabolism of planktonic log phase cultures is increased compared to biofilms. The physiological state of cells can lead to considerable variation in the receptiveness of bacteria to bactericides [18]. Reduction of viability seems to take place during active metabolic states like growth and cell division. Biofilm-associated tolerance is different from resistance displayed by planktonic cultures of bacteria, because of modified gene expression, which reduces metabolism and growth rate [19]. Therefore, it can be supposed that biofilm specific factors influenced the effect of PDMS-coating on bacterial growth. It is relevant to mention, that we only have examined single-species biofilms. Presumably, combinations of various species like in natural biofilm communities can lead to improved resistance [20].

Traditionally, biofilm formation is divided into at least three steps: Phase of primary attachment, accumulative phase, and disassembly of biofilm structure [21]. Physical forces like van der Waals forces, steric and electrostatic interactions, bacterial appendages like flagella, pili or fimbriae, as well as adhesive surface structures are essential for initial adherence to surfaces and following biofilm formation [22]. *S. epidermidis* harbors adhesins like the polysaccharide intercellular adhesin (PIA) which is required for enforced biofilm accumulation [21]. Due to hydrophobic and van der Waals interactions it adheres strongly to hydrophobic surfaces like plastics [23]. Autolysin AtlE, a bacterial peptidoglycan-hydrolase, is also of importance for binding on unmodified polystyrene [21]. These characteristics enabled *S. epidermidis* to be the most potent biofilm producer on plastic surface in our study, and explain why *S. epidermidis* is one of the most common cause of biofilm-associated opportunistic infections on indwelling medical devices [24]. However, *S. epidermidis* biofilm growth was most severely affected by 2 vol-% PDMS which led to a reduction of growth for more than 60% compared to the growth without PDMS. In contrast, 2 vol-% PDMS caused an increased biofilm growth of *E. coli* and *P. aeruginosa* while *P. aeruginosa* still exhibiting the stronger growth of both. These results are confirmed by the study of Ozdamar et al. [25] which also approved *S. epidermidis* less resistant to PDMS than *P. aeruginosa*. They addressed the effect of 10 vol-% silicone oil on

different bacteria and after 12 hrs of growth on brain heart infusion agar at 35°C, *S. epidermidis* and *P. aeruginosa* showed an apparent decrease in the number of CFUs, whereby *S. epidermidis* was able to survive for only 5–7 days and *P. aeruginosa* for 7–10 days.

In *P. aeruginosa* at least three polysaccharides (alginate, Pel and PsI) mainly determinate stability of the biofilm structure [22, 26]. Alginate interacts with water and nutrients and supplies nutrients to cells in biofilm [26]. Additionally it reduces the access of toxins like antibiotics to the bacteria and blocks host immune reactions [27].

Strains of *E. coli* are genotypic and phenotypic heterogeneous [28]. Biofilm formation is mediated through motility by flagella, attachment and microcolony formation by type 1 fimbriae, curli and exopolysaccharides like PGA and colonic acid, antigen 43 as well as other factors for later biofilm maturation [29]. Increased biofilm growth after PDMS exposition might have been caused by the large number of hydrophobic amino acid residues on *E. coli* flagella, which makes the surface of *E. coli* very hydrophobic promoting contact of *E. coli* to hydrophobic surfaces like polystyrene and other polymers [30, 31].

B. subtilis is a robust model organism for examination of biofilm formation of Gram-positive bacteria that manifests in highly structured floating pellicles in liquid cultures [32, 33]. However, laboratory 'domesticated' strains of *B. subtilis* are known to form weaker biofilms in contrast to environmental strains [32]. Also in our study, *B. subtilis* ssp. *spizizenii* was a weak biofilm producer. PDMS coating reduced biofilm formation significantly but not as powerful as in *S. epidermidis*. Additionally it is known that in submerged cultures of *B. subtilis* BL53 PDMS enhances oxygen supply improving cell metabolism and growth [34]. Therefore the activity of PDMS as oxygen carrier might have been advantageous for the resistance of *B. subtilis* against the adverse effects of PDMS.

5. Conclusion

PDMS had a significant impact on growth of nosocomial bacteria in planktonic and biofilm culture assays. In planktonic cultures, PDMS exposition resulted in a decreased growth of all tested bacteria in a dose-dependent manner in the used 300 min assay. This antimicrobial effect of PDMS was strongly related to the type of bacteria. Biofilm culture resulted in a modified growth behavior and PDMS resistance, whereby the growth of *E. coli* and *P. aeruginosa* were not adversely affected any more by PDMS as in the planktonic assay. However, these results are based on tests with single species biofilms. Previous to practical application it is necessary to confirm these results by tests in which different bacteria are able to interact like in natural biofilms, because this can cause modified bacterial growth and resistance [20]. Furthermore, research on the interactions on the molecular level could provide more insight into how antimicrobial effects can be explained and exploited.

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