Microcalorimetric detection of staphylococcal biofilm growth on various prosthetic biomaterials after exposure to daptomycin

Ravn, Christen; Ferreira, Inês Santos; Maiolo, Elena; Overgaard, Søren; Trampuz, Andrej

Published in:
Journal of Orthopaedic Research

DOI:
10.1002/jor.24040

Publication date:
2018

Document version:
Accepted manuscript

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:
• You may download this work for personal use only.
• You may not further distribute the material or use it for any profit-making activity or commercial gain.
• You may freely distribute the URL identifying this open access version.

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk

Download date: 28. May. 2021
Research Article

Microcalorimetric detection of staphylococcal biofilm growth on various prosthetic biomaterials after exposure to daptomycin†

Running title: Daptomycin against prosthetic biofilm

Christen Ravn1,2, Inês Santos Ferreira3, Elena Maiolo4, Søren Overgaard1,2, Andrej Trampuz4

1 Department of Orthopaedic Surgery and Traumatology, Odense University Hospital, Denmark
2 Department of Clinical Research, University of Southern Denmark, Denmark
3 Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Portugal
4 Center for Musculoskeletal Surgery, Charité - University Medicine Berlin, Berlin, Germany

Corresponding author:
Christen Ravn (MD): christen.ravn@rsyd.dk, +45 6541 3889
Department of Orthopedic Surgery and Traumatology, Odense University Hospital
Sdr. Boulevard 29, DK-5000 Odense C, Denmark

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jor.24040]

Additional Supporting Information may be found in the online version of this article.

Received 12 October 2017; Revised 3 April 2018; Accepted 17 April 2018
Journal of Orthopaedic Research®
This article is protected by copyright. All rights reserved
DOI 10.1002/jor.24040
Contribution of authors

All authors contributed in protocol writing. Laboratory work was mainly conducted by CR with help from ISF and EM. CR wrote the first draft of the manuscript and all authors have revised it and approved the final version.

Conflict of interest

No competing interests declared
ABSTRACT

Primary aim of this *in vitro* study was to test the efficacy of daptomycin to eradicate staphylococcal biofilms on various orthopedic implant materials. Secondary aim was to quantitatively estimate the formation of staphylococcal biofilm. We tested six clinically important biomaterials: cobalt chrome, pure titanium, grid-blasted titanium, porous plasma-coated titanium with/without hydroxyapatite, and polyethylene. Biofilms of *S. aureus* and *S. epidermidis* were formed on the samples and thereafter exposed to daptomycin. Samples were subsequently sonicated in order to detect dislodged biofilm bacteria and transferred to a microcalorimeter for real-time measurement of growth-related heat flow. Minimal biofilm eradication concentration (MBEC) was determined as the lowest concentration of daptomycin required to eradicate biofilm bacteria on the sample. Median MBEC of *S. aureus* biofilm on smooth metallic surfaces was lower than the rough metallic surfaces. In experiments with *S. epidermidis*, no pattern was seen in relation to the surface roughness. Regarding the quantitative estimation of staphylococcal biofilm formation on the sample, we found a significantly higher amount of biofilm growth on the rough surfaces than the smooth samples and polyethylene.

In conclusion, the presented study showed that daptomycin could eradicate *S. aureus* biofilm at lower concentrations on the smooth surfaces compared to the rough surfaces, as well as polyethylene. In experiments with daptomycin against *S. epidermidis* biofilms, no pattern was seen in relation to the surface roughness. Furthermore, we demonstrated a faster detection of staphylococcal heat flow due to higher biofilm quantity on the rough surfaces compared to smooth samples and polyethylene. This article is protected by copyright. All rights reserved.

**Keywords:** Biofilm, prosthesis, sonication, microcalorimetry, daptomycin
INTRODUCTION

Prosthetic biomaterials are increasingly implanted to restore joint function in a growing elder population.\textsuperscript{1,2} Developments in orthopedic arthroplasty surgery have not only increased the mechanical properties and biocompatibility of implant materials, but also improved the surface texture for tissue integration.\textsuperscript{3,4}

Factors responsible for metallic implant fixation include physico-chemical bonding mechanisms such as surface charge, hydrophobicity, roughness and porosity.\textsuperscript{3,5} These surface-related properties for implant-tissue integration are also believed to influence the ability for microbial adhesion in the ‘race for the surface’.\textsuperscript{6,7}

Bacteria that adhere to a prosthetic implant grow predominantly in slime-enclosed biofilms. These adherent biofilms are inherently resistant to host defenses and to conventional antibiotic therapy.\textsuperscript{8} Comparison and quantification of surface-adherent bacteria on different prosthetic materials was previously performed in experimental and clinical studies.\textsuperscript{9,10} In these studies sonication was used for biofilm dislodgement in order to make the surface-attached bacteria eligible for incubation and quantification.

Isothermal microcalorimetry and sonication were recently used to analyze the influence of bone graft material properties on the initial adhesion and formation of biofilm under \textit{in vitro} and \textit{in vivo} settings.\textsuperscript{11-14} Microcalorimetry is a highly sensitive and accurate method for the detection of slow-growing microorganisms and reduced bacterial numbers after antibiotic pre-exposure.\textsuperscript{15-17} The principle of microcalorimetry relies on microbial heat production related to bacterial growth and metabolism. Without disturbing the growth process, real-time heat flow measurement can estimate bacterial quantity in surface-related biofilms. Decreasing number of replicating bacteria, e.g. due to inhibition after antimicrobial exposure, is equivalent to decreased calorimetric heat production and/or delayed heat flow. Furthermore, our study group recently applied this analytical tool when a strong suppressive effect of daptomycin was observed.
against staphylococcal biofilm growth on porous glass beads was demonstrated under in vitro conditions.\textsuperscript{18}

Daptomycin is a fast acting, concentration dependent lipopeptide with potent activity against Gram-positive bacteria. Daptomycin targets the cell membrane rather than the metabolic active pathways, making it more active against metabolically stationary bacteria, such as biofilm bacteria.\textsuperscript{19} Though previous in vitro studies have demonstrated superior activity of daptomycin against staphylococcal biofilms,\textsuperscript{20-22} the influence of physicochemical properties of orthopedic implant materials on the efficacy of daptomycin to eradicate staphylococcal biofilms has not been systematically elucidated. In the present study we directly investigate the ability for daptomycin to inhibit (delayed heat production) and eradicate (no heat production) mature staphylococcal biofilms after 24 hours.

The primary aim of this in vitro study was to test the efficacy of daptomycin to eradicate staphylococcal biofilms on various orthopedic implant surfaces and materials. The secondary aim was to quantitatively estimate the formation of staphylococcal biofilm on various implant materials with different surface properties.

We hypothesized that bacterial growth and biofilm formation on prosthetic materials would vary with the different surface properties, and also be variably influenced by exposure to the potent antimicrobial effect of high-dose daptomycin.
MATERIALS AND METHODS

Biomaterials and test organisms.

We tested six clinically available orthopedic implant materials (Table 1) that were purchased at the manufacturers (Biomet Aps, Horsens, Denmark; Ortotech, Kolding, Denmark).

Cylindrical test samples were custom-made to fit in a microcalorimeter ampoule (L 10 mm, Ø 6 mm, surface area 270 mm$^2$) and sterilized at 121°C for 20 minutes.

Staphylococcal sp. are the most common findings in culture of periprosthetic tissue samples during PJI revision surgery.23 We investigated two well-characterized reference strains of S. aureus (ATCC 29213, methicillin-susceptible) and S. epidermidis (ATCC 35984, methicillin-resistant).13 These ATCC-strains, capable of biofilm production through adherence to and aggregation on biomaterial surfaces, have been used in numerous experimental studies,12,13,24 and were considered representative of staphylococcal species commonly causing PJI. The susceptibility of these strains has been determined in a previous experimental study analyzing the inhibitory effect of daptomycin against a 24 hours biofilm compared to the minimal inhibitory concentration (MIC) of planktonic bacteria.18 MIC for daptomycin against S. aureus and S. epidermidis was assessed as 0.5 µg/ml and 1.0 µg/ml respectively, whereas the minimal heat inhibitory concentration (MHIC) measured by microcalorimetry with sonication fluid was respectively 128 and 64 times higher than MIC.

The bacterial strains were stored at -80°C and cultured overnight on blood agar plates before each experiment. Hereafter a bacterial test-suspension was prepared by 1:100 dilution of a 0.5 McFarland solution. Hence bacterial concentrations in each test-suspension were approximately 1x10$^6$ CFU/ml of S. aureus and 3x10$^5$ CFU/ml of S. epidermidis respectively.

Biofilm formation on test samples. An illustration of the experimental protocol is found in the Electronic supplementary material. For each experiment 6 samples were placed in a 50 ml Falcon tube containing 5 ml tryptic soy broth (TSB), inoculated with 0.25 ml of the
bacterial test-suspension and incubated aerobically for 24 hours at 37°C. After the incubation, samples were washed 5 times through rinsing with 20 ml saline and gentle shaking in order to minimize carry-over of planktonic and loosely attached bacteria on the biomaterial surface. After the washing step, one of the samples was selected to act as growth control during the rest of the experiments.

**Antibiotic exposure of biofilm.** Serial two-fold dilutions of daptomycin (Novartis Pharma AG, Bern, Switzerland) were prepared in TSB with concentrations ranging from 4x MIC up to 256x MIC. As daptomycin belongs to a calcium-dependent antibiotic group \(^{25}\), the TSB that received daptomycin, was enriched with calcium chloride (0.3 µg/ml [equivalent to 0.1 µg/ml of Ca\(^{2+}\)]). Samples with biofilm embedded bacteria were transferred with sterile forceps to seven individual 4 ml tubes (containing 2 ml TSB and different antibiotic concentrations) and further incubated for 24 hours at 37°C. The eighth tube contained TSB without daptomycin, and the submerged sample acted as growth control when continued incubation resulted in formation of a 48-hour biofilm. Experiments were performed independently in triplicate and accompanied with a negative control of one sterile sample in antibiotic-free medium.

**Biofilm dislodgement by sonication.** After antibiotic exposure test samples were transferred to individual 15 ml Falcon tubes with 3 ml saline, vortexed 30 s with maximum power (Vortex Genie 2, Scientific Industries, Bohemia NY, USA), sonicated for 60 s (BactoSonic™, Bandelin electronic, Berlin, Germany) and vortexed for 30 s again to dislodge biofilm embedded bacteria. The mild sonication process made loosely attached biofilm bacteria eligible for plate incubation, whereas more firmly attached biofilm bacteria remained on the material samples for subsequent microcalorimetric evaluation. Sonication fluid in 100 µl aliquots was plated on blood agar plates to detect growth of dislodged biofilm bacteria that survived the antibiotic exposure. Biofilm bactericidal concentration (BBC) was measured as +/- growth in sonication fluid after 24 hours incubation on blood-agar plates.

This article is protected by copyright. All rights reserved
Detection of biofilm bacteria by microcalorimetry. The sonicated test samples were transferred to individual 4 ml glass ampoules containing 1 ml TSB. After sealing the ampoules, they were lowered into a 48-channel batch microcalorimeter (thermal activity monitor, model 3102 TAM III; TA Instruments, New Castle, DE). The initial 15 minutes of equilibration ensured a temperature of 37.0000°C to minimize exogenous heat disturbance before the measurements started. Heat was measured continuously for 24 hours with an analytical sensitivity of ±0.2 µW. The results were plotted as heat flow (in µW) over time using the manufacturer’s software (TAM Assistant; TA Instruments) and analyzed with Microsoft Excel 2013 (Microsoft Corporation, Richmond, USA).

The minimal biofilm eradication concentration (MBEC) was defined as the lowest antimicrobial concentration killing biofilm bacteria on the sample, leading to absence of regrowth after 24 hours of incubation in the microcalorimeter, indicated by the absence of growth-related heat flow.\textsuperscript{26}

To distinguish microbial heat production from the thermal background an experimental threshold was set at 10 µW and all measurements below this value were recorded as negative. The time to detection (TTD-50) was defined as the time from the insertion of the ampoule into the calorimeter until the exponentially rising heat flow signal exceeding 50 µW. This measure indirectly quantifies the amount of biofilm bacteria, with a shorter TTD-50 representing a larger amount of bacteria.\textsuperscript{11} The maximum heat flow during the experiment, heat flow peak (HFP), is specific to the strain and reduced with decreasing biofilm fitness due to growth inhibition factors, such as antibiotic exposure.\textsuperscript{27}
STATISTICS

MBEC-data on the ordinal scale from 2-256 µg/ml daptomycin are expressed as median and range. Heat flow (µW) and time (hours) are continuous numeric data presented as mean ± standard deviation (SD) and compared between groups by standard (non-parametric) ANOVA. Post-hoc mutual comparisons were performed by unpaired t-test and Wilcoxon test adjusting for multiple comparisons using the statistical software R (www.R-project.org) and Prism 7.0 (GraphPad Software, San Diego, CA, USA) for comparison of mean HFP and mean TTD-50 of S. aureus and S. epidermidis biofilms. P-value <0.05 was accepted as significant.
RESULTS

In the present study we directly investigate the efficacy of daptomycin to inhibit (delayed heat production) and eradicate (no heat production) mature staphylococcal biofilms after 24 hour incubation on various orthopedic implant surfaces and materials.

The efficacy of daptomycin to eradicate staphylococcal biofilms. Figure 1 A-D show four representative heat flow curves of viable biofilm bacteria on the samples after antibiotic exposure. The time shift of curves to the right shows the delayed bacterial detection due to lower quantity of biofilm bacteria on the test samples after exposure to increasing concentrations of daptomycin. This picture shows that bacterial regrowth was successively inhibited until the lowest antimicrobial concentration killing biofilm bacteria on the sample, indicated by absence of growth-related heat flow, which is defined as the MBEC. Median MBEC of daptomycin against staphylococcal biofilms on various orthopedic implant surfaces and materials is displayed in Table 2.

Comparison of the MBEC of daptomycin against S. aureus biofilms on various test samples show that variations are related to the surface structure of the material used (Figure 2A). With smooth metallic surfaces of cobalt-chrome and pure titanium regrowth of S. aureus biofilm was absent at 4-8 µg/ml, equivalent to daptomycin concentrations 8-16 times higher than the MIC (0.5 µg/ml). With the remaining test samples having a macroscopic rougher surface structure the MBEC of S. aureus biofilms to daptomycin was higher (32-256 µg/ml). In experiments with S. epidermidis biofilms (Figure 2B) the MBEC variated from 8 to 256 µg/ml, but no pattern was seen in relation to the surface roughness.

Quantitative estimation of staphylococcal biofilm. The heat flow curves of growth controls without previous antibiotic exposure (broken lines in Figure 1 A-D) have a characteristic
shape for each test bacterium, including the heat flow peak. Table 3 shows heat flow of staphylococcal biofilm on different materials without antibiotic exposure. HFP did not vary significantly in experiments with the same bacterial strain on different test materials. On the contrary when comparing growth-related heat flow of *S. aureus* and *S. epidermidis*, mean (±SD) HFP showed significant difference among the strains (202 ±22 µW and 105 ±9 µW respectively; *p*<0.001).

The bacterial amount on the biofilm embedded test samples was indirectly quantified by microcalorimetric analysis of the time to reach 50 µW (TTD-50) after 24 hour incubation. A highly viable and quantitatively strong biofilm has faster initial heat flow development due to growth and metabolism than a weak and more dormant biofilm. Initial heat flow developed faster with *S. epidermidis* biofilms (mean TTD-50: 2.9 ±1.3 hours) compared to *S. aureus* (4.2 ±2.2 hours).

**Figure 3A** shows variations of TTD-50 in triplicate experiment with *S. aureus* biofilm on various materials. The heat flow developed differently between the surfaces (Friedman’s test, *p*<0.01) and post hoc comparisons showed that the heat flow developed faster with the rough surfaces (gbTi, pcTi and pcTi-HA) in comparison to the smooth surfaces (CoCr, puTi) and polymer (UHMWPE) (*p*<0.001, Bonferroni corrected). This indicates a higher quantity of *S. aureus* biofilm on the macroscopically rough surfaces solely, as the smooth surfaces (CoCr, puTi) and polymer (UHMWPE) did not indicate any difference (*p*>0.2). Regarding *S. epidermidis* (**Figure 3B**), we also observed significantly different heat flow development between the surfaces (Friedman's test, *p*=0.03). Post hoc comparisons showed that the heat flow developed faster with the rough surfaces (gbTi and pcTi-HA) in comparison to 2/3 of the smooth surfaces (CoCr, puTi) and polymer (UHMWPE) (*p*<0.01, Bonferroni corrected). TTD-50 for CoCr, puTi, pcTi and UHMWPE did not indicate any difference (*p*>0.40).
The vortex-sonication method allowed visual growth-detection of dislodged bacteria on blood agar plates after 24 hour incubation. These results confirmed those obtained by microcalorimetry (data not shown).
DISCUSSION

In the present *in vitro* study we directly investigated the efficacy of daptomycin to eradicate mature staphylococcal biofilms after 24 hour incubation on various orthopedic implant surfaces and materials. With *S. aureus* biofilm on smooth metallic surfaces, the MBEC of daptomycin was 4-8 µg/ml, whereas the efficacy of daptomycin against *S. aureus* biofilm on test samples with rough surface structure was lower (MBEC 32-256 µg/ml). In experiments with *S. epidermidis* biofilms, MBEC varied from 8 to 256 µg/ml, but no pattern was seen in relation to the surface roughness.

We furthermore examined microbial heat production of the antibiotic-free growth controls in order to investigate quantitative variations of staphylococcal biofilm formation. With *S. aureus* biofilms we found a significantly higher heat flow rate on the rough surfaces than the smooth samples and polyethylene. In experiments with *S. epidermidis* biofilms, the heat flow rate showed less variation, but again we observed a significantly higher heat flow rate on 2/3 of the rough samples.

We investigated mature staphylococcal biofilms on six clinically important orthopedic implant materials, namely cobalt chrome alloy, pure titanium, grid-blasted titanium, porous plasma-coated titanium without/with hydroxyapatite, and ultra-high molecular weight polyethylene. We hypothesized that bacterial growth and biofilm formation on prosthetic materials would vary with the different surface properties, and would also be variably influenced by the exposure to the potent antimicrobial effect of daptomycin.

The biofilm evaluations were performed according to a well-established protocol including sonication, culture and microcalorimetric heat flow measurement previously used to analyze the influence of physicochemical biomaterial properties, as well as studies of antimicrobial susceptibility of biofilm bacteria.

This article is protected by copyright. All rights reserved
We were able to demonstrate microbial heat production during growth and regrowth of biofilm bacteria on all biomaterials after 24 hour incubation. In order to minimize the influence of free-floating and loosely attached planktonic bacteria, samples underwent a 5-step washing procedure. After a 24-hour antibiotic exposure the more loosely attached bacteria were removed during a mild sonication process. Thus leaving the more firmly attached biofilm bacteria for subsequent microcalorimetric investigation. We benefited from the ability to insert the biofilm-covered samples into the microcalorimeter, as we measured the heat flow directly from bacterial growth on the samples rather than detached bacteria in sonication fluid.

Limitations of this study design include lack of important in vivo conditions, such as antimicrobial pharmacokinetics (dose, tissue penetration, repeated administration, duration of treatment) and host factors (tissue reaction, immune response). Furthermore different production methods were applied by the manufacturers, hence the test samples of four materials were provided with a threaded canal with resulting alteration in the macroscopic surface structure and surface area. Finally, during microcalorimetric measurement of real-time heat flow, direct demonstration of biofilm bacteria by traditional fluorescent staining and microscopy is not applicable.

**The efficacy of daptomycin to eradicate staphylococcal biofilms.** The range of tested daptomycin concentrations in this study runs from 4-256 µg/ml in two-fold steps of categorical variables, and interpretation of MBEC results should be seen in this light. Thus median MBEC of 128 µg/ml (range 64-256) correlate with median plus/minus one concentration step. Seen from a clinical point of view, a local tissue concentrations of daptomycin above e.g. 20 µg/ml is hardly achievable, though.29

This article is protected by copyright. All rights reserved
Median MBEC of daptomycin was found at 128 µg/ml in 7 out of 12 different combinations of material and staphylococcal spp. Similar levels of daptomycin susceptibility have been reported in other experimental studies of staphylococcal biofilms.\textsuperscript{18,21,30} The experimental detection limit was determined at 10 μW to distinguish microbial heat production from the thermal background (e.g., nonspecific heat flow generated by degradation of the growth medium). We cannot exclude that discrete metabolism in viable but non-culturable bacteria is neglected due to this detection limit, and complete bacterial eradication hence was not achieved at the reported MBEC levels.

Biofilms of \textit{S. aureus} on smooth metallic surfaces were eradicated at lower daptomycin concentrations (8-16 times MIC) compared to rough/porous metallic surfaces (64-512 times MIC). This finding is in line with theories of biofilm resistance and tolerance mechanisms including incomplete penetration and altered chemical microenvironment in the extracellular biofilm matrix.\textsuperscript{27,31,32} With \textit{S. epidermidis} biofilms, heterogeneity was observed in daptomycin susceptibility and variations could not be correlated to the surface structure in our study. Whether this difference between the bacterial strains is an effect of physicochemical properties which has not been taken into account or simply a matter of measuring variation is however unclear. Biofilm formation is a multifactorial process, and opposing physicochemical properties related to the sample materials (e.g. hydrophobicity, charge and pH) or unintendedly by the study protocol (e.g. chemical composition of culture medium) may blur the overall results in this experimental study.

**Quantitative estimation of staphylococcal biofilm.** In order to investigate quantitative variations of staphylococcal biofilm formation on different biomaterials we examined microbial heat production of the antibiotic-free growth controls. When studying biofilm with microcalorimetry, duration of an experiment to reach a certain heat flow limit or heat flow peak are inversely proportional to the bacterial quantity and growth rate.\textsuperscript{13,15,33} TTD-50 was
defined as the time from insertion of an ampoule into a microcalorimeter until exponential
growth produced a heat flow value of 50 μW. This initial lag phase exceeds the experimental
threshold of 10 μW in order to take into account the heterogeneous starting point of those
experiments that already produced high amounts of heat flow when measurements started
after the equilibration period.\textsuperscript{11}

TTD-50 was used to compare biofilm performance on different biomaterials. We found
significantly lower time variables when grid-blasted and porous titanium samples were used
with \textit{S. aureus} and \textit{S. epidermidis} (except the combination of \textit{S. epidermidis} and \textit{pcTi}). This
indicates a higher surface area, biofilm affinity and holding capacity of rough/porous metallic
surfaces in comparison to smooth surfaces and polyethylene. Our findings confirmed previous
reports showing a higher staphylococcal biofilm production on biomaterials with rough and
porous topography due to more available biofilm binding-sites.\textsuperscript{9,13,34}

Factors determining biofilm formation specifically on polyethylene includes altered
hydrophobicity and surface charge, which in turn is differently influencing bacterial
subspecies.\textsuperscript{7,35} Clinical reports in the literature do not agree whether polyethylene components
of infected joint prosthesis should be regarded as more or equally prone to biofilm formation
than other prosthetic materials.\textsuperscript{10,36,37} Recent \textit{in vitro} studies found reduced adhesive ability of
\textit{S. aureus} and \textit{S. epidermidis} on vitamin E blended UHMWPE in comparison to standard
UHMWPE samples.\textsuperscript{38,39}

In conclusion, the presented study showed that daptomycin could eradicate \textit{S. aureus} biofilm
at lower concentrations on the smooth surfaces of CoCr and puTi compared to the rough
surfaces of gbTi, pcTi and pcTi-HA, as well as polyethylene. In experiments with daptomycin
against \textit{S. epidermidis} biofilms, no pattern was seen in relation to the surface roughness.
Furthermore, we demonstrated a significantly faster detection of staphylococcal heat flow due
to higher biofilm quantity on the rough surfaces compared to smooth samples and

This article is protected by copyright. All rights reserved
polyethylene. This is an indication of a higher biofilm affinity and holding capacity of rough surfaces. Findings in the present study indicate that orthopedic biofilm infections may not be sufficiently treated with parenteral administration of daptomycin in clinically achievable doses alone.
Acknowledgements

We thank Ulrika Furustrand Tafin, Sabrina Cabric and Bertrand Bétrisey for valuable laboratory assistance. We also thank for statistical advice from Professor Jacob Hjelmborg, Department of Public Health, Epidemiology and Biostatistics, University of Southern Denmark.

Conflict of interest

Financial support was provided by the Regional Foundation of Southern Denmark, and the Danish Rheumatism Association. None of the authors have professional or financial affiliation to bias the presentation.
Reference list:


This article is protected by copyright. All rights reserved


Figure legends:

**Electronic supplementary material:** Schematic overview of the study protocol. A: Biofilm formation (24 h). B: Gentle washing. C: Exposure to daptomycin (24 h). D: Sonication. E: Culture of sonication fluid. F: Microcalorimetry of samples (24 h).

**Figure 1 A-D:** Heat flow-time curves generated by *S. aureus* (A+B) and *S. epidermidis* (C+D) biofilm on pure titanium and porous plasma-coated titanium after exposure to increasing daptomycin concentrations (8-256 µg/ml). Patterns indicate daptomycin concentrations (in µg/ml). GC denotes growth control (biofilm-covered sample submerged in TSB without daptomycin).

**Figure 2 A-B:** Minimal biofilm eradication concentration (MBEC, median and range) of daptomycin against staphylococcal biofilms in triplicate experiment (Exp. 1-3).

**Figure 3 A-B:** Microcalorimetric analysis of staphylococcal biofilm in triplicate experiment. Time to detection (TTD-50, mean ± SD) indicates the time needed to reach the heat flow of 50 µW.
**Table legends**

**Table 1:** Specifications of the 6 implant materials.

*Four test samples were due to the production method provided with a threaded canal.*

**Table 2:** Minimal biofilm eradication concentration of daptomycin

Results from sonication and microcalorimetry on biofilm embedded test samples after exposure to increasing daptomycin concentrations (µg/ml), displayed as median (range). Test samples consisted of various biomaterials: cobalt-chrome (CoCr), pure titanium (puTi), grid blasted titanium (gbTi), porous plasma coated titanium (pcTi), porous plasma coated titanium with hydroxyapatite (pcTI-HA), and ultra-high molecular weight polyethylene (UHMWPE).

**Table 3:** Microcalorimetric analysis of biofilm formation on different biomaterials

Heat flow of bacterial growth on sonicated samples with no antibiotic exposure. Time to detection (TTD-50): Experimental duration (hours) when heat flow exceeds 50 µW. Heat flow peak (HFP): Maximum heat flow peak reached during the experiment. Cobalt-chrome (CoCr), pure titanium (puTi), grid blasted titanium (gbTi), porous plasma coated titanium (pcTi), porous plasma coated titanium with hydroxyapatite (pcTI-HA), ultra-high molecular weight polyethylene (UHMWPE).
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Material</th>
<th>Surface structure</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCr</td>
<td>Cobalt-chrome alloy</td>
<td>Smooth, metal*</td>
<td>Biomet</td>
</tr>
<tr>
<td>puTi</td>
<td>Pure titanium</td>
<td>Smooth, metal</td>
<td>Ortotech</td>
</tr>
<tr>
<td>gbTi</td>
<td>Grid blasted titanium</td>
<td>Rough, metal*</td>
<td>Biomet</td>
</tr>
<tr>
<td>pcTi</td>
<td>Porous plasma coated titanium</td>
<td>Porous, metal*</td>
<td>Biomet</td>
</tr>
<tr>
<td>pcTi-HA</td>
<td>Pc titanium w/ hydroxyapatite</td>
<td>Porous, metal*</td>
<td>Biomet</td>
</tr>
<tr>
<td>UHMWPE</td>
<td>Ultra-high molecular weight</td>
<td>Rough, hydrophobic</td>
<td>Biomet and Ortotech</td>
</tr>
</tbody>
</table>

*Four test samples were due to the production method provided with a threaded canal.
### Table 2: Minimal biofilm eradication concentration of daptomycin

<table>
<thead>
<tr>
<th>MBEC (mg/l)</th>
<th><em>S. aureus</em> (ATCC 29213)</th>
<th><em>S. epidermidis</em> (ATCC 35984)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCr</td>
<td>8 (8;8)</td>
<td>128 (64;128)</td>
</tr>
<tr>
<td>puTi</td>
<td>4 (4;8)</td>
<td>128 (128;256)</td>
</tr>
<tr>
<td>gbTi</td>
<td>128 (32;128)</td>
<td>16 (8;64)</td>
</tr>
<tr>
<td>pcTi</td>
<td>128 (128;256)</td>
<td>128 (128;256)</td>
</tr>
<tr>
<td>pcTi-HA</td>
<td>128 (128;128)</td>
<td>64 (64;64)</td>
</tr>
<tr>
<td>UHMWPE</td>
<td>64 (64;64)</td>
<td>128 (128;128)</td>
</tr>
</tbody>
</table>

Results from sonication and microcalorimetry on biofilm embedded test samples after exposure to increasing daptomycin concentrations (µg/ml), displayed as median (range). Test samples consisted of various biomaterials: cobalt-chrome (CoCr), pure titanium (puTi), grid blasted titanium (gbTi), porous plasma coated titanium (pcTi), porous plasma coated titanium with hydroxyapatite (pcTI-HA), and ultra-high molecular weight polyethylene (UHMWPE).
Table 3: Microcalorimetric analysis of biofilm formation on different biomaterials

<table>
<thead>
<tr>
<th></th>
<th>TTD-50 (hour)</th>
<th>HFP (µW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. aureus (mean ±SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoCr</td>
<td>6.7 ±0.2</td>
<td>180 ±3</td>
</tr>
<tr>
<td>puTi</td>
<td>6.8 ±0.5</td>
<td>180 ±22</td>
</tr>
<tr>
<td>gbTi</td>
<td>3.6 ±0.5</td>
<td>200 ±7</td>
</tr>
<tr>
<td>Rough/porous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pcTi</td>
<td>1.9 ±0.1</td>
<td>228 ±13</td>
</tr>
<tr>
<td>pcTi-HA</td>
<td>1.2 ±0.6</td>
<td>208 ±19</td>
</tr>
<tr>
<td>Polymer</td>
<td>me</td>
<td></td>
</tr>
<tr>
<td>UHMWPE</td>
<td>5.3 ±0.5</td>
<td>214 ±9</td>
</tr>
<tr>
<td>Overall, S. aureus</td>
<td>4.2 ±2.2</td>
<td>202 ±22</td>
</tr>
</tbody>
</table>

|                  |               |          |
| **S. epidermidis (mean ±SD)** |               |          |
| Smooth           |               |          |
| CoCr             | 3.7 ±0.2      | 103 ±6   |
| puTi             | 4.1 ±1.4      | 113 ±10  |
| gbTi             | 1.5 ±0.6      | 103 ±6   |
| Rough/porous     |               |          |
| pcTi             | 3.2 ±0.1      | 103 ±7   |
| pcTi-HA          | 1.3 ±0.8      | 111 ±3   |
| Polymer          | me           |          |
| UHMWPE           | 3.4 ±0.2      | 98 ±8    |
| Overall, S. epidermidis | 2.9 ±1.3 | 105 ±9   |

Heat flow of bacterial growth on sonicated samples with no antibiotic exposure. Time to detection (TTD-50): Experimental duration (hours) when heat flow exceeds 50 µW. Heat flow peak (HFP): Maximum heat flow peak reached during the experiment. Cobalt-chrome (CoCr), pure titanium (puTi), grid blasted titanium (gbTi), porous plasma coated titanium (pcTi), porous plasma coated titanium with hydroxyapatite (pcTi-HA), ultra-high molecular weight polyethylene (UHMWPE).
S. aureus biofilm on pure titanium

Figure I(A)
Figure 1(B)

S. aureus biofilm on porous coated titanium

Heat flow (µW)

Time (hr)
Figure 1(C)

S. epidermidis biofilm on pure titanium

Heat flow (µW)

Time (hr)
Figure 1(D)

S. epidermidis biofilm on porous coated titanium
Figure 2(A)

S. aureus biofilm
MBEC of daptomycin

MBEC (µg/ml)

0 32 64 96 128 160 192 224 256

CoCr pTi gbTi pcTi pcTi-HA UHMWPE

Smooth surfaces Rough surfaces Polymers

Exp. 1 Exp. 2 Exp. 3

This article is protected by copyright. All rights reserved

Page 33 of 36
Figure 2(B)

S. epidermidis biofilm
MBEC of daptomycin

MBEC (µg/ml)

CoCr  puTi  gbTi  pcTi  pcTi-HA  UHMWPE

Smooth surfaces  Rough surfaces  Polymers

Exp. 1  Exp. 2  Exp. 3

This article is protected by copyright. All rights reserved
Figure 3(A)

S. aureus biofilm
mean time to detection ±SD

<table>
<thead>
<tr>
<th>CoCr</th>
<th>pTi</th>
<th>gbTi</th>
<th>pcTi</th>
<th>pcTi-HA</th>
<th>UHMWPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth surfaces</td>
<td>Rough surfaces</td>
<td>Polymer</td>
<td>Smooth surfaces</td>
<td>Rough surfaces</td>
<td>Polymer</td>
</tr>
</tbody>
</table>

TTD-50 (hr)
Figure 3(B)

S. epidermidis biofilm
mean time to detection ±SD

![Graph showing S. epidermidis biofilm mean time to detection for different surfaces.](image-url)