Preventing Biofilm Formation and Associated Occlusion by Biomimetic Glycocalyxlike Polymer in Central Venous Catheters

Ashwini Chauhan, Aude Bernardin, Windy Mussard, Irène Kriegel, Marc Estève, Jean-Marc Ghigo, Christophe Beloin, Vincent Semetey

Preventing biofilm formation and associated occlusion by biomimetic glycocalyx-like polymer in central venous catheters

Ashwini Chauhan[a], Aude Bernardin[b], Windy Mussard[b], Irène Kriegel[c], Marc Estève[c], Jean-Marc Ghigo[a], Christophe Beloin[a,1,*] and Vincent Semetey[b,d,1,*]

[a] Institut Pasteur, Unité de Génétique des Biofilms. 25 rue du Dr. Roux, 75724 Paris cedex 15 France
[b] Institut Curie, Centre de Recherche, France.
[c] Institut Curie, Hôpital, Service Anesthésie-Réanimation-Douleur, Paris, France
[d] CNRS, UMR 168, Laboratoire de Physicochimie « Curie », Paris, France.

[1] These authors contributed equally to this work

[*] To whom correspondence should be addressed to C.B. (cbeloin@pasteur.fr) or V.S. (vincent.semetey@curie.fr).

Authors do not declare any conflict of interest

Funding statement: This work was supported by ANR grants (ANR-07-EMPB-004-01), (ANR-08-PCVI-0012), the Institut Curie/Institut Pasteur (PIC/PTR maladies nosocomiales) and by the French Government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant n°ANR-10-LABX-62-IBEID).

Running title: Biomimetic bacterial anti-adhesive strategy

Abstract- 150 words

The use of catheters and other implanted devices is constantly increasing in modern medicine. Although catheters improve patients' healthcare, the hydrophobic nature of their surface material promotes protein adsorption and cell adhesion. Catheters are therefore prone to complications such as colonization by bacterial and fungal biofilms, associated infections and thrombosis. Here we describe the *in vivo* efficacy of bio-inspired glycocalyx-like anti-adhesive coatings to inhibit *Staphylococcus aureus* and *Pseudomonas aeruginosa* colonization on commercial totally implantable venous access ports (TIVAP) in a clinically relevant rat model of biofilm infection. While non-coated TIVAP implanted in rats were heavily colonized by the two biofilm-forming pathogens with high percentage of occlusion, coating of TIVAP reduced their initial adherence and subsequently led to 4-log reduction in biofilm formation and reduced occlusion. Our anti-adhesive approach is a simple and generalizable strategy that could be used to minimize clinical complications associated with the use of implantable medical devices.

Key words: Methylcellulose, biofilms, medical devices, anti-adhesion

Text-3401 words

Introduction

The use of totally implantable venous access ports (TIVAP) has led to great improvement in healthcare of patients admitted to oncology, nephrology and intensive care units due to its ease when administering fluids and performing blood sampling. However, its use may be associated with significant complications, most notably infection, occlusion and thrombosis [1, 2]. TIVAP can be colonized by pathogenic microorganisms mainly *via* endoluminal contamination, which leads to development of complex bacterial and fungal biofilm communities. Biofilms display high tolerance towards the immune system and various antimicrobials and are thus difficult to eradicate [3, 4]. Moreover, biofilms present on the surfaces of medical devices are potential sources of bloodstream infection, a leading cause of healthcare-associated infection in critically ill patients [5]. Currently, there is no fully efficient method for treating catheter-related biofilms besides traumatic and costly removal of colonized devices [6]. Recent clinical practice guidelines recommend the use of antibiotic lock therapy for uncomplicated long-term catheter-related infections [7], and various antimicrobial lock solutions have been developed with promising results [8-11]. Alternative preventive strategies make use of modifications in medical device surfaces designed to kill bacteria upon surface attachment due to impregnation/incorporation of antibiotics or other antimicrobials such as silver salts, chlorhexidine [12-14], minocycline and rifampin [15, 16]. Although some of these biocidal coatings have shown promise *in vitro* and could reduce catheter-related biofilm infections, they often present substantial limitations, including leaching (limiting protection over time), antibiotic resistance [17] and safety and regulatory problems [18, 19]. Recent advances have led to the emergence of promising non-biocidal anti-adhesive strategies applied to biomaterial in the context of implantation aimed at reducing protein adsorption and cell adhesion implicated in numerous clinical complications

[20-23]. The external layer of a cell membrane, known as the glycocalyx, is composed of polysaccharides and prevents undesirable protein adsorption and non-specific cell adhesion *via* steric repulsion [24]. Others and we previously showed that treatment of abiotic surfaces *via* the adsorption of different bacterial polysaccharides has a long-lasting effect to significantly inhibit mature biofilm development of a broad-spectrum of pathogenic bacteria [25-27]. Thus, mimicking the non-adhesive properties of a glycocalyx may provide a solution to the clinical problem associated with device colonization [28]. In this context, regarding the advantage of optimizing the polysaccharidic structure [29], we recently reported the preparation of glycocalyx-like hydrophylic methylcellulose (MeCe) polymer nanofilms grafted onto polydimethoxysiloxane (PDMS, silicone) and characterized their anti-adhesive properties *in vitro* [30]. Such an approach offers numerous advantages and should prove to be a cost-effective, wide-spectrum, long lasting anti-adhesive coating.

In this study, we describe the surface modification of commercial TIVAP composed of PDMS and titanium using MeCe as well as polyethylene glycol (PEG) and investigate the ability of this glycocalyx-like polymer to prevent *in vivo* biofilm development using a clinically relevant rat model of biofilm infections with trackable bioluminescent bacteria [31, 32]. We show that modified TIVAP strongly reduced *in vivo* biofilm development as well as clot formation associated with TIVAP colonization. These results prove that an anti-adhesive approach could constitute an efficient prophylactic strategy for controlling infections in medical devices.

Methods

Bacterial strains. We used luminescent variants of two clinically relevant pathogens, i.e. *S. aureus* (*S. aureus* Xen36, purchased from Caliper) and *P. aeruginosa* Lm1, a bioluminescent derivative of the PAK clinical strain [33]. *E. coli* MG1655 (*E. coli* Genetic stock center, #CGS6300) was used to evaluate surface modification efficiency after sterilization. *S. aureus* was grown in tryptic soy broth (TSB) supplemented with 0.25% glucose, while *P. aeruginosa* and *E. coli* were grown in lysogeny broth (LB) at 37°C.

MeCe derivative 1 synthesis. Synthesis of MeCe **1** was described previously [30]. Briefly, 1.5 g of MeCe (8000 cPs) was dissolved in water (150 mL) at 0°C for 1 h. Then, sodium hydroxide (435 mg, 10.9 mmol) was added to the solution. After complete dissolution of NaOH, allyl bromide (4 mL, 46.3 mmol, 5 eq.) was added to the solution and the mixture was stirred vigorously for 24 h at room temperature. The MeCe derivative was flocculated by heating the solution at 80°C and the off-white gel was isolated by filtration. The residual product was then dialyzed against water for 2 days and lyophilized to make the polymer into a transparent solid (yield: 64-72%). ^{1}H-NMR (300 MHz, D_2O) : 5.80 (m, 1H), 5.30 (m, 2H), 2.9-4.5 (m, 227 H).

TIVAP surface modifications. Pediatric TIVAP (POLYSITE® 2005ISP, Perouse Medical) were fully disassembled prior to surface treatment adapted for each material: silicone elastomers (polydimethylsiloxane) or titanium. After surface modifications, parts made of silicone elastomer or titanium and the fully connected medical devices were sterilized using ethanol. Several sterilization procedures (ethanol, steam, ethylene oxide sterilizations) were evaluated and found to preserve anti-adhesive surface properties (**Fig. S1**).

Tubing and septum treatment with MeCe derivative 1. Tubings and septa were placed in a 1 mg/mL MeCe derivative **1** aqueous solution at 65°C and tubings were perfused continuously via a syringe pump with the same solution at a flow rate of 500 µL/min for 6 h. Then the medical devices were removed from the bath and the solution left inside expelled, followed by extensive washing with 200 mL water.

Grafting of poly(ethylene glycol) coating on the titanium port of TIVAP. The titanium ports were exposed, under Argon for 120 min at room temperature, to a monolayer deposition solution prepared by mixing 100 µL of 10-undecenyltrichlorosilane with 100 mL of dry toluene solvent. Then samples were withdrawn from the silane solutions and washed several times with toluene and $CHCl_3$, ethanol and then dried under a nitrogen stream. Ports were immersed in an aqueous solution containing MeO-PEG-SH (MW = 5000 g/mol, 5 mg/mL) and a photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure-2959, Ciba-Geigy, 0.1 wt-%) exposed to UV light for 1 h at room temperature using a CL-1000L cross-linker (UVP, USA), providing 5.2 mW/cm^2 (λ = 365 nm) at the surface. The treated titanium ports were removed from the solution and rinsed extensively several times with ethanol and water.

***In vitro* bacterial adhesion on unmodified and modified silicone or titanium disassembled parts of TIVAP.** *In vitro* adhesion properties of modified and unmodified silicone or titanium surfaces were assessed using classical adhesion assay with fluorescently labeled *S. aureus* Xen36 or *P. aeruginosa* Lm1. Bacterial densities were calculated by counting bacteria per image using Image-Pro Plus (Media Cybernetics). The detailed experiments is described in Supplementary Methods.

***In vitro* bacterial adhesion inhibition efficacy of MeCe and PEG coating on reconstituted TIVAP.** To evaluate the *in vitro* antibiofilm efficacy of the MeCe and PEG coating on TIVAP, a previously described continuous flow system [31] was used with a slight modification and is described in Supplementary Methods. Adhesion experiments were carried out at least in quadruplicate.

Animal model. Male CD/SD (IGS:Crl) rats purchased from Charles River weighed 275-300 g and were allowed to acclimatize using 12 h day/night cycles for one week before use at the Institut Pasteur animal facilities accredited by the French Ministry of Agriculture to perform experiments on live rodents (accreditation #A75-15 27, issued on November 12, 2004 and #A75-15 04, issued on May 22, 2008). Work on animals was performed in compliance with French and European regulations on care and protection of laboratory animals (EC Directive 2010/63, French Law 2013-118, February 76th, 2013). The protocols used in this study were approved by the ethics committee of "*Paris Centre et Sud N°59*" under reference N°2012-0045 and are described in Supplementary Methods.

Statistical analysis. Results for CFU are mean +/- standard deviation. Statistical differences were evaluated using one-way ANOVA (Tukey multiple comparison test) or a two tailed t-test when comparing two sets of data included in Graphpad Prism Version 5.0c. The treatment groups were considered statistically different if p-values were lower than 0.05.

Results

Preparation and characterization of MeCe-coated silicone from TIVAP. In light of the previously demonstrated anti-adhesive efficacy of glycocalyx-like compounds [28, 30], we hypothesized that modifying TIVAP with polysaccharides or polyethylene glycol could inhibit protein and cellular adhesion. However, one major challenge in using polymers to engineer anti-adhesive surfaces in biomedical devices lies in achieving total high-density surface modifications of the different materials composing the device. TIVAP is composed of a titanium port closed with a silicone septum and is connected to a catheter made of silicone elastomer (PDMS). To ensure chemical modification of the entire commercial device, we completely disassembled the TIVAP to enable surface treatment adapted to each material (PDMS or titanium) of its components (**Fig. 1**). First, we used a MeCe derivative bearing alkene groups to modify the inner and outer surface of the septum and catheter made in silicone (polydimethylsiloxane, PDMS) *via* hydrosilylation in one step in water [30]. This MeCe derivative **1,** shown in **Fig. 1C**, is synthesized from MeCe (number-average molecular mass Mn ≈ 110 000 daltons). The grafting of **1** on PDMS leads to a more wettable surface with θ_{adv} = 90±1° and θ_{rec} = 35±2° contact angle as opposed to θ_{adv} = 110±1° and θ_{rec} = 75±2° for the untreated PDMS. Characterization of these modified surfaces by ToF-SIMS confirmed the presence of a MeCe layer with characteristic key ions of the saccharide residues (**Table S1**). We modified the titanium reservoir surface extracted from the port in two steps, with first the formation of an alkene-terminated self-assembled monolayer (SAM) by liquid phase deposition of undecenyltrichlorosilane, followed by the immobilization of the polyethylene glycol derivative **2** *via* a thiol-ene reaction (number-average molecular mass Mn ≈ 5 000 daltons)[34]. Surface analysis of the polyethylene glycol-terminated SAM by high-resolution XPS of the C 1s core level region exhibited two features: the first, at 285.0 eV, was characteristic of internal units of the polymethylene chain (-$CH_2CH_2CH_2$-); the

second, at 286.8 eV, corresponded to methylene groups adjacent to an oxygen (-OCH_2-) (Supplementary **Fig. S2**). Integration of these signals indicated that the surface density of polyethylene glycol is 0.13 chains/nm^2.

MeCe derivative 1 and PEG derivative 2 are not toxic to bacteria or eukaryotic cells. Before evaluating the anti-adhesive efficacy of MeCe derivative **1** and PEG derivative **2,** we assessed their toxicity against two bacterial pathogens frequently associated with catheter-related infections, i.e. *Staphylococcus aureus* and *Pseudomonas aeruginosa* [31, 35, 36], and against eukaryotic cells, i.e. fibroblast L929 mouse cell line that is widely used and recommended for testing toxicity of medical-device-associated material [37] (Supplementary Fig. S1). None of the two polymeric derivatives exhibited bacterial toxicity in solution. Bacterial growth was observed even at high polymer concentration 1 mg/mL with *P. aeruginosa* (Lm1) and *S. aureus* (Xen36), whereas the minimal inhibitory concentration of chlorhexidine was 8 µg/mL for both bacterial strains. The MeCe-modified catheter had no measurable zones of growth inhibition when they were placed on agar plates inoculated with the same bacterial strains. Moreover, the two tested polymers were not cytotoxic to fibroblast L929 cell cultures (100 µg/mL final concentration).

Biomimetic modification reduced bacterial colonization of TIVAP *in vitro*. We first evaluated anti-adhesive efficacy on separated parts of the TIVAP, *i.e.* the port septum and catheter in PDMS (Si) modified with MeCe derivative **1** and the titanium port (Ti) modified with PEG derivative **2**. After 24 h of static contact, both *P. aeruginosa* and *S. aureus* adhesion to MeCe- and PEG-modified materials was strongly reduced compared to unmodified TIVAP materials (75- to 480-fold reduction, **Fig. 2A-E**). We then decided to evaluate the ability of MeCe and PEG modifications to inhibit subsequent steps in bacterial adhesion leading to

biofilm formation on fully reconstituted TIVAP. We adopted a previously developed *in vitro* continuous flow system, using TIVAP to grow biofilm of bioluminescent *P. aeruginosa* and *S. aureus* strains for 48 h [31]. Both *P. aeruginosa* and *S. aureus* developed high-cell-density biofilms on non-modified TIVAP, as indicated by high luminescent signals (**Fig. 2FG**), which correlated with high colony-forming unit (CFU) counts (**Fig. 2H**). In contrast, MeCe derivative **1** modified TIVAP (septum+catheter, Si) showed strongly reduced biofilm formation, as indicated by the absence of luminescence signals, which was further confirmed by a marked reduction (~ 7-log) in CFU counts obtained for both *P. aeruginosa* and *S. aureus* (**Fig. 2H**). Further modifications in the titanium part by PEG derivative **2** (Si-Ti) did not increase the anti-adhesive effect, suggesting that *in vitro* biofilm formed on the titanium part of the TIVAP is negligible when compared to biofilm formed on silicone parts (**Fig. 2H**).

MeCe and PEG grafting strongly inhibits bacterial biofilm colonization in TIVAP implanted in rats. We tested the *in vivo* efficacy of the modified TIVAP using a previously described clinically relevant *in vivo* rat model of biofilm-associated infection in a TIVAP [31]. In clinical settings, TIVAP dwell for a long time in the blood flow and their surfaces are progressively conditioned by a film composed of blood components such as proteins and blood cells. This conditioning film can promote bacterial adhesion and impair the efficiency of the coated molecules [38]. We mimicked this clinical situation by manipulating implants using the flush and draw method to ensure blood inflow followed by heparin lock for 4 days before inoculating bacteria into the port of the device [31]. Initial adherence of bacteria is a critical step in biofilm formation on a surface. Thus, we first estimated the difference in number of bacteria that adhered to the implanted coated *vs.* the non-coated TIVAP endoluminal surface. For this, the inoculum was left to dwell in the implanted TIVAP for 1 h or 3.5 h for *P. aeruginosa* and *S. aureus*, respectively. The rats were euthanized, non-adherent

planktonic bacteria were removed from the implanted TIVAP and we determined the quantity of bacteria adhering to the endoluminal part of the explanted TIVAP (**Fig. 3A**). Coating of the PDMS parts of TIVAP with MeCe derivative **1** led to a twofold reduction in both *S. aureus* and *P. aeruginosa* adherence. Additional coating of the titanium part with PEG derivative **2** further reduced initial adhesion of the two bacteria. These results demonstrated that despite prolonged contact of the TIVAP with host proteins, modification of TIVAP by derivatives **1** and **2** was still capable of strongly inhibiting initial adhesion of two major biofilm-forming pathogens. We then evaluated the effect of these coatings on later stages of biofilm formation by monitoring TIVAP colonization for 5 days (**Fig. 3B-H**). While bioluminescence signals appeared at day 1 for uncoated TIVAP infected with both *S. aureus* and *P. aeruginosa*, no *S. aureus*-associated bioluminescence was observed in TIVAP coated only with MeCe derivative **1** (treatment of PDMS), nor in TIVAP coated with both MeCe and PEG derivative polymers (treatment of PDMS and titanium). In the case of *P. aeruginosa*, bioluminescence signals appeared at day 5 for the MeCe-coated catheter, but were still absent at day 5 for PDMS and titanium coated TIVAP. Since biofilms are heterogeneous environments containing subpopulations of poorly metabolic bacteria, absence of detectable bioluminescence after 5 days of biofilm formation does not reflect total absence of viable bacteria. We therefore measured viable bacteria colonizing the coated and uncoated TIVAP by counting colony-forming units from the endoluminal part of extracted TIVAP after 5 days of biofilm formation. MeCe derivative **1** coating of TIVAP PDMS parts resulted in a 2- to 3-log reduction, for both pathogens, of bacteria colonizing the TIVAP after 5 days. Additional coating of titanium with PEG derivative **2** further inhibited bacterial colonization, with the reduction reaching 4 to 5 logs.

MeCe grafting prevents biofilm-associated clogging of TIVAP implanted in rats. Previous studies have shown that biofilm formation increases risk of blood coagulation within implanted catheters, which may be a potential source of thrombosis [39]. Our results also suggest that the efficiency of the MeCe coating could be due to reduced interactions of host proteins with the TIVAP surface, therefore avoiding formation of a conditioning film. To test whether coating of the septum and catheter PDMS parts of the TIVAP with a MeCe derivative **1** could prevent biofilm-associated clogging *in vivo*, implanted TIVAP were monitored every day for patency. Interestingly, in the case of colonization by *P. aeruginosa*, while 66% of uncoated TIVAP lost patency due to clogging within 24 h of biofilm formation, MeCe-coated TIVAP prevented biofilm-associated clogging in all coated TIVAP. Similarly, the number of patent TIVAP increased to 78% when MeCe-coated TIVAP were infected with *S. aureus* as compared to non-coated TIVAP, that showed only 10% patency (**Table 1**). In order to confirm that the loss of patency in implanted devices was due to biofilm formation, we performed electron microscopy imaging on coated and non-coated TIVAP isolated after 5 days of infection (**Fig. 4**). Scanning electron microscopy analysis clearly demonstrated the massive and distinct rod-like biofilm structures formed in the endoluminal part of catheters from non-coated TIVAP. These biofilms were associated with the presence of high numbers of host cells (red blood cells, platelets) entangled in a dense fibrin network likely leading to device occlusion. In contrast, catheters from TIVAP that received the MeCe graft showed many fewer bacteria and host cells scattered on the surface of the device (**Fig. 4**), thereby pointing to the efficiency of MeCe coating at reducing *in vivo* host cell, protein and bacterial cell interactions with the luminal part of the catheter.

Discussion

TIVAP have considerably improved management of patients by facilitating chemotherapy, supportive therapy and blood sampling over a long period of time. However, the interaction between the implant and surrounding tissues can lead to complications such as thrombosis and infections due to the hydrophobic nature of the material composing most medical devices. Suppression of such deleterious interactions *via* an anti-adhesive strategy is of particular interest by offering a universal solution so as to avoid medical complications, while also preventing the emergence of drug resistance. However, the efficiency of such anti-adhesive strategy in the case of fully functional implantable medical devices must be demonstrated *in vivo*, a setting in which coated surfaces can become conditioned by host factors, reducing its efficacy. In this study, we demonstrate an anti-adhesive strategy developed using a rational design by grafting, in solution, a monolayer of polymers on the surface of a commercial TIVAP: methylcellulose (MeCe) **1** on the septum and the catheter and polyethylene glycol (PEG) **2** on the titanium reservoir. The polymeric monolayers immobilized on medical device surfaces are meant to protect the surface from non-specific protein adsorption and cell adhesion, similarly to the steric repulsion provided by the glycocalyx on the surface of eukaryotic cells. While neither free nor immobilized polymers (**1** and **2**) display biocidal activity, these monolayers significantly reduce *in vitro* biofilm growth of *S. aureus* and *P. aeruginosa,* lowering surface bacterial density inside the TIVAP by ca. 7-logs compared to commercial uncoated TIVAP. Interestingly, although MeCe coating of septum and catheter TIVAP components very significantly reduce biofilm formation *in vivo*, maximal efficiency is reached when the tubing and septum are coated with MeCe and the titanium port is modified with PEG, suggesting that the untreated titanium port is an important site of infection. These experiments indicate that, in the case of medical devices composed of several materials, use of multiple non-adhesive strategies is needed to treat all available surfaces and obtain

satisfactory results, and new grafting procedures should be developed in the future to allow the coating of all different parts composing the TIVAP if possible.

We show that the efficiency of the developed coating was partly due to its capacity to reduce initial adhesion of both *S. aureus* and *P. aeruginosa* biofilm-forming pathogens. In light of the previously described anti-fouling activity of methylcellulose against host proteins and cell adhesion [30], and its herein excellent *in vivo* anti-biofilm efficacy, we also hypothesized that this coating could present strong anti-clogging activity. Consistent with the fact that occlusion and associated thrombosis increase the risk of clinical complications [39-41], a high percentage of implanted TIVAP clogging was observed after colonization with both bacterial strains. These observations were further confirmed by SEM, which revealed substantial biofilm mass formation, with characteristic fibrin networks in untreated TIVAP leading to clot formation. Such infectious clots were strongly reduced by coating of the catheter with MeCe, as shown by the reduction in loss of TIVAP patency and SEM observations.

Thus, we believe that our study provides *in vivo* proof that anti-adhesive strategy on a fully functional implantable TIVAP is efficient enough to potentially lower the drastic complications associated with their use, i.e. infections and thrombosis. This approach is particularly attractive, since it offers a general solution to numerous complications in the field of implantable medical devices, including infections, thrombosis and fibrosis, by suppressing protein adsorption and cell adhesion. Efforts will be made to evaluate the described anti-fouling strategy to other types of composite medical devices.

Acknowledgements

This work was supported by ANR grants (ANR-07-EMPB-004-01), (ANR-08-PCVI-0012), the Institut Curie/Institut Pasteur (PIC/PTR maladies nosocomiales) and by the French Government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant n°ANR-10-LABX-62-IBEID).

Author contributions

A.C., C.B. and V.S. conceived and designed the experiments, analyzed the data and wrote the paper. W.M. and A.B. performed surface modifications and analyses of implantable medical devices. A.C. performed *in vivo* experiments. I.K. and M.E. contributed to data analyses and discussion. J.M.G. contributed to discussion and paper writing.

Competing financial interests

The authors declare no competing financial interests.

References

1. Baskin JL, Pui CH, Reiss U, et al. Management of occlusion and thrombosis associated with long-term indwelling central venous catheters. Lancet **2009**; 374:159-69.
2. Hartkamp A, van Boxtel AJ, Zonnenberg BA, Witteveen PO. Totally implantable venous access devices: evaluation of complications and a prospective comparative study of two different port systems. The Netherlands journal of medicine **2000**; 57:215-23.
3. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International journal of antimicrobial agents **2010**; 35:322-32.
4. Romling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of internal medicine **2012**; 272:541-61.
5. Maki DG, Kluger DM, Crnich CJ. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. Mayo Clinic proceedings Mayo Clinic **2006**; 81:1159-71.
6. Sticca RP, Dewing BD, and, Harris JD. Totally Implantable Venous Access Devices: Management in Mid- and Long-term clinical Setting. Springer-VErlag, Berlin, 2012 **2012**; Ch.36.
7. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis **2009**; 49:1-45.
8. Ferreira Chacon JM, Hato de Almeida E, de Lourdes Simoes R, et al. Randomized study of minocycline and edetic acid as a locking solution for central line (port-a-cath) in children with cancer. Chemotherapy **2011**; 57:285-91.
9. Safdar N, Maki DG. Use of vancomycin-containing lock or flush solutions for prevention of bloodstream infection associated with central venous access devices: a meta-analysis of prospective, randomized trials. Clin Infect Dis **2006**; 43:474-84.

10. Chatzinikolaou I, Zipf TF, Hanna H, et al. Minocycline-ethylenediaminetetraacetate lock solution for the prevention of implantable port infections in children with cancer. Clin Infect Dis **2003**; 36:116-9.

11. Wolf J, Shenep JL, Clifford V, Curtis N, Flynn PM. Ethanol lock therapy in pediatric hematology and oncology. Pediatric blood & cancer **2013**; 60:18-25.

12. Maki DG, Stolz SM, Wheeler S, Mermel LA. Prevention of central venous catheter-related bloodstream infection by use of an antiseptic-impregnated catheter. A randomized, controlled trial. Annals of internal medicine **1997**; 127:257-66.

13. Heard SO, Wagle M, Vijayakumar E, et al. Influence of triple-lumen central venous catheters coated with chlorhexidine and silver sulfadiazine on the incidence of catheter-related bacteremia. Archives of internal medicine **1998**; 158:81-7.

14. Darouiche RO, Raad, II, Heard SO, et al. A comparison of two antimicrobial-impregnated central venous catheters. Catheter Study Group. N Engl J Med **1999**; 340:1-8.

15. Hanna H, Benjamin R, Chatzinikolaou I, et al. Long-term silicone central venous catheters impregnated with minocycline and rifampin decrease rates of catheter-related bloodstream infection in cancer patients: a prospective randomized clinical trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology **2004**; 22:3163-71.

16. Raad I, Darouiche R, Hachem R, Mansouri M, Bodey GP. The broad-spectrum activity and efficacy of catheters coated with minocycline and rifampin. The Journal of infectious diseases **1996**; 173:418-24.

17. Sampath LA, Tambe SM, Modak SM. *In vitro* and *in vivo* efficacy of catheters impregnated with antiseptics or antibiotics: evaluation of the risk of bacterial resistance to the antimicrobials in the catheters. Infect Control Hosp Epidemiol **2001**; 22:640-6.

18. US_Food_and_Drug_Administration. Potential Hypersensitivity Reactions To Chlorhexidine-Impregnated Medical Devices. Available at: http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/PublicHealthNotifications/ucm062306.htm.

19. Guleri A, Kumar A, Morgan RJ, Hartley M, Roberts DH. Anaphylaxis to chlorhexidine-coated central venous catheters: a case series and review of the literature. Surgical infections **2012**; 13:171-4.

20. Busscher HJ, van der Mei HC, Subbiahdoss G, et al. Biomaterial-associated infection: locating the finish line in the race for the surface. Science translational medicine **2012**; 4:153rv10.

21. Epstein AK, Wong TS, Belisle RA, Boggs EM, Aizenberg J. Liquid-infused structured surfaces with exceptional anti-biofouling performance. Proc Natl Acad Sci U S A **2012**; 109:13182-7.

22. Hook AL, Chang CY, Yang J, et al. Combinatorial discovery of polymers resistant to bacterial attachment. Nature biotechnology **2012**; 30:868-75.

23. Smith RS, Zhang Z, Bouchard M, et al. Vascular catheters with a nonleaching poly-sulfobetaine surface modification reduce thrombus formation and microbial attachment. Science translational medicine **2012**; 4:153ra32.

24. Jeon SL, Lee JH, Andrade JD, De Gennes PG. Protein-surface interactions in the presence of polyethylene oxide: I. Simplified theory. J Colloid Interface Sci **1991**; 142:149-58.

25. Rendueles O, Kaplan JB, Ghigo JM. Antibiofilm polysaccharides. Environ Microbiol **2013**; 15:334-46.

26. Rendueles O, Travier L, Latour-Lambert P, et al. Screening of *Escherichia coli* species biodiversity reveals new biofilm-associated antiadhesion polysaccharides. MBio **2011**; 2:e00043-11.

27. Valle J, Da Re S, Henry N, et al. Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. Proc Natl Acad Sci U S A **2006**; 103:12558-63.

28. Holland NB, Qiu Y, Ruegsegger M, Marchant RE. Biomimetic engineering of non-adhesive glycocalyx-like surfaces using oligosaccharide surfactant polymers. Nature **1998**; 392:799-801.

29. Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM. A Survey of Structure-Property Relationships of Surfaces that Resist the Adsorption of Protein. Langmuir **2001**; 17:5605-20.

30. Mussard W, Kebir N, Kriegel I, Esteve M, Semetey V. Facile and efficient control of bioadhesion on poly(dimethylsiloxane) by using a biomimetic approach. Angew Chem Int Ed Engl **2011**; 50:10871-4.

31. Chauhan A, Lebeaux D, Decante B, et al. A rat model of central venous catheter to study establishment of long-term bacterial biofilm and related acute and chronic infections. PloS one **2012**; 7:e37281.

32. Chauhan A, Lebeaux D, Ghigo JM, Beloin C. Full and broad-spectrum *in vivo* eradication of catheter-associated biofilms using gentamicin-EDTA antibiotic lock therapy. Antimicrob Agents Chemother **2012**; 56:6310-8.

33. Ramphal R, Balloy V, Jyot J, Verma A, Si-Tahar M, Chignard M. Control of *Pseudomonas aeruginosa* in the lung requires the recognition of either lipopolysaccharide or flagellin. J Immunol **2008**; 181:586-92.

34. Oberleitner B, Dellinger A, Deforet M, Galtayries A, Castanet AS, Semetey V. A facile and versatile approach to design self-assembled monolayers on glass using thiol-ene chemistry. Chemical communications **2013**; 49:1615-7.

35. Adler A, Yaniv I, Steinberg R, et al. Infectious complications of implantable ports and Hickman catheters in paediatric haematology-oncology patients. The Journal of hospital infection **2006**; 62:358-65.

36. Sotir MJ, Lewis C, Bisher EW, Ray SM, Soucie JM, Blumberg HM. Epidemiology of device-associated infections related to a long-term implantable vascular access device. Infect Control Hosp Epidemiol **1999**; 20:187-91.

37. International_Organization_for_Standardization. Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. Vol. ISO10993-5. Geneva: International Organization for Standardization, **1999**.

38. Raad I, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. The Journal of infectious diseases **1993**; 168:400-7.

39. van Rooden CJ, Schippers EF, Barge RM, et al. Infectious complications of central venous catheters increase the risk of catheter-related thrombosis in hematology patients: a prospective study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology **2005**; 23:2655-60.

40. Crowley AL, Peterson GE, Benjamin DK, Jr., et al. Venous thrombosis in patients with short- and long-term central venous catheter-associated *Staphylococcus aureus* bacteremia. Critical care medicine **2008**; 36:385-90.

41. Fitzgerald JR, Loughman A, Keane F, et al. Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcgammaRIIa receptor. Molecular microbiology **2006**; 59:212-30.

Tables

Table 1. Biofilm-associated clogging of TIVAP is reduced *in vivo* in MeCe-**1** modified implants. (Percent non-patent/blocked TIVAP is mentioned).

Organism	**Treated (n=9)**	**Untreated (n=9)**
S. aureus	**22%**	**90%**
P. aeruginosa	**0%**	**66%**

Figure Legends

Figure 1. Schematic view of the approach used to prepare anti-adhesive TIVAP. (***A***), Commercially available TIVAP. (***B***), disassembled TIVAP (a: catheter; b and c: envelope of the port; d: sealing ring of the port; e: septum; f: port). (***C***), chemistry used to modify TIVAP parts. Catheter and septum made of PDMS were modified using (**1**) methylcellulose (MeCe) derivative while port made of titanium was modified using (**2**) poly(ethylene glycol) (PEG) derivative.

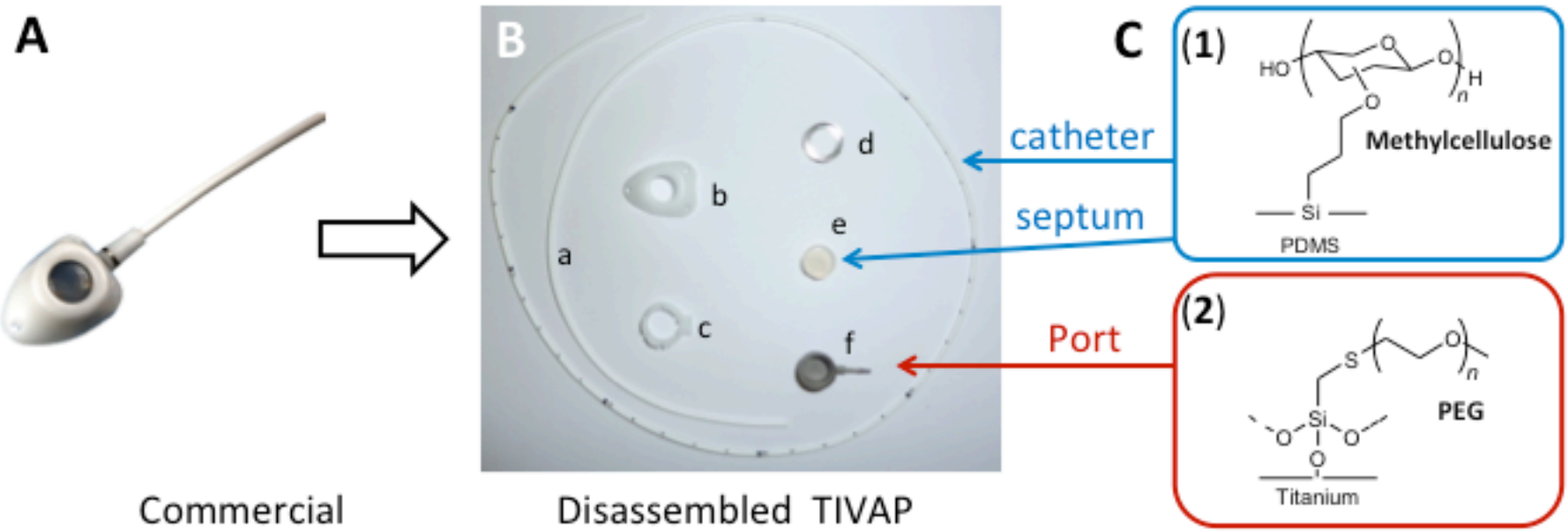
Figure 2. *In vitro* inhibition of bacterial adhesion by grafted compounds MeCe-1 and PEG-2. (***A-D)*** Bacterial adhesion (*P. aeruginosa*, *S. aureus*) on unmodified and modified PDMS septum (Si) with MeCe-**1** observed using epifluorescence microscopy. (***A***), *S. aureus* adhesion to the PDMS surface. (***B)***, *S. aureus* adhesion to PDMS surface grafted with MeCe-**1**. (***C***), *P. aeruginosa* adhesion to the PDMS surface. (***D)***, *P. aeruginosa* adhesion to to PDMS surface grafted with MeCe-**1**. (***E***), cells harvested from grafted and non-grafted PDMS or Titanium surface and plated on LB agar (*P. aeruginosa*) or TSB agar (*S. aureus*) for CFU/cm^2. (***F-H***), modified and unmodified TIVAPs were reconstituted and an *in vitro* continuous flow system was developed to grow biofilms. Bioluminescence activity was acquired after 48 h using an IVIS 100 camera with TIVAP injected with *P. aeruginosa* (***F***) and with *S. aureus* (***G)***. (***H***), biofilm biomasses were analyzed by counting CFU/cm^2 on LB agar for *P. aeruginosa* or TSB agar plates for *S. aureus* (n = 3-4 per group). Control: non-modified TIVAP, Si: PDMS/silicone elastomer (septum+catheter) grafted with MeCe-**1** and Si-Ti: PDMS/silicone elastomer (septum+catheter) grafted with MeCe-**1** and titanium grafted with PEG-**2**. Statistical analysis was done using one-way analysis of variance (ANOVA) with

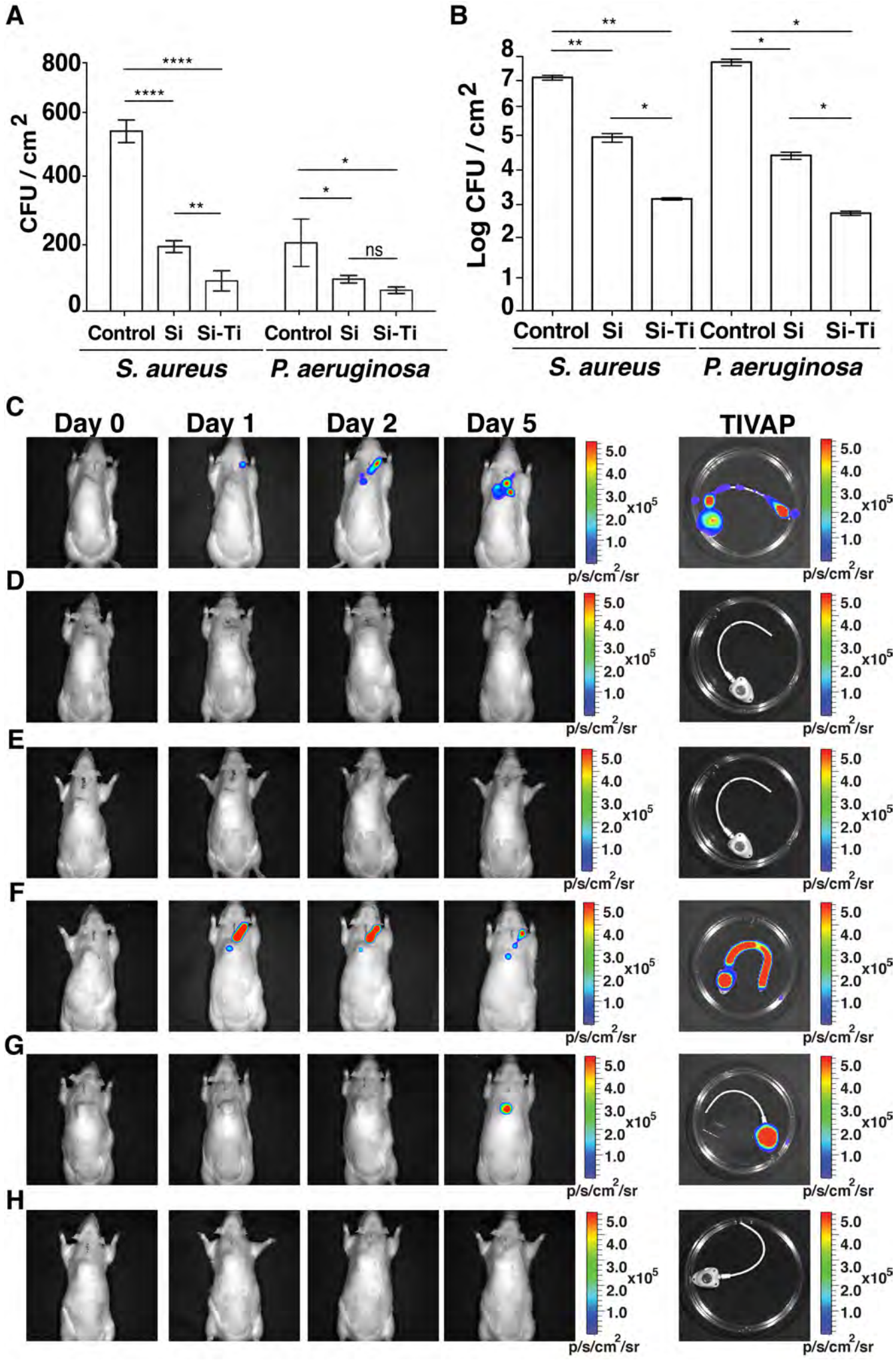
Graphpad Prism version 5.0c. A P value < 0.05 was considered significant; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns (non-significant).

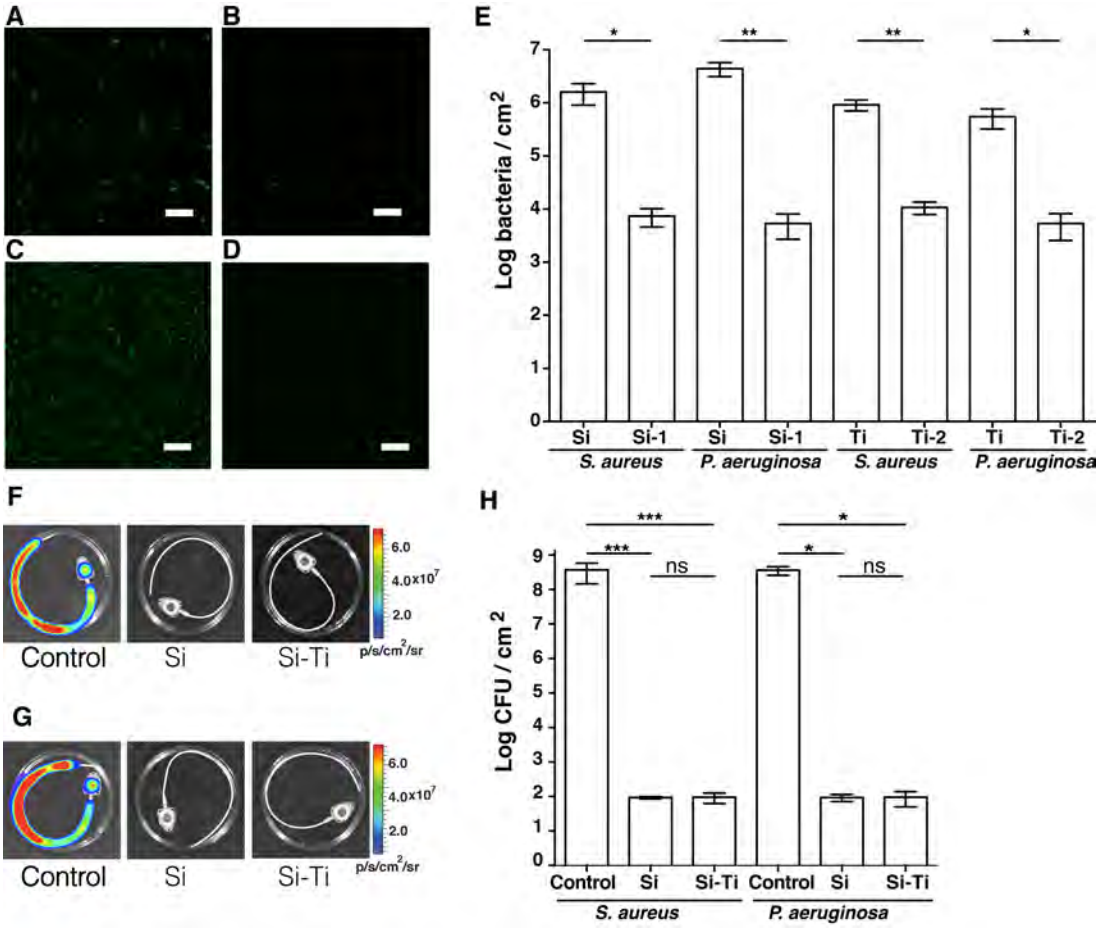
Figure 3. *In vivo* inhibition of initial adhesion and mature bacterial biofilm development on TIVAP by biomimetic glycocalyx-like polymers. Rats with modified or unmodified implanted TIVAP were maintained by the flush and draw technique for 4 days prior to bacterial inoculation. An inoculum of 10^6 CFU *S. aureus* or 10^3 CFU *P. aeruginosa*/50 µL 1X PBS was injected into implanted TIVAP. Bacteria were allowed to adhere to the PDMS/silicone surface for 3 h (*S. aureus*) or 1.5 h (*P. aeruginosa*). (***A***), planktonic bacteria were removed and rats (n = 3 rats per group) were either sacrificed to aseptically remove the implanted TIVAP and evaluate the initial adherent population or (***B***), biofilms were left to form for 5-days and TIVAP extracted to measure bacterial biofilm colonization. Adherent bacteria were harvested and counted by plating on TSB agar for *S. aureus* or LB agar plates for *P. aeruginosa*. Control, Si and Si-Ti as described in Figure 2. Statistical analysis was done using one-way analysis of variance (ANOVA) with Graphpad Prism version 5.0c. P value < 0.05 was considered significant; * $P \leq 0.05$, ** $P \leq 0.01$, **** $P \leq 0.0001$ and ns (non-significant). (***C to H)*** Rats were monitored for bioluminescence up to day 5 using Xenogen IVIS 100. *S. aureus* biofilm formation with: (***C***), control rats with unmodified TIVAP; (***D***), rats with Si TIVAP and (***E)***, rats with Si-Ti TIVAP. *P. aeruginosa* biofilm formation with: (***F***), control rats with unmodified TIVAP ; (***G***), rats with Si TIVAP and (***H***), rats with Si-Ti TIVAP.

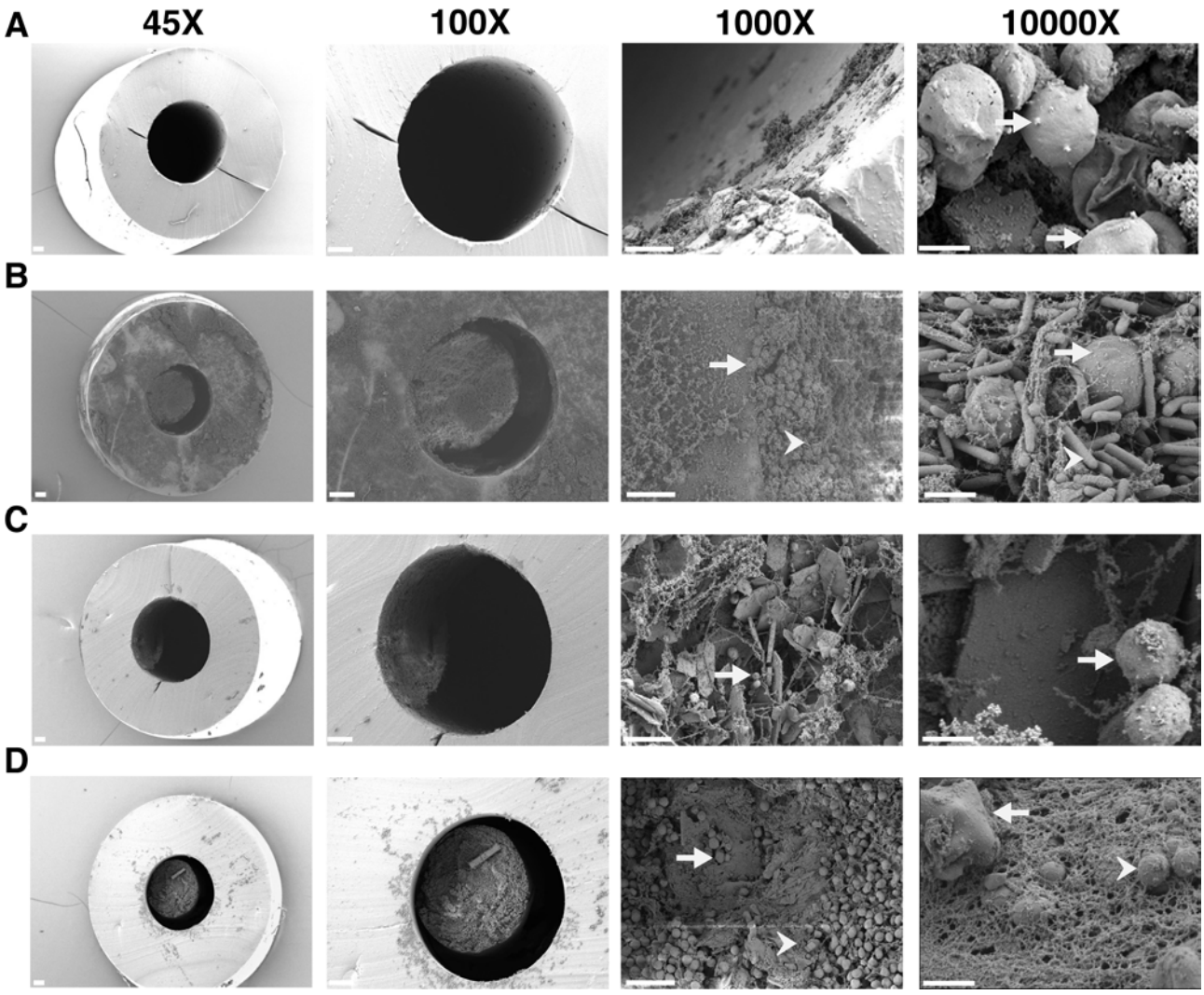
Figure 4. MeCe modification prevents biofilm-associated clogging of TIVAP, as revealed by scanning electron microscopy. MeCe-**1** modified and unmodified TIVAP implanted in rats and inoculated with either *P. aeruginosa* or *S. aureus* were harvested on day 5 and analyzed

by scanning electron microscopy. Arrow: blood cells; arrowhead: bacteria in biofilm. Bars (100 μm for 45 X and 100 X, 10 μm for 1000 X and 2 μm for 10000 X magnification). (***A***), MeCe-**1** modified TIVAP inoculated with *P. aeruginosa*. (***B***), unmodified TIVAP inoculated with *P. aeruginosa*. (***C***), MeCe-modified TIVAP inoculated with *S. aureus*. (***D***), unmodified TIVAP inoculated with *S. aureus*.


A
B
C
(1)
HO
O
O
H
n
O
Methylcellulose
Si
PDMS
catheter
septum
d
b
e
a
c
f
Port
(2)
S
O
n
Si
O
O
O
PEG
Titanium
Commercial
Totally implantable
venous access port (TIVAP)
Disassembled TIVAP


A
CFU / cm2
800
600
400
200
0
****
****
**
*
*
ns
Control Si Si-Ti Control Si Si-Ti
S. aureus
P. aeruginosa
B
Log CFU / cm2
8
7
6
5
4
3
2
1
0
**
**
*
*
*
*
Control Si Si-Ti Control Si Si-Ti
S. aureus
P. aeruginosa
C
D
E
F
G
H
Day 0
Day 1
Day 2
Day 5
TIVAP
5.0
4.0
3.0
2.0
1.0
x10^5
p/s/cm2/sr


A
B
C
D
E
Log bacteria / cm2
Si
Si-1
Si
Si-1
Ti
Ti-2
Ti
Ti-2
S. aureus
P. aeruginosa
S. aureus
P. aeruginosa
F
Control
Si
Si-Ti
6.0
4.0x10^7
2.0
p/s/cm2/sr
G
Control
Si
Si-Ti
H
Log CFU / cm 2
ns
ns
Control
Si
Si-Ti
Control
Si
Si-Ti
S. aureus
P. aeruginosa


A
45X
100X
1000X
10000X
B
C
D