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

Ashwini Chauhan<sup>a</sup>, Aude Bernardin<sup>b</sup>, Windy Mussard<sup>b</sup>, Irène Kriegel<sup>c</sup>, Marc Estève<sup>c</sup>, Jean-Marc Ghigo<sup>a</sup>, Christophe Beloin<sup>a,1,\*</sup> and Vincent Semetey<sup>b,d,1,\*</sup>

<sup>a</sup> Institut Pasteur, Unité de Génétique des Biofilms. 25 rue du Dr. Roux, 75724 Paris cedex 15 France

<sup>b</sup> Institut Curie, Centre de Recherche, France.

<sup>c</sup> Institut Curie, Hôpital, Service Anesthésie-Réanimation-Douleur, Paris, France

<sup>d</sup> CNRS, UMR 168, Laboratoire de Physicochimie « Curie », Paris, France.

<sup>1</sup> These authors contributed equally to this work

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

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**Running title:** Biomimetic bacterial anti-adhesive strategy

1 **Abstract- 150 words**

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

15

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

17

18

1 **Text-3401 words**

2 **Introduction**

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

1 [20-23]. The external layer of a cell membrane, known as the glycocalyx, is composed of  
2 polysaccharides and prevents undesirable protein adsorption and non-specific cell adhesion  
3 *via* steric repulsion [24]. Others and we previously showed that treatment of abiotic surfaces  
4 *via* the adsorption of different bacterial polysaccharides has a long-lasting effect to  
5 significantly inhibit mature biofilm development of a broad-spectrum of pathogenic bacteria  
6 [25-27]. Thus, mimicking the non-adhesive properties of a glycocalyx may provide a solution  
7 to the clinical problem associated with device colonization [28]. In this context, regarding the  
8 advantage of optimizing the polysaccharidic structure [29], we recently reported the  
9 preparation of glycocalyx-like hydrophilic methylcellulose (MeCe) polymer nanofilms  
10 grafted onto polydimethoxysiloxane (PDMS, silicone) and characterized their anti-adhesive  
11 properties *in vitro* [30]. Such an approach offers numerous advantages and should prove to be  
12 a cost-effective, wide-spectrum, long lasting anti-adhesive coating.

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

21

## 1 **Methods**

2

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

9

10 **MeCe derivative 1 synthesis.** Synthesis of MeCe **1** was described previously [30]. Briefly,  
11 1.5 g of MeCe (8000 cPs) was dissolved in water (150 mL) at 0°C for 1 h. Then, sodium  
12 hydroxide (435 mg, 10.9 mmol) was added to the solution. After complete dissolution of  
13 NaOH, allyl bromide (4 mL, 46.3 mmol, 5 eq.) was added to the solution and the mixture was  
14 stirred vigorously for 24 h at room temperature. The MeCe derivative was flocculated by  
15 heating the solution at 80°C and the off-white gel was isolated by filtration. The residual  
16 product was then dialyzed against water for 2 days and lyophilized to make the polymer into a  
17 transparent solid (yield: 64-72%). <sup>1</sup>H-NMR (300 MHz, D<sub>2</sub>O) : 5.80 (m, 1H), 5.30 (m, 2H),  
18 2.9-4.5 (m, 227 H).

19

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

1

2 **Tubing and septum treatment with MeCe derivative 1.** Tubings and septa were placed in a  
3 1 mg/mL MeCe derivative 1 aqueous solution at 65°C and tubings were perfused  
4 continuously via a syringe pump with the same solution at a flow rate of 500  $\mu\text{L}/\text{min}$  for 6 h.  
5 Then the medical devices were removed from the bath and the solution left inside expelled,  
6 followed by extensive washing with 200 mL water.

7

8 **Grafting of poly(ethylene glycol) coating on the titanium port of TIVAP.** The titanium  
9 ports were exposed, under Argon for 120 min at room temperature, to a monolayer deposition  
10 solution prepared by mixing 100  $\mu\text{L}$  of 10-undecenyltrichlorosilane with 100 mL of dry  
11 toluene solvent. Then samples were withdrawn from the silane solutions and washed several  
12 times with toluene and  $\text{CHCl}_3$ , ethanol and then dried under a nitrogen stream. Ports were  
13 immersed in an aqueous solution containing MeO-PEG-SH (MW = 5000 g/mol, 5 mg/mL)  
14 and a photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone  
15 (Irgacure-2959, Ciba-Geigy, 0.1 wt-%) exposed to UV light for 1 h at room temperature using  
16 a CL-1000L cross-linker (UVP, USA), providing 5.2  $\text{mW}/\text{cm}^2$  ( $\lambda = 365 \text{ nm}$ ) at the surface.  
17 The treated titanium ports were removed from the solution and rinsed extensively several  
18 times with ethanol and water.

19

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

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***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:CrI) 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.



## 1 **Results**

2 **Preparation and characterization of MeCe-coated silicone from TIVAP.** In light of the  
3 previously demonstrated anti-adhesive efficacy of glycocalyx-like compounds [28, 30], we  
4 hypothesized that modifying TIVAP with polysaccharides or polyethylene glycol could  
5 inhibit protein and cellular adhesion. However, one major challenge in using polymers to  
6 engineer anti-adhesive surfaces in biomedical devices lies in achieving total high-density  
7 surface modifications of the different materials composing the device. TIVAP is composed of  
8 a titanium port closed with a silicone septum and is connected to a catheter made of silicone  
9 elastomer (PDMS). To ensure chemical modification of the entire commercial device, we  
10 completely disassembled the TIVAP to enable surface treatment adapted to each material  
11 (PDMS or titanium) of its components (**Fig. 1**). First, we used a MeCe derivative bearing  
12 alkene groups to modify the inner and outer surface of the septum and catheter made in  
13 silicone (polydimethylsiloxane, PDMS) *via* hydrosilylation in one step in water [30]. This  
14 MeCe derivative **1**, shown in **Fig. 1C**, is synthesized from MeCe (number-average molecular  
15 mass  $M_n \approx 110\,000$  daltons). The grafting of **1** on PDMS leads to a more wettable surface  
16 with  $\theta_{adv} = 90 \pm 1^\circ$  and  $\theta_{rec} = 35 \pm 2^\circ$  contact angle as opposed to  $\theta_{adv} = 110 \pm 1^\circ$  and  $\theta_{rec} =$   
17  $75 \pm 2^\circ$  for the untreated PDMS. Characterization of these modified surfaces by ToF-SIMS  
18 confirmed the presence of a MeCe layer with characteristic key ions of the saccharide  
19 residues (**Table S1**). We modified the titanium reservoir surface extracted from the port in  
20 two steps, with first the formation of an alkene-terminated self-assembled monolayer (SAM)  
21 by liquid phase deposition of undecenyltrichlorosilane, followed by the immobilization of the  
22 polyethylene glycol derivative **2** *via* a thiol-ene reaction (number-average molecular mass  
23  $M_n \approx 5\,000$  daltons)[34]. Surface analysis of the polyethylene glycol-terminated SAM by  
24 high-resolution XPS of the C 1s core level region exhibited two features: the first, at 285.0  
25 eV, was characteristic of internal units of the polymethylene chain ( $-\text{CH}_2\text{CH}_2\text{CH}_2-$ ); the

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

#### 4 **MeCe derivative 1 and PEG derivative 2 are not toxic to bacteria or eukaryotic cells.**

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

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

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

12  
13 **MeCe and PEG grafting strongly inhibits bacterial biofilm colonization in TIVAP**  
14 **implanted in rats.** We tested the *in vivo* efficacy of the modified TIVAP using a previously  
15 described clinically relevant *in vivo* rat model of biofilm-associated infection in a TIVAP  
16 [31]. In clinical settings, TIVAP dwell for a long time in the blood flow and their surfaces are  
17 progressively conditioned by a film composed of blood components such as proteins and  
18 blood cells. This conditioning film can promote bacterial adhesion and impair the efficiency  
19 of the coated molecules [38]. We mimicked this clinical situation by manipulating implants  
20 using the flush and draw method to ensure blood inflow followed by heparin lock for 4 days  
21 before inoculating bacteria into the port of the device [31]. Initial adherence of bacteria is a  
22 critical step in biofilm formation on a surface. Thus, we first estimated the difference in  
23 number of bacteria that adhered to the implanted coated *vs.* the non-coated TIVAP  
24 endoluminal surface. For this, the inoculum was left to dwell in the implanted TIVAP for 1 h  
25 or 3.5 h for *P. aeruginosa* and *S. aureus*, respectively. The rats were euthanized, non-adherent

1 planktonic bacteria were removed from the implanted TIVAP and we determined the quantity  
2 of bacteria adhering to the endoluminal part of the explanted TIVAP (**Fig. 3A**). Coating of the  
3 PDMS parts of TIVAP with MeCe derivative **1** led to a twofold reduction in both *S. aureus*  
4 and *P. aeruginosa* adherence. Additional coating of the titanium part with PEG derivative **2**  
5 further reduced initial adhesion of the two bacteria. These results demonstrated that despite  
6 prolonged contact of the TIVAP with host proteins, modification of TIVAP by derivatives **1**  
7 and **2** was still capable of strongly inhibiting initial adhesion of two major biofilm-forming  
8 pathogens. We then evaluated the effect of these coatings on later stages of biofilm formation  
9 by monitoring TIVAP colonization for 5 days (**Fig. 3B-H**). While bioluminescence signals  
10 appeared at day 1 for uncoated TIVAP infected with both *S. aureus* and *P. aeruginosa*, no *S.*  
11 *aureus*-associated bioluminescence was observed in TIVAP coated only with MeCe  
12 derivative **1** (treatment of PDMS), nor in TIVAP coated with both MeCe and PEG derivative  
13 polymers (treatment of PDMS and titanium). In the case of *P. aeruginosa*, bioluminescence  
14 signals appeared at day 5 for the MeCe-coated catheter, but were still absent at day 5 for  
15 PDMS and titanium coated TIVAP. Since biofilms are heterogeneous environments  
16 containing subpopulations of poorly metabolic bacteria, absence of detectable  
17 bioluminescence after 5 days of biofilm formation does not reflect total absence of viable  
18 bacteria. We therefore measured viable bacteria colonizing the coated and uncoated TIVAP  
19 by counting colony-forming units from the endoluminal part of extracted TIVAP after 5 days  
20 of biofilm formation. MeCe derivative **1** coating of TIVAP PDMS parts resulted in a 2- to 3-  
21 log reduction, for both pathogens, of bacteria colonizing the TIVAP after 5 days. Additional  
22 coating of titanium with PEG derivative **2** further inhibited bacterial colonization, with the  
23 reduction reaching 4 to 5 logs.  
24

1 **MeCe grafting prevents biofilm-associated clogging of TIVAP implanted in rats.**

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

23

## 1 **Discussion**

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

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

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

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

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6

7 **Author contributions**

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

12

13 **Competing financial interests**

14 The authors declare no competing financial interests.

15



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25  
26

1 **Tables**

2

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

5

<b>Organism</b>	<b>Treated (n=9)</b>	<b>Untreated (n=9)</b>
<i>S. aureus</i>	<b>22%</b>	<b>90%</b>
<i>P. aeruginosa</i>	<b>0%</b>	<b>66%</b>

6

7

8

## 1 **Figure Legends**

2

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

9

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

1 Graphpad Prism version 5.0c. A  $P$  value  $< 0.05$  was considered significant; \*  $P \leq 0.05$ , \*\*  $P \leq$   
2  $0.01$ , \*\*\*  $P \leq 0.001$  and ns (non-significant).

3

4 **Figure 3. *In vivo* inhibition of initial adhesion and mature bacterial biofilm development**  
5 **on TIVAP by biomimetic glycocalyx-like polymers.** Rats with modified or unmodified  
6 implanted TIVAP were maintained by the flush and draw technique for 4 days prior to  
7 bacterial inoculation. An inoculum of  $10^6$  CFU *S. aureus* or  $10^3$  CFU *P. aeruginosa*/50  $\mu$ L 1X  
8 PBS was injected into implanted TIVAP. Bacteria were allowed to adhere to the  
9 PDMS/silicone surface for 3 h (*S. aureus*) or 1.5 h (*P. aeruginosa*). (**A**), planktonic bacteria  
10 were removed and rats (n = 3 rats per group) were either sacrificed to aseptically remove the  
11 implanted TIVAP and evaluate the initial adherent population or (**B**), biofilms were left to  
12 form for 5-days and TIVAP extracted to measure bacterial biofilm colonization. Adherent  
13 bacteria were harvested and counted by plating on TSB agar for *S. aureus* or LB agar plates  
14 for *P. aeruginosa*. Control, Si and Si-Ti as described in Figure 2. Statistical analysis was done  
15 using one-way analysis of variance (ANOVA) with Graphpad Prism version 5.0c.  $P$  value  $<$   
16  $0.05$  was considered significant; \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*\*  $P \leq 0.0001$  and ns (non-  
17 significant). (**C to H**) Rats were monitored for bioluminescence up to day 5 using Xenogen  
18 IVIS 100. *S. aureus* biofilm formation with: (**C**), control rats with unmodified TIVAP; (**D**),  
19 rats with Si TIVAP and (**E**), rats with Si-Ti TIVAP. *P. aeruginosa* biofilm formation with:  
20 (**F**), control rats with unmodified TIVAP ; (**G**), rats with Si TIVAP and (**H**), rats with Si-Ti  
21 TIVAP.

22

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



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

6

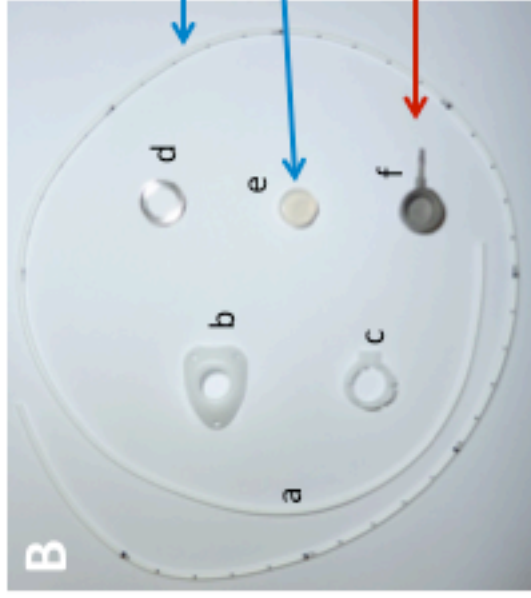
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**A**

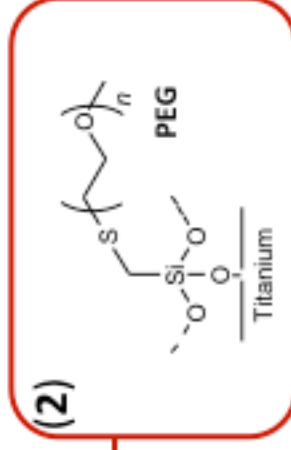
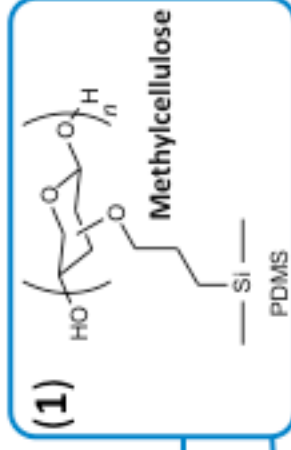


Commercial  
Totally implantable  
venous access port (TIVAP)



Disassembled TIVAP

**C**



catheter

septum

Port

