Society For Biomaterials

Improved *ex vivo* blood compatibility of central venous catheter with noble metal alloy coating

Manijeh Vafa Homann,[1] Dorota Johansson,[2] Håkan Wallen,[3] Javier Sanchez[2,3]

[1]Infectious Disease Unit, Karolinska University Hospital, Stockholm, Sweden
[2]Research and Development Department, Bactiguard AB, Stockholm, Sweden
[3]Division of internal and Cardiovascular Medicine, Department of Clinical Science Danderyd Hospital, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden

Received 11 August 2014; revised 22 December 2014; accepted 16 February 2015
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.33403

Abstract: Central line associated bloodstream infections (CLABSIs) are a serious cause of morbidity and mortality induced by the use of central venous catheters (CVCs). Nobel metal alloy (NMA) coating is an advanced surface modification that prevents microbial adhesion and growth on catheters and thereby reduces the risk of infection. *In vitro* microbiological analyses have shown up to 90% reduction in microbial adhesion on coated CVC compared to uncoated ones. This study aimed to assess the blood compatibility of NMA-coated CVC according to ISO 10993-4. Hemolysis, thrombin–antithrombin (TAT) complex, platelet counts, fibrin deposition, and C3a and SC5b-9 complement activation were analyzed in human blood exposed to the NMA-coated and control CVCs using a Chandler-loop model. NMA-coated CVC did not induce hemolysis and fell in the "nonhemolytic" category according to ASTM F756-00. Significantly lower amounts of TAT were generated and less fibrin was deposited on NMA-coated CVC than on uncoated ones. Slightly higher platelet counts and lower complement markers were observed for NMA-coated CVC compared to uncoated ones. These data suggest that the NMA-coated CVC has better *ex vivo* blood compatibility compared to uncoated CVC. © 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2015.

Key Words: blood compatibility, central line associated blood stream infection, central Venous catheter, nobel metal alloy coating, surface modification

How to cite this article: Vafa Homann M, Johansson D, Wallen H, Sanchez J. 2015. Improved *ex vivo* blood compatibility of central venous catheter with noble metal alloy coating. J Biomed Mater Res Part B 2015:00B:000–000.

INTRODUCTION

Healthcare-associated infections (HAIs) are a major cause of morbidity and mortality, with a high economic impact worldwide. As reported by the American Center for Disease Control and Prevention, the approximate annual occurrence of HAIs is about 1.7 million, which gives rise to nearly 99,000 deaths and overall direct hospital costs of $36–45 billion.[1,2] Similar estimates in Europe indicate that a yearly incidence of 4 million HAIs leads to approximately 37,000 deaths, 16 million added days of hospitalization, and a direct cost of about €7 billion.[3]

Large proportion of HAIs is related to the use of medical devices such as catheters and ventilators.[4,5] Catheter-related bloodstream infections (CRBSIs) also called CLABSI, catheter-associated urinary tract infections (CAUTIs), and ventilator-associated pneumonia (VAP) are HAIs associated with the application of specific medical devices.

CLABSI is the most serious and life-threatening blood stream infection (BSI), and it is associated with the use of central access devices such as central venous catheter (CVC). CLABSIs are found to be the most costly,[6] the second-most deadly, and the third-most frequent HAI.[1] CVCs are widely used in critically ill patients to administer medication, blood products, and fluids as well as to obtain blood samples. An estimated three to five million CVCs were employed in medical care each year in the USA alone,[7] leading to about 41,000 CLABSI episodes with a mortality rate of 1–4.[8] Recent studies in Germany[9] and the USA[6] have reported median attributable additional costs of €29,909 and $45,814 per case of CLABSI, respectively. Both studies indicated a median of seven days length of stay (LOS) for each CLABSI episode.

The application of CVC in clinical care is unavoidable, whereas the incidence of CVC-associated infections, to a large extent, is preventable.[8] Several reports have shown that despite a general decline in HAI incidence after the employment of intervention programs,[9] catheter-related infections remain a burden on the global health system. Thus, for effective intervention, it is important to understand the mechanism underlying catheter-related pathogenesis. Catheters provide microbes with a surface for adhesion, which is crucial for the growth and persistence of the infection. Moreover, catheter as foreign material induces host immunological/inflammatory responses and, if in

Correspondence to: J. Sanchez; e-mail: javier.sanchez@bactiguard.se

contact with blood, also initiates the clotting cascade, which in turn can lead to serious complications. Hence, a key approach to reduce the risk of catheter-related infection is to employ catheters with anti-microbial properties that are highly biocompatible.

Bactiguard® (BG) has developed an advanced technology to coat medical devices with a thin layer of noble metals to achieve modified surfaces that reduce microbial adhesion and microbial growth while being biocompatible and devoid of antibiotics. BG-coating was first applied on latex and silicone Foleys (Bardex I.C. and Lubri-Sil I.C.) together with CR Bard® in the mid-1990s. BG-coated Foleys have been tested in several clinical trials,[10] were approved by the FDA (Food and Drug Administration of the USA) in 1994, and have been in the market ever since. Extra caution should be taken when employing any CVC, a class III product for use in the blood stream, as they may enhance the risk of thrombosis. Bactiguard (called Metacot at the time), together with Fresenius, developed a noble metal coated polyurethane CVC. Clinical trials of the product indicated that BG-coated CVCs not only reduced the risk of infection but also resulted in less thrombosis (0.8%) as compared to uncoated CVC (2.7%); however, the difference did not reach statistical significance due to low numbers.[11,12]

BG has recently developed a new BG-coated CVC, the Bactiguard Infection Protection (BIP) CVC, intended to have increased anti-microbial properties without compromising blood compatibility. A modified Ahearn test[13] was performed to evaluate the anti-microbial properties of the BIP-CVC in *in vitro* and up to 90% reduction in bacterial adhesion on BIP-CVC was observed (Persson L. et al. unpublished).

This study aimed to assess blood compatibility of the BIP-CVC according to ISO 10993-4. For this purpose, hemolytic characteristics and the capacity of the BIP-CVC to activate coagulation (as defined by thrombin–antithrombin (TAT) generation, fibrin deposition, and platelet counts) and the complement system (using measures for C3a and SC5b-9) cascades were investigated and compared to values obtained from analysis of commercially available uncoated CVCs currently in clinical use.

MATERIALS AND METHODS

Bactiguard surface and control materials

The Bactiguard coating was applied on standard (commercially available) polyurethane (PU) CVC according to Bactiguard AB's patented standard coating protocol, which includes a validated set of dipping steps in metal solutions of silver (Ag), gold (Au), and palladium (Pd), producing a submicron layer of metals firmly attached to the surface without significant release during use. The coating metal composition at the surface follows standardized specifications approved by regulatory authorities and maintained for each batch. This composition has been optimized for the CVC application with the goal of achieving an optimal combination of anti-infective properties and good blood compatibility.

The uncoated control was a commercial PU standard multi-lumen central venous catheter purchased from Biosensors International™, and the Chlorhexidine Silver Sulfadiazine (CSS) catheter was an ARROWg+ard Blue multilumen antibacterial central venous catheter purchased from Arrow® International. The CSS-CVC and the uncoated CVC are presently applied in medical care.

Blood collection

This study was approved by the National Ethics Committee at Karolinska Institute in Stockholm, Sweden (Dnr 2010/1627-31/3), and informed consent was given by all participants.

Twenty-three healthy adult volunteers, who had not taken any medication for at least two weeks prior to the blood sampling, were included in each experiment. "Healthy" was defined as the absence of any kind of autoimmune diseases, asthma, coagulation disorders, ongoing ulcer, or pregnancy as well as known hepatitis and HIV infections. Blood was drawn from the median cubital vein using an 18-G (1.2 mm ∅) needle (Trumo Europe, Leuven Belgium) connected to 30 cm of platinum silicone tubing, allowing the blood to flow gently into the collecting tube. After discarding the first 2 mL, 40 mL blood was collected into a 50 mL Falcon® tube (Sarsted, Numbrecht, Germany) with heparin added to a final concentration of 0.1 U/mL (Leo Pharma, 100IE/KY/mL), which was then immediately aliquoted in Chandler loops.

Sample preparation

For measurement of baseline values, ethylene-diamine-tetra-acetic acid (EDTA)- and citrate-anticoagulated aliquots ("00") were separated from each blood sample prior to circulation in the Chandler loop. The remainder of the blood was aliquoted in loops containing testing materials. In our *ex vivo* model, 7.5 cm^2 of CVC (7 Fr) is exposed to 4.5 mL blood, giving rise to an exposure ratio of 1.125 cm^2/mL, which is [mt]500 times the exposure ratio in *in vivo*. The first loop was left with no device and served as a negative control or blank to measure the background effect of the bare loop on tested parameters. Uncoated CVC and CSS-CVC were used as controls. Plasma was collected after immediate centrifugation of blood and saved in 200 μL aliquots at −70 °C for further analyses.

Chandler loop model

A modified Chandler loop model was used to assess the blood compatibility of BIP coating materials as previously described.[14–16] Briefly, polyvinyl chloride (PVC) tubes (Medtronic, Minneapolis, USA), internally coated with Carmeda BioActive Surface (CBAS), with an internal diameter of 6.5 mm and 30 cm length, pre-treated with saline for 5 min, were filled with 4.5 mL fresh whole blood. The tubing was closed into loop with CBAS coated-Medtronic Intersept® connector (Medtronic, Minneapolis, MN). The loops were then mounted on a Plexiglas plate and rotated vertically in a 37 °C water bath at 10 rpm for 60 min as shown in Figure 1. After circulation, blood was removed from loops, and 3 mL was transferred into EDTA- (BD Vacutainer®, K2E 5.4 mg) and 1 mL into citrate- (BD Vacutainer®, 9NC

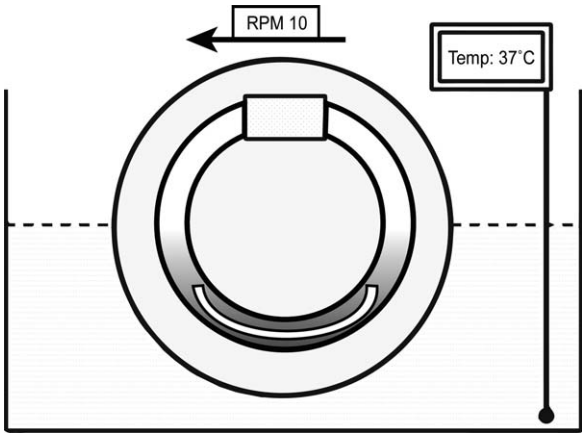

FIGURE 1. Chandler loop diagram model used in the present investigation. The CVC materials were placed into the loop and carefully filled with fresh blood. The loops were closed and placed in a Plexiglas table (10 rpm) and rotated vertically in a water bath at 37 °C for 60 min.

0.129*M*) containing tubes. Plasma was collected and stored as previously stated.

Blood compatibility assays

Blood compatibility tests, including measures of coagulation and complement activation markers, hemolysis, and platelet counts as well as fibrin deposition, were performed according to ISO 10993-4 (Biological evaluation of medical devices-Part 4: Selection of tests for interactions with blood, ISO 10993-4:2002, Amd 1:2006).

Coagulation and hemolysis assays

Induction of coagulation was assessed by applying measures for platelet counts, TAT, and fibrin deposition. Whole-blood count was measured in EDTA anticoagulated blood according to the standard procedure for clinical evaluation of blood in the Clinical Chemistry Department at Karolinska University Hospital.

TAT was measured in plasma collected from citrated blood, using a commercially available ELISA kit (Enzygnost® TAT micro, Siemens AG, Muenchen, Germany), following the instructions provided by the manufacturer.

The endpoint product of the coagulation cascade is a fibrin clot. After blood incubation in the loops, tested CVCs were washed with saline (0.9%) and stained with a suspension of hematoxylin (0.1%) at room temperature for 30 min. Fibrin deposition on CVCs was evaluated visually before and after staining.

The hemolysis assay was performed using a QuantiChrome ™ Hemoglobin assay kit (Bioassay Systems, Hayward, USA) to estimate the release of hemoglobin to plasma due to possible damage of red blood cells (RBCs) caused by tested materials.

Complement activation

The activation of the complement system is one of the most significant challenges when blood is exposed to foreign materials.[16–19] Measures of C3a and SC5b-9, also called Terminal Complement Complex (TCC), are widely used as complement activation markers. In plasma samples collected from EDTA anticoagulated blood, C3a and SC5b-9 levels were measured using a MicroVue™ C3a and SC5b-9 Plus EIA Kit (San Diego, USA).

Analysis of TAT, hemolysis, and the complement system were carried out at the laboratories of the Clinical Research Center at Danderyd Hospital. The tests were conducted following the assay procedure specified for each analysis.

Statistical analysis

The GraphPad Prism software package (version 5, GraphPad Software, La Jolla, USA) was used to perform all statistical analyses. The results shown in figures are expressed as mean (M) ± standard deviation (SD). The unpaired t test was used to analyze the confidence interval between the means of different groups. The non-parametric rank sum Mann–Whitney test was performed to compare the groups. Values of $p < 0.05$ were considered significant, $p < 0.01$ and $p < 0.001$ were considered highly significant, and values of $p \geq 0.05$ were considered not significant.

RESULTS

In most of the experiments two of each uncoated and BIP-coated CVCs were tested. Most of the assessments were not feasible for the CSS-CVC, due to the pronounced hemolysis induced by this material (see Hemolysis test).

Hemolysis test

Hemolytic properties of the BIP CVC ($n = 29$) were tested and compared to hemolytic values of blank ($n = 9$), uncoated CVC ($n = 23$), and CSS-CVC ($n = 3$). The hemolytic index or % hemolysis was calculated as the ratio of free plasma hemoglobin to total blood hemoglobin concentration and expressed as a mean percentage ± SD. The mean hemolytic index of the blank was subtracted from those of tested materials to define the hemolytic grades according to the standard protocol for testing hemolytic properties of materials (ASTM F756-00).

Hemolytic indexes and grading are presented in Figure 2. No statistically significant differences in hemolytic properties of BIP-CVCs and uncoated ones (mean 2.96 ± 0.5 and 2.77 ± 0.65%, respectively) were observed when compared to the blank (mean 2.28 ± 0.61%). Hemolysis values were significantly enhanced in the samples incubated with CSS-CVC (mean 29.33 ± 8.92%, $p < 0.0001$) as compared to blank and other tested CVCs [Figure 2(A)]. As shown in Figure 2(B), uncoated and BIP-CVC (hemolytic grade 0.7 and 1.0, respectively) were classified as "nonhemolytic" according to ASTM F756-00 protocol, whereas the CSS-CVC with a hemolytic grade of 25.8, was categorized as "hemolytic." The pronounced hemolytic characteristics of CSS-CVC interfered with the results of coagulation and complement activation analysis, leading to its exclusion from the rest of the experiment.

TAT generation

TAT generation was measured in 38 and 43 samples exposed to BIP- and uncoated CVCs respectively, as well as

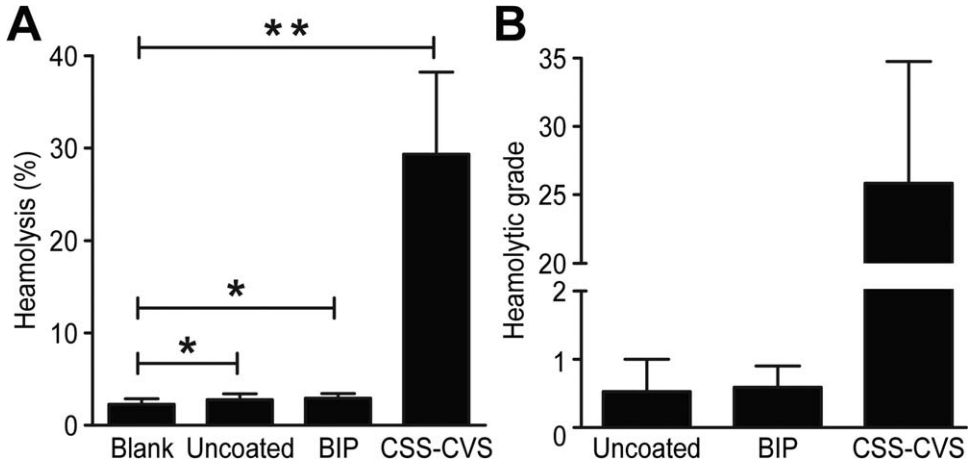

FIGURE 2. Hemolytic index (A) and grades (B) in blank ($n = 9$), uncoated-CVC ($n = 23$), BIP-CVC ($n = 29$), and CSS-CVC ($n = 3$). Uncoated and BIP-CVC showed no hemolytic properties, while a pronounced level of hemolysis was seen in the samples in contact with CSS-CVC. According to ASTM F756-00, haemolytic index 0-2% is classified as "nonhemolytic," 2–5% as "slightly hemolytic" and >5% as "hemolytic." Based on this, BIP-CVC was graded as "Nonhemolytic," where CSS-CVC was categorized as "Hemolytic." *$p > 0.05$, **$p < 0.0001$.

in 17 blank controls. Results are summarized in Figure 3. Statistically significantly less TAT was generated after incubation with BIP-CVC as compared to the uncoated control (mean 337.4 ± 269 vs. 590.4 ± 541 μg/L, $p = 0.02$). Circulation of blood in a bare loop led to a significant production of TAT when compared to the "00" control (mean 26.8 ± 11.9 vs 9.1 ± 7.7 μg/L, $p < 0.0001$). Exposure to both coated and uncoated CVCs increased the TAT production significantly ($p < 0.0001$).

Fibrin deposition

Visual inspection of control and coated CVCs before and after staining showed a pronounced difference in fibrin deposition on tested groups. None to minimal fibrin was deposited on BIP CVC surfaces, whereas detectable amount of fibrin was formed on the surface of uncoated CVCs (Figure 4).

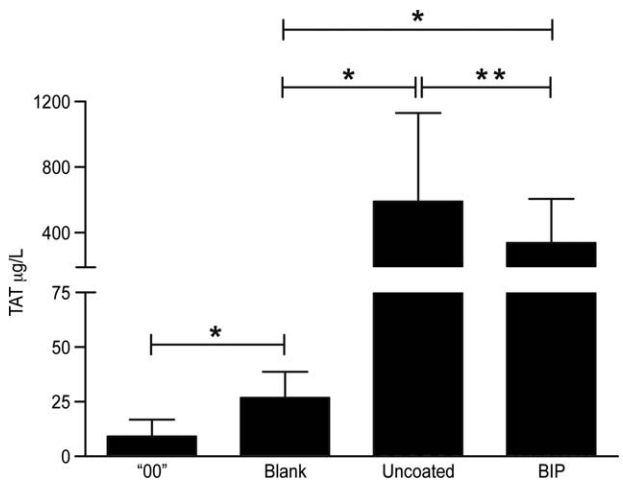

FIGURE 3. Measures of TAT generation induced by control ($n = 43$) and BIP-CVC ($n = 38$) in comparison to blank ($n = 17$) and "00" control ($n = 22$), presented as mean ± SD in mg/L. Significantly lower amount of TAT was generated in exposure to BIP-CVC than uncoated ones. Blood circulation in bare loop induced the TAT generation and exposure to both tested CVCs enhanced the TAT production significantly. *$p < 0.001$, **$p = 0.02$.

Platelet counts

Platelet counts were determined in 38 and 43 blood samples exposed to BIP- and uncoated CVCs respectively, as well in 14 blank controls. As can be seen in Figure 5, the median number of remaining platelets in samples exposed to BIP-CVC (80.9%) was slightly higher than in those incubated with uncoated CVC (77.2%), however the difference did not reach statistical significance ($p > 0.05$). These values were significantly lower than remaining platelet counts after circulation in the bare loop (median 95.5%, $p < 0.0001$).

Complement activation

Activation of the complement was assessed by measuring C3a and SC5b-9. Circulation of blood in the bare loop caused a significant increase in C3a (mean 805.6 ± 84.9 vs. 110.5 ± 57.9 ng/mL, $p = 0.001$) and SC5b-9 levels (mean 507.6 ± 126.9 vs. 122.0 ± 43.79 ng/mL, $p < 0.0001$) as compared to the "00" control (Figure 6). Production of C3a was significantly amplified after exposure to uncoated (mean 1403 ± 413.4 ng/mL, $p = 0.001$) and BIP-CVC (mean 1355 ± 517.9 ng/mL, $p = 0.03$) when compared to the blank. SC5b-9 production was not significantly boosted after blood contact with either BIP (mean 540 ± 178.4 ng/mL) or uncoated CVC (582 ± 229.0 ng/mL).

FIGURE 4. This figure shows the status of fibrin deposition on the catheter material after being exposed to blood for 60 min and then stained with hematoxylin. The control catheter is in the top part of the picture while the BIP catheter is placed on bottom.

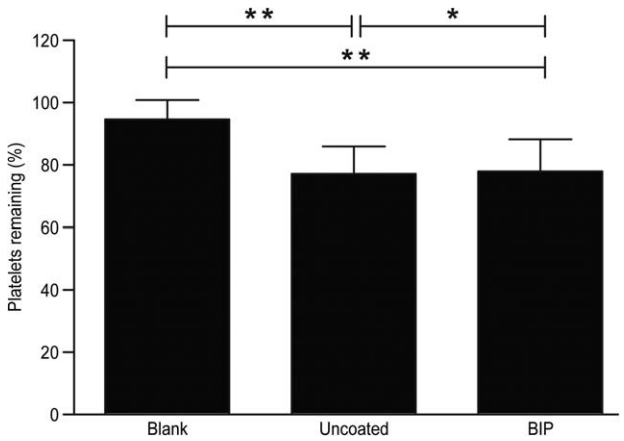

FIGURE 5. Platelet counts remaining in the circulation after 1 h exposure to BIP- ($n = 38$) as compared to uncoated-CVC ($n = 43$) and blank ($n = 14$), presented as median in percentage. No significant difference was observed between tested CVCs whereas significantly higher platelet counts were remained in the blank. *$p > 0.05$, **$p < 0.0001$.

No significant difference in inflammatory markers (C3a and SC5b-9) was seen between samples exposed to BIP- and uncoated CVCs. However, BIP-CVC activated the complement to a slightly lower extent as compared to uncoated ones.

DISCUSSION

Surface modification of medical devices may influence their biocompatibility, and thus, they must be cautiously assessed in this respect. Biocompatibility of invasive devices is vital when a device is in contact with blood, due to the risk of thrombus formation. The present study analyzed the impact of Bactiguard noble metal coating (surface modification) on the blood compatibility properties of a CVC according to ISO 10993-4.

Hemolytic characteristics of a biomaterial are fundamental features in defining its blood compatibility. A blood compatible device, by definition, should not induce hemolysis. Based on our results, BIP- and uncoated CVC did not differ with regard to their hemolytic properties and both were found to be "nonhemolytic," indicating that BIP-surface modification does not cause any damage to erythrocytes' membrane that can lead to their lysis. CSS-CVC, on the contrary, induced prominent hemolysis and was classified as "hemolytic."

The coagulation cascade protects and maintains the integrity of blood vessels, can be initiated by different factors and ends in the conversion of fibrinogen into fibrin by thrombin. Coagulation is tightly regulated by deactivation of thrombin through complex binding with antithrombin to form a TAT complex. Measurement of TAT levels is used as an indicator of thrombin generation, as thrombin is a protease central to coagulation but also deeply involved in other processes, such as inflammation and platelet activation. Notably, BIP-CVC showed a significantly lower TAT, and thereby lower potential for coagulation activation when compared to original CVC with unmodified surface. Similarly, very little to no fibrin formation was noted on BIP-CVCs, suggesting that thrombin is not produced to a sufficient level to form a visible clot.

Platelets are crucial elements for hemostasis due to their ability to adhere and aggregate as well as to support thrombin generation and subsequently blood coagulation. It has been found that contact with foreign materials activates platelets and subsequently leads to their removal from circulation.[20] The magnitude of platelet counts remaining in circulation after exposure to medical devices reflects the blood compatibility of the biomaterial. We observed that contact of blood with BIP-CVC led to a small depletion (< 20%) in platelets from circulation. The impact of BIP-CVC on platelet consumption was slightly smaller than that of uncoated CVC. However, the remaining platelet count in the blank was significantly more than those of BIP- and uncoated CVCs. Activated platelets have been shown to promote thrombin generation.[21] This corresponds with the TAT analysis where we observed considerably less thrombin production in response to BIP-CVC compared to the uncoated CVC. Thrombotic complications are a fatal outcome associated with the use of vascular devices.[20] Our results from coagulation activation analysis suggest that BIP coating may potentially lower the risk of thrombotic complications. This idea is supported by the results from a clinical study in which only 0.8% of patients using BG-coated CVCs developed thrombosis, while 2.7% of patients using uncoated

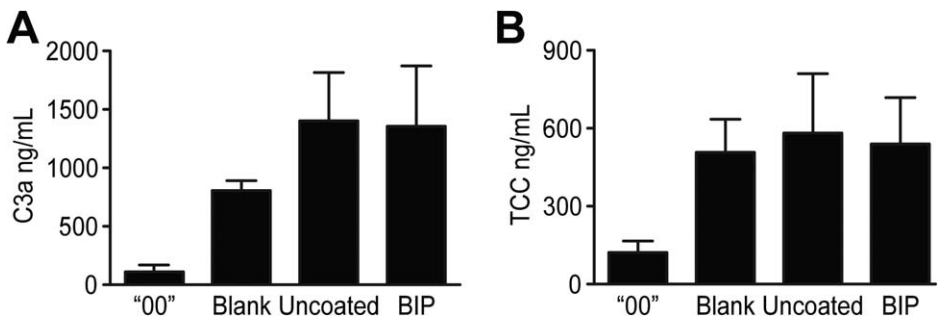

FIGURE 6. C3a (A) and TCC (B) generations, induced by uncoated ($n = 37$) and BIP-CVC ($n = 32$) compared to blank ($n = 5$) and "00" control ($n = 16$), presented as mean ± SD ng/mL. Both C3a and TCC were elevated significantly after circulation in bare loop ($p < 0.001$). C3a generation was amplified in exposure to both uncoated- ($p = 0.001$) and BIP-CVC ($p = 0.03$) while TCC production did not increase markedly ($p > 0.05$). BIP-CVC induced insignificantly lower amount of C3a and TCC in comparison with uncoated-CVC ($p > 0.05$).

CVC had this complication.[9] However, further clinical trials in a larger study population are required to confirm this idea.

The presence of foreign material in the blood activates the complement system as part of the host immune response and initiates a systemic inflammatory reaction, which can lead to biomaterial-associated complications. Contact activation of complement by artificial surfaces gives rise to a rapid release of C3a and C3b. The activation of the cascade through all pathways proceeds to the generation of SC5b-9/TCC. C3a and C5a have been reported to be the most potent anaphylatoxic proteins in blood, which recruit and activate polymorphonuclear leukocytes (PMNs) and monocytes and coordinate the inflammatory reaction originating from the material surface. Together with soluble C3a and C5a, production of SC5b-9 leads to chemotaxis and cytokine release as well as generation of prostaglandins and leukotrienes, which may further enhance inflammation.[22] In addition, SC5b-9 induces platelet activation, resulting in procoagulant activity of platelets and expression of P-selectin, the latter of which mediates the binding of platelets to leukocytes.[23,24] Measures of C3a and SC5b-9/TCC can be used as indicators of acute and complete activation of complement via various pathways. In the present study, contact of blood with the bare loop activated the complement. The C3a production was increased in contact with uncoated CVC and to a lower extent in response to the BIP-CVC. SC5b-9 formation was not significantly amplified after contact with any of tested CVCs. These data suggest that the whole cascade was not activated and the acute amplification of complement response induced by CVCs did not proceed to terminal steps, which is the actual initiator of the inflammatory response. The discrepancy between C3a and SC5b-9 levels may also be explained by the fact that thrombin can induce C3 cleavage and the release of C3a, resulting in a background level of this component,[25] in which case the initial complement activation might not be directly induced in contact with CVCs. Moreover, studies by Gorbet and Sefton suggest that the terminal pathway of complement (C5b-9) is more important in leukocyte activation than the common pathway (C3a and C3b).[25] SC5b-9 is also suggested as a remarkably stable and therefore more reliable indicator of complement activation.[26] Our results indicated that the BIP-CVC is more blood compatible with respect to complement activation as compared to the uncoated CVC.

Hulander et al.[27,28] used whole blood in a "slide chamber model" to study the immune complement activation, generation of thrombin/antithrombin (TAT) complexes, and platelet depletion from blood upon contact with silver (Ag), palladium (Pd), gold (Au), titanium (Ti), and the Bactiguard surface. In this publication, the TAT generation and platelet depletion was lower on the Bactiguard surface, and the microbalance adsorption studies showed that the fibrinogen adsorption was lowest on the Bactiguard surface. The author concluded that the individual noble metals could not alone explain the low coagulation on the Bactiguard surface, and a synergistic effect of the chemistry, galvanic effects, and structure would be a likely explanation. Further research should focus on investigating the importance of the different factors.

In our *ex vivo* model, almost 500 times the exaggerated ratio of CVC surface exposed to blood was applied compared to the actual exposure ratio in *in vivo*. This suggests that even lower values of coagulation and complement activation markers than observed in the present study should be expected *in vivo*. All together, our results revealed that BIP coating has potentially improved blood compatibility properties of the CVC. Results from the Ahearn test showed up to a 90% decrease in bacterial adhesion on the BIP-CVC (Persson et al., unpublished). Preliminary data from the Tube method[29] to detect biofilm formation has indicated that those bacteria that manage to adhere to the BIP-CVC do not go on to colonize or form biofilms (personal communication with Javier Sanchez). However, further safety and clinical trials are required to confirm the anti-infective and blood compatibility properties of the BIP-CVC. The safety trial of the BIP-CVC has been approved by the National Ethics Committee at Karolinska Institute in Stockholm, Sweden (Dnr 2013/622-31/4) and is in process.

CONCLUSION

Despite a remarkable decline in the number of CLABSI during the last two decades, owing to the employment of different prevention strategies,[8] CVC-related infection/complications remain a major issue confronting health care systems worldwide, emphasizing the urgent need for devices with anti-infective properties that are devoid of antiseptics and antibiotics and are blood compatible. Results of the present study together with biofilm formation prohibition and potent adhesion-inhibition observed in *in vitro* studies suggest that BIP surface treatment can be a key approach to battle HAI in general and CLABSI in particular.

ACKNOWLEDGMENTS

The authors especially thank the blood donors who participated in the present study as well as Graciela Elgue, Lena Gabrielsson, Catarina Aguilera and Sofia Sjöberg from Clinical Research Center, Danderyd Hospital, for their cooperation and assistance in the execution of the experiments.

REFERENCES

1. Klevens RM, Edwards JR, Richards CL Jr, Horan TC, Gaynes RP, Pollock DA, Cardo DM. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep 2007; 122:160–166.
2. ScottRD, II. The Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention. Atlanta, GA: Centers for Disease Control and Prevention; 2009.
3. European Centre for Disease Prevention and Control. Surveillance of Healthcare-Associated Infections in Europe. Stockholm: ECDC; 2007.
4. Di Filippo A, Casini A, de Gaudio AR. Infection prevention in the intensive care unit: Review of the recent literature on the management of invasive devices. Scand J Infect Dis 2011;43:243–250.
5. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol 2010;59:227–238.
6. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, Keohane C, Denham CR, Bates DW. Health care-associated

infections: A meta-analysis of costs and financial impact on the US health care system. JAMA Intern Med 2013;173:2039–2046.
7. Chugh TD, Khan ZU. Intravascular device-related infections: Antimicrobial catheters as a strategy for prevention. J Hosp Infect 2001;49:1–3.
8. World Health Organization. Preventing Bloodstream Infections From Central Line Venous Catheters. Geneva: WHO; 2011.
9. Leistner R, Hirsemann E, Bloch A, Gastmeier P, Geffers C. Costs and prolonged length of stay of central venous catheter-associated bloodstream infections (CVC BSI): A matched prospective cohort study. Infection 2014;42:31–36.
10. Beattie M, Taylor J. Silver alloy vs. uncoated urinary catheters: A systematic review of the literature. J Clin Nurs 2011;20:2098–2108.
11. Goldschmidt H, Hahn U, Salwender HJ, Haas R, Jansen B, Wolbring P, Rinck M, Hunstein W. Prevention of catheter-related infections by silver coated central venous catheters in oncological patients. Zentralbl Bakteriol 1995;283:215–223.
12. Harter C, Salwender HJ, Bach A, Egerer G, Goldschmidt H, Ho AD. Catheter-related infection and thrombosis of the internal jugular vein in hematologic-oncologic patients undergoing chemotherapy: A prospective comparison of silver-coated and uncoated catheters. Cancer 2002;94:245–251.
13. Gabriel MM, Sawant AD, Simmons RB, Ahearn DG. Effects of silver on adherence of bacteria to urinary catheters: In vitro studies. Curr Microbiol 1995;30:17–22.
14. Andersson J, Sanchez J, Ekdahl KN, Elgue G, Nilsson B, Larsson R. Optimal heparin surface concentration and antithrombin binding capacity as evaluated with human non-anticoagulated blood in vitro. J Biomed Mater Res A 2003;67:458–466.
15. Fink H, Hong J, Drotz K, Risberg B, Sanchez J, Sellborn A. An in vitro study of blood compatibility of vascular grafts made of bacterial cellulose in comparison with conventionally-used graft materials. J Biomed Mater Res A 2011;97:52–58.
16. Sinn S, Scheuermann T, Deichelbohrer S, Ziemer G, Wendel HP. A novel in vitro model for preclinical testing of the hemocompatibility of intravascular stents according to ISO 10993-4. J Mater Sci Mater Med 2011;22:1521–1528.
17. Harboe M, Ulvund G, Vien L, Fung M, Mollnes TE. The quantitative role of alternative pathway amplification in classical pathway induced terminal complement activation. Clin Exp Immunol 2004; 138:439–446.
18. Lappegard KT, Bergseth G, Riesenfeld J, Pharo A, Magotti P, Lambris JD, Mollnes TE. The artificial surface-induced whole blood inflammatory reaction revealed by increases in a series of chemokines and growth factors is largely complement dependent. J Biomed Mater Res A 2008;87:129–135.
19. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. The role of complement in biomaterial-induced inflammation. Mol Immunol 2007; 44:82–94.
20. Gorbet MB, Sefton MV. Biomaterial-associated thrombosis: Roles of coagulation factors, complement, platelets and leukocytes. Biomaterials 2004;25:5681–5703.
21. Sefton MV, Gemmell CH, Gorbet MB. What really is blood compatibility? J Biomater Sci Polym Ed 2000;11:1165–1182.
22. Engberg AE, Nilsson PH, Huang S, Fromell K, Hamad OA, Mollnes TE, Rosengren-Holmberg JP, Sandholm K, Teramura Y, Nicholls IA, et al. Prediction of inflammatory responses induced by biomaterials in contact with human blood using protein fingerprint from plasma. Biomaterials 2015;36:55–65.
23. Rinder C, Fitch J. Amplification of the inflammatory response: Adhesion molecules associated with platelet/white cell responses. J Cardiovasc Pharmacol 1996;27(Suppl 1):S6–S12.
24. Zhang W, Lachmann PJ. Neutrophil lactoferrin release induced by IgA immune complexes can be mediated either by Fc alpha receptors or by complement receptors through different pathways. J Immunol 1996;156:2599–2606.
25. Gorbet MB, Sefton MV. Complement inhibition reduces material-induced leukocyte activation with PEG modified polystyrene beads (Tentagel) but not polystyrene beads. J Biomed Mater Res A 2005;74:511–522.
26. Mollnes TE. Complement and biocompatibility. Vox Sang 1998; 74(Suppl 2):303–307.
27. Hulander M, Hong J, Andersson M, Gerven F, Ohrlander M, Tengvall P, Elwing H. Blood interactions with noble metals: Coagulation and immune complement activation. ACS Appl Mater Interfaces 2009;1:1053–1062.
28. Hulander M, Lundgren A, Berglin M, Ohrlander M, Lausmaa J, Elwing H. Immune complement activation is attenuated by surface nanotopography. Int J Nanomed 2011;6:2653–2666.
29. Singhai M, Malik A, Shahid M, Malik MA, Goyal R. A study on device-related infections with special reference to biofilm production and antibiotic resistance. J Glob Infect Dis 2012;4:193–198.