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Hydrophilic surface coatings with embedded biocidal silver nanoparticles and sodium heparin for central venous catheters

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ABSTRACT

Central venous catheters (CVCs) have become indispensable in the treatment of neonates and patients undergoing chemotherapy or hemodialysis. A CVC provides easy access to the patient's circulation, thus enabling facile monitoring of hemodynamic parameters, nutritional support, or administration of (cytostatic) medication. However, complications with CVCs, such as bacterial bloodstream infection or thromboembolism, are common. Bloodstream infections, predominantly caused by *Staphylococcus aureus*, are notoriously difficult to prevent and treat. Furthermore, patients receiving infusion therapy through a CVC are at risk for deep-vein thrombosis, especially of the upper limbs. Several recent clinical trials have shown that prophylactic anticoagulation (low-molecular-weight heparin or vitamin K antagonists) is not effective. Here, we report on the systematic development of a new bifunctional coating concept that can –uniquely– be applied to make CVC surfaces antimicrobial and antithrombogenic at the same time. The novel coating consists of a moderately hydrophilic synthetic copolymer of N-vinylpyrrollidinone (NVP) and n-butyl methacrylate (BMA), containing embedded silver nanoparticles (AgNPs) and sodium heparin. The work demonstrates that the AgNPs strongly inhibit adhesion of *S. aureus* (reference strain and clinical isolates). Surprisingly, heparin not only rendered our surfaces practically non-thrombogenic, but also contributed synergistically to their biocidal activity.

1. Introduction

Central venous catheters (CVCs) are used ubiquitously during treatment of critically ill cancer patients. According to recent estimates, more than 5 million cancer patients in the US require central venous access each year [1]. A similar estimate was made for Europe. CVCs offer important advantages, such as facile sustained administration of cytostatic or pain-killing medication, infusion of stem cells, continuous measurement of hemodynamic parameters, or sustained nutritional support. However, application of CVCs is associated with a significant risk for adverse effects, particularly bloodstream infection [2–5] and thromboembolism [6–10]. In the US, approximately 80,000 CVC-related nosocomial bloodstream infections occur annually. The associated extra cost is in the range of $300 million to $2.3 billion per year, and the attributable mortality is around 20%. On an average, survivors usually remain one extra week in the intensive care unit, or 2–3 additional weeks in the hospital.

Hence, prevention of CVC-related complications is of paramount importance. Regarding infection, preventive strategies include the use of (i), a maximum sterile barrier during CVC insertion; (ii), innovative catheter hubs, and (iii), chlorhexidine-containing cutaneous antiseptics [2–5,11]. Moreover, strict adherence to evidence-based protocols for hygiene and sterility proved highly successful [11]. Prevention of thrombotic complications is mostly attempted through administration of anticoagulants during treatment [6–10]. Despite all efforts, it is evident that there is a need for improved biomaterials for the manufacture of safer catheters. Engineering into this direction must focus of the catheter's surface, which must have broad-spectrum antimicrobial activity as well as excellent blood compatibility. We describe the systematic development of new bifunctional surface coatings that –uniquely– meet these requirements.

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Table 1
Atomic concentrations (percentages) measured by XPS. All data represent averages of two independent measurements.

Entry	Surface	C1s	N1s	O1s	Ag3d	S2p ($-SO_3H$)
1	SS	79.7	4.6	14.4	–	0.21
2	SS-Hep	78.6	4.3	14.5	–	0.43
3	SS-Ag6V	80.4	4.2	12.6	0.22	0.21
4	SS-Ag6V-Hep	79.2	5.0	14.2	0.11	0.42
5	SS-Ag4E	79.3	6.6	13.0	0.15	0.24
6	SS-Ag4e-Hep	78.9	5.3	14.6	0.19	0.35

2. Materials and methods

2.1. Formulation

Six different coating solutions were prepared as follows: (i), 600 mL of 10% solution of the hydrophilic copolymer (SS) in NMP was equally divided over six 500 mL glass bottles. (ii), Sodium heparin (3 × 1.5 g, purchased from Celsus Laboratories, Cincinnati, OH, USA) was dissolved (mechanical stirring) in formamide (75 mL). The solution was split into 3 equal parts, and these were mixed with 3 of the SS solutions as indicated in Table 1. (iii), AgNPs (Ag6V or Ag4E; 3.0 g, purchased from Metalor SA, Neuchâtel, Switzerland; Ag6V and Ag4E differed with respect to the hydrophilic surface coating used for their stabilization) were dispersed in NMP and mixed with 2 of the SS solutions as follows: **Coating #1** (SS): SS solution (100 mL) + NMP (50 mL). **Coating #2** (SS-Hep): SS solution (100 mL) + NMP (25 mL) + 1.5 g heparin dissolved in formamide (25 mL). **Coating #3** (SS-Ag6V): SS solution (100 mL) + NMP (50 mL) + nanosilver (1.5 g). **Coating #4** (SS-Ag6V-Hep): SS solution (100 mL) + NMP (25 mL) + nanosilver (1.5 g) + 1.5 g heparin dissolved in formamide (25 mL). **Coating #5** (SS-Ag4E): SS solution (100 mL) + NMP (50 mL) + nanosilver (1.5 g). **Coating #6** (SS-Ag4E-Hep): SS solution (100 mL) + NMP (25 mL) + nanosilver (1.5 g) + 1.5 g heparin dissolved in formamide (25 mL). Coatings had a thickness of 3.0 μm, and differed only with respect to the embedded species. Fabrication of the specimens involved three consecutive steps: (i) formulation of coating suspensions through mixing of the NVP/BMA copolymer (designated SS) + AgNPs suspended in N-methylpyrrolidone and/or sodium heparin dissolved in formamide; (ii) application of the coating onto a long (approximately 300 m) and thin (178 μm diameter) stainless steel wire in a continuous process; (iii), coiling of the coated wire around a rotating mandril of 600 μm diameter [12–14].

2.2. Methods and equipment

The platelet-stabilizing anticoagulant mixture, citrate-theophylline-adenosine-dipyridamole (CTAD) was purchased from Becton–Dickinson (Alphen a/d Rijn, Netherlands). Brain Heart infusion broth and Mueller-Hinton agar were from Oxoid BV (Badhoevedorp, Netherlands). The lactate dehydrogenase (LDH) assay was performed using the CytoTox 96 Non-Radioactive Cytotoxicity Assay from Promega Benelux BV (Leiden, Netherlands). Platelet activation was quantified with the Assachrom β-TG linked immunosorbent assay (ELISA), obtained from Roche Diagnostics Nederland BV (Almere, Netherlands). Fluorescence tracings were recorded on a SpectraMax M2 spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA). Absorbances were measured on an ELx808 Absorbance Microplate Reader (BioTek Instruments Inc., VT, USA). Sputter coating for electron microscopy was performed with a Sputter Coater 108/SE (Cressington Scientific Instruments Ltd., Watford, UK). Scanning electron microscopy was performed with a Philips XL30 instrument (Philips, Eindhoven, Netherlands). X-ray Photoelectron Spectroscopy measurements were carried out in a Quantera SXM instrument from Ulvac-PHI (Q2). During the measurements, the angle between the axis of the analyzer and the sample surface was 45°. The information depth is then appr. 6 nm. The measurements have been performed using monochromatic AlKα radiation in High Power mode (100 Watt, measuring spot 100 μm, scanned over 1400 μm × 500 μm).

2.3. Experiments with bacteria

Overnight cultures were prepared by inoculation of a bacterial colony into 10 mL growth medium (37 g/L heart infusion broth). From this culture, a bacterial suspension of 10^7 CFU/mL was prepared in 0.9% NaCl. Coated coils (length 30 mm) were incubated at 37 °C for 60 min in 1.5 mL bacterial suspension, in a shaking incubator. The samples were subsequently transferred to a fresh volume of 1.5 mL 0.9% NaCl, and vortexed for 1 s. Vortexing resulted in detachment of loosely adhered bacteria. Then, the samples were transferred again to fresh volumes of 1.5 mL of 0.9% NaCl, and left for 4 h under continuous shaking. Subsequently, the catheter samples were carefully removed, washed in 0.9% NaCl, and rolled over a Mueller-Hinton blood agar plate (38 g/L Mueller-Hinton agar, 5% defibrinated sheep blood). Plates were incubated overnight and photographed and evaluated on the next day. The six sample groups were subjected to these tests in three-fold.

2.4. Thrombin generation experiments

Freshly prepared human platelet-rich blood plasma (PRP) was used in the thrombin generation assays. There were two donor groups: a first group consisting of 5 healthy male volunteers (ages 22, 23, 24, 25, and 51 years), who were non-smokers and non-users of any drugs that could possibly influence hemostasis. The second group consisted of a cohort of 9 patients who were under treatment in the Maastricht University Medical Centre. These patients all received high-dose cytostatic medication through a central venous catheter; patient data are summarized in Table 2. Informed consent was obtained in accordance with the Declaration of Helsinki, and the study was approved by the Maastricht University Medical Centre ethical committee. The volunteers each donated appr. 40 mL blood through venipuncture. The collection tubes contained citrate for anticoagulation (end-concentration 0.013 M citrate). The patients donated 20 mL blood each. In these cases, Vacutainers containing citrate for anticoagulation were used. PRP was isolated through centrifugation (200 g, 15 min, room temperature). PRP was carefully transferred into new tubes and kept at 37 °C until further use. Thrombin generation experiments were done according to a one-donor-on-one-day scheme. On such an experimental day, 30 thrombin generation curves were measured (6 experimental coatings, each experiment in five-fold). The experiments were done in 4 consecutive steps: (i), Five pieces of 25 mm were cut out of each of the six different coils. Then, each piece was cut further into five approximately equal pieces, and these were transferred into one single well of a 96 well plate, (ii), The fluorogenic substrate for thrombin, Z-Gly-Gly-Arg-AMC (a product of Bachem Holding AG (Bubendorf, Switzerland; ref. I-1140) was added to the citrated PRP to a final concentration of 400 μM. Then, the PRP was "recalcified" through adding $CaCl_2$ stock solution (0.5 M) up to a final concentration of 20 mM Ca^{2+}. Consequently the mechanism for intrinsic (i.e., biomaterial-surface-induced) coagulation is no longer inhibited, (iii), The PRP was then rapidly distributed over the wells; 200 μL was transferred into each well. All pieces of the coiled wire were submersed in PRP. Wells without coil-samples served as controls. (iv), Fluorescence tracings were recorded at 37 °C. Wavelengths of excitation and measuring were 368 and 460 nm, respectively. Data were collected every 30 s; the plate was gently shaken for 2 s prior to each measurement. The fluorescence intensity was converted into nanomolar concentrations of thrombin. This technique resulted in a thrombin generation curve for each well.

Table 2
Data on patients/blood donors.

Patient	Age (gender)	Length (m)/mass (kg)	Specified malignancy	Thrombocyte counts (10^9/mL)[b]	Leukocyte counts (10^9/mL)[c]
1	50 (f)	1.64/47	AML[a]	134	1.4
2	61 (m)	1.72/64	Histiocytic sarcoma	52	0.2
3	61 (f)	1.58/53	AML	145	3.0
4	60 (m)	1.85/104	AML	51	1.0
5	58 (f)	1.63/69	Amyloidosis	107	10.5
6	66 (m)	1.83/65	MGUS + C1-esterase deficiency	281	4.5
7	76 (m)	1.75/60	Mantle cell non-Hodgkin's lymphoma	120	0.8
8	44 (m)	1.80/77	AML	252	6.2
9	55 (m)	1.72/109	Multiple myeloma	27	1.5

Patient #5 was the only patient receiving anticoagulant medication (low-molecular-weight heparin) at the time of blood sampling.
[a] AML = acute myeloid leukemia.
[b] Normal thrombocyte concentration: 150–450 × 10^9/L.
[c] Normal leukocyte concentration: 4.5–10 × 10^9/L.