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RESEARCH ARTICLE

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Antimicrobial and Anti-Thrombogenic Features Combined in Hydrophilic Surface Coatings for Skin-Penetrating Catheters. Synergy of Co-embedded Silver Particles and Heparin

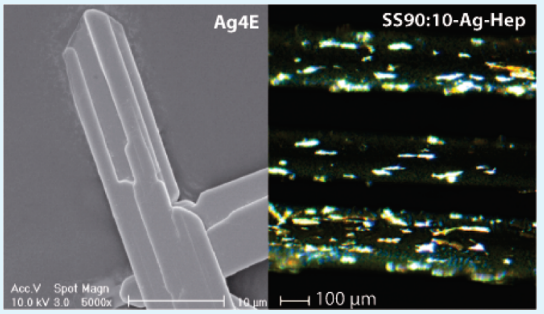
Sander Croes,[†,‡] Ellen E. Stobberingh,[†] Kris N.J. Stevens,[§] Menno L.W. Knetsch,[§] and Leo H. Koole*[,§]

[†]Department of Medical Microbiology, School for Public Health and Primary Care (CAPHRI), and [‡]Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, the Netherlands

[§]Department of Biomedical Engineering/Biomaterials Science, Maastricht University, P.O. Box 616, 6200 MD Maastricht, the Netherlands

Supporting Information

ABSTRACT: Percutaneous (skin-penetrating) catheters such as central venous catheters (CVCs), are used ubiquitously in the treatment of critically ill patients, although it is known that the risks for serious complications, particularly bloodstream infection and thromboembolism, are high. Materials science and engineering offer important new perspectives regarding further improvement of CVCs. A promising approach is the use of synthetic biocompatible hydrogel coatings with both silver particles and heparin embedded therein. Such formulations combine the well-known broad-spectrum antimicrobial features of silver with the anticoagulant activity of immobilized heparin. Previous work revealed that heparin augments antimicrobial activity of silver, while maintaining its anticoagulant function. This study set out to investigate the synergy of heparin and silver in more detail. Exit-challenge tests, experiments on bacterial killing and adherence, as well as in vitro challenge tests with three *Staphylococcus aureus* strains (one reference strain, and two clinical isolates) consistently showed the synergistic effect. In addition, the impact of changing the coating's hydrophilicity, and changing the silver concentration in the coatings, were examined. The experimental results, taken together and combined with data from the literature, point out that synergy of heparin and silver is best explained by binding of Ag^+ ions to heparin within the swollen coating, followed by release of heparin-Ag^+ complexes upon immersion of the coatings in an aqueous environment such as blood. Possible implications of this work regarding the development of improved/safer CVCs are briefly discussed.



KEYWORDS: antimicrobial coatings, bacterial adherence, sodium heparin, silver, catheter, hydrophilicity

1. INTRODUCTION

Central venous catheters (CVCs) play a key-role in the treatment of critically ill patients. For example, CVCs are commonly applied for intravenous delivery of cytostatic agents, blood transfusion, hemodialysis, blood sampling, parenteral nutrition, or plasmapheresis. Yet, the use of CVCs is associated with a high incidence of serious complications, particularly bloodstream infection.[1−6] Especially indigenous micro-organisms such as coagulase-negative staphylococci (CoNSs) and the more virulent *Staphylococcus aureus* are involved.[7,8] Infections mostly occur from the port of entry along the CVC's surface.[8] Another important source of complications with CVCs is thromboembolism,[4,9−11] because of imperfect blood-compatibility of the blood-contacting interface of the CVC.[12,13]

Biomaterials science and engineering can contribute to further technical improvements of CVCs. Evidently, such endeavors must concentrate on the CVC surface. Ideally, the surface should inhibit bacterial adhesion and feature perfect or near-perfect hemocompatibility. In our previous work, we have reported on new synthetic hydrophilic biomaterials with both silver particles and heparin embedded therein.[14] These materials were applied as thin surface coatings, with the obvious aim to exploit both the broad-spectrum antimicrobial activity of silver[15−17] and heparin's inhibitory effect on the intrinsic coagulation pathway. Our experiments revealed an unexpected synergistic effect: coatings containing both silver particles and heparin were much more effective in preventing bacterial adhesion, compared to coatings containing merely silver or heparin.

The objective of the present study was to further explore this synergy by: (i), examining different *S. aureus* strains, including clinical isolates; (ii) studying the effect of silver particles and heparin on bacterial killing, and by performing exit-site and in vitro challenge tests; (iii) studying the effect of the hydrophilicity of the coating matrix; (iv) studying the effect of the silver content of the coating.

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Table 1. Composition of the Seven Solutions That Were Used in the Preparation of the Specimens for This Study

no.	coating	SS90:10/ SS70:30 (mL)	Ag (g)	NMP (mL)	sodium-heparin (g)	formamide (mL)
1	SS90:10	100		50		
2	SS90:10-Hep	100		25	1.5	25
3	SS90:10-Ag	100	1.5	50		
4	SS90:10-Ag-Hep	100	1.5	25	1.5	25
5	SS70:30-Ag	100	1.5	50		
6	SS70:30-Ag-Hep	100	1.5	25	1.5	25
7	SS70:30-Ag2×-Hep	100	3.0	25	1.5	25

2. EXPERIMENTAL SECTION

2.1. Materials. Hydrophilic SlipSkin-coating biomaterials were purchased from Interface Biomaterials BV (Geleen, the Netherlands). Two versions were used: copolymer SS90:10, prepared from N-vinyl-2-pyrrolidinone (NVP) and *n*-butylmethacrylate (BMA) in a molar ratio 90:10, and the less hydrophilic copolymer SS70:30, prepared from NVP and BMA in a molar ratio 70:30. The static water contact angles of SS90:10 and SS70:30 have been reported previously: 22−26° for SS90:10 and 38−40° for SS70:30.[18] Sodium heparin was supplied by Celsus Laboratories (Cincinatti, OH, USA). Silver particles were purchased from Metalor SA (Neuchâtel, Switzerland). Two different types were used: Ag4E (stabilized by poly(ethylene glycol), and Ag6V (stabilized with poly (vinylpyrrolidone). All experiments were done with Ag4E, in some cases parallel experiments with Ag6V were done as well. Data on Ag6V are described in the Supporting Information. Commercial silver-impregnated catheters were purchased from Vygon BV (Valkenswaard, the Netherlands). The Vygon Multicath Expert 2 lumen catheter (ref. 8157.207) was used throughout this work for benchmark referencing. The active antimicrobial constituent is the silver-based agent AgION, which is not just a surface coating, but is incorporated into the material from which the tube is made.

2.2. Formulation. Seven different coating solutions were prepared as is compiled in Table 1. First, 400 mL of a 10 % solution of SS90:10 in N-methylpyrrolidone (NMP) was prepared. This solution was split into 4 equal parts. Secondly, 300 mL of a 10 % solution of SS70:30 in NMP was prepared; this solution was split into 3 equal parts. Thirdly, sodium heparin (6.00 g) was dissolved in 100 mL of formamide. This solution was split into 4 equal parts and these were mixed with two of the SS90:10 solutions (#s 2 and 4, Table 1) and two of the SS70:30 solutions (#s 6 and 7, Table 1). Fourthly, silver particles (3.00 g) were dispersed in NMP (100 mL) through mechanical stirring, split into 2 equal parts, and combined with SS90:10 solution #3 and SS70:30 solution #5. Analogously, 3.00 g silver was dispersed in NMP (50 mL), split into 2 equal parts and combined with SS90:10 solution #4 and SS70:30 solution #6. Finally, silver (3.00 g) was dispersed in NMP (25 mL) and combined with SS70:30 solution #7. Note that in this case a double content of silver was achieved.

All coating solutions were used in MCTec BV's procedure for the continuous and precisely controlled application of thin adherent polymer coatings on long metallic wires, as described previously.[18] Stainless steel wires with a diameter of 178 μm were used. The thickness of the coatings was 3−4 μm. Coated wires were coiled, using a rotating mandril of 600 μm diameter.

2.3. Bacterial Strains. The *Staphylococcus aureus* reference strain ATCC 29213, and two clinical *S. aureus* isolates (coined BF110 and D107) were tested. BF110 (meticillin-resistant, MRSA), was previously shown to be a strong biomass producer,[19] with the genetic background associated with multilocus sequence typing (MLST) clonal complex (CC)8. D107 (meticillin-susceptible, MSSA), was obtained from a patient with a CVC-related bloodstream-infection.

2.4. Exit Site Challenge. An in vitro method was adopted that determines the ability of antimicrobial coatings to prevent migration of bacteria along the external surface of a CVC after cutaneous colonization and invasion of the insertion site. Coils (30 mm length) were inserted half into 35-mm thick solidified 28 g L^{-1} nutrient (Oxoid) agar, which was in advance poured into containers (4 cm diameter). Afterwards, the surrounding surface of the insertion site was inoculated with *S. aureus* ATCC 29213, 1×10^6 CFU mL^{-1} using a swab, mimicking the circumstances in which an insertion site on the skin is adequately disinfected before entrance of a CVC and the cutaneous port of entry later becomes re-colonized. To mimic improperly disinfected skin surface, coils were also inserted after inoculation of the agar surface, as described previously.[20] Migration of bacteria down the coils along the abluminal coating surface was assessed visually for up to 7 days during incubation at 37°C.

2.5. Bacterial Killing. Coils were segmented into 30 mm long test-samples by cutting under aseptic conditions. The length of the Vygon catheter pieces was corrected for external and internal surface area. The specimen parts were added separately to 10 mL NaCl 0.9% tubes containing a bacterial inoculum of 1×10^6 CFU mL^{-1} and incubated at 37 °C. At t = 0, 2, 4, 8, 12, 24, 48, and 72 h samples of 100 μL were drawn to count viable bacteria. The lower limit of detection was 2.73 log(10) CFU mL^{-1}, using a spiralplater (Eddy Jet; IUL Instruments, Barcelona, Spain) as part of the enumeration procedure.

2.6. Bacterial Adherence. Specimen pieces of 30 mm were separately exposed to bacterial suspensions to assess adherence of ATCC 29213, BF110 and D107 to the different coating surfaces. The length of the Vygon catheter pieces was corrected for external surface area. To mimic infusion through and/or flushing of CVC prior to colonization or infection, a part of the specimens was pre-washed in Milli-Q water or blood plasma (Sanquin, Amsterdam, the Netherlands). The duration of pre-wash varied between 0 and 72 h. The bacterial exposure time (1×10^5, 1×10^6, or 1×10^7 CFU mL^{-1} in NaCl 0.9% (1.5 mL)) varied between 1 and 8 h at 37 °C. The inoculum was maintained at the same level by replacement of the inoculum each hour and was checked before and after each replacement.

Non-adhered bacteria were removed by vortexing each specimen for 1 s in fresh NaCl 0.9% (1.5 mL) and transferring it to another tube with NaCl 0.9% (1.5 mL), which was kept in a shaking incubator at 37°C for 4h. Subsequently, specimens were rolled over Mueller-Hinton agar plates containing 5% defibrinated sheep blood, according to a roll-plate assay described previously.[21] After overnight incubation at 37°C, the numbers of CFU per cm^2 of each roll track (33 cm^2) were determined.

2.7. In vitro Challenge. To determine which coating provided the best anti-adhesive properties over a prolonged period of time, a repeating set-up of the adherence assay was used in which exposure to *S. aureus* ATCC 29213 (1×10^5 CFU mL^{-1} NaCl 0.9% at 37°C) was alternated with roll-plate assessment.[21] The series was terminated after 25 challenges.

2.8. Statistical Analysis. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Independent t-tests were used to compare bacterial adherence data. A P value of ≤ 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

Materials and Specimens. Figure 1 shows a typical example of the specimens that were used in this study. These are flexible coils, manufactured from a surface-coated thin metallic wire; coating of these wires was performed as described previously.[14] Seven different coatings were used; these are all based on a copolymer of N-vinylpyrrollidone (NVP) and n-butyl methacrylate (BMA). These copolymers are biocompatible materials, also known as SlipSkin (SS, vide infra). Two different SS compositions

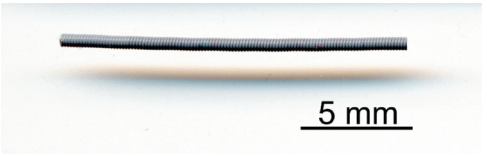

Figure 1. Scanning electron micrograph of a typical specimen, showing the coiled structure.

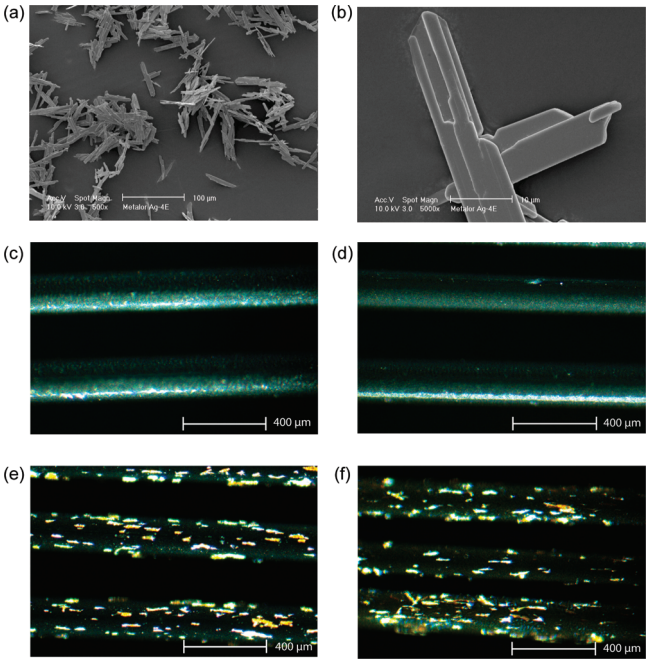

Figure 2. (a,b) Scanning electron micrographs of the silver particles Ag4E, which were used throughout this work. (c) Light-microscopic images of the coated wires SS90:10, (d) SS90:10-Hep, (e) SS90:10-Ag, and (f) SS90:10-Ag-Hep. Note that (i) the wires in (c−f) were photographed prior to coiling; (ii) the extrusion-like coating procedure results in some alignment of the silver particles in the direction of the wire.

were used: either 90:10 NVP:BMA or 70:30 NVP:BMA (molar ratios); these coatings materials are denoted as SS90:10 and SS70:30, respectively. Furthermore, the coatings contain either silver particles (SS90:10-Ag and SS70:30-Ag), or heparin (SS90:10-Hep), or silver particles + heparin (SS90:10-Ag-Hep, SS70:30-Ag-Hep and SS70:30-Ag2×-Hep). The precise formulations of the 7 coatings are compiled in Table 1. The advantage of our procedure to first formulate the coatings, then to apply them to thin metallic wires, followed by coiling is that numerous identical specimens can be produced for each surface coating. Images a and b in Figure 2 show scanning electron micrographs of the silver particles (Ag4E). Note that these are microparticles rather than nanoparticles, since their smallest dimension is approximately 1 μm. Figure 2c−f shows light-microscopic images of our wires prior to coiling: SS90:10 (c), SS90:10-Hep (d), SS90:10-Ag (e), and SS90:10-Ag-Hep (f). Note the alignment of the rod-shaped silver particles at the wire's surface (e and f).

Exit Site Challenge. This test essentially mimics the situation in which the insertion site on the skin is first disinfected, and later becomes invaded by bacteria after cutaneous recolonization. For

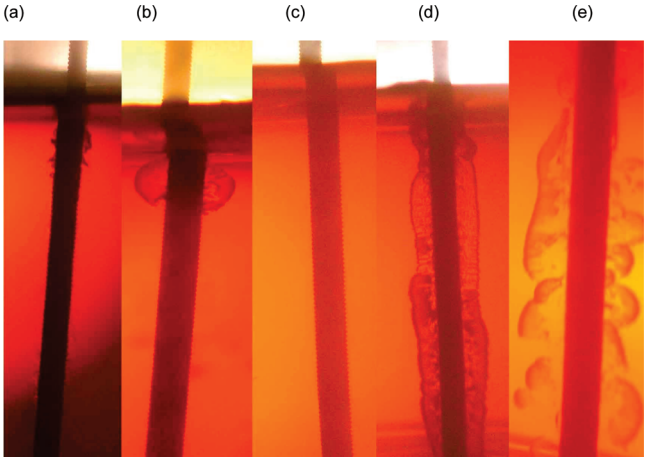

Figure 3. (a, b) Exit site challenge. First signs of ATCC 29213 migration extending along the coating surfaces of SS90:10 and SS90:10-Hep, observed 24 h after inoculation of the surrounding agar surface at the insertion site, respectively. (c) No migration of ATCC 29213 along the coating surface (existing of SS90:10-Ag-Hep) was observed over 168 h. (d) Growth of inserted bacteria at the surface of SS90:10. (e) Vertical movement and/or growth of bacteria directed away from the coating surface (SS90:10-Ag-Hep) through the agar, when specimens were inserted after inoculation of the insertion site.

the SS90:10 and SS90:10-Hep coatings, clear bacterial migration along the specimens was observed after 24 h (Figure 3a,b). Growth extended further down the wires within 72 h. Coatings SS90:10-Ag and SS90:10-Ag-Hep did not show bacterial migration under the same conditions, indicating that the silver particles effectively inhibit movement and growth of bacteria. Still, no bacterial invasion was noticed after 168 h of incubation (Figure 3c). Changing the matrix into the less hydrophilic version (SS70:30) influenced the outcome of the test. The surfaces SS70:30-Ag and SS70:30-Ag-Hep prevented bacterial migration within 48 h, but some signs of migration were observed after 72 h.

The test was also performed in such a way that the agar surface was first inoculated with *S. aureus* ATCC 29213, as described previously.[20] Essentially, this would mimic the situation in which the skin surface is not properly disinfected before insertion. For all specimens, it was observed that bacteria were transferred with the specimen into the agar. Subsequently, bacterial growth occurred in all cases, but there was a clear distinction between the reference coatings on one hand, and the silver-containing coatings on the other hand. The reference coatings showed bacterial growth along their surfaces (Figure 3d), whereas the silver-containing coatings directed bacterial growth more away from the specimens, i.e., perpendicular to their surfaces (Figure 3e).

Bacterial Killing. Figure 4 shows data on survival of bacteria of the *S. aureus* reference strain ATCC 29213 (Figure 4a), and the clinical isolates BF110 and D107 (Figure 4b,c) upon incubation with the different test coatings, up to 72 h. Figure 4a reveals that SS90:10 and SS90:10-Hep did not influence the survival of ATCC 29213. Incorporation of silver resulted in killing of a substantial portion of the bacteria, such that the number of bacteria dropped with 2−3 orders of magnitude (SS90:10-Ag). Embedding the same amount of silver particles in the less hydrophilic matrix (SS70:30) did not introduce a major difference. Strikingly, introduction of silver particles and heparin led to

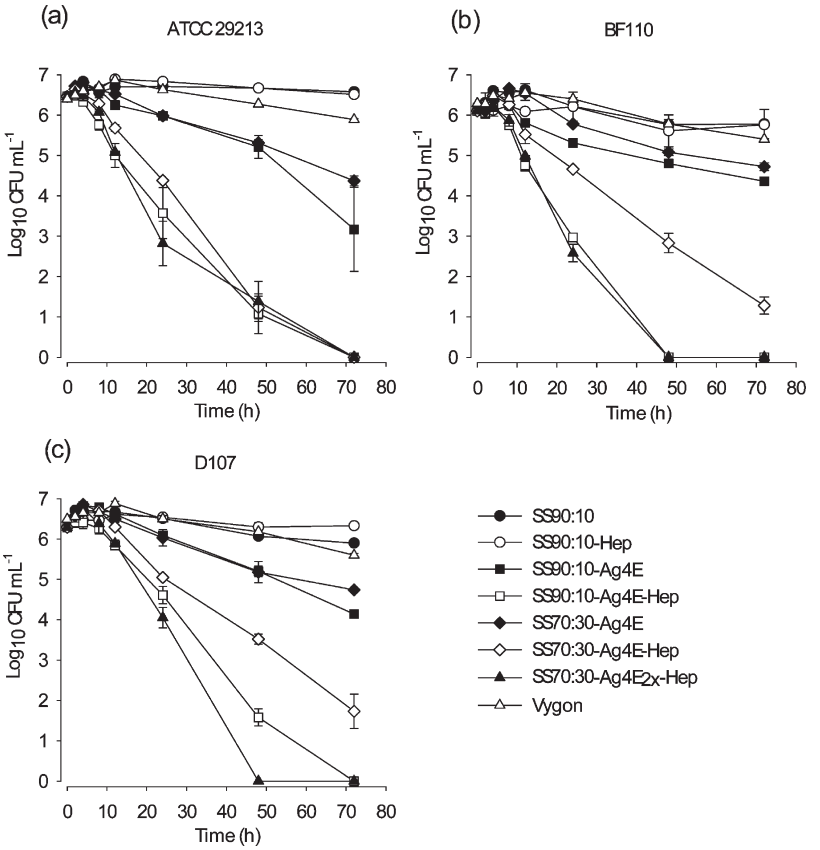

Figure 4. Antibacterial activity of different coating compositions over time against inocula of 1×10^6 CFU mL^{-1} *S. aureus*: (a) ATCC 29213, (b) BF110, and (c) D107. Error bars indicate standard errors (SE).

almost complete killing of ATCC 29213 within 72 h. This effect was independent of the coating's hydrophilicity (SS90:10 vs. SS70:30). Doubling of the silver content (in SS70:30-Ag2×-Hep) reduced the viable count similarly. Noteworthy, the commercial Vygon catheter, which is claimed to have an antibacterial surface,[22] had no impact on the survival of ATCC 29213 within 72 h. The experiments with the *S. aureus* clinical isolates BF110 and D107 led to highly similar bacterial time-kill curves. Again, no variation in bacterial counts occurred upon incubation with SS90:10 or SS90:10-Hep. Furthermore, combining silver particles and heparin (SS90:10-Ag-Hep) substantially improved the bactericidal activities, achieving practically complete kill (99.99%) within 24-48 h. Although eradication of the reference strain seemed independent of the amount of embedded silver and the coating hydrophilicity, substantial differences were seen regarding the clinical isolates BF110 and D107. Compared to SS90:10-Ag-Hep, SS70:30-Ag-Hep reduced the viable count of these isolates more slowly. However, whenever a double amount of silver particles was incorporated in the less hydrophilic coating (SS70:30-Ag2×-Hep), similar reductions in viable count of BF110 were obtained as with SS90:10-Ag-Hep. Moreover, the fastest killing of D107 was observed with SS70:30-Ag2×-Hep, which exceeded even the eradication time of SS90:10-Ag-Hep. No bactericidal effect was noticed when testing the Vygon catheter pieces.

Highly similar antimicrobial effects were observed with the PVP-stabilized silver particles. Generally, the biocidal effects of PEG-stabilized silver particles were slightly stronger than those of their PVP stabilized counterparts, see the Supporting Information.

Bacterial Adherence. Adherence to different specimens was examined after various washing periods and incubation times. Figure 5a shows data on the adherence of ATCC 29213 to different specimens after incubation in a constant inoculum of 1×10^5 CFU mL^{-1} for 1, 2, 4, or 8 h. The adherence to the SS90:10 coating was influenced by the incubation time; the longer the exposure the more adherence of ATCC 29213 was seen. Considerably, more bacteria adhered to this coating compared to the other ones, which occurred already from onset. Initial adherence was much lower for coatings containing both silver particles and heparin compared to silver particles alone. There was also a difference in initial adherence when the hydrophilicity of the coating was taken into account. The more hydrophilic coatings SS90:10-Ag and SS90:10-Ag-Hep adhered less bacteria compared to SS70:30-Ag and SS70:30-Ag-Hep, respectively. Especially the difference in initial adherence between the SS90:10-Ag and SS70:30-Ag coatings is noteworthy. The anti-adhesive effects of the more SS90:10 silver-containing containing coatings deteriorated more quickly in comparison to their less hydrophilic SS70:30 silver-containing counterparts. The quicker reduction in anti-adhesive efficacy of the more hydrophilic coatings became more obvious when the data was presented as a normalized effect score, whereby the adherence within 1 h was set to an arbitrary value of 1.0 (Figure 5b). Although this relative increase was more pronounced for the more hydrophilic matrixes, absolute adherence was sustained at a slightly lower level for SS90:10-Ag-Hep compared to SS70:30-Ag-Hep. No influence of more embedded silver was observed since adherence to SS70:30-Ag-Hep and SS70:30-Ag2×-Hep

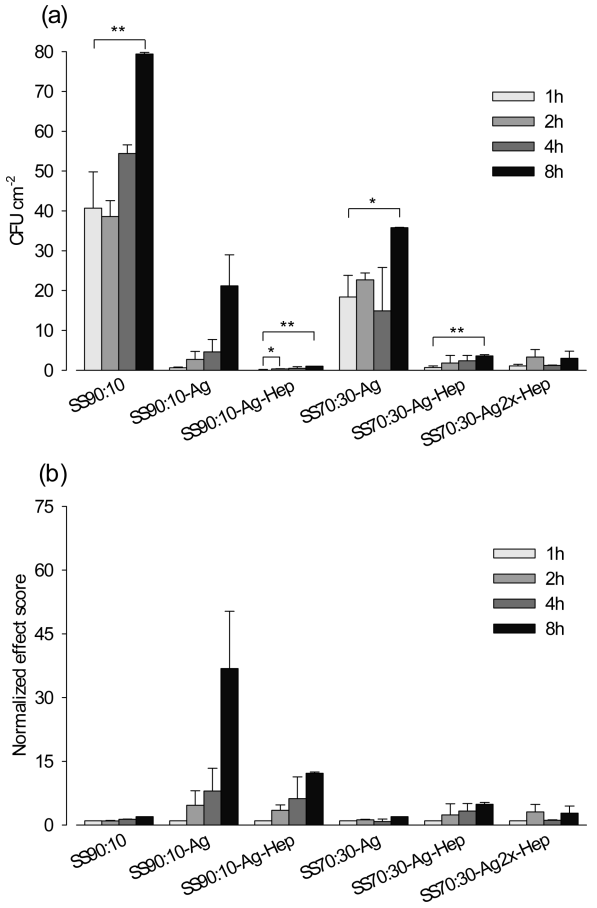

Figure 5. (a) Adherence of *S. aureus* ATCC 29213 to different specimens during incubation for 1-8 h in 1 $\times 10^5$ CFU mL^{-1}. The same data expressed as normalized effect score. (b) Adherence was assessed by roll-plate analysis. Asterisks denote statistically significant difference, (*) $P < 0.05$ and (**) $P < 0.01$.

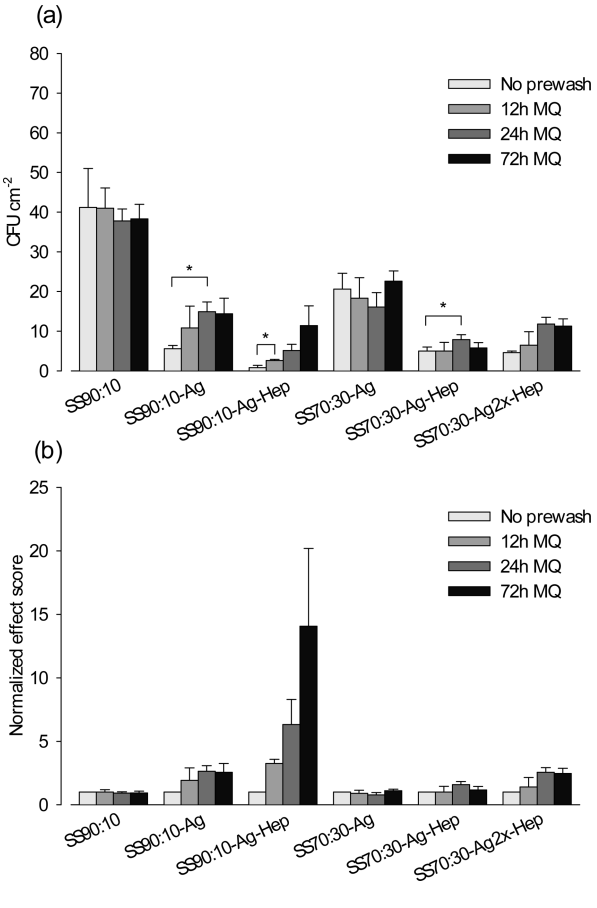

Figure 6. (a) Adherence of *S. aureus* ATCC 29213 to different specimens during incubation for 1 h in 1 $\times 10^6$ CFU mL^{-1} after pre-wash of the specimens with Milli-Q water (MQ) for 12−72 h. The same data expressed as normalized effect score. (b) Adherence was assessed by roll-plate analysis. Asterisks denote statistically significant difference, (*) $P < 0.05$ and (**) $P < 0.01$.

reached the same level. Bacterial adhesion to the different surfaces was also assessed after preceding washing periods. This essentially mimics the situation whereby a CVC is routinely used for a certain period of time and later becomes contaminated with bacteria. As shown in Figure 6a, even after 72 h pre-wash, adherence of ATCC 29213 was more pronounced when only silver particles (without heparin) were incorporated in the coating. Pre-wash with Milli-Q water seemed to have no effect on the adherence of ATCC 29213 to the less hydrophilic coatings SS70:30-Ag and SS70:30-Ag-Hep. On the other hand, the anti-adhesive features of the coatings SS90:10−Ag and SS90:10-Ag-Hep declined when pre-wash was extended (Figure 6b). Remarkably, although the effect of pre-washing was negligible for the coatings having SS70:30 as the matrix, SS70:30-Ag2×-Hep displayed a tendency towards more adherence of ATCC 29213 when a longer pre-wash program was applied. To explore whether fouling of human plasma proteins would influence bacterial adherence to the specimens, Milli-Q water as a washing fluid was replaced by drug-free human plasma from healthy volunteer blood donors. It appeared that plasma incubated specimens containing heparin in its coating layer, i.e., SS90:10-Hep and SS90:10-Ag-Hep, displayed increased adherence of ATCC 29213 (Figure 7a,b). Still, adherence with SS90:10-Ag-Hep was far below the level achieved with the reference coatings SS90:10 and at the same level as the coating containing silver particles alone, SS90:10-Ag. The coatings without heparin, i.e., SS90:10-Ag lost repulsion activity when prewashed during 12 h with Milli-Q water in the same order as when plasma was used.

In vitro Challenge. Upon multiple challenges, bacterial adherence to all specimens containing silver particles gradually increased over time, but remained relatively low, especially for the ones with heparin (Figure 8). In contrast, soon after the first few challenges, roll-plate analysis of SS90:10 and SS90:10-Hep displayed a strong rise in the number of adhered bacteria. Already after 4-5 challenges the limit of detection, i.e. 100 CFU cm^{-2}, was exceeded (Figure 8a). After eight challenges it was estimated that specimens SS90:10 and SS90:10-Hep achieved a maximum of adhered cells of approximately 230 CFU cm^{-2} (data not shown). However, it has to be noticed that during the first four challenges, it was clear that specimens containing heparin alone, SS90:10-Hep, demonstrated anti-adhesive properties on interaction with ATCC 29213. From the beginning, bacterial adherence to the Vygon catheter segments was excessive compared to all our silver-containing specimens. After the last challenge, adherence to the Vygon segments was approximately six times more compared to all silver-containing specimens. Whereas SS90:10-Ag and SS90:10-Ag-Hep demonstrated low adherence from the onset, SS70:30-Ag, SS70:30-Ag-Hep, and Vygon catheter segments

showed initially a higher adherence, while anti-adhesiveness improved soon thereafter (Figure 8b). Compared to matrixes with both heparin and silver particles in them, adherence to silver-containing specimens remained slightly higher over multiple challenges. This observation was independent of the coating's hydrophilicity. The less hydrophilic coating with the double amount of silver, SS70:30-Ag2×-Hep, displayed more decline in its anti-adhesive capacities than the one with the reference content, SS70:30-Ag-Hep.

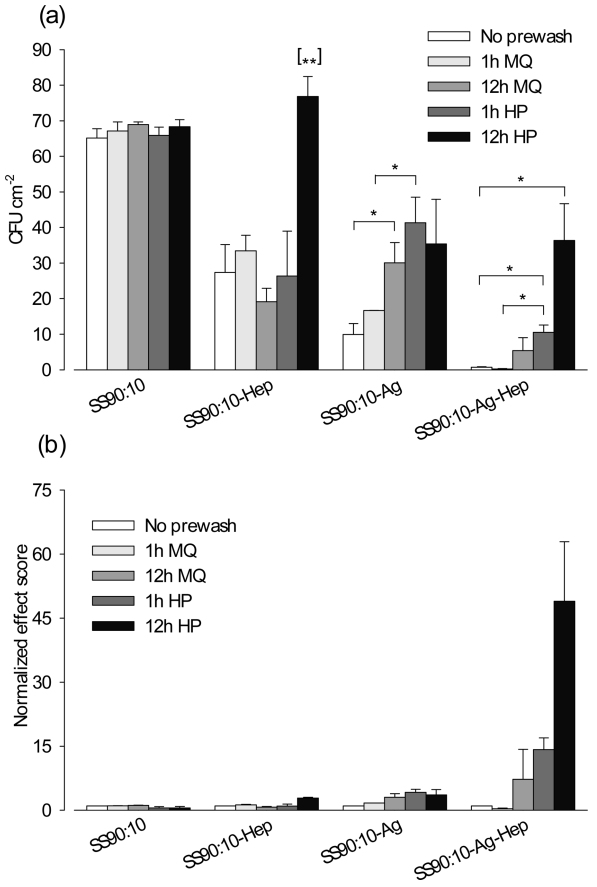

Figure 7. (a) Adherence of *S. aureus* ATCC 29213 to different specimens during incubation for 1 h in 1×10^7 CFU mL^{-1} after pre-wash of the specimens with Milli-Q water (MQ) for 1-12h or with human plasma (HP) for 1−12 h. (b) The same data expressed as normalized effect score. Adherence was quantified by roll-plate analysis. Asterisks denote statistically significant difference, (*) $P < 0.05$ and (**) $P < 0.01$. Asterisks between brackets denote statistically significant difference of individual conditions versus all other circumstances (except SS90:10).

Our experimental data confirm that embedding of silver particles in hydrophilic surface coatings is a viable strategy toward biocidal surfaces for medical devices such as catheters.[23] The experiments on bacterial killing and adhesion showed strong synergistic effects, if silver particles and heparin are co-embedded in the coating. This is the key message of this work. The effect was found with three different *S. aureus* species: a reference strain (ATCC 29213) and two clinical isolates.

The observed more-than-additive effect of silver particles and heparin on bacterial killing and adhesion clearly asks for an explanation. First, it is important to consider the mechanistic background of the antimicrobial activity of surfaces containing silver. It is well-known that silver particles expose Ag^+ ions at their periphery.[24] Furthermore, it is important to realize that all our media contained chloride ions, and that the solubility of AgCl in aqueous media is extremely low (a Ksp of 1.77×10^{-10}).[25] Hence, dissolution of Ag^+ ions from the silver particles—most probably—does not play a major role in the observed biocidal effects. In our earlier work, we have corroborated this through a series of titration experiments, using the soluble silver salt $AgNO_3$. We found that dissolved Ag^+ ions only start to become biocidal if their concentration exceeds 100 nM.[26] We hypothesize, therefore, that antimicrobial effects of our silver-containing surfaces can be attributed to collisions of bacteria with the surface. During such a collision, transfer of Ag^+ ions can occur from a silver particle's surface to the bacterium's membrane, e.g., by binding to negatively charged phospatidyl serine moieties. These membrane-bound Ag^+ ions can subsequently induce rupture of the bacterial wall,[27,28] eventually killing the bacterium. In essence, this mechanism is heterogeneous, as the critical step is collision between suspended bacteria, and a solid surface that exposes Ag^+ ions at its surface.

The question now is how this mechanism will change if heparin is introduced in the surface coating, next to the silver particles. Clearly, collisions and transfer of Ag^+ ions as described above can still occur. But in addition there is release of heparin

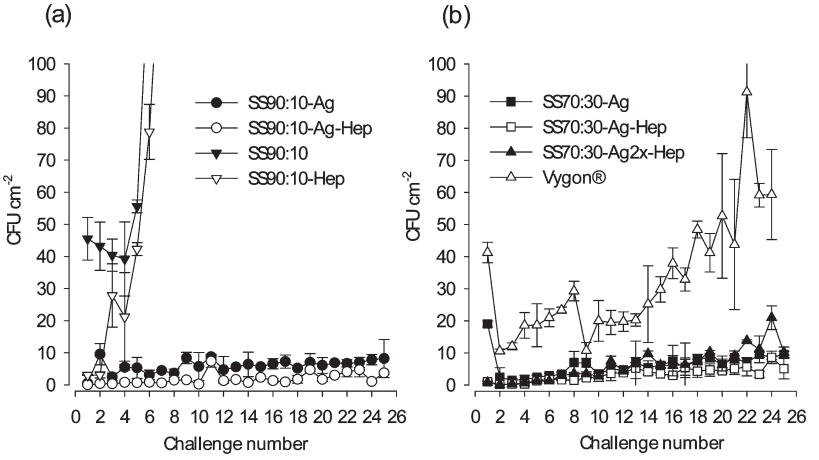

Figure 8. In vitro challenge results of all specimens. The roll-plate assay was used to assess adherence (CFU cm^{-2}) of ATCC 29213 after each challenge, consisting of incubation in 1×10^5 CFU mL^{-1} during 1 h at 37°C and removement of non-adhered bacteria afterwards. Error bars indicate standard errors (SE). The detection limit was approximately 80−100 CFU cm^{-2}.

molecules from the coating's surface occurring, especially if the matrix is hydrophilic.[29] Heparin molecules, escaping from the coating can drag silver ions with them; affinity of heparin for monovalent cations is well documented.[30] So, in essence there is now a second mechanism by which Ag^+ ions can reach the bacterium, based on dissolved heparin molecules working as a carrier for Ag^+ ions. This second mechanism is homogeneous, and heparin/Ag^+ complexes could bind to the outer surface of bacteria, or be internalized.

The present experimental data can not be used to prove our hypothesis right or wrong, but it is obvious that most of our observations are in line with it. Bacterial killing with the coatings SS90:10-Ag-Hep, SS70:30-Ag-Hep and SS70:30-Ag2×-Hep against inocula of *S. aureus* was much stronger, in comparison with counterpart coatings that contained only silver particles *or* heparin (Figure 4). Analogously, adherence of *S. aureus* ATCC 29213 was much lower for the coatings containing both silver particles and heparin (Figure 5); prewashing with MQ water or with human blood plasma did not change this picture (Figures 6, 7). The in vitro challenge tests also pointed out that the coatings SS90:10-Ag-Hep, SS70:30-Ag-Hep and SS70:30-Ag2×-Hep outperformed their counterparts containing either silver particles or heparin. Interestingly, the adherence tests allowed us to compare the long term effects between SS90:10-Ag-Hep and SS70:30-Ag-Hep coatings (up to 72 h prewash). These coatings differ only regarding the hydrophilicity of the matrix. The less hydrophilic material SS70:30 is expected to have slower release of embedded heparin, and hence a more sustained antimicrobial effect. This is exactly what is seen in Figure 6.

4. CONCLUSIONS

In summary, we found that embedding of silver particles in SlipSkin (NVP/BMA) hydrophilic surface coatings leads to pronounced antimicrobial effects in experiments with three strains of *S. aureus* (one reference strain and two clinical isolates). Heparin potentiates the biocidal activity of the silver particles. A tentative explanation for this effect of heparin is based on two premises: (i) the observed biocidal effects of silver per se relate to a collision mechanism, and (ii) silver ions are transported by heparin molecules that are released from the coating's surface. The present results, taken together with our previously reported data, demonstrate for the first time that it is possible to design surface coatings with strong antimicrobial and antithrombogenic features.[14] Most probably, rational design of such coatings becomes possible if the mechanistic details are explored further. We expect that his work will open a new route to safer (percutaneous) blood-contacting medical devices such as CVCs, which have become indispensable in the treatment of critically ill patients.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional information as noted in the text (antimicrobial activity of the Ag6V silver particles). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +31-43-3881531. Fax: +31-43-3881725. E-mail: l.koole@maastrichtuniversity.nl.

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■ REFERENCES

(1) Hockenhull, J.C.; Dwan, K.M.; Smith, G.W.; Gamble, C.L.; Boland, A.; Walley, T.J.; Dickson, R.C. *Crit. Care Med.* **2009**, *37*, 702–712.
(2) Rosenthal, V.D. *Clin. Infect. Dis.* **2009**, *49*, 1899–1907.
(3) O'Grady, N.P.; Chertow, D.S. *Cleve. Clin. J. Med.* **2011**, *78*, 10–17.
(4) McGee, D.C.; Gould, M.K. *N. Engl. J. Med.* **2003**, *348*, 1123–1133.
(5) Frasca, D.; Dahyot-Fizelier, C.; Mimoz, O. *Crit. Care* **2010**, *14*, 212.
(6) Mermel, L.A.; Allon, M.; Bouza, E.; Craven, D.E.; Flynn, P.; O'Grady, N.P.; Raad, I.I.; Rijnders, B.J.; Sherertz, R.J.; Warren, D.K. *Clin. Infect. Dis.* **2009**, *49*, 1–45.
(7) Gilbert, R.E.; Harden, M. *Curr. Opin. Infect. Dis.* **2008**, *21*, 235–245.
(8) Walz, J.M.; Memtsoudis, S.G.; Heard, S.O. *J. Intensive Care Med.* **2010**, *25*, 1–8.
(9) Debourdeau, P.; Kassab Chahmi, D.; Le Gal, G.; Kriegel, I.; Desruennes, E.; Douard, M.C.; Elalamy, I.; Meyer, G.; Mismetti, P.; Pavic, M.; Scrobohaci, M.L.; Lévesque, H.; Renaudin, J.M.; Farge, D. *Ann. Oncol.* **2009**, *20*, 1459–1471.
(10) Baskin, J.L.; Pui, C.H.; Reiss, U.; Wilimas, J.A.; Metzger, M.L.; Ribeiro, R.C.; Howard, S.C. *Lancet* **2009**, *374*, 159–169.
(11) Verso, M.; Agnelli, G. *J. Clin. Oncol.* **2003**, *21*, 3665–3675.
(12) Boersma, R.S.; Jie, K.S.; Verbon, A.; van Pampus, E.; Schouten, H.C. *Ann. Oncol.* **2008**, *19*, 433–442.
(13) Wang, H.; Huang, T.; Jing, J.; Jin, J.; Wang, P.; Yang, M.; Cui, W.; Zheng, Y.; Shen, H. *J Hosp. Infect.* **2010**, *76*, 1–11.
(14) Stevens, K.N.J.; Croes, S.; Boersma, R.S.; Stobberingh, E.E.; van der Marel, C.; van der Veen, F.H.; Knetsch, M.L.W.; Koole, L.H. *Biomaterials* **2011**, *32*, 1264–1269.
(15) Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; Kim, Y.K.; Lee, Y.S.; Jeong, D. H.; Cho, M.H. *Nanomedicine* **2007**, *3*, 95–101.
(16) Rai, M.; Yadav, A.; Gade, A. *Biotechnol. Adv.* **2009**, *27*, 76–83.
(17) Bayston, R.; Vera, L.; Mills, A.; Ashraf, W.; Stevenson, O.; Howdle, S.M. *J. Antimicrob. Chemother.* **2010**, *65*, 258–265.
(18) Hanssen, J.H.L.; Wetzels, G.M.; Benzina, A.; van der Veen, F. H.; Lindhout, T.; Koole, L.H. *J. Biomed. Mater. Res.* **1999**, *48*, 820–828.
(19) Croes, S.; Deurenberg, R.H.; Boumans, M.L.L.; Beisser, P.S.; Neef, C.; Stobberingh, E.E. *BMC Microbiol.* **2009**, *9*, 229.
(20) Bayston, R.; Fisher, L.E.; Weber, K. *Biomaterials* **2009**, *30*, 3167–3173.
(21) Maki, D.G.; Weise, C.E.; Sarafin, H.W. *N. Engl. J. Med.* **1977**, *296*, 1305–1309.
(22) Trogolo, J.A. *MD&DI Mag.* **2010**, *32*, 52–58.
(23) Casey, A.L.; Mermel, L.A.; Nightingale, P.; Elliott, T.S. *Lancet Infect. Dis.* **2008**, *8*, 763–776.
(24) Lok, C.N.; Ho, C.M.; Chen, R.; He, Q.Y.; Yu, W.Y.; Sun, H.; Tam, P.K.; Chiu, J.F.; Che, C.M. *J. Biol. Inorg. Chem.* **2007**, *12*, 527–534.
(25) Weast, R.C. In *Handbook of Chemistry and Physics*, 70th ed.; Lide, D.R., Ed.; CRC Press: Boca Raton, FL, ; pp 207−208.
(26) Stevens, K.N.J.; Crespo-Biel, O.; van den Bosch, E.E.; Dias, A. A.; Knetsch, M.L.W.; Aldenhoff, Y.B.J.; van der Veen, F.H.; Maessen, J. G.; Stobberingh, E.E.; Koole, L.H. *Biomaterials* **2009**, *30*, 3682–3690.
(27) Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; Kim, Y.K.; Lee, Y.S.; Jeong, D. H.; Cho, M.H. *Nanomedicine* **2007**, *3*, 95–101.

(28) Tolaymat, T.M.; El Badawy, A.M.; Genaidy, A.; Scheckel, K.G.; Luxton, T.P.; Suidan, M. *Sci. Total Environ.* **2010**, *408*, 999–1006.
(29) Aldenhoff, Y.B.J.; Hanssen, J.H.L.; Knetsch, M.L.W.; Koole, L. H. *J. Vasc. Interv. Radiol.* **2007**, *18*, 419–425.
(30) Rudd, T.R.; Guimond, S.E.; Skidmore, M.A.; Duchesne, L.; Guerrini, M.; Torri, G.; Cosentino, C.; Brown, A.; Clarke, D.T.; Turnbull, J.E.; Fernig, D.G.; Yates, E.A. *Glycobiology* **2007**, *17*, 983–993.