

Chlorhexidine-silver sulfadiazine-impregnated central venous catheters: in vitro antibacterial activity and impact on bacterial adhesion

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SUMMARY

The aim of this study was to compare the in vitro activity and the impact on bacterial adhesion of two different catheters, one impregnated with chlorhexidine-silver sulfadiazine (C-SS) and the other not impregnated with antibacterial agents. The antimicrobial coating prevented the bacterial colonization by slime positive *Staphylococcus epidermidis* in the first two days. The antibacterial activity of the effluents from catheters impregnated with C-SS dissipated by day seven. Our results demonstrated that the surface treatment modified the composition of impregnated catheters and determined different contact angle values of the two catheters (impregnated and not impregnated). Examination of coated and uncoated catheter segments by scanning electron microscopy showed a good correlation with the results of adherence experiments. In conclusion, the findings suggest that C-SS coated catheters prevent in vitro bacterial adhesion.

KEY WORDS: Bacterial adhesion, Chlorhexidine-silver sulfadiazine-impregnated catheter, Slime producing *Staphylococcus epidermidis*, Catheter-related infections, Antibacterial activity

Received March 04, 2011

Accepted November 22, 2011

INTRODUCTION

Intravascular catheter-related infections are a major cause of morbidity and mortality. The incidence of central venous catheter-related sepsis can be relatively high, ranging from 3.8% to 20% depending on the physicochemical properties of the devices, their implantation time, their insertion site and finally on the care unit (Wenzel *et al.*, 2001; Blot *et al.*, 2005; Maki *et al.*, 2006; Warren *et al.*, 2006). Many of these infections are difficult to treat with antimicrobial agents and often require removal of the device (O'Grady *et al.*, 2011). The microorganisms involved in central

venous catheter infections are in two thirds of cases *Staphylococcus epidermidis*, other coagulase negative staphylococci and *Staphylococcus aureus* (Donelli *et al.*, 2001). A large percentage of strains of *S. epidermidis* and other coagulase negative staphylococci produce amounts of extracellular slime in which cells are embedded and covered (Christensen *et al.*, 1982; Fey *et al.*, 2010). In particular the polysaccharide intercellular adhesin (PIA) is important in the pathogenesis of intravascular catheter-associated infection, and has an essential role in cellular aggregation and biofilm formation (Olson *et al.*, 2006). This slimy substance protects bacteria against host defense mechanisms and antimicrobial agents (Hoyle *et al.*, 1992; Costerton *et al.*, 1999).

Electron microscopy studies show that all catheters eventually become colonized after insertion. However, the probability of infection is proportional to the number of organisms multiplying on the intravascular segment of the catheter surface. Therefore, factors that would

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favour the multiplication and spread of organisms from the biofilm environment would also increase the risk of blood-stream infections (Raad *et al.*, 1992).

In the last years, two main strategies have been employed in the prevention of catheter-related infections:

- 1) the use of preventive measures including maximal sterile barriers, the institution of an experienced infusion team, optimal local care of the catheter insertion site and educational programs for clinical practitioners;
- 2) the antimicrobial-utilizing approach for modifying the catheter surface (the so-called second generation vascular catheters).

A variety of second generation vascular catheters have been investigated. Most studies have demonstrated a striking reduction in the number of colonized catheters, the prelude of catheter-related infections (Bosma *et al.*, 2010; Crnich *et al.*, 2004). The present study assessed the *in vitro* adhesion of slime producer *S. epidermidis* on chlorhexidine and silver sulfadiazine (C-SS) impregnated catheters in comparison with untreated control catheters.

MATERIALS AND METHODS

Microorganism: slime producer strain of *S. epidermidis* isolated from a central venous catheter removed 14 days after insertion in a patient with catheter-related bacteraemia. *S. epidermidis* was identified by conventional laboratory procedures and speciation was performed by standard biochemical tests. Slime production was evaluated with the Christensen method (Christensen *et al.* 1982).

Catheters: the following devices were included in the study:

1. polyurethane central venous catheters surface treated with chlorhexidine and silver sulfadiazine (C-SS) (Arrow International®, Italy);
2. polyurethane central venous catheters were used as a control (Arrow International®, Italy). All catheters were divided into 0.5 cm segments under aseptic conditions.

Evaluation of bacterial adherence on the catheter surface

Segments of catheters 0.5 cm long of both types

were immersed separately in tubes containing an overnight culture of slime producer *S. epidermidis* diluted 1:1000 in tryptone-soy broth (TSB, Oxoid LDT, UK) with 0.25% casamino acids (Becton Dickinson, France). After 24 hours incubation at 37°C the catheters were removed from the broth, washed 3 times with phosphate-buffered-saline solution (PBS, Biomérieux, France), placed in 1 ml of PBS and sonicated for 2 min in a sonicating bath at 35 KHz. The collected liquid of sonication was suitably diluted and the number of bacteria was determined by a colony count method (Pasqual, 2002). The experiment was performed simultaneously with four identical segments for each type of catheter and incubation time. A fifth segment of catheter was processed after incubation for scanning electron microscope (SEM) observation. Further segments of both types of catheter were incubated at 37°C in PBS, which was replaced daily with fresh PBS solution. The removed PBS (effluent samples) was frozen at -20°C in order to determine its antibacterial activity. The catheters immersed in PBS were removed after 24h, 48h, 7days, 14 days, 21 days and 28 days of incubation and colonization with *S. epidermidis* as mentioned above.

Determination of the antibacterial activity of the PBS effluent samples

For this purpose a modified version of the Kelsey-Sykes method was used (Kelsey *et al.*, 1969). Twofold dilutions of PBS effluent samples were prepared in 96 well microtiter plates. Bacterial suspension of the *S. epidermidis* was made in trypticase soy broth, so that the final concentration was 5×10^5 cfu/ml. The bacteriostatic concentration was read as the highest effluent dilution yielding no visible growth of the test organism. Each well that showed no visible growth was subcultured (10 µl) onto trypticase soy agar. The highest dilution of effluent yielding no growth upon subculture was considered the bactericidal activity.

Surface analysis with X-ray photoemission spectroscopy

The surface chemical composition of the treated and untreated catheters was assessed with X-ray photoemission spectroscopy (XPS) (Seah *et al.*, 1983). A non-monochromatized MgK_α source was used to excite the photoelectrons, which were collected by the entrance lens of a hemispherical elec-

tron analyzer at an emission angle of 45 degrees. The probing depth of this technique is about 2-3 nm, depending on the kinetic energy of the line selected for each element, and the analysis was performed by averaging over regions of about 1 mm radius on the external surface of the catheters. To obtain the surface concentration the area of the various XPS lines was calculated after Shirley background subtraction (Seah *et al.*, 1983) and then normalized with the standard sensitivity factors (Moulder *et al.*, 1992). The so-called surface concentration, as atomic percentage, obtained in this way is accurate for samples which are uniform in the analyzed depth and can be used for relative comparisons in all the other cases. For the analysis the catheters were cut into segments 1 cm long (5 samples for each type), which were mounted with their long axis oriented parallel to the axis of the analyzer. All the manipulations of the catheters were performed with stainless steel cutters and tweezers after extracting the catheters from the sterile package.

Contact angle measurement

Contact angle measurements of demineralized water were performed on the external surface of the catheters (Zisman, 1964) A 3 μ l drop was deposited from a calibrated micropipette and the profile of the drop was observed with an optical microscope. The internal contact angles of the drops were measured on the image obtained from the camera connected to the microscope. Ten drops were deposited on each type of catheter.

Scanning electron microscopy

Scanning electron microscopy observations were carried out on specimens fixed for 2 h in 5% glutaraldehyde solution in a 0.2 M cacodylate buffer (pH 7.2) with the addition of 0.1% ruthenium red. This colouring is added to a cacodylate buffer and to ethanol solutions until 60% to stabilize the slime and to prevent its collapse during dehydration. After repeated rinses in the buffer, the specimens were post-fixed for 2 h with 2% OsO₄ in the cacodylate buffer and dehydrated in increasing concentrations of ethanol solutions. The catheters were then processed by the critical point drying method in an Emitech K-850 apparatus. The dried specimens were placed on a mounted base, coated with gold and examined with a Philips XL 20 scanning electron microscope.

RESULTS

Evaluation of the adherence of *S. epidermidis* to the catheter

No bacterial growth was observed on antiseptic coated catheters at day 0 after 24 hours and 48 hours. In control group catheters, the mean initial bacterial adherence of *S. epidermidis* was 2.04×10^5 cfu per catheter fragment at day 0, 1.41×10^5 after 24 hours and 2×10^5 after 48 hours (Table 1 and Figure 1). Adherence of the microorganism to coated catheters was reduced by 95.4%, 98.4 %, 99.4% and 99.6% on days 7, 14, 21, and 28 respectively. The results of the present investigation clearly demonstrate that the treatment of the catheter with sulphadiazine and chlorexidine drastically reduces the formation of biofilm for all the considered time lapses. A two-sample Student's t-test confirms that the difference between the microorganism adherence on the coated and uncoated catheters is very highly significant (with $p < 0.001$) for all incubation times. The number of microorganisms adhering to the untreated catheter fragments varies between 1.41×10^5 to 2.09×10^6 per fragment at various time durations. In contrast, in the initial 48 hours no microorganism showed adherence to the treated catheters and only after 7 days did the number of adhering staphylococci reach 3.09×10^4 per fragment.

TABLE 1 - Colonization of catheter surface by slime positive *S. epidermidis*. [Time (days of immersion in PBS before the infection): 0 catheter fragments not immersed in PBS; 1, 2, 7, ... days of immersion].

Number of adhering microorganisms /catheter fragment		
Time (days in PBS)	Polyurethane catheter	C-SS impregnated catheter
0	2.04×10^5	0
1	1.41×10^5	0
2	2.00×10^5	0
7	6.76×10^5	3.09×10^4
14	8.32×10^5	1.29×10^4
21	2.09×10^6	1.17×10^4
28	9.33×10^5	3.72×10^3

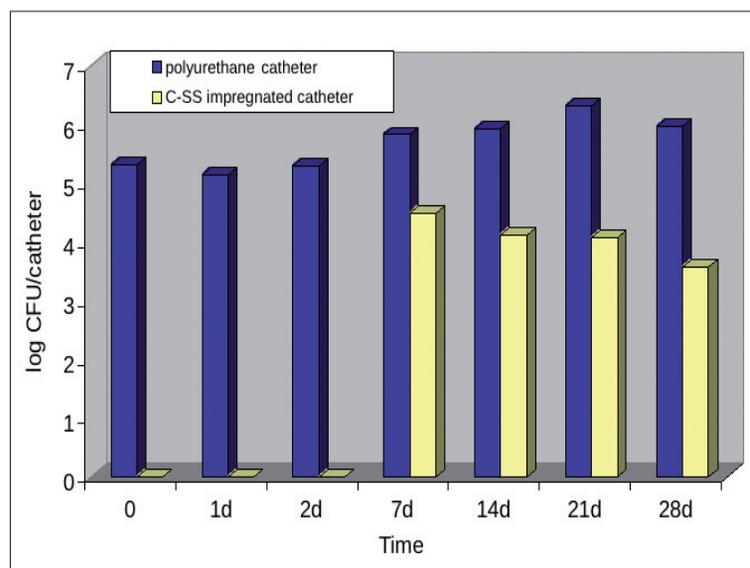


FIGURE 1 - Colonization of the catheters by slime producing *S.epidermidis*.

Evaluation of antibacterial activity of the PBS solution removed after incubation of the catheter

The antibacterial activity of the PBS solution, which contained the antimicrobial agents previously released by the catheter fragments at various times, was expressed as the highest dilution with bactericidal activity against the *S.epidermidis* strain used for the present study (Table 2).

The antiseptic coated catheters lost 75% of their eluted antibacterial activity by day 2, and 87.5% by day 4.

Eluted activity disappeared completely by day 6. No eluted antibacterial activity was measurable in the effluents of uncoated catheters.

The results of the bacteriostatic and bactericidal effluent studies suggest that coated catheters expressed bactericidal activity.

XPS analysis results

The results of the surface chemical composition as obtained by XPS analysis are summarized in figure 2. On all the catheters the surface chemical composition is dominated by carbon (between 70 and 80%) and oxygen (10-20%). The main differences between the impregnated and non-impregnated samples consisted in the higher percentage of N and the presence of Cl, which was found for the impregnated ones. Cl is very likely a fingerprint of the chlorhexidine used for the impregnation and the higher level of N might be related either to the presence of sulphadiazine or to a difference in the polymer blend of the catheter.

Traces of Si, probably as residues from the manufacturing procedure were found on all the samples. No traces of Ag or S were found with the sensitivity of this technique, which can be esti-

TABLE 2 - Antibacterial activity of the PBS solution containing the antimicrobial agents released by the catheter fragments at various times. Activity expressed as the highest dilution with bactericidal activity.

Catheter	Time of incubation in PBS							
	24h	48h	4d	6d	8d	10d	12d	14d
Polyurethane Catheter	<1/2	<1/2	<1/2	<1/2	<1/2	<1/2	<1/2	<1/2
C-SS impregnated Catheter	1/16	1/4	1/2	<1/2	<1/2	<1/2	<1/2	<1/2

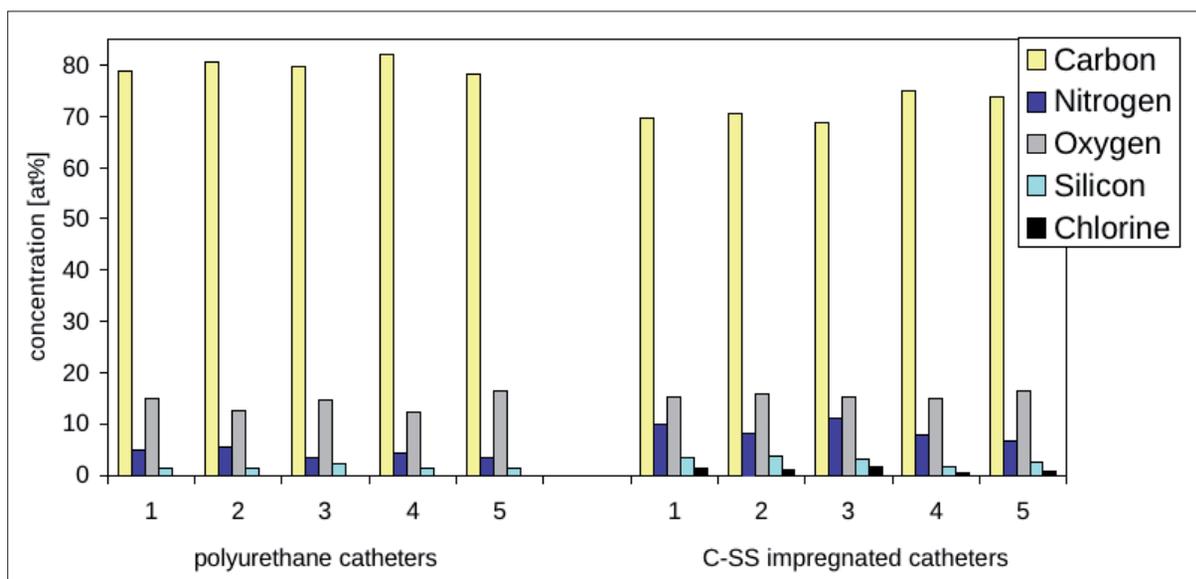


FIGURE 2 - Catheter surface composition obtained by XPS analysis

mated at 0.5-1% in the analyzed probing depth. The surface composition is quite uniform within the series of 5 samples belonging to the same material and therefore it can be assumed as uniform along the 10 cm of catheters which were selected to extract the segments for analysis.

Contact angle results

The difference in the surface composition is reflected by the contact angle of demineralized water. The average values are 85.2 and 96.6 degrees for the impregnated and non-impregnated samples, respectively (Figure 3). The difference is well beyond the standard deviation, which amounts

to 5 degrees and is clearly related to the surface treatment.

Scanning electron microscopy

The surfaces of the two types of catheters were examined by scanning electron microscopy after incubation at various times in PBS and then infection with slime producer *S.epidermidis*. Analysis of both catheters showed a good correlation with the results of adherence experiments. The organisms adhered to the outer surface of the non impregnated catheter fragments in large numbers, often in clusters embedded in slime. Fewer bacteria were present on the

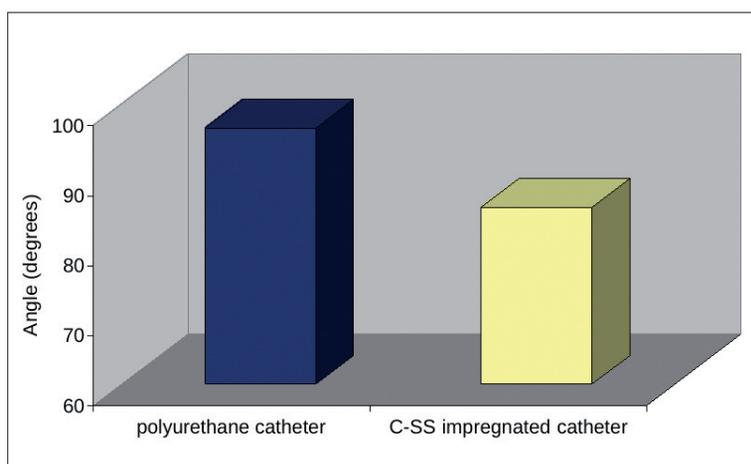


FIGURE 3 - Contact angle of polyurethane catheter versus C-SS impregnated catheter.

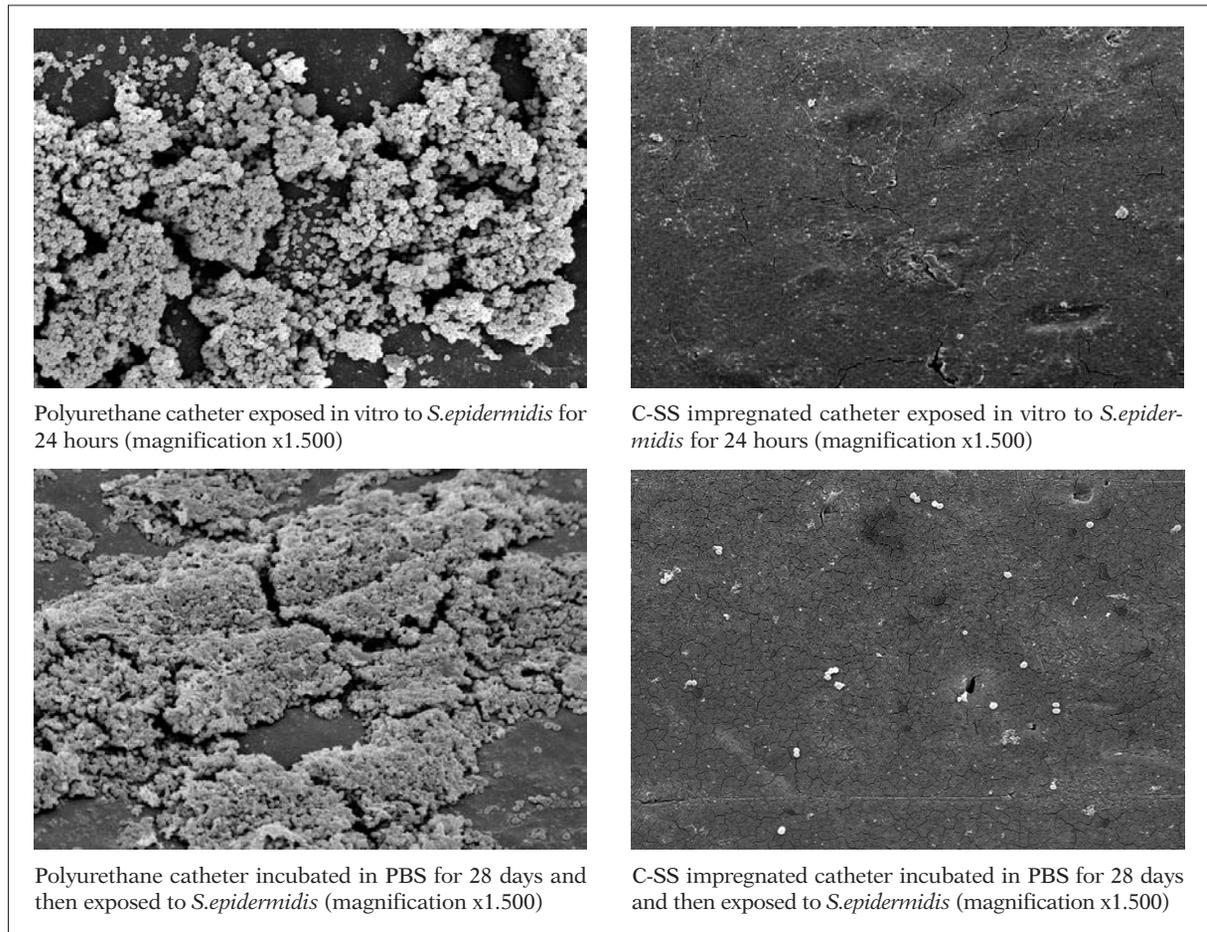


FIGURE 4 - Scanning electron micrograph of non-impregnated polyurethane catheters and C-SS impregnated catheters exposed in vitro to slime positive *S.epidermidis* after 24 hours and 28 days. The non-impregnated catheters show numerous bacteria attached to the surface at 24 hours and 28 days. In contrast, very few bacteria are present on the C-SS impregnated catheters after 24 hours and 28 days.

catheters impregnated with C-SS after 7, 14, 21 and 28 days, as compared to the non impregnated ones (Figure 4).

DISCUSSION

Adhesion of microorganisms to the catheter surface is considered the initial step in the process leading to catheter-related infections (Darouiche, 2001). Antimicrobial-coated catheters have been developed to reduce the initial adherence and colonization of these devices. The term antimicrobial-coated catheters is generally used to include all catheters that have antibiotics or antiseptics and catheters that are modified at the bedside

(Liang *et al.*, 2007; Pasquardini *et al.*, 2008). The first and most commonly studied type of chlorhexidine/silver sulfadiazine-coated catheter had antimicrobial agents incorporated only along the external surface of the catheter. A meta-analysis of 12 clinical trials showed that such antimicrobial-coated catheters result in a significant reduction in the rate of both catheters colonization and catheter-related bloodstream infections (Veenstra *et al.*, 1999). These types of catheters provide a short-lived antimicrobial activity (about 1 week), which could explain the results of a prospective randomized clinical trial showing that placement of clorhexidine/silver sulfadiazine-coated catheters for an average of 20 days in patients with hematological malignancy did not re-

duce the rate of catheter-related bloodstream infections as compared with uncoated catheters (Logghe *et al.*, 1997). A second type of chlorhexidine/silversulfadiazine-coated catheters has the antimicrobial agents combination incorporated onto both the external and internal surfaces. A study showed that these catheters had a longer in vitro durability of antimicrobial activity than the catheters with antimicrobial agents incorporated only along the external surface. The results of an animal study (Raad *et al.*, 1996) showed that polyurethane vascular catheters coated with minocycline and rifampin protected against *S. aureus* infection more than catheters coated with chlorhexidine/silver sulfadiazine. A large randomized clinical trial showed a superior clinical efficacy of minocycline/rifampin-coated versus clorexidine/silver sulfadiazine coated catheters (Darouiche *et al.*, 1999). There are three other types of second generation catheters coated with:

- 1) a low concentration of chlorhexidine;
- 2) benzalkonium chloride;
- 3) silver.

Since the studies with the three catheters have yielded conflicting results, at the present time the clinical efficacy of these antimicrobial-coated catheters remains unknown. Bedside dipping of catheters in antibiotic solutions and flushing catheter-lumen with antibiotic/anticoagulant solutions remain controversial (Schwartz *et al.*, 1990; Kamal *et al.*, 1991; Thornton *et al.*, 1996). Although the majority of clinical trials with the second generation vascular catheters have not been perfect in terms of experimental and statistical analyses, nearly all have demonstrated a significant reduction in the rate of catheter colonization and some large and well-controlled studies have shown a significant reduction in catheter-related bloodstream infections.

The optimal characteristics of antimicrobial coated catheters that manufacturing companies should consider in developing new intravascular devices are:

- 1) broad spectrum antimicrobial activity against most potential pathogens;
 - 2) durable antimicrobial activity;
 - 3) antimicrobial activity along both the external and internal catheter surface;
 - 4) activity against biofilm embedded organisms.
- The present study evaluated the efficacy of a catheter containing chlorhexidine and silver sul-

phadiazine for the prevention of staphylococcal colonization. Our results demonstrate that the antimicrobial coating reduced the number of organisms that could colonize the catheter surface. Adherence of the strain of *S. epidermidis* to a coated catheter was significantly reduced in comparison to a non-coated catheter. No bacterial growth was observed on a coated catheter after 48 hours. The eluted antibacterial activity persisted in the first 4 days and disappeared completely by day 6. Several factors determine the capacity of an antimicrobial-coated catheter to prevent initial bacterial adherence and colonization. Not only the antibacterial activity but also the presence of hydrophilic molecules, as demonstrated in our study by contact angle measurements, reduced the adherence of bacteria to the catheter surfaces. Finally, the analysis by scanning electron microscopy showed a good correlation with the results of adherence experiments.

In conclusion, the results of this and previous studies demonstrated that a chlorhexidine-silver sulfadiazine-coated catheter is effective in preventing early bacterial colonization by slime positive *S. epidermidis*. Further clinical trials of antibiotic and antiseptic coated catheters need to be carried out to determine their relative benefits and drawbacks.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dr. Mauro Taborelli for the surface analysis and the contact angle measurements. This study was supported by grant: Ricerca corrente 2008, Fondazione IRCCS S. Matteo, Pavia, Italy.

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