Pathogenesis of catheter-related infections: lessons for new designs

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In the last decade, two main strategies have been employed in the prevention of catheter-related infections: the creation of anti-adhesive biomaterials using physicochemical methods, and the incorporation of antimicrobial or antiseptic agents into current polymer biomaterials. There has been limited success with the first approach. Intravascular catheters and cuffs with an antimicrobial coating have been developed in recent years. Nevertheless, preventive strategies should avoid the use of therapeutic antibiotics. Exposure to antimicrobial agents could favor the development of resistance or the expression of genes responsible for biofilm formation. The use of these catheters should be restricted to situations where the rate of infection is high despite adherence to other strategies that do not incorporate antimicrobial agents. Better knowledge of the pathogenesis of catheter-related infections will facilitate the design of new devices that avoid the use of antimicrobial agents and decrease the risk of associated bloodstream infections. This could include the use of 'biospecific polymers' coated with anti-adhesive molecules or the use of agents which might block the expression of genes controlling biofilm formation for the most prevalent pathogens.

Keywords Catheter, pathogenesis, slime, biofilms

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Independent of the source of microorganisms colonizing intravascular catheters, many factors have been shown to affect the risk of catheters becoming infected. These include the unique capability that some microorganisms have of causing catheter-related infections. In fact, a series of interactions takes place between microorganism, biomaterial and host that determines the development of infection. A fourth relevant factor is the antimicrobial agent that develops its activity against sessile bacteria by forming a biofilm. The purpose of this review is to describe the interaction among these four essential factors in the development of catheter-related infections.

SOURCES OF MICROORGANISMS

The routes by which bacteria reach the surface of intravenous devices have remained constant [1] over time. In the short term (<8 days), catheters are colonized by skin microorganisms (70–90%), followed by bacteria from the hub/lumen (10–50%), the bloodstream (3–10%) and infusate (< 3%). In the case of long-term catheters (> 8 days), the most frequent source of colonization is the hub [2,3], followed by the skin [2]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the microorganisms most frequently involved in catheter-related infections.

BACTERIA–BIOMATERIAL INTERACTION

The probability of bacterial adhesion is dependent on the surface characteristics of the plastic biomaterial and the microorganism, while the kinetics of the process are affected by environmental factors. The initial bacterial adhesion depends on long-range bacteria–biomaterial surface interactions, including van der Waals forces, electrostatic interactions and hydrophobic interactions [3,4]. These forces also affect protein adhesion on the surface of the biomaterial. The correlation between bacterial hydrophobicity and adhesion to hydrophobic biomaterials has been demonstrated for *S. epidermidis*,

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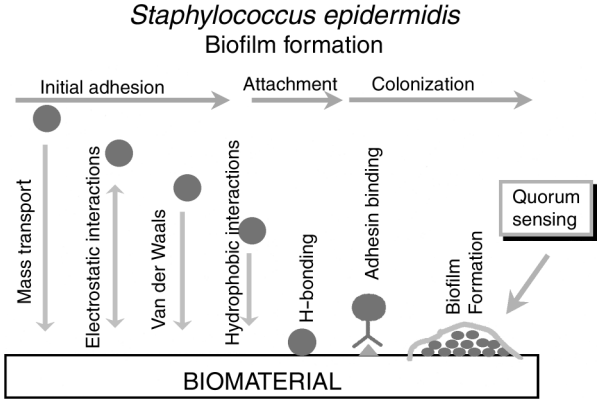

Figure 1 Factors involved in the colonization of a plastic biomaterial by *S. epidermidis*.

the most frequent bacterium causing this type of infection (Figure 1) [3].

The adherence of a hydrophobic strain of *S. epidermidis* to teflon catheters is significantly greater than that of a hydrophilic strain (Figure 2). Similar results were obtained when several strains with different hydrophobicity values were tested [4]. Moreover, most plastic biomaterials commonly used to make intravascular devices are hydrophobic, thus facilitating the initial attachment of bacteria.

Many bacteria also have hydrophobic components at their surface. When two hydrophobic surfaces interact in an aqueous environment, they tend to become attached by removing the water molecules between them. This initial bacterial adhesion, which is non-specific and reversible, is followed by a specific adhesion, mediated by an adhesin receptor, which strongly fixes the microorganisms to the surface of the biomaterial [5].

Staphylococci express specific adhesins on their surface which can recognize some major extracellular host proteins. Host proteins able to promote the adherence of *S. aureus* include protein and glycoprotein components from plasma and connective tissue. Of these, the most important glycoprotein is fibronectin, followed by fibrinogen and fibrin [6]. Collagen, laminin, vitronectin, elastin and von Willebrand factors have also been involved [6]. Fibronectin is a large multidomain glycoprotein which can be found, in soluble form, in blood and other body fluids or, in insoluble form, in connective tissues and cell surfaces.

In contrast, coagulase-negative staphylococci, particularly *S. epidermidis*, exhibit weaker interactions with host proteins, and seem to have a lower number of adhesins. While the adherence of *S. aureus* is stimulated by protein-coated polymers, the adherence of *S. epidermidis* to plastic biomaterials depends more on hydrophobic interaction and is usually impaired by plasma proteins [7].

The adherence of *S. aureus* to fibrinogen is mediated by a fibrinogen-binding protein (FBe) [8]. The Fbe gene is only expressed in a few *S. epidermidis* strains [9]. The site of *S. aureus* recognition on fibronectin is located in the N-terminal half of the protein. This binding domain is close to a heparin domain of fibronectin, leading to the inhibition of

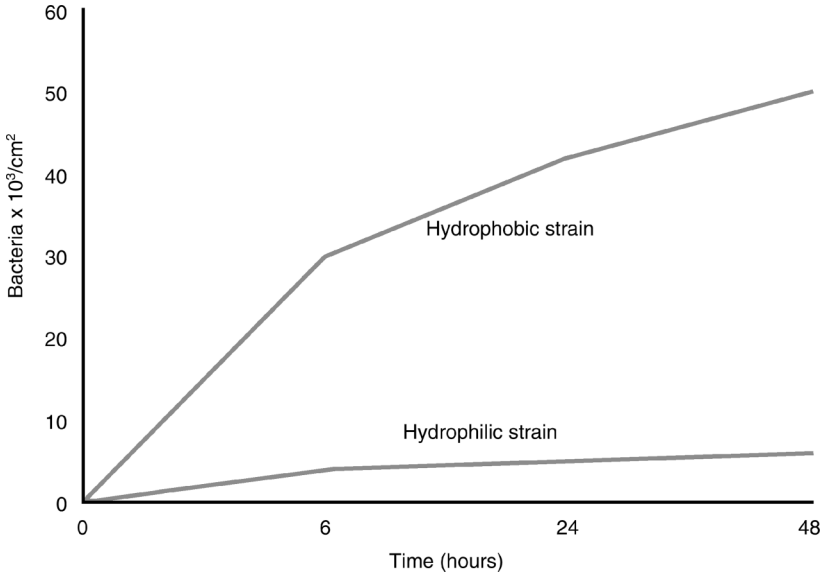

Figure 2 In vitro adherence of a hydrophobic and a hydrophilic *S. epidermidis* strain to teflon catheters.

the interaction with bacteria in the presence of heparin [9].

The role of fibronectin in *S. epidermidis* adherence is controversial. Some authors postulate that fibronectin mediates *S. epidermidis* attachment, although the adhesin involved is currently unknown. In our experience, fibronectin-coated catheters did not increase the adherence of *S. epidermidis* in vitro [4].

During the course of polymer colonization, *S. epidermidis* and other staphylococci produce large amounts of extracellular slime in which cells are embedded and covered. This slimy substance protects bacteria against host defense mechanisms and antimicrobial agents [10]. Many other biological activities have been attributed to slime [11]. Slime is an extensive and diffuse polyanionic matrix that surrounds the cells [10]. The composition of slime is controversial.

Early studies purified a substance that was contaminated with components of the medium and led to false results [7]. Several studies have tried to elucidate the real composition of slime. Probably, what have been called variously, 'capsular polysaccharide adhesin' (PS/A) [12], 'slime-associated antigen' (SAA) [13] and 'polysaccharide intracellular adhesin' (PIA) are in fact the same substance [7].

The most important study on the composition of slime was published by Mack et al. [14]. This substance, which is composed of two polysaccharide fractions I and II, is referred to as PIA. The core polymer of PIA has a ∃ 1,6-linked *N*-acetylglucosamine structure [7]. PIA expression is mediated by the *ica* gene [15]. It has been shown that *S. epidermidis* strains expressing PIA had 3–10-fold increased capability for biofilm formation.

Recent studies suggest that a relevant factor in biofilm formation is a phenomenon called quorum sensing [16]. This is a cell-to-cell communication device used by bacteria to regulate different processes, including biofilm formation. Quorum sensing has been shown to occur in vivo on urinary catheters with *Pseudomonas aeruginosa* [17]. A deeper knowledge of this process in vascular catheter infection may lead to new strategies for preventing it.

An ongoing question is whether there is any correlation between catheter-related infection and catheter-related thrombosis. It has been shown that heparin-coated central venous catheters lead to reductions in both catheter-related thrombosis and catheter-related infection [18]. Heparin is attached to the catheter via benzalkonium chloride, a substance that shows antibacterial activity and may be responsible for the lower risk of infection. Whether the heparin or the benzalkonium chloride is responsible for the reduced risk of thrombosis remains to be evaluated [19].

The nature and chemical composition of the biomaterials commonly used to make medical devices differ, and many of them contain different additives and plasticizers to improve their physicochemical properties and biocompatibility.

The adherence of different microorganisms to, and their survival in, catheters is promoted not only by bacterial factors, but also by bacterium–device interactions [20]. It has been proposed that some microorganisms, such as coagulase-negative staphylococci, could metabolize some of the components of plastic catheters in the absence of other nutrients and use them to sustain growth on the surface of the biomaterial [21].

We evaluated the kinetics of adherence of different microorganisms to catheters made of polyvinyl chloride (PVC), teflon, siliconized latex, polyurethane and Vialon [21–23]. Bacterial adherence to PVC and siliconized latex was significantly higher than adherence to the other biomaterials for all the strains tested (Table 1). Lowest adherence values were observed with polyurethanes for the staphylococci. Bacterial viability and growth

Table 1 Maximum levels of bacterial adherence to plastic catheters during incubation for 72 h (modified from [21])

	Mean (SD) number of adherent bacteria $\times 10^3/cm^2$			
Catheter	***S. aureus***	***S. epidermidis***	***Escherichia coli***	***P. aeruginosa***
Siliconized latex	27 (3)	18 (2)	19 (2)	24 (3)
PVC	42 (5)	23 (3)	18 (2)	41 (4)
Teflon	11 (1)	7 (0.4)	3 (0.1)	10 (1)
Polyurethane	15 (1)	4 (0.6)	9 (0.5)	13 (1)
Vialon	7 (1)	5 (0.3)	4 (0.2)	11 (1)

Table 2 Growth of bacteria in eluates from different catheters in PBS

Catheter	Mean (SD) CFU $\times 10^4$/mL			
	S. aureus	*S. epidermidis*	*Escherichia coli*	*P. aeruginosa*
None	0.1	0.6	1.5	600
Siliconized latex	0.2	0.5	0	1150
PVC	0.2	0.2	11	3150
Teflon	0.2	0.1	160	2980
Polyurethane	0.2	0.6	250	3250
Vialon	0.2	0.4	60	3506

were evaluated in eluates obtained from the incubation of segments of each catheter in buffer for 24 h. None of the eluates affected the viability of the staphylococci. However, all of them significantly increased the growth of *Escherichia coli* and *P. aeruginosa* (Table 2). Bacterial adherence to catheters may depend, in part, on the nature of the biomaterial, and some substances eluted from the catheter may affect the viability and growth of different microorganisms [21].

BIOMATERIAL–HOST DEFENSE MECHANISM INTERACTION

Adhesion of bacteria to a catheter surface is a critical step in the development of catheter-associated infection. Persistence of the adherent bacteria is essential for colonization and biofilm formation. A significant factor in this persistence is depressed immunity induced by the presence of the device. Both the complement system and polymorphonuclear leukocyte (PMN) activity may be affected by the presence of an implanted device [6]. Activation of the complement system by a plastic biomaterial generally occurs via the alternative pathway.

The capacity to activate complement depends upon the properties of the surface. Initiation of the complement cascade may result in the activation of leukocytes [6]. Activation of leukocytes is also mediated by the adhesion of the biomaterial. Initial activation in response to the biomaterial is followed by the depression of leukocyte functions (chemotaxis, uptake, microbicidal mechanisms) and a state of refractoriness with respect to other stimuli.

We evaluated the effect of five types of plastic biomaterials (polyvinyl chloride, teflon, polyurethane, Vialon and siliconized latex) on the phagocytic and bactericidal functions of human PMN [24]. Superoxide radical production by PMN was significantly inhibited in the presence of PVC, teflon and siliconized latex (Table 3).

Table 3 Superoxide production of PMN after incubation for 30 min in the presence of different catheters

Catheter	nmol superoxide radicals/mg protein	
	PMA	Zymosan
None (control)	196	86
Siliconized latex	62	32
PVC	104	50
Teflon	127	51
Polyurethane	180	87
Vialon	167	80

The effect of siliconized latex was presumably mediated by products eluted from the catheter into the medium, since the incubation of PMN in eluates, obtained from the incubation of this catheter in buffer, induced a similar inhibitory effect. This phenomenon was not observed with polyurethanes. None of the catheters affected the uptake of *S. aureus* by human PMN.

ANTIMICROBIAL AGENTS WITH ACTIVITY AGAINST BIOFILM-PRODUCING BACTERIA

Many research groups have investigated the potential of antimicrobial agents to prevent biofilm formation or to cure catheter-related infection [7]. Little attention has been paid, however, to the effect of biomaterials or their components on the activity of antibiotics against bacteria. We observed that MICs of amikacin and carbapenem against *P. aeruginosa* increased at least 4-fold and 16-fold, respectively, in the presence of siliconized latex, a biomaterial commonly used to make urinary

catheters [25]. This effect was not strain-dependent and was similar for different branches of catheters. In fact, this phenomenon was induced by substances eluted from the biomaterials [25].

These substances did not inactivate carbapenems or increase β-lactamase activity [26]. The outer membrane of *P. aeruginosa* grown in the presence of the biomaterial lacked an OprD-like protein and expressed a new 50-kDa protein. The substance involved in this effect is currently under investigation [26]. The clinical importance of these findings is unknown but, assuming that the concentrations of substances eluted from this biomaterial are high in the microenvironment of the bacterial biofilm, we may suppose that it is an advantageous situation for *P. aeruginosa* to be attached to this biomaterial, because of its ability to grow using the eluate as a nutrient and to evade the activity of some antimicrobial agents [26].

S. epidermidis is an important cause of infection in patients with intravascular catheters. Treatment of these infections often includes the removal of the device, since a cure cannot usually be effected by antimicrobial therapy alone.

S. epidermidis grows on the surface of plastic catheters, forming bacterial biofilms that hinder the activity of most antimicrobial agents. It has been reported that bacterial biofilms on plastic surfaces are more resistant to antimicrobial agents than are planktonic bacteria. We evaluated the activity of different antimicrobial agents against bacterial biofilms produced by slime-producing and non-slime-producing *S. epidermidis* strains on teflon catheters [27]. The increase in MBC values when sessile and planktonic bacteria were compared is shown in Table 4. The MBC values of amikacin, clindamycin, cloxacillin, ciprofloxacin, vancomycin, daptomycin and rifampin were 32–32.768 times greater when bacterial biofilms were used as inoculum for both strains.

At high concentrations ($16 \times$ MBC), none of these agents was able to sterilize the surface of the catheters. Cloxacillin and quinolone induced the greatest reduction in bacterial viability [28,29].

Previous reports have demonstrated differences in interaction between bacteria and different biomaterials [30]. The responses of bacterial biofilms developed on the surface of several biomaterials to antimicrobials may in fact be different. We evaluated the susceptibility of *S. epidermidis* attached to two different polyurethane catheters [30]. MBCs markedly increased in the presence of 6- and 48-h bacterial biofilms. These increases in MBC values occurred when either slime-producing or non-slime-producing strains were used and, in most cases, were much higher for one catheter than for the other (Table 5). This phenomenon was shown not to be due to differences in bacterial adherence. The activity of certain antimicrobials decreases markedly when bacteria are attached to plastic catheters, but this effect may have been partly dependent on the nature of the catheters [29].

Since single-antibiotic therapy has little effect in curing biofilm-associated infections, combinations of different antibiotics have been tested. Antibiotics in the cell wall-active class, such as glycopeptides, are synergistic with rifampin, completing the bactericidal action [7]. For treatment of implant-associated infections, therefore, a combination of vancomycin and rifampin has been successfully used. This was corroborated in another study, where biofilm infection was never cured by vancomycin alone and where the highest cure rate was achieved with a combination of vancomycin and rifampin [31]. We evaluated the in vitro activity of vancomycin and teicoplanin ($4 \times$ MBC), both alone and in combination with amikacin (16 mg/L) or

Table 4 Activity of eight antimicrobial agents against *S. epidermidis*: planktonic bacteria versus biofilm on teflon (modified from [27])

	$MBC_{biofilm}/MBC$	
Antimicrobial agent	**Slime (–)**	**Slime (+)**
Amikacin	>256	>128
Clindamycin	>1024	>128
Cloxacillin	>512	>256
Ciprofloxacin	>1024	>256
Vancomycin	>256	32
Teicoplanin	>64	>16
Daptomycin	>64	>32
Rifampin	>32768	>8192

Table 5 MBCs (mg/L) of seven antimicrobial agents agains planktonic bacteria and biofilms (modified from [30])

Antimicrobial agent	**Planktonic**	**Cavafix**	**Vialon**
Amikacin	2	>512	1
Clindamycin	2	>512	32
Cloxacillin	1	>512	128
Ciprofloxacin	0.5	>512	0.5
Vancomycin	4	>512	>512
Teicoplanin	16	>512	64
Daptomycin	8	>512	64

Table 6 Effect of glycopeptides (4 × MBC) alone or in combination with amikacin or rifampicin on *S. epidermidis* (slime+) biofilms (modified from [28])

Antimicrobial agent	CFU × 10^3/catheter segment	
	Teflon	Polyurethane
Vancomycin	1860	3270
Teicoplanin	363	316
Vancomycin + amikacin	0.2	242
Vancomycin + rifampicin	0.6	0
Teicoplanin + amikacin	3.6	0
Teicoplanin + rifampicin	3.1	0

rifampin (1 mg/L), against *S. epidermidis* biofilms on different plastic catheters. The addition of amikacin or rifampin significantly increased the activity of glycopeptides against sessile bacteria [28]. With slime-producing strains, these combinations were able to sterilize the surface of a polyurethane catheter (Table 6). This effect is not due to the activity of amikacin or rifampin alone.

Resistance in the biofilm population is probably not genetically encoded or due to the selection of resistant bacterial subpopulations, since the resistance disappears when bacteria are removed from the catheter. Resistance is probably due to the physiologic state of the individual cells rather than a function of biofilm formation or slime production [32]. Adherent bacteria grow more slowly than planktonic bacteria as a result of the adherence process rather than of nutrient depletion.

It has also been postulated that only the surface layers of a biofilm are exposed to a lethal dose of the antibiotic, due to a reaction–diffusion barrier which limits the transportation of the antibiotic to the biofilm. The absorption of the antibiotic to biofilm components could also give rise to such a situation [33]. In fact, the transportation properties of many antibiotics through biofilms have been evaluated, with controversial results.

Many antimicrobial agents at subinhibitory concentrations have shown to inhibit slime production and the adherence of *S. epidermidis* [34]. This phenomenon could theoretically be used to prevent the adherence of bacteria to intravascular catheters. Nevertheless, it has been recently reported that the Subinhibitory Concentrations (subMIC) of some antimicrobial agents (tetracycline and streptogramins) enhanced 9–11-fold the expression of the *ica* gene encoding biofilm formation in *S. epidermidis* [35]. Other agents did not affect *ica* expression.

NEW STRATEGIES IN INTRAVASCULAR CATHETER DESIGN

Two main strategies have been employed in the prevention of catheter-related infections: the creation of anti-adhesive biomaterials using physicochemical methods, and the incorporation of antimicrobial agents into current polymer biomaterials [36]. The first approach must prevent not only unspecific bacterial adhesion, but also the adsorption of host components which promote bacterial adhesion.

By modifying biomaterial surfaces with highly hydrated molecules, such as heparin or polyethylene oxide, anti-adhesive properties can be obtained [37]. This kind of hydrophilic surface has also been shown to decrease protein absorption. The development of a simple surface modification technique that creates a hydrophilic bacterial-resistant surface has been attempted but with limited success.

Intravascular catheters and cuffs with an antimicrobial coating have been developed in recent years. A silver-impregnated, subcutaneous collagen cuff attached to a central venous catheter just before insertion has been used as a tissue interface barrier [38]. Most studies showed no reduction in the incidence of catheter-related bacteremia when this cuff was used. Based on published studies, the use of this cuff is not recommended [39]. There is no doubt that silver ions are active against a broad spectrum of bacteria. However, in an environment containing albumin and halide ions, the antibacterial activity of silver ions will be decreased as a result of specific adsorption processes with albumin and precipitation of insoluble silver chloride. Extrusion of the silver cuff to the skin and a reduced subcutaneous anchorage of silver-cuffed, central venous tunneled catheters have been reported, possibly due to silver-induced cytotoxicity [40].

The use of heparin-bonded pulmonary catheters has been associated with a lower risk of catheter-related bloodstream infection. In most of these catheters, heparin is bonded with benzalkonium chloride, thus providing them with antimicrobial activity [41]. According to these data, heparin-bonded pulmonary artery catheters should be used.

The use of exidine- and silver sulfadiazine-impregnated catheters reduces the incidence of short-term (<2 weeks) central venous catheter-related bloodstream infections [42]. These catheters

have been shown to be less effective when catheters are in place for 3 weeks or longer. This is probably due to the lack of protection against bacteria invading the lumen of the catheter from a contaminated hub. Moreover, some in vitro studies have shown development of bacterial resistance to clorhexidine that was sometimes extended to other antimicrobial agents. Anaphylactic reactions due to clorhexidine impregnation have also been described [43].

Central venous catheters impregnated intra- and extraluminally with minocycline and rifampin have been shown to reduce the incidence of catheter-related bloodstream infection compared with clorhexidine–silver sulfadiazine-impregnated catheters [44]. Nevertheless, preventive strategies should avoid the use of therapeutic antibiotics. In fact, exposure to antimicrobial agents could favor the development of resistance. Tetracycline at subinhibitory concentrations has been shown to increase 10-fold the expression of the *ica* gene responsible for biofilm formation in *S. epidermidis* [35]. On the other hand, the use of these antibiotic-impregnated catheters could reduce the use of systemic antibiotics for presumed or real catheter-related infections. Before more studies are performed, the use of this catheter should be restricted to short-term central venous catheters if the rate of infection is high despite adherence to other strategies that do not incorporate antimicrobial agents.

Future strategies include the use of catheters with silver incorporated throughout the biomaterial [45]. Preliminary studies using this device showed reduced catheter colonization. Another possibility is the use of catheters with covalently linked heparin on the surface, which seems to reduce the rate of bloodstream infection [46].

Electrically charged catheters reduced catheter colonization [47]. The production of hydrogen peroxide and free chlorine by electrolysis at the catheter surface avoids bacterial colonization. This new approach is extremely interesting, since it avoids the use of topical antimicrobial agents.

Increasing knowledge of biofilm formation will allow new strategies for catheter design. It will be possible to coat biomaterials with anti-adhesive molecules, such as antifibronectin antibodies. These so-called 'biospecific polymers' will certainly reduce catheter colonization by specific microorganisms. The use of agents which might block the messengers involved in quorum-sensing-dependent biofilm formation may also be possible. Future methods blocking the expression of genes controlling *S. epidermidis* adherence to biomaterials could prevent this type of infection [39].

In conclusion, better knowledge of the pathogenesis of catheter-related infections will facilitate the design of new devices that avoid the use of antimicrobial agents and decrease the risk of associated bloodstream infections.

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