Catheters: a suitable surface for biofilm formation

J. Treter¹, A. J. Macedo¹,²

¹ Faculdade de Farmácia;² Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

The successes of modern medicine are intimately connected to the ever-increasing use of biomedical devices such as those used for vital functions management. Catheters of different types are the most common assist devices utilized every year. Current materials from which such medical devices are made include polyurethane, Teflon®, silicone rubber, polyethylene, polypropylene, polyvinyl chloride, and polytetrafluoroethylene. After exposure to body fluids the device becomes an environment suitable to support biofilm growth and subsequent infection. Even today, the treatment of choice for patients with infected devices is the catheter removal. Bacterial biofilms cause chronic infections due to the increased tolerance to antibiotics and disinfectants, resistance to phagocytosis and to the human defence system. Depending on the type and composition of the medical device, some pathogens are more prevalent. Staphylococcus aureus, S. epidermidis, Escherichia coli and Pseudomonas aeruginosa are the microorganisms most frequently involved in catheter-related infections.

Keywords Bacterial adhesion; surface characteristic

1. Introduction

The successes of the modern medicine are intimately connected to the ever-increasing use of biomedical devices such as those used for vital functions management. Conceptually, a biomedical device is an apparatus used provisionally or permanently, externally or internally, to diagnose conditions or to preserve, restore, or augment structure or function in the body. Biomaterial is defined as a single element or a compound, or mixture of elements which are used to construct medical devices [1]. These new technologies not only improve the patient quality of life, but also increase their survival time.

Medical devices such as prostheses, bone replacement implants, drug delivery, tissue engineering and catheters are composed by polymers made mostly of the following biomaterials: poly(dimethyl siloxane) (SR), polyethylene (PE), poly(ethylene terephthalate) (PET), silicone rubber, poly(methyl methacrylate) (PMMA), polypropylene (PP), poly(tetrafluoroethylene) (PTFE or Teflon®), polyvinyl chloride (PVC), polystyrene (PS), and polyurethane (PU). Desirable properties of these biomaterials are their biocompatibility, mechanical properties, ease of molding into desirable shapes and low cost.

In spite of the considerable success achieved with new material devices, these abiotic surfaces are susceptible to bacterial colonization which creates an important public health problem. When any biomaterial is implanted, it can become a site for bacterial adhesion, colonization, and infection. More than 60% of hospital-acquired infections worldwide are accredited to bacteria forming biofilms on medical devices [2,3]. In most of the cases the occurrence of an infection and re-infection obligate the removal of the device. Microbial biofilm formation is directly involved in this process, reducing the efficiency of the antimicrobial treatment and extending the length of stay in hospitals, costs and morbidity [4].

2. Biofilm formation

Microbial activities control or influence all aspects of biosphere functioning, including health, nutrition and disease of animals, plants and environmental quality. Since biofilms are ubiquitous in nature, considerable efforts have been made to understand this complex mode of life. Biofilm is a microbial lifestyle, in which the microbes attach to surfaces, allowing this community to survive in hostile environments.

Biofilm formation follows the steps represented in the Fig. 1. Briefly, the planktonic cells approach the surface (①), attach to it (②), and start the formation of microcolonies, also called bacterial aggregates. Up to step ② the process might be considered reversible. Bacterial cell-cell communication begins in the next step, a phenomenon known as Quorum Sensing, where bacterial cells produces and feels chemical signals in order to organize themselves and start the production of exopolysaccharides (EPS) (③), in what becomes an irreversible system. Biofilm maturation occurs as represented in step ③, when the characteristic “mushroom shape” is observed. The shape of the mature biofilm is appropriate to enable the exit of metabolic wastes and circulation of nutrients and oxygen. The last step of biofilm formation is dispersion (④). This is the stage when bacterial cells in the biofilm feel adverse environmental conditions (such as presence of high concentration of antibiotics) and might then disperse rapidly and colonize new surfaces [5].
In a human health context, biofilms are important and represent a challenge to one of the greatest achievements in modern medicine, which is the possibility to use antibiotics to effectively treat most acute infections. However, there are two important exceptions to this rule: bacteria resistant to antibiotics, and bacteria that reside within a biofilm. A biofilm can be up to 1000 folds more resistant to antibiotic treatment than the same organism growing planktonically. Thus, it is not surprising that a remarkable number of chronic bacterial infections involve bacterial biofilms. Indeed, the biofilm lifestyle increases the virulence of many other infectious diseases [6] which are not easily exterminated by conventional antibiotic therapy [7].

Biofilms are responsible for a variety of infectious diseases. Some typical examples are: dental caries (caused by acidogenic Gram-positive cocci Streptococcus sp.), periodontitis (Gram-negative anaerobic oral bacteria), otitis media, or middle ear infection (non-typeable Haemophilus influenza), chronic tonsillitis (by various species), cystic fibrosis pneumonia (Pseudomonas aeruginosa, Burkholderia cepacia), endocarditis (streptococci, staphylococci), necrotizing fasciitis (Group A streptococci), musculoskeletal infections (Gram-positive cocci), osteomyelitis (various species), biliary tract infection (enteric bacteria), infectious kidney stones (Gram-negative rods), bacterial prostatitis (Escherichia coli and other Gram-negative bacteria). Organisms like P. aeruginosa, Staphylococci and E. coli are associated to infections caused by foreign body materials, such as contamination of implants, catheters, contact lenses and prostheses [6].

In this context, several aspects have to be considered:
(a) pathogenic bacteria are becoming more adapted to antibiotics;
(b) bacterial communities living in biofilms are less susceptible to antibiotic treatments and at the same time maintain an enhanced level of virulence to the host;
(c) most infections and a variety of diseases involve bacterial biofilms and;
(d) the EPS matrix provides a protective environment for pathogenic microbes, facilitating the accumulation of autoinducers, and the attack to the host.

3. Biomedical devices – Catheters
Reliable vascular access is an essential feature of modern day health care [8] and for decades biomaterials have played an important role in disease management [9]. Among biomaterials, the most widely employed are catheters - simple tubing for the delivery of substances over time, implanted from the external surface into the organ or vessel of interest. Intravascular catheters are used mainly in the administration of fluids, medications, parenteral nutrition, and blood products, as well as in the monitoring of hemodynamic status and in chronic outpatient hemodialysis [12]. Unfortunately, the use of devices for vascular access is associated with an underappreciated risk of intravascular device-related bloodstream infection, caused by microorganisms that colonize the implanted device or contaminate the fluid pathway, at the time of insertion or during its use [8]. Two barriers are violated during vascular catheterization: the skin...
and the vein wall (Fig. 3B). This enables the contact of the surrounding heavily contaminated with the blood flow along two interfaces: the outer surface of the catheter and the catheter lumen [10].

There are four principal ways for microorganisms to reach and contaminate catheters [11]:

1) migration of skin microorganisms from the insertion site into the cutaneous catheter tract and along the surface of the catheter tip (most common);
2) direct contamination of the catheter or catheter hub by contact with hands or contaminated fluids or devices (common);
3) bacterial contamination spread by blood from another focus of infection (less common);
4) an infusiate contamination (rare).

The risk factors of intravascular catheter-related infections comprise type of catheter; the hospital size, unit, or service; the location of the site of insertion; and the duration of catheter placement [12]. In this sense, it is important to highlight some of the different terminologies that may possibly be applied to identify different types of catheters [11]:

- the type of vessel in which the catheter is inserted: peripheral venous, central venous, or arterial.
- intended life span: temporary or short-term versus permanent or long-term.
- site of insertion: subclavian, femoral, internal jugular, peripheral, and peripherally inserted central catheter.
- pathway from skin to vessel: tunneled versus nontunneled.
- physical length: long versus short
- some special characteristics of the catheter: presence or absence of a cuff, impregnation with heparin, antibiotics or antisepsics, and the number of lumens.

Devices used for short-term vascular access [13]:

- Peripheral venous catheter: It is the most commonly used intravascular device. Currently made of PU, Teflon® or steel.
- Peripheral arterial catheters: Are widespread in critical care units to monitor blood pressure and obtain arterial samples for blood gas determination.
- Midline catheter: Peripheral catheter is inserted via the antecubital fossa into the proximal basilica or cephalic veins, but it does not enter central veins.
- Nontunneled central venous catheter (CVC): CVCs are estimated to account about 90% of all catheter-related bacteremias. The site of insertion will resonate at higher or lower rate of infection.
- Pulmonary artery catheters: Short period of duration

Devices used for long-term vascular access [13]:

- Tunneled CVC: Surgically implanted with a tunneled portion exiting the skin and a cuff just inside the exit site.

3.1 Magnitude of catheter-related infections

The first scientific report of the use of catheters dates from the year 1823 [14], when a bladder catheter was used to support the extraction of bladder stones. Since then, the use of medical devices increased rapidly. From the 1990’s, new materials and technologies enable the routine use of catheters in hospitals, to a relative low cost. As a consequence of this increase, hospitals are nowadays systematically challenged to combat the catheter-associated infections.

Figure 2A shows the scientific reports with the terms “catheter and infection” for the last five decades, demonstrating that infection is constantly being related to catheter placement. The first direct association of biofilm infection in a catheter was presented by Costerton in 1984 [15]. In that study, intravenous and intraarterial catheters subjected to electron microscopy showed surface irregularities and extensive bacterial adhesion. Remarkable was the presence of *S. epidermidis*, which is frequent, and possesses an increased antibiotic resistance when in biofilm lifestyle [16,17]. Since then, and in particular in the last five years, a vertiginous increase of reports relating biofilms and infection is observed (Fig. 2B), corroborating the idea of high index of catheter placement in hospitals.

Intravascular catheters have become a leading cause of health-care-associated bloodstream infections. The infection will not only result in prolonged hospitalization and associated increased health care costs, but also in higher morbidity. From a prevention standpoint, the need for the development of catheters using materials that prevent microbial attachment and biofilm formation is paramount [18].
A. Méndez-Vilas (Ed.)

Science against microbial pathogens: communicating current research and technological advances

The International Nosocomial Infection Control Consortium (INICC) - the only source of aggregated international data on the epidemiology of device-associated infections - reports that in developing countries rates of device-associated infections and bacterial resistance are 3 to 5 times higher than international standards, leading to an increased length of stay (10 days), costs (US $5000 to US $12,000), and mortality by 2-fold. Approximately 80,000 catheter-related infections are reported in intensive care units (ICU) each year in the United States (US) but it is believed that the real numbers are much higher, about 250,000 to 500,000 cases per year, when it takes into account the data from entire hospital [8].

Annually, in the US, the mortality of critical ill patients due to bacterial infections ranges from 12% to 25% [19]. Considering urinary tract infections (UTIs) alone, more than 8 million visits to physician’s offices, 1.5 million emergency room visits, and 300,000 hospital admissions are reported in the US annually [20]. In European countries mortality attributable to catheter-related infections reaches 10-12% in Germany, 17.1% in Italy, and 11% in France. Lastly, a study estimated the increased risk from ICU-acquired bloodstream infections at 24.8% [21].

*Staphylococcus aureus* and *S. epidermidis* are the microorganisms frequently involved in catheter-related infection [22] with coagulase-negative being the most common among them. Although patients with catheter-related coagulase-negative staphylococcal bacteremia can be treated successfully while the catheter remains in place, with the majority of patients remaining free of recurrence [19] for *S. aureus* the removal of infected catheters has been associated with a more rapid response to therapy and/or a higher cure rate [23].

In chronic kidney disease, it is estimated that 25% of the patients use catheters as vascular access [24]. The use of tunneled dialysis catheter is an important source of infection with an incidence of bacteremia reported as 0.8-5.5/1000 catheter days [25] Staphylococcal tunneled dialysis catheter infection, particularly with *S. aureus*, is highly prevalent in a hospital setting. Infection with either coagulase-negative staphylococcus or *S. aureus* accounts for 40–81% of cases of catheter-related infections in reported studies [26].

*Escherichia coli*, an important member of the normal intestinal microflora in humans and other mammals is a highly versatile bacterium. It may act as harmless gut commensal to intra- or extraintestinal pathogens, being also a frequent colonizer of medical devices and the primary causes of recurrent urogenital infections [27]. UTIs are among the most common infectious diseases of humans, and the most common urological disease in the US, with a total annual cost of more than $3.5 billion [28]. *Escherichia coli* is reported to be responsible for >80% of all cases [29]. Catheterization of the urinary tract increases the risk of bacteriuria in up to 10% per day [30], and the majority of the patients with an indwelling urinary catheter for 30 days or longer develops bacteriuria [31]. Considering other infections, not related to the urinary tract, a study about *E. coli* showed that 63% the episodes of bacteremia were caused by the insertion of peripheral or central vascular catheter, being considered one of the six risks factors for *E. coli* infection, together with emergency, malignancy, cytoreductive or immunosuppressive therapy. The results evidence the importance of the control of risks at early stages of infection [32].

*P. aeruginosa*, an opportunistic pathogen, is the third most common pathogen related with hospital-acquired catheter associated UTIs due to its ability to cause infections, as well as its propensity to form biofilm [33] leading to higher incidence in patients with long-term indwelling bladder catheters. The virulence of *P. aeruginosa* is attributed to a series of factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores [34]. A 2-year study performed in Italian hospitals with 106 patients showed that risk factors for the isolation *P. aeruginosa* bloodstream infections (BSIs), even by multidrug-resistant (MDR) or non-MDR were: presence of CVC, previous antibiotic therapy, corticosteroid therapy, previous BSI,
neutrophil count <500/mm$^3$, urinary catheterization. The results showed that the use of catheters is a very important issue to be controlled. As consequence, the 21-day mortality rate considering all patients was 33.9%, due to septic shock, infection due to MDR $P.$ aeruginosa, and inadequate initial antimicrobial therapy [35].

4. Multifactor bacterial adherence

Clinically, different biomedical devices or even identical devices differing in polymeric composition seem to pose considerably different infection risks [36]. It is important to highlight the fact that the formation of an infectious biofilm is initiated by bacterial adhesion to surface material, a phenomenon governed by physicochemical properties of the microorganism, environment and biomaterial characteristics [37,38] as can be seen in Fig. 3A and 3B.

![Figure 3: Catheter-related infections involving the A) triad interaction: microorganism, environment and material B) dual interaction microorganism and material.](image)

4.1. Material Factors

The presence of a device – catheter insertion – by itself can enhance bacterial virulence. Some principles must be taken into account in the analysis of the bacterial adhesion to a material surface: [39].

1) different bacteria may adhere differently to the same material
2) the same bacteria may adhere differently to different device materials
3) the same bacteria may adhere differently to the same device material placed under different circumstances (medium, type of flow, temperature)

In general, surface properties such as chemical composition of the material, surface free energy, charge, hydrophobicity, roughness and porosity directly affect bacterial adhesion [37]. Functional groups present on the biomaterial surface will determine the hydrophobicity and surface charge of the abiotic target [38]. It is known that hydrophobic materials favor bacterial adherence more than hydrophilic ones [22,38,39]. A significant correlation between surface free energy of a material and its plaque-retaining capacity has been established, with the higher energies showing a favorable effect on bacterial adherence [40]. On the other hand, it was demonstrated that low surface free energy materials attracted microorganisms with a relatively low surface free energy [41].

It has been found that irregular polymeric surfaces promote bacterial adhesion and biofilm deposition, and that infection rates are higher in porous material than in dense materials, indicating that bacteria adhere preferentially to the pores [39]. In this sense, bacteria have different preferences to attach to polymeric materials of different constitutions [22,39,42,43]. It is well established in the literature that Teflon® is one of the polymeric materials to which microorganisms in general do not easily adhere. However, small variations in preference depend on the bacterial genus and species. For example, $S.$ epidermidis prefers to attach to Teflon®, while $S.$ aureus, $P.$ aeruginosa and $E.$ coli prefer PU [22, 44].

Bacterial adherence to PVC and siliconized latex was significantly higher than adherence to other biomaterials like Teflon® and PU, for all strains tested, including Gram positive staphylococci and Gram negative $P.$ aeruginosa and $E.$ coli. [22]. It is common to observe that $S.$ epidermidis adhesion to less hydrophobic materials, like acrylics such as PMMA, for example, is lower than adhesion to the more hydrophobic ones, such as silicone [45]. Corroborating this idea, bacteria prefer to attach to silicone rather than PU and hydrophilic PU [46,47]. A general evaluation of the
performance of distinct materials against bacterial adhesion is difficult to be established, since research groups use different experimental conditions and strains.

4.2. Environment Factors

Certain environmental factors, including the presence of a conditioning film, temperature, concentration of electrolytes, bacterial concentration, the presence of antibiotics and fluid flow affect bacterial adhesion [37,38]. When a foreign material is placed inside the body, host molecules interact with the surface to form a conditioning film [38]. Molecules initially interact with surface nonspecifically through electrostatic, Van der Waals and hydrophobic forces leading to reversible adsorption to the material. Once in contact with the material, the bacterium is able to engage in interactions dependent on the surface characteristics of both bacteria and material surface. And the number of bonds between bacterium and surface will determine the shear stress that the attached bacterium is able to sustain [37].

Among the environmental factors, flow condition is considered the dominant factor that influences strongly the number of attached bacteria as well as biofilm structure and performance [37]. Concentration of electrolytes such as KCl and NaCl, and pH of the culture medium influence bacterial adhesion. pH and ionic strength of a suspending surface will alter the cell surface hydrophobicity [48]. Electrostatic interactions contribute to biofilm cohesion, as can be demonstrated for divalent cations which include Mg\(^{2+}\) and Ca\(^{2+}\), which influence directly biofilm formation and indirectly via a physiology dependent attachment process by acting as an important cellular cation and enzyme cofactor [38].

4.3 Microorganisms Factors

It is known that bacteria with hydrophobic properties prefer hydrophobic material surfaces, just like hydrophilic bacteria prefer hydrophilic surfaces. However, it is observed that hydrophobicity of the material plays a more prominent role in bacterial adherence than the hydrophobicity of the bacteria [38]. Bacterial surface charges in aqueous suspension are almost always negative. Charge varies according to bacterial species and strains, and is influenced by growth medium, pH, ionic strength of the suspending buffer, bacterial age and surface structure [37].

The presence of antibiotics decreases bacterial adhesion, depending on bacterial susceptibility and antibiotic concentration [49], although adherent cells were less susceptible to antibiotic therapy than non-adherent cells [50]. This last fact is due to the capacity some microorganisms have to produce extracellular polysaccharide and, consequently, form biofilm [51].

5. Preventive measures

Some measures that proved to have preventive effect on catheter-related bloodstream infections will be mentioned.

- To prevent extraluminal contamination: maximal aseptic barriers (large drape, sterile gloves, gown, cap, and mask) must be adopted at insertion time, accompanied by skin antisepsis (chlorhexidine-containing antiseptic showing superior effects) [10]. Depending on the site of insertion, the catheter can be easily contaminated; in this sense, subclavian site should be the first choice, followed by femoral and finally jugular site [52]. It is vital that the procedure be performed by a trained team.

- To prevent of endoluminal contamination: an aseptic hub handling is necessary, including several steps as a reduction in the number of connections, separation of the hub from the patient’s skin, and protection of the hub using a povidone/iodine-impregnated foam. Other strategies to prevent contamination are external hub protection, spacing changes of the infusion sets, and reducing the number of catheter lumens/ports. Simple measures, like reducing the number of catheter manipulations, are also important. Intraluminal antibiotics and antiseptic connectors also help to control catheter-related infections. Similarly to the intraluminal contamination, a trained team is essential [10].

Some strategies have proven ineffective in the prevention of catheter related-infection. In urinary tract infections, the use of antimicrobial agents either systemically or instilled directly into the bladder and catheter irrigation were not effective in preventing bacterial colonization [53]. Chronic antibiotic suppression of asymptomatic bacteriuria of a patient that is catheterized to prevent urinary tract infection leads to the emergence of resistant flora and to adverse drug effects [54].

Another way to prevent infection that is gaining ground in recent years is the modification of materials already being used by the medical industry [55]. This approach is an economic and effective method by which biocompatibility and biofunctionality can be achieved while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness [9]. It is based on the modification of the device surfaces in such a way that no bacterial adhesion can occur [56]. The proposal is to achieve a biomaterial with nonfouling properties, meaning that protein adsorption and subsequent microbial adhesion - biocontaminants - are minimized.
Most approaches for preventing bacterial adhesion involve at least one of the following two mechanisms, as reviewed by Banerjee and coworkers (2011) [57]:

- Resistance to adhesion of biocontaminants:
  - PEG or oligo(ethylene glycol) groups
  - Smart or stimuli-responsive materials
- Degradation of the biocontaminants:
  - Released-based approach
    - Silver.
    - Antibiotics or antiseptics:
    - Nitric oxide.
  - Non-released-based approach
    - Surfaces functionalized with polycations
    - Surfaces functionalized antimicrobial peptides (AMPs)
    - Photoactive surfaces
    - Coatings containing enzymes

Most of these strategies are helpful in combating the problem of adherent biocontaminants, although they are still under development and study. The active search for new strategies and materials encourages and gives hope that better materials will be developed and the problem of contamination will be solved in future.

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