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1 **"Understanding biofilm formation in intravascular device-related infections"**

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Introduction

The use of indwelling devices is constantly increasing in modern medicine. Although they improve patients' care, it is recognised that their use is associated with the risk of infection. Device-related infections represent a significant part of hospital-acquired infections and are due to device contamination by microorganisms subsequently forming biofilms. These biofilms can be defined as sessile communities encased in a self-produced matrix that protects them from external insults including the host immune system and antimicrobial agents. Historically the first description of the involvement of biofilms in a device-related infection was provided in 1982 by the observation of a pacemaker colonised by *Staphylococcus aureus* [1]. Since then, formation of biofilms has been described in all indwelling devices and is especially problematic in intensive care units (ICU) where intravascular (including intracardiac) devices are extensively used. Among them, intravascular catheters are used for different purposes in ICU including infusion, haemodialysis, parenteral nutrition, hemodynamic monitoring or support such as during extracorporeal membrane oxygenation (ECMO) or extracorporeal life support (ECLS). In part because of their repeated manipulation, the use of such devices is associated with infection incidence densities ranging from 0.8 to 3.7 episodes per 1000 catheter-days, depending on ICU units [2]. Main microorganisms responsible for intravascular catheter colonisation and subsequent infections depend on local epidemiology and type of ICU but mostly include *Staphylococcus* spp, Gram-negative rods and yeasts. Most of the time, clinical symptoms of intravascular catheter-related infections are unspecific, such as fever, chills or hypotension, and local cutaneous signs such as redness or pain are seen in ~20% of the cases. Here we will describe the different steps of bacterial biofilm formation on intravascular catheters and strategies that can be developed to avoid or reduce their clinical consequences (Figure 1). Such a physiopathologic scheme can also be applied to the formation of biofilm on other intravascular devices such as cardiac prosthetic valves or pacemakers.

Targeting early steps of colonisation

While initial contamination of intravascular catheters can occur through bacterial translocation from the gut (typically in critically ill and oncology patients) or bloodstream infection (BSI) from another infectious source (Figure 1A), the main route of contamination is the patient's skin bacterial flora or healthcare workers' hand manipulating the catheter hub (Figure 1B and C, respectively). In this case, contamination occurs mainly because of suboptimal aseptic measures and can lead to intra- or extra-luminal colonisation [3]. Initial events of adhesion on abiotic surfaces such as intravascular catheters that will further lead to colonisation are dependent on electrostatic interactions between bacteria and surfaces as well as more specific interactions between bacterial surface appendages and host proteins that can rapidly cover the implanted material (Figure 1D). These interactions are mediated through different types of adhesins both in Gram-positive and Gram-negative bacteria such as flagella, homo- or hetero-polymeric fimbriae and large adhesins [4]. In Gram-negative bacteria, many of these biofilm-promoting factors are activated by cyclic-di-GMP (c-di-GMP), an allosteric activator controlling free-swimming to sessile lifestyle transition [5]. The best prevention approach comes with hand hygiene and skin antiseptics for catheter insertion and handling. Skin disinfection with chlorhexidine-alcohol has been proven to be the more active [6]. If incidence of catheter-related infections is still high, other prophylactic strategies can be proposed including chlorhexidine-impregnated sponges or microbicides-impregnated catheters such as those containing minocycline-rifampin [7]. While not in clinic yet, devices coated with natural or synthetic antimicrobial peptides might provide in the future novel preventive solutions [8]. In the setting of ICU, as catheters are continuously used, preventive lock therapies are of limited help. The principle of such locks is to inject a small volume of highly concentrated antibiotics in the catheter lumen in order to prevent or eradicate biofilm. One trial demonstrated that a short-course ethanol lock did not prevent dialysis catheter-related infections [9]. Furthermore, the use of such ethanol locks may be associated with catheter clotting and should therefore be carefully used. An alternative to biocidal treatment might be translated in the future into the clinic by the use of anti-adhesive molecules avoiding initial attachment such as coating of catheters with polymers like poly-ethylene-glycols or polysaccharides (methyl-cellulose [10], ulvans) [11], or by interfering with the transition from free-swimming to sessile lifestyle using c-di-GMP inhibitors (for example [12]).

Targeting mature biofilms

74 After initial attachment, maturation of biofilm essentially relies on communication between bacteria
75 through “quorum-sensing” (QS) molecules and the production of the biofilm matrix. The latter is
76 composed essentially of water and is structured by various macromolecules such as extra-cellular
77 DNA, polysaccharides and proteins (Figure 1E). This matrix not only governs biofilm architecture but
78 also provides protection against both the immune system and antimicrobial agents by reducing their
79 diffusion. At that stage, pieces of biofilms can shed in the bloodstream, leading to sepsis, septic
80 thrombophlebitis, or distant infections such as infective endocarditis or bone and joint infections
81 (Figure 1F). Growth of biofilms in the lumen of the catheter can also be responsible for catheter
82 clotting limiting its use.

83 The presence of the matrix within mature biofilms imposes gradients of nutrients, oxygen and waste
84 products leading to an important chemical heterogeneity [13] and thus promotes emergence, at a low
85 frequency, of phenotypic variants including the so-called persisters. Persisters, unlike most of the cells
86 composing the biofilms, can survive to extremely high levels of antimicrobial agents, and are thus
87 considered the main cause of biofilm antibiotic tolerance and of chronic and recurrent infections [14].

88 The diagnosis of catheter-related BSI (C-RBSI) is established when the same microorganism grows in
89 percutaneous blood cultures and on the catheter tip. It can also be made without removing the
90 catheter by means of the differential time to positivity or quantitative blood cultures techniques [15]. In
91 the last, blood samples from all catheters lumens must be cultured in order to increase sensitivity [16].

92 Catheter withdrawal and systemic antimicrobials are the cornerstones of C-RBSI treatment. Antibiotic
93 lock therapy plus systemic antimicrobials is an effective and safe strategy to treat uncomplicated C-
94 RBSI without catheter removal but should be restricted to long-term central venous catheters in stable
95 patients. As a consequence, it cannot be recommended routinely in ICU patients [17].

96 In the future, however, some promising approaches might translate into the clinics. For example,
97 together with a systemic treatment, antibiotic adjuvants that destroy or destabilise the matrix and thus
98 weaken the biofilms such as enzymes (DispersinB, DNaseI) or cations chelators (EDTA [18]), or
99 molecules that directly target persister cells (sugars, ADEP4, antimicrobial peptides, etc) [19], could be
100 used as short-course catheter locks for catheter salvage.

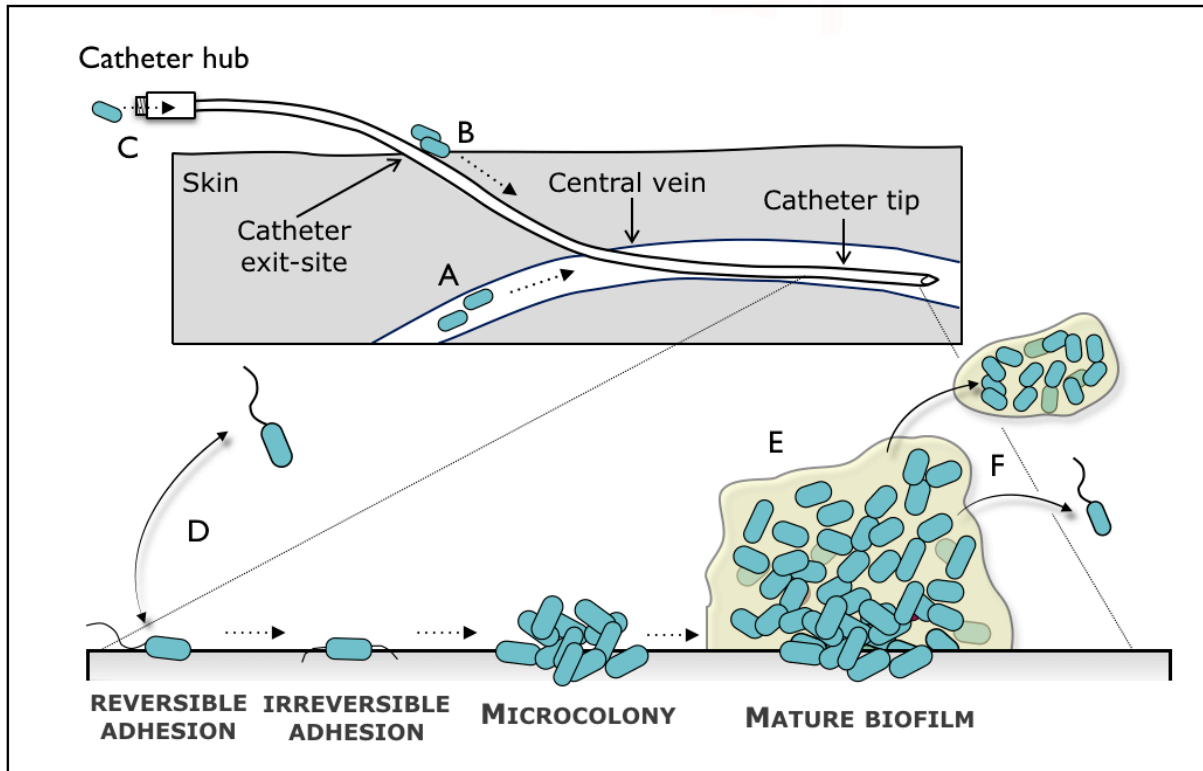
101 **Conclusion and future challenges.**

102 Intravascular catheter-related infections remain a major health issue especially in the ICU. Despite
103 promising approaches developed in research laboratories, in part because of molecule development
104 cost issues, limited relevance of some *in vitro* and *in vivo* animal models and the severity of patients,
105 the physicians in ICU still have very little options when facing such infections.

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108 When an ICU patient suffers from a C-RBSI, the safest option today remains removal of the colonised
109 catheter. Rapid improvements will probably come from preventive strategies firstly based on enhanced
110 hygiene. The next challenges for researchers will be to develop novel strategies to improve early
111 detection of biofilm formation before any symptoms are visible and to increase knowledge and
112 mitigation of polymicrobial biofilm infections that are now often detected in intravascular catheters and,
113 especially, intracardiac devices.

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Figure 1. Schematic view of biofilm formation on the surface of a central venous catheter. **a** Microbial contamination can come from a bloodstream infection (defining an hematogenous contamination) but must most frequently originates from the patient's skin (because of a lack of skin antiseptics) (**b**) or from the catheter hub (**c**). These last two routes of contamination lead to extraluminal and endoluminal colonisation, respectively. After contamination, reversible and irreversible adhesion allow the microorganism to stick to the surface of the catheter (**d**). Microorganisms multiply, form a microcolony and then evolve to mature biofilm (**e**) through matrix production and quorum-sensing signals. **f** Lastly, dispersal of biofilm pieces or individual cells is responsible for bloodstream infection and subsequent infection dissemination.



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127 **Conflicts of interest.** The authors declare no conflicts of interest.

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