REVIEW ARTICLE

Mechanisms of bacterial adhesion to biomaterials

Mecanismos de adesão bacteriana aos biomateriais

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ABSTRACT

Bacterial adhesion to biomaterials is a highly complex process that warrants careful medical scrutiny given not only its obvious influence on morbidity and mortality of patients who use dentures, but also the substantial costs involved. In this literature-based review we present the main microorganisms involved in adhesion to biomaterials and discuss the pathogenesis involved, addressing each of its stages, including biofilm formation, which is a crucial step in the the establishment of infection. We also highlight the main factors influencing the adhesion mechanism, including bacterial, materials and medium properties.

Key words: Bacterial Adhesion; Biofilms; Biocompatible Materials.

RESUMO

A adesão bacteriana aos biomateriais é processo de alta complexidade que demanda importante preocupação médica dada a sua evidente influência sobre a morbimortalidade dos pacientes que fazem uso de próteses, além dos vultosos gastos que o envolvem. Nesta revisão com base na literatura médica são apresentados os principais microrganismos envolvidos na adesão aos biomateriais, discutindo a patogênese desse processo, sendo abordada cada uma de suas etapas, inclusive a formação do biofilme, que é etapa ímpar para o estabelecimento da infecção. São indicados, também, os principais fatores que influenciam o mecanismo de adesão, incluindo as características bacterianas e dos materiais, assim como as propriedades do meio.

Palavras-chave: *Aderência Bacteriana; Biofilmes; Materiais Biocompatíveis.*

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INTRODUCTION

Use of temporary or permanent implants has become frequent in current medical practice. Joint and vascular prostheses, catheters, lenses and dental implants are increasingly being used, with a 124% rise being registered between the years of 1990 and 1999 in the US.[1]

Infection is one of the main limitations for keeping implants in the body.[2,3] Despite its still low incidence, a growing number of patients with implants have experienced infections. The use of endotracheal tubes, vascular and urinary catheters, and hip prostheses account for more than half of the cases of nosocomial infections in the US[4], and around 1.32 million implants present with infections every year (Table 1)[5] and amount to devastating consequences that expose patients to high morbidity and mortality.[6]

The implant works as a "foreign body", modifying the local microenvironment and facilitating bacterial contamination by direct or hematogenous means, creat-

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ing fertile ground for invasion and colonization on the biomaterial's surface. Periprosthetic infection becomes resistant to host defense mechanisms and to the action of antimicrobial medication, making prosthesis removal a frequent condition for cure.[3-5]

The cost of infection associated with biomaterials is very high. Studies carried out in the US estimate that the cost to treat an infected arthroplasty is of around US$ 50,000.00 per patient, with a 10% risk of relapse.[7-9]

Bacterial adhesion and biofilm formation on prosthetic surfaces are key steps in the pathogenesis of those infections, even if the entire mechanism of molecular and physical interaction between bacteria and biomaterial is not fully known.[10]

This literature-based review describes the pathophysiology of these infection and the physical, chemical and molecular factors associated with microorganism-biomaterial interactions.

Table 1 - Expected use of implants and risk of infections in the US

Implants	Number / Year	Risk of Infection (%)
Central Venous Catheter	5 million	3-8
Gall bladder catheter	10 million	10-30
Heart Valve	85,000	1-3
Vascular Prosthesis	450,000	2-10
Joint prosthesis	600,000	1-3
Implants for fracture fixation	2 million	5-10
Dental Implants	2 million	5-10

Adapted from Ehrlich *et al.*[5]

MICROORGANISMS

Staphylococci are the most commonly diagnosed microorganism in biomaterial-associated infections, with *S. epidermidis* and *S. aureus* accounting for 60 to 70% of cases. [8-13]

Rarely associated with community-acquired infections, *S. epidermidis* is nonetheless frequently observed in infections in intensive care units, probably due to its ability to adhere and form a thick multilayer extracellular matrix on the surface of polymers.[3,11] *In vitro* studies show that *S. epidermidis* adheres primarily to polymer surfaces, while *S. aureus* adheres to metal surfaces.[7]

Other species of bacteria are also related to these infectious processes, such as contact lens colonization by *P. aeruginosa*. *Streptococcus spp.* and *Enterococcus faecalis,* as well as anaerobic bacteria and in special *Proprionobacterium acne,* which have figured with increasing incidence. [13]

S. aureus and Gram-negative bacilli predominate in early post-surgical infections (up to three months after surgery), while less virulent microorganisms such as coagulase-negative staphylococci and *Proprionobacterium acne* are more often associated with late surgical infections[4,13,14] (Table 2).

Some infectious processes are of polymicrobial origin. An epidemiological survey of nearly 800 cases of infection associated with orthopedic prostheses (including knee, hip, tendon and ligament prostheses, among others) revealed that in approximately 16% of cases the source of infection was polymicrobial.[8]

Table 2 - Importance of different microorganisms in implant-related infections by type of infection

Microorganisms	EPI	AHI	LCI	Global (%)
Staphylococcus aureus	+++	+++	+	25
Staphylococcus epidermidis (SCN)	+	-	+++	35
Streptococcus / Enterococcus spp.	+	++	+	10-15
Enterobacteria / Pseudomonas aeruginosa	++	+	+	10-15
Anaerobic (*Proprionobacterium acnes*)	-	-	+	>5
Polymicrobial infection	++	-	±	>10

Key: EPI: early postsurgical infection; AHI: acute hematogenous infection; LCI: late chronic infection; SCN: Staphylococcus coagulase negative. Adapted from Ariza et al. (2008).[13]

PATHOGENESIS OF THE PERIPROSTHETIC INFECTION

Bacterial adhesion to various biomaterials and the formation of biofilm on implant surfaces are key steps in the pathogenesis of this kind of infection. Most cases of contamination happen while the prosthesis is put in place, by direct contact between the biomaterial and either the external environment or colonized tissues such as skin. Adhesion after contact happens in two distinct stages; the early stage is marked by the physical attraction between germs and the implant (reversible stage) and the late by the cellular and molecular interactions with the surface of the biomaterial (irreversible stage).

Stage 1

In this stage, physical forces are more active than the chemotaxis or molecular effects.[3] The attraction is

caused by "long range" (>50 nm) attraction forces, described as mutual forces, which depend on distance and free energy, and "short range" forces, such as the Wan der Walls, electrostatic (ionic, bipolar and hydrophobic interactions) and covalent forces.[15,16] Thus, the biophysical aspects of the implant surface and bacterial concentration are key factors for the definitive adhesion of microorganisms, which may interact directly with the surface or with host macromolecules (e.g: proteins, glycoproteins) adhered to that surface.[9]

At this point, there has been no molecular interaction between the biomaterial and microorganisms and there is no formation of biofilm. The use of electric currents <100μA in this initial stage of adhesion can reduce the number of viable bacteria on the surface of the biomaterial by more than 75% of the initial concentration.[17,18] As adhesion is not yet definitive, this stage is considered "reversible", and physical measures such as washing, the host's own immune defenses, and use antimicrobials can prevent the infection from developing.

Stage 2

The second stage represents molecular and cellular interaction between microorganisms and the surface of the biomaterial or proteins adsorbed to it, including fibronectin and collagen. Molecular reactions are dominant in this stage and allow bacteria to adhere firmly to the implant surface. The structures responsible for adhesion are called adhesins and produce molecular interactions with the host's surrounding tissues, allowing an extracellular matrix known as biofilm to be formed.[19,20] Specific pathogens, such as *S. epidermitis*, have theirvirulence increased by the genes that encode adhesins. During the various stages of the infection, different genes are expressed to enable: a) microorganic tropism toward specific anatomic locations, b) the cells' ability to organize themselves in biofilms with extracellular matrices, and c) disruption of the biofilm once the adequate cell density is reached, so as to allow the infection to spread to adjacent sites.[1]

Biofilm

Biofilm is composed of a layer of extracellular matrix protecting bacteria microcolonies adhered to solid surfaces. Biofilm formation plays an important role in implant surface colonization, reducing the efficiency of host immunological response and of antimicrobial treatment.[6] Many features of biofilms remain unknown.

Cells correspond to almost 15% of the biofilm volume, while the matrix represents the remaining 85%.[21,22] It is formed following the sequential steps starting when bacteria adhere to the substrate, followed by proliferation and the multi-layer accumulation of extracellular matrix, culminating in a bacterial community that is supported by the matrix thus produced. Some microorganisms from the colony will then detached themselves and be transported to neighboring areas, spreading over the surface of the biomaterial[3,24] (Figure 1).

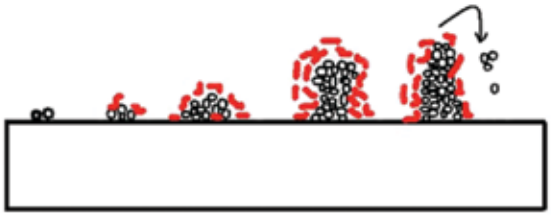

Figure 1 - Systematic Model of the steps involved in biofilm formation.

The composition of biofilms is complex and variable, depending on the species of bacteria and local environment conditions. Despite their heterogeneous structures, the main component of the biofilm matrix are exopolysaccharides such as cellulose and acetylglucosamide.[25,26] These molecules provide protection and help in colony formation and intracellular adhesion.[7] In addition to polysaccharides, surface proteins also help form the matrix's multiple layers.[26]

After adhesion, colonization and biofilm formation, a microenvironment is created that allows these organisms to obtain nutrients and protects them against phagocytes, interfering with the immune system's cellular response and acting as a barrier to antibiotics. Some mechanisms responsible for the increased resistance of microorganisms present on a biofilm include: a) slower penetration of antimicrobial medication into the biofilm; b) modified growth rate within the biofilm; c) other physiological changes in cellular growth within the biofilm. A bacterial colony may, moreover, lie dormant for years before manifesting itself in times lowered immunity.[4]

Once microcolonies are formed, bacterial propagation continues on the biomaterial when peripheral cells break down to start new adhesion processes. The

infection thus becomes chronic and resistant, and implant removal the only option to control infection.

FACTORS INFLUENCING BACTERIAL ADHESION

Bacterial adhesion is a highly complex process, influenced by several micro-environmental factors as temperature, microorganism concentration, length of exposure, tissue conditions, tissue and plasma proteins, characteristics of bacteria and materials, pH level, and use of antimicrobials (Table 3).

Table 3 - Factors influencing bacterial adhesion to biomaterials

Increase adhesion	Inhibit adhesion
Hydrophobic surface	Hydrophilic surface
Fibrinogen, fibronectin, thrombin	Albumin
Porous or rough surfaces	Polished surfaces
Cobalt-chromium alloys	Titanium alloys
Acrylic components	Components with long chain ethylene glycol
Hydroxyapatite coating	Silver coating

Bacterial Factors

The main bacterial factors that influence microorganism adhesion are the membrane's electric charge, hydrophilicity and ability to interact with host proteins.

The electric charge of the bacterial membrane varies according to species, environmental conditions, and membrane composition. In general, bacteria suspended in aqueous media tend to have a negatively charged surface. This profile appears to influence early adhesion (reversible stage). Biofilm formation reduces the action of electric currents on adhesion.[27] The importance of electrical charges on the bacterial surfaces, however, is still not very well understood.[3]

Hydrophilicity can also influence bacterial adhesion. In general, bacteria with hydrophilic characteristics prefer surfaces with hydrophilic properties whereas hydrophobic ones prefer surfaces with characteristics similar to their own.[3] Hydrophobic surfaces seem to be more susceptible to colonization.[3]

The ability to interact with serum or tissue proteins present when bacterial contamination occurs seems have a significant influence on adhesion, considering that the majority of these interactions happen in the presence of specific receptors.[28] Some interactions increase adhesion, such as those involving fibronectin, fibrinogen and thrombin, while others inhibit adhesion, such as those involving albumin.[29-32] Fibronectin is more frequently involved in the adhesion of *S. aureus* to the surface of substrates[29,33] through specific, time-dependent, and irreversible bonds.[3] The influence of fibronectin on *S. epidermidis adhesion* is still controversial.[29] Fibrinogen promotes bacterial adhesion to biomaterials, especially when it comes to staphylococci. Thrombin, in turn, promotes bacterial adhesion when converting fibrinogen to fibrin, a process that involves platelet aggregation, because it stabilizes the thrombus.[32] Albumin adsorbed to the material's surface has been shown to inhibit adhesion in ceramic, metal, and polymer surfaces.[3]

Biomaterial Factors

The two characteristics of biomaterials that most influence their ability to interact with microorganisms are chemical composition and surface type.[15,34]

The chemical components of the implants can increase bacterial adhesion or inhibit it. Overall, implants are made of polymers (polyurethane, polyethylene, acrylic) or metallic alloys (steel, titanium, cobalt chromium). Polymers can be affected by infection at lower bacterial concentrations than metals.[35] A preference for polymers has also been noted in adhesion by *S. epidermidis* and *Escherichia coli*, while metals produce more adhesion by *S. aureus*.[36] Acrylic components are more easily colonized compared with their polyethylene counterparts.[37]

Among metals, titanium alloys are less prone to infections than steel or cobalt chromium,[35-38] given that titanium achieves better biointegration and is more inert when implanted in human tissues.[36] Alloys with limited host cell integration, such as cobalt chromium, have more free energy on their surfaces, which increases bacterial colonization.[7,36] Silver (Ag) have been shown to inhibit colonization of biomaterials.[39]

Characteristics of the implant's surface are also an important contributing factor to adhesion mechanisms. Porous surfaces are more prone to adhesion than polished ones,[3,7,38,40] due to both an increase in the total area and to better bacterial adaptation to porosities of similar size.[41]

Microenvironment Factors

Antibiotic drugs can influence bacterial adhesion depending on microorganisms susceptibility and on drug

concentration.[42] Adhered *S. epidermidis* are less susceptible to treatment with antibiotics than non-adhered cells.[43] Adhesion by this kind of bacteria can be reduced by coating catheters with rifampicin-sparfloxacin.[44] Electrolyte concentration, such as KCI and NaCI, as well as culture medium pH, also influence bacterial adhesion.[45-47] Ionic force and pH levels affect both the characteristics of bacterial surfaces and of materials (hydrophobic index – charge), thus modifying stage 1 interactions.[3]

CONCLUSION

Bacterial adhesion to biomaterials is a complex event that culminates in biofilm formation and irreversible colonization of the biomaterial surface. This process is influenced by several factors and further studies should increase our understanding of bacteria/implant interactions and lead to advances in the manufacture of medical prostheses so as to reduce the risk of periprosthetic infections.

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