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Review Article

Bacterial adhesion to biomaterials: What regulates this attachment? A review

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ABSTRACT

Bacterial attachment to biomaterials is of great interest to the medical and dental field due to its impact on dental implants, dental prostheses, and others, leading to the need to introduce methods for biofilm control and mitigation of infections. Biofilm adhesion is a multifactorial process and involves characteristics relevant to the bacterial cell as well as biological, chemical, and physical properties relative to the surface of biomaterials. Bacteria encountered different environmental conditions during their growth and developed interspecies communication strategies, as well as various mechanisms to detect the environment and facilitate survival, such as chemical sensors or physical detection mechanisms. However, the factors that govern microbial attachment to surfaces are not yet fully understood. In order to understand how bacteria interact with surfaces, as well as to characterize the physical-chemical properties of bacteria adhesins, and to determine their interrelation with the adhesion to the substrate, in recent years new techniques of atomic force microscopy (AFM) have been developed and helped by providing quantitative results. Thus, the purpose of this review is to gather current studies about the factors that regulate microbial adhesion to surfaces in order to offer a guide to studies to obtain technologies that provide an antimicrobial surface.

1. Introduction

Biofilms play a significant role in infections associated with biomaterials [1–4], and when it comes to dental implants, peri-implantitis may be the main cause of failure in rehabilitation [4–7].

Biofilms consist of sessile bacterial communities protected by a self-produced polymer matrix that may contain proteins, carbohydrates, nucleic acids, and molecules [3,8–18]. Communities are formed by varied interactions between species and genera, which include physical cell-cell associations, known as coaggregation, interspecies signaling, secretion and renewal of antimicrobial compounds, and sharing of an extracellular matrix [19]. This matrix protects microorganisms against antimicrobial agents, facilitating the transfer of nutrients to support their survival, mediating cell-cell and cell-surface adhesion to form 3D polymeric networks, and assisting microbes to adhere and remain attached to surfaces [3,14,16,20–22]. The matrix is differently constituted depending on the specific bacterial species. The main components are polysaccharides, proteins, nucleic acids, and lipids, interacting within each other to constitute an aqueous gel-like material with proper mechanical properties and resistance to external shocks [23]. And yet, in more mature biofilms, the matrix provides physicochemical forces for biofilm adhesion to the substrate [8].

Bacterial adhesion to the surface of biomaterials involves different types of physical-chemical interactions and biological processes, with mechanisms specific to the bacteria or the substrate [1,2,17]. Bacteria promote adhesion through the development of cells that detect signals, production of extracellular polysaccharides (EPS), metabolic activity, cell viability [3] charge, hydrophobicity, cell wall stiffness, receptor-ligand binding mediated by adhesins, which are protein complexes that recognize and bind to protein receptors on the host cell surface, and appendages (pili and curli) [13,14,24]. Adhesion refers to the attachment of cells to a substrate, while cohesion is the bond between cells [20,22]. As well as the characteristics of bacteria, the physical-chemical parameters of the substrate [4,13] also regulate the adhesion and initial formation of biofilm and include surface charge, surface free energy, hydrophobicity, roughness, specific surface geometry (macro, micro, and nano), topography, and chemistry [1,13,15].

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In order to prevent infections, efforts are made to introduce antimicrobial surfaces [25–32] including the incorporation of antibiotics [26,30], several antimicrobial agents [25,28,29,31,33], and micro- and nano-patterned surface technologies [25,27,29,32], which seek to employ antibacterial or anti-encrusting activity, based on features found in nature [34,35]. However, this goal has not been fully achieved yet, considering that bacteria are highly developed beings that adapt quickly to environmental signals (light, temperature, magnetic fields, and oxygen) [2], changing the constitution of their membrane, changing their surface receptors and gene expression patterns, which is the process by which the hereditary information contained in a gene, such as the DNA sequence, is used to form a functional gene product, such as proteins or RNA [22]. In addition, they have an efficient means of communication without direct physical contact, through the secretion of extracellular chemical signals, known as quorum sensing [11,13].

A breakthrough in the study of interactions between bacteria and substrate was the introduction of atomic force microscopy (AFM), which allows the analysis of the connection of a biofilm or a single cell with a substrate, making it possible to better explain the steps in the formation of biofilm that go beyond mass transport (when bacteria are transported to surfaces by aerosols, sedimentation or diffusion when in aqueous suspension) and initial adhesion [36]. AFM provides quantitative maps and spatial patterns of the mechanical properties of cells in a liquid environment, making it possible to assess membrane stiffness, which is a determining factor in biological response and survivability [13].

Thus, the goal of this review was to gather information on the characteristics of bacteria to understand their attachment to substrates, with a focus on surface identification and the adhesion process. The introduction of AFM as a method of quantifying the strength of adhesion is also addressed, followed by the formation of biofilm, colonizing species in teeth and implants, and communication mechanisms. Understanding the adhesion of bacteria to the substrate will make it possible to guide studies towards the search for antimicrobial surfaces.

2. Introduction and applications of atomic force microscopy (AFM) for measuring adhesion force

Bacterial adhesion to abiotic surfaces is of great interest to the scientific community because it marks the beginning of biofilm formation, and in that sense, atomic force microscopy (AFM) has opened the way for a detailed understanding of biofilms [2]. In addition to being compatible with aqueous solution environments, which is an important factor for medical research, and reaching a nanoscale resolution, AFM delivers high-resolution nanometric images [13,15,24,37,38], as well as quantitative measurements of the mechanical forces involved in cell adhesion, which can vary from 5 pN to 100 nN [12], making it possible to probe interactions at the molecular level between different species [2,19,37] (Fig. 1).

The AFM consists of a nanometric tip connected to the end of a highly flexible cantilever that sweeps the sample in the x and y directions, and due to the interactions between the tip and the sample, the cantilever bends vertically (z-direction) [2,13,15,24,39], thus the force required to move the bacteria attached to a surface can be registered by a laser beam focused on the cantilever and reflected on a photodiode [24,40]. A piezoelectric motor maintains a defined level of deflection in the cantilever ensuring that the force applied to the sample is constant and controlled to avoid sample damage [40]. The cantilever can have a sharp tip (standard), a colloidal tip (to deliver a more defined geometry), or a functionalized tip, to probe specific chemical interactions [13]. The adhesion is recorded in newtons and determined by the force exerted by the tip on the sample [24,37,40]. The scan can be performed in constant contact, intermittent contact, or without contact [24,40] (Fig. 2).

In addition, in force spectroscopy, the tip is approximated and retracted from the surface, and the force-distance (FD) curves this generates provide measurements of the physical properties of the sample, such as stiffness, elasticity, deformation, and adhesion [13,24,37]. FD-based images make it possible to map the spatial distribution of these properties in nanoscale [24]. Probe functionalization with ligands allows probing, for example, a single bacterial adhesin exposed in a live bacterium and a single protein in the extracellular matrix; as well as probing the interactions of force between a single cell and a substrate [24,37].

The adhesion strength of bacteria to certain surfaces is 106–108 times greater than its gravitational force, so it is not surprising that bacteria die from damage to the cell wall as a result of adhesion strength experiments [14,15]. Another factor observed was the existence of viscoelastic deformation of the bacterial cell wall, which was small due to the stiffness provided by the peptidoglycan layer that surrounds the membrane [15]. This layer is substantially thicker in Gram-positive bacteria than in Gram-negative bacteria. Thus, it is believed that membrane stiffness should be greater for Gram-positive cells. However, although Gram-negative cells have a much thinner layer of peptidoglycan, they have an outer membrane that acts as an extra layer and can provide additional strength [13].

Studying bacterial adhesion makes it possible to understand the mechanisms involved in attachment and helps in the search for technologies that promote antibacterial surfaces. Wang et al. [38] assessed the role of gtfB and gtfC virulence factors in the adhesion strength of *Streptococcus mutans* to tooth enamel and found that they are essential for adhesion. Regarding dental materials, the adhesion strength of some bacteria was measured for resinous composites, polymers used in prosthetic bases, Cobalt-Nickel-Chromium alloy, feldspar, and titanium alloys, and the adhesion strength differed according to the substrate, microorganism, and presence of salivary film [41–43].

The following three strength regimes were reported: (1) weak adherence, when adhesion strength is less than 1 nN. In these cases, bacteria do not realize that they are attached, and do not show any adaptive response to a substrate surface; (2) strong adherence when the adhesion strength is above 10 nN; and (3) intermediate adherence, comprising adhesion forces between 1 and 10–15 nN [15]. More recently, non-covalent protein complexes and folds with high mechanical stability have been discovered, ranging from 800 pN to 2.000 pN, found in the extracellular space, and responsible for anchoring bacteria to certain surfaces [39].

AFM has enabled advances in understanding the mechanisms of adhesion and continues to open the way to identify adhesins specific to certain bacteria [39], those involved in molecular interactions [24], as well as possible inhibitors capable of preventing adhesion and invasion of pathogens.

3. How does a bacterium know when it is near a surface?

A key element in bacteria's adhesion process is the development of mechanisms to sense the environment, which can be by detecting chemical signals, biological molecules, or physical detection mechanisms (Table 1) [44].

Chemical sensing depends on the presence of specific molecules, such as H^+ ions, antimicrobials, or biological signaling molecules [44]. The ability of bacteria to mechanically sense physical contact with substrates [36,44–46] is referred to as surface mechanosensing [1,15], and filamentous appendages are used by various bacteria as mechanosensors [12,17]. One possible mechanism for surface detection occurs when contact inhibits flagellar rotation [45]. Type IV pili, for example in *P. aeruginosa*, after contact with the sur-

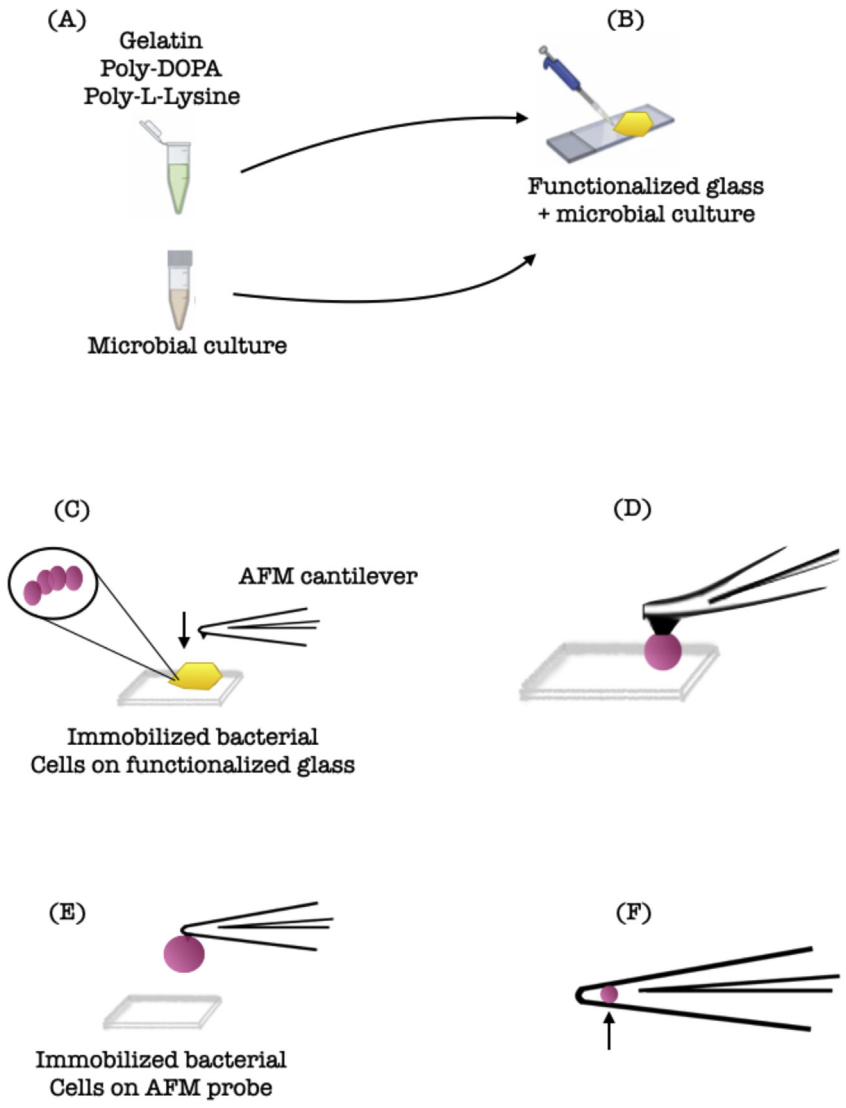

Fig. 1. Schematic representation of single-bacterial-contact probe atomic force microscopy.
(A) The bacterial culture and the functionalizing coating are ready to be placed on the glass; (B) The glass is functionalized; (C) The bacterial cells are Immobilized on the functionalized glass; (D) The AFM probe is approached until a certain degree of indentation occurs on the cell surface; (E) Force spectra are typically captured as a cycle of tip approach and tip retraction. The tip is retracted from the surface; (F) Bacterium is attached to a tipless AFM cantilever.

Table 1
Mechanisms to sense environment and facilitate adhesion.

Mechanisms	Description
Chemical Signals	pH, ionic strength
Biological molecules	Quorum-sensing
Physical sensing	Surface appendages, bacterial cell wall deformation, envelope proteins, and secondary messengers

face, appear to inhibit retraction, which may result in a biological response [45]. Another mechanism is the deformation of the lipid membrane when in contact with surfaces, as it is loaded with environmental sensors (stress-sensitive proteins) that are activated by contact pressure, and also have mechanosensitive channels that act as interpreters of the membrane tension, and mechanical stimuli can be translated into a biological response [1,14,15,44,45]. The cell wall deformation not only increases the surface contact area but favors the physical–chemical bond [36,44].

Other suggested mechanosensors are the envelope protein system, such as PilY1 for *P. aeruginosa* [45] and NlpE-Cpx for *Escherichia coli* [12] and three major nucleotide-based secondary messengers, cyclic dimeric guanosine monophosphate (C-di-GMP), cyclic diadenosine monophosphate (C-di-AMP), and guanosine pentaphosphate and guanosine tetraphosphate ((p)ppGpp) [9,45,47–49]. In the envelope protein system, the deformation of the bacterial cell membrane is detected by the protein present in the outer membrane, which activates the protein in the inner membrane, triggering signal transduction [12,45]. For NlpE-Cpx, CpxA undergoes autophosphorylation and transfers its phosphate groups to the protein that regulates cytoplasmic response, CpxR, which activates the transcription of target genes [45]. The secondary messenger C-di-GMP is a master bacterial signaling molecule that controls, among other things, motility and biofilm formation. Cyclic diadenosine monophosphate C-di-GMP is involved in metabolic processes, controls essential cellular pathways such as ion transport and potassium homeostasis [9,45,47,49]. Bacteria use a high level of C-di-GMP to stimulate adhesin and EPS production [45]. (p)ppGpp is considered the master regulator of the stringent response, i.e., it helps bacteria survive under stressful conditions such as nutrient limitation. And further, (p)ppGpp plays a substantial role in modulating bacterial growth rate, regulation of many physiological processes such as protein biosynthesis

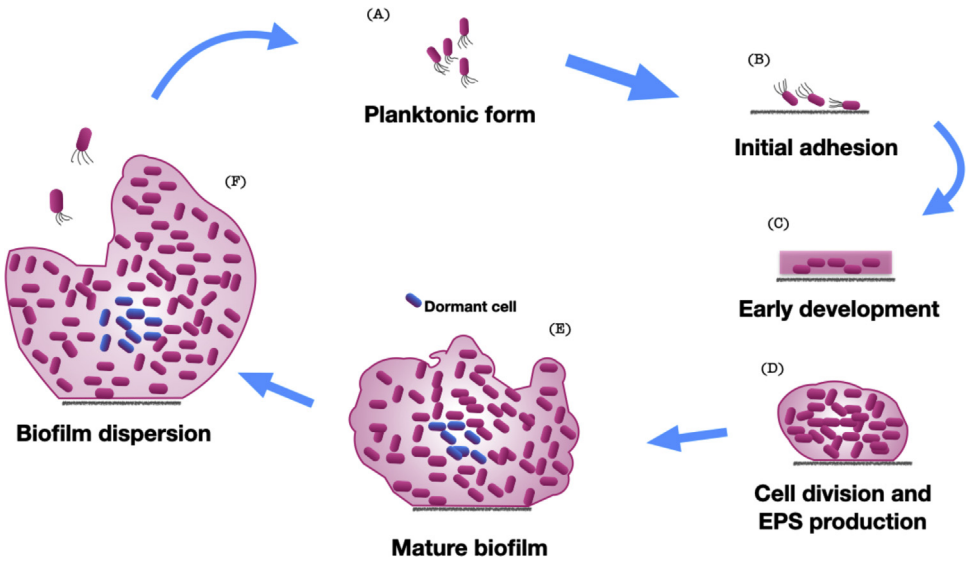

Fig. 2. Stages of biofilm formation on (bio)materials surfaces.
(A) Planktonic form of bacteria; (B) Bacteria adhere to the surface in a dynamic process; (C) Cells aggregating and bacterial attachment becomes irreversible; (D) Bacterial form microcolonies, and start secreting extracellular polymeric substance; (E) Cells form multi-layered clusters, and maturation of the biofilm occurs; (F) Biofilm reaches a critical mass and disperses planktonic bacteria that may colonize other surfaces.

including transcription, translation, replication, viability and virulence, acid stress response, polyphosphate metabolism, nucleotide biosynthesis, and uptake [48,50]. In *E. coli*, (p)ppGpp is produced in response to severe environmental factors (environmental stress sensors) such as lack of fatty acid, amino acid, and iron [51].

4. How does the process of surface adhesion occur?

Bacterial adhesion to a surface is a multifactorial process [2], which is affected by various factors such as duration of the bacterial exposure to surfaces, bacterial characteristics (cell wall components, appendages, and motility), nutrients, and bacterial density [52]. The cell-cell bond between two genetically distinct microorganisms is known as coaggregation, and cohesion when a cell is attached to the surface [24].

According to Straub et al., [1] the interactions involved in bacterial adhesion can be classified into three different levels, being (1) nonspecific physical-chemical interactions, (2) specific interactions, and (3) surface mechanosensing.

In non-specific physical–chemical interactions, adhesion occurs through non-covalent interactions where appendages and proteins interact with certain chemical fractions on a surface. That is, they use van der Waals forces (usually attractive), electrostatic charges (usually repulsive), or acid-base interactions (attractive or repulsive), and their characteristics are influenced by the composition of the medium, environmental pH, pressure, nutrient availability, oxygen, and surface properties [1,2,13,20,22,44,53]. Van der Waals forces have the longest range and act at distances up to 1 μm and become increasingly stronger as they get closer to the interaction surfaces [15]. Considering that the bacteria are approximately 1 μm in size, mechanosensing must be sensitive to mechanical stimuli or variations in a ~1 μm scale [45].

In specific interactions, the adhesion mechanisms involve specific appendages capable of binding to some chemical species on certain surfaces. Coaggregation interactions are highly specific and involve the recognition of receptors in a cell type by adhesins in the partner cell. One example is when the adhesin on the cell surface of a certain bacterium recognizes a polysaccharide containing glucose, mannose, and galactose on the surface of another bacterium [1,24]. *Streptococcus sanguinis* recognizes salivary film receptors on teeth surface and forms initial bonds [21]. In addition to these receptors, *S. sanguinis* recognizes multiple types of fixation receptors that can bind to several components. One such example is SsaB, described as a saliva-binding protein that can mediate binding to saliva-coated hydroxyapatite via a pH-sensitive receptor [21]. Another example is Steric forces, which assist in the adhesion of some bacteria such as *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *E. coli*, whose surfaces house a network of long chains of polysaccharides and biopolymers, responsible for generating these forces [2]. Geng et al. [46] demonstrated that in addition to detecting contact with a certain surface, *E. coli* can decrease breathing in response to that contact, triggering a signal [46].

In surface mechanosensing, the interaction implies the active detection of a bacterium when it comes in contact with a surface [1], and adhesion is mediated by sensory organs, such as flagella, and tension in the pili retraction. One characteristic of this adhesion is the involvement of signal transduction and response from the organism [1].

The process of adhering to a surface involves the three types of interactions (specific, nonspecific, and mechanosensing), and happens with the formation of the conditioning film, which is the base on which biofilm grows, formed by organic and inorganic particles [22,52]. The particles present in the fluid that bathes the surface can settle and form a part of this conditioning film. Microbes adhere to the substrates present in this layer with the aid of the appendages, by forces of attraction and/or through adhesins that interact with substances present on the surface [22,45]. Many types of bacteria have more than one type of adhesin [45]. In this phase, adhesion is reversible [13,36], especially for mobile organisms that can retain appendages, when the repulsive forces are greater than the forces of attraction, or when they do not find a suitable surface for growth, thus being able to leave the surface [22].

Irreversible adhesion progresses through the synthesis and secretion of EPS which is an essential component of the extracellular matrix [10]. EPS plays critical roles in surface adhesion, cell recognition, biofilm formation and structure, water retention, signaling, cell protection, symbiosis, nutrients, and genetic exchange [10]. The major constituents of EPS include polysaccharides, proteins, DNAs, lipids, and other polymeric compounds, and are dependent on bacterial species and environmental conditions. The EPS matrix also contains considerable amounts of proteins that are critical for adhesion and colonization, such as

enzymes and protein structures like pili and fimbriae [8,10]. Microbial surface proteins can be generally divided into two groups: functional surface proteins like adhesins, and long-chain surface-bound macromolecules like pili [40].

The cell surface proteins of Gram-positive bacteria play crucial roles in their adhesion to abiotic and biotic surfaces [55]. In this regard, there is Sortase A, which are enzymes produced by the bacteria responsible for covalent attachment of surface-exposed proteins to the cell wall envelope of Gram-positive bacteria [56]. It plays a critical role in Gram-positive bacterial pathogenesis because it is involved in the first step of bacterial adhesion [51].

4.1. What is the role of appendages?

Appendages (pili, flagella, curli, and fimbriae) promote adhesion to surfaces through specific and nonspecific interactions [2,24], as described in the previous chapter. The small diameter of the appendages (flagellum, pili, fimbria) allows them to overcome repulsive electrostatic interactions, actively assisting in adhesion, as well as protein loops, DNA polysaccharides present in EPS, and patches of lipoteichoic acid that act as bonds to assist in bacterial adhesion to a surface [2,36].

Pili are long, flexible helical protein filaments that protrude out of bacterial cell walls. [2,55,57] They are found on the surface of Gram-negative and positive bacteria [55], and responsible for increasing initial bacterial adhesion [55], and in some organisms, they also play a role in motility within biofilms [24,45,58]. Although thin, flagella (filamentous appendix) and pili (Latin for fur) can have lengths equal to or greater than the bacteria [45]. On hydrophobic surfaces, pili strengthen adhesion by acting as nano-springs, which allows bacteria to resist high shear forces under physiological conditions [57].

The pili in Gram-negative and Gram-positive bacteria differ in how one subunit (pilins) binds to another, and how the subunits attach to the cell wall [55,57]. For Gram-negative bacteria, the most studied pili are type I, type IV, and P, which lengthens when subjected to a certain adhesion strength, giving rise to strength plateaus [12]. These pili, whose subunits are held together through non-covalent interactions, can withstand forces in the 250 pN range, and multiple pili often work together [12,24].

In Gram-positive bacteria, the pili consist of a main subunit containing one or two accessory subunits [57], which are generally linked to each other by means of covalent bonds [55], and due to such bonds, these pili behave like nano-springs, resisting forces higher than 500 pN [24]. And yet, pili can act as adhesins [53].

The presence of pili on the surface of Gram-positive pathogens is known to interact with other extracellular matrix proteins influencing bacterial adhesion and biofilm structure on biotic surfaces [55]. In a study on the function of pili on a group of non-pathogenic lactic acid bacteria, Dramé et al. [55], suggested that pili are involved in cell-cell interactions as well as the number of pili on the bacterial surface is not constant and they may influence adhesion abilities.

Most bacteria exhibit swimming filamentous mobility to generate an active self-propelled movement (which has its own means of propulsion), but even non-motile bacteria are subject to physical forces that bring them close to the surface through gravity [2,17]. Bacteria exhibit circular trajectories when close to surfaces, and straighter trajectories when distant [2,58], with this circular locomotion promoting a tendency for bacteria to be attracted to the surface, since an impelling dipole pulls the fluid present around the bacteria, and therefore directs the bacteria to the surface [58].

Flagella also play a key role in sensing and responding to surface topographies, either for swimming or adhering [9,52]. Some bacteria such as *E. coli* have multiple evenly distributed flagella responsible for controlling motility, with some groups rotating clockwise (which causes *E. coli* to approach the surface) and others counterclockwise (causes running) [9,52]. Bacteria with polar flagella, such as pseudomonads, are able to alter the swimming mode by reversing the motor [9]. This suggests that the clockwise and counterclockwise rotation of the flagellum has an important effect on cell adhesion [52]. It has been seen that flagella of certain bacteria have adhesion preferences according to surface features, with *E. coli* preferentially adhering in narrow line patterns [59]. And further, pili and flagella on hydrophobic surfaces can act as nano-springs, causing bacteria to resist high shear forces under physiological conditions [57].

5. Development of biofilm

The biofilm development process differs between motile and non-motile bacteria, with five stages generally involved: (1) Initial attachment of the bacteria to the surface, known as reversible adhesion; (2) Formation of a monolayer, irreversible attachment phase, which involves interaction between bacterial cells and a surface using bacterial adhesins, such as fimbriae and lipopolysaccharide; (3) Formation of multi-layered colonies, production of extracellular polymeric substances (EPS) by the resident bacterial cells; (4) biofilm maturation phase, in which bacterial cells synthesize and release signaling molecules to sense each other's presence, leading to microcolony formation and biofilm maturation; and (5) Dispersal phase, where the bacterial cells leave the biofilms and return to an independent planktonic lifestyle (Fig. 3) [4,10,21,22,36].

Teeth and implants exhibit a similar pattern of biofilm formation, with it taking 2–6 h after species colonization, which is faster where pellicle is present, like in freshly-cleaned natural teeth [19,54]. This pellicle contains proteins, salivary glycoproteins, and gingival fluid, and in implants, the formation begins 30 min after exposure in the oral cavity [6,54]. In this phase, biofilm is reduced due to the low albumin adsorption capacity of the film [54]. In this stage of development, the characteristics of the surface of the implants interfere with the adhesion, or not, of biofilm [54].

When an implant is implanted within the human body, they are coated with blood proteins and interstitial fluids, and this process is determined by the implant's surface chemistry and wettability. Once implanted, bacteria use adhesins to attach to the implant surface. *S. aureus* and *S. epidermidis* have multiple biofilm attachment and formation mechanisms that contribute to their virulence in chronic infections in implants [53].

To make the film attractive, bacteria modify its surroundings. For example, early colonizers have the ability to induce conformational changes in the protein film that surrounds them [15], as well as in the production of EPS, which is another cooperative phenomenon that offers advantages in adherence to neighboring bacteria [15].

Regarding oral bacterial species, it was seen that they have undergone genetic adaptation to suit the oral cavity. This includes specialized adhesion mechanisms to allow attachment to tooth surfaces and oral mucosal tissues, metabolic coherence with the nutritional properties of saliva and gingival crevicular fluid, and the ability to interact with host defense cells [60].

An important factor in biofilm formation is hydrodynamics, which can interfere with surface detection by bacteria affecting the architecture, composition, and mechanical strength of the biofilm [52]. An example of hydrodynamics in the oral cavity is the bacterial plaque that forms on teeth that is subject to the flow of salivary and gingival crevicular fluid [52]. And yet, for *S. aureus,* shear flow enhances biofilm formation by increasing the production and strength of the EPS matrix [61]. And this resulting matrix plays a protective role as it allows the biofilm to recover from the mechanical challenges induced by pressure and flow, resulting in more resistant, compressible biofilms that favor bacterial growth [52].

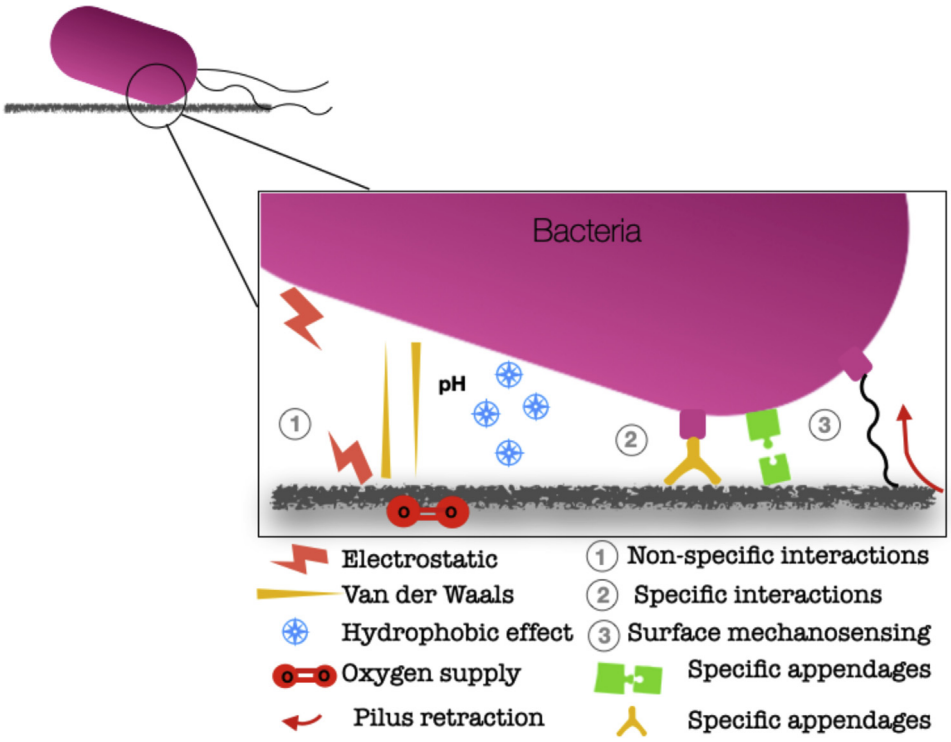

Fig. 3. Schematic representation of bacteria proposed adhesion mechanisms. This image was adapted from Ref. [1].

The matrix also provides limited molecular dispersion, makes the biofilm a protected environment, facilitates communication, metabolite exchange, and protection from external threats [23]. The extracellular matrix, made of polysaccharides, proteins, and extracellular DNA, can prevent some antibiotics from successfully penetrating cells, inducing antibiotic tolerance [62].

5.1. Are initial colonizers that adhere to teeth the same as those that adhere to implant surfaces?

Initial colonizers make the attachment of successive organisms possible, so that biofilm is formed. In this sense, inter-bacterial coaggregation is well established for *Streptococcus*, *Actinomyces*, and *Veillonella* [63]. Enamel contains *Streptococcus* spp., *S. sanguinis*, *Streptococcus mitis*, and *Streptococcus oralis* [54,64] which adhere to the acquired film components through selective binding of the adhesin receptor [19,65]. *Actinomyces*, *Gemella*, *Neisseria*, and *Veillonella* can also be found. During the first 48 h of growth, there is a change in the balance of the first colonizers, with an increase in the presence of *Streptococcus* spp. and a decline in the amount of *Actinomyces* spp. [19]. If gingivitis is established, there is an increase in *Fusobacterium*, *Lachnospiraceae*, *Lautropia,* and *Prevotella* species [65,66], whereas, in periodontitis, *P. gingivalis*, *T. forsythia*, *Prevotella intermedia*, and *T. denticola* are observed [19,66].

Gram-negative oral bacteria produce a variety of adhesins that contribute to both polymicrobial biofilm formation and host cell interactions. For example, long (FimA) and short (MfaI) pili of *P. gingivalis* are involved in host cell coaggregation and adhesion [8].

One microorganism considered to be a key factor in the development of oral biofilm for helping in the attachment of successive organisms is *S. sanguinis*. It is an optional Gram-positive anaerobic [4], which uses a wide range of carbohydrate sources for survival [21] and is abundant in supragingival and subgingival plaque [21]. It is usually reported as non-motile, although *S. sanguinis* produces short type IV pili that do not confer motility but are important for adhesion to host cells [8].

The main secondary colonizers include species of *Actinomyces*, *S. mutans*, and *S. sobrinus*. Some bacteria, like *Fusobacterium nucleatum*, can bind to the initial and secondary colonizers, multiplying and coaggregating with other species [54].

Bacteria that infect implants usually present as bacterial aggregates involved in an abundant EPS matrix [53]. The microbiota of healthy implants is composed of Gram-positive rod cells and cocci [6,54] and in peri-implant infections, there is the presence of Gram-negative bacteria such as *Veillonella* sp. and spirochete including *Treponema denticola* [54]. It has recently been demonstrated that the peri-implantitis microbiome differs from the periodontitis microbiome [5]. According to DAUBERT & WEINSTEIN [6], there is still no consensus on the specific microbial profile associated with peri-implant diseases. However, it is suggested that factors such as surface roughness, free energy, chemistry, and titanium purity, as well as the patient's periodontal condition, might influence the microbiome [6].

Three key factors can be considered important for the microbiota shift during periodontal disease. First is the presence of organisms that subvert the inflammatory response by triggering a state of dysbiosis and inflammation, and these influence a change in the entire bacterial population. The second is an elevated commensal microbial population activity is observed and the third is the ability of the oral microbial population to form biofilms enabling multiple species to exist [65]. And yet, in addition to these factors, there are the individual characteristics of the oral cavity of each person, and that influence the bacterial environment of the mouth, such as diet and consequently the availability of nutrients, host immune response, saliva pH, hydrogen ion concentration, and hygiene [64].

A change from a healthy peri-implant groove to a peri-implant pouch is associated with an increased presence of cocci, motile bacilli, and spirochetes [67]. Do Nascimento et al. [68] have found relevant microbial counts containing the species *T. denticola*, *T. forsythia*, *P. gingivalis*, *F. nucleatum*, *P. intermedia* and, *A. actinomycetemcomitans*, but with significant differences depending on

Table 2
Quorum sensing (QS) system in Gram-negative and Gram-positive bacteria.

Microorganism	Type	Mechanism of QS
Staphylococcus aureus	Gram-positive	Production of proteases, lipases, and nucleases
Pseudomonas aeruginosa	Gram-negative	Virulence factors and biofilm formation
Escherichia coli	Gram-negative	Motility and biofilm formation
Enterococcus faecalis	Gram-positive	Virulence factors
Porphyromonas gingivalis	Gram-negative	Production of proteases, Virulence factors and biofilm formation
A. actinomycetemcomitans	Gram-negative	Virulence factors, metabolic activity, microbial cell growth
Streptococcus mutans	Gram-positive	Biofilm formation, stress response, and bacteriocin production

the material used for the implant and the collection site [68]. The genus *Eubacterium*, *Staphylococcus aureus*, and *Filifactor alocis* were also found in peri-implantitis lesions [5].

6. Quorum sensing

Bacteria have a communication mechanism that allows them to produce, detect and respond to signals produced by other microorganisms of the same or different species [11,13,18,22,36,54,69]. This is called quorum sensing and consists of an enzyme that catalyzes the synthesis of chemical signals, and a receptor that binds to the signal and induces the expression of genes responsible for various physiological mechanisms, such as sporulation, biofilm production, conjugation, and motility, in addition to virulence factors, such as proteases, toxins, and adhesins [11,18,62]. Biofilm formation is directly regulated by this quorum sensing activity, with the matrix optimizing and detecting the signaling [8,54]. Bacterial quorum sensing depends on a series of events such as signal production, signal dissemination, signal receptors, signal detection, gene expression, and signaling response [10] (Table 2).

There are at least three classes of quorum detection mechanisms, also called inducers: (1) LuxI/LuxR detection in Gram-negative species with acyl-homoserine lactone (AHL) signals, with these Lux proteins producing a specific AHL for each bacterial species, and variations in AHL occurring according to the length of the carbon chain [11,20,70]; (2) detection of peptides produced by Gram-positive bacteria (AIPs), which are species- and strain-specific; and (3) Lux-S encoded autoinducer-2 (AI-2), which is another class of signaling molecules and can be found in Gram-positive and Gram-negative bacteria [11,18,22,51,54,62,69,70]. Because it is widely used for interspecies communication, AI-2 is considered a signal for universal communication between different species [8,10,11]. A wide variety of other signaling molecules have also been identified and include fatty acids used by *Xanthomonas* spp., *Burkholderia* spp., *Xylella* spp. ketones, epinephrine, norepinephrine, and AI-3, or quinolones [62] (Table 3).

Some bacteria have their own quorum-sensing signaling systems. Signaling among oral *Streptococcus* spp. strains involve peptides, such as the competence-stimulating peptide or the X-inducing peptide of *S. mutans* [8]. *Pseudomonas aeruginosa* employs a quorum-sensing system via the Pseudomonas quinolone signal (PQS) [51,71].

The quorum allows bacteria to coexist in a community and express phenotypes that are advantageous for the group and ensure survival [11,18], since this system begins with the production and release of autoinducers into the environment, whether by the pathogen or the resident microbiota. It has been found that communication is effective among Gram-negative bacteria that are up to 78 μm apart [18]. However, the most effective distances for communication occur between 4 and 5 μm [15,18].

Quorum sensing systems can be expressed in different bacterial pathogens, and some examples occur in *P. aeruginosa*, where the quorum sensor plays a role in the generation of extracellular DNA, controlling the production of rhamnolipid biosurfactant, and of siderophores such as pyoverdine and pyochelin, which are important for biofilm formation [15,16]. Another example is hydrogen peroxide (H_2O_2) produced *in vitro* by some oral streptococci, and when present in sublethal concentrations, they trigger signaling responses in *C. albicans* [19].

Due to the need to seek antimicrobial therapies alternative to antibiotics, quorum-quenching is studied as an alternative to inhibit biofilm formation by inhibiting or interrupting quorum detection [15,20,69,70]. Interferences with quorum sensing are called quorum quenching, a natural phenomenon where an enzyme degrades AHL signals and leads to the disruption of the quorum-sensing signal [51,62]. There are some bacteria called non-conformists, which represent a subpopulation that does not obey quorum detection commands. They act by interfering with the binding of the receptor signal, decreasing its concentration, or inhibiting certain enzymes capable of degrading signaling molecules [20,69]. In the environment, there are many compounds that affect communication, and, based on their molecular weight and chemical composition, these compounds can be macromolecular enzymes or microparticulate quorum-quenching inhibitors [20]. Some examples may be natural products, such as polyphenols isolated from tea or honey, ajoene from garlic, eugenol from clove, or many others produced by marine organisms and fungi [62]. And yet, products arising from bacteria, plant, and animal derivatives are also studied, which include enzymes such as AHL Acylases, AHL Lactonases, and Oxidoreductases, as well as alternative materials that include nano-molecules, and nano- and micro-composites, based for example, on Ag or ZnO, or chemicals with small molecules, such as 5-Fluorouracil (5-FU) and halogenated furanones [11,20,70].

7. Strategies for interrupting biofilm formation

Some approaches are studied to disrupt biofilm formation or to prevent its diffusion. Some strategies are based on materials engineering, where anti-adhesive surfaces are created or antibacterial additives are incorporated into substrates [52]. Other areas research ways to act directly on the bacteria, either by inhibiting the quorum sensing system, preventing the formation of extracellular matrix, and, among others, inhibiting the signaling pathways of secondary messengers [51].

Regarding the material's topography, it was seen that characteristics concerning the size, shape, and distribution of roughness patterns affect both the attachment and biofilm formation of different bacterial strains on various substrates. Bacterial adhesion decreases as the size of the topographical pattern get smaller [72–74], and in this sense, topographies on a micron-scale mainly affect bacterial fixation, whereas topographies on a nanoscale may have bactericidal effects [9].

Nanometric surfaces were inspired by antibacterial activities seen in nature, such as those that occur in lotuses, cicadae, sharks, butterflies, and among others, dragonfly wings [35,52]. Recent studies [75,76] attempt to replicate these patterns by developing surfaces for future application in the biomedical field, such as PMMA surfaces designed as shark skin-patterned [75], and zirconium-based bulk metallic glasses [76]. However, this area of

Table 3
Main strategies to interrupt biofilms.

Target	Description/ Effects on biofilm
Matrix components	Enzimas de decomposição matricial
Autoinducers of quorum sensing system (AHL, AIP, AI-2)	Enzimas supressoras de quórum ou inibidoras de detecção de quórum agem inativando a moléculas de acyl homoserine lactona (AHLs, QPS, AIP e AI-2)
Second messengers c-di-GMP, c-di-AMP and ppGpp	Small organic molecules capable of inhibiting secondary messenger signaling pathways
Enzymes (Sortase A, proteases)	Small molecule inhibitors may be able to block SrtA by disrupting protein attachment
Incorporation of antibacterial agents	TiO2, SiO2, ZnO, $AgVO_3$, fluorapatite, quaternary ammonium resin monomer, silver NPs, fluorohydroxyapatite, and others. Most are bactericides and act by contact.
Anti-adhesive surfaces	Nano-scale topographies; bio-inspired; act by making it impossible for bacteria to adhere or cause death by puncture.

research is still in its infancy and further studies are needed before these materials can be used.

Regarding the incorporation of antimicrobial agents to biomaterials, specifically in dentistry, different nanoparticles and agents have been incorporated in order to prevent biofilm formation without changing the physicochemical and mechanical properties.

Nanometric materials have increased antimicrobial activity due to their larger surface area and chemical reactivity [77]. Silver vanadate decorated with silver nanoparticles ($AgVO_3$) is an antimicrobial with the advantage of being stable and not forming agglomerations. When incorporated into acrylic resin at low concentrations (0.5%; 1%; 2.5%; 5%; and 10%) it inhibited the growth of *Candida albicans*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [78,79]. When incorporated into a soft denture liner, 5% was effective against *Pseudomonas aeruginosa, Enterococcus faecalis*, *Candida albicans*. However, none of the concentrations tested (1% and 2.5% 5% and 10%) was effective against *S. aureus* [33]. $AgVO_3$ at a concentration of 2.5% incorporated into irreversible hydrocolloid acted as an antimicrobial agent without affecting the physicomechanical properties [80]. Furthermore, the incorporation of $AgVO_3$ into endodontic sealers did not induce DNA breaks in human gingival fibroblast or cell death by apoptosis [81].

The white spots formation is an undesirable side effect of orthodontic therapy [82], however, it is known that such appliances can affect hygienic ability, alter the oral microflora, and increase levels of acidogenic bacteria such as *streptococcus mutans* [82]. The addition of 0.11%, 0.18%, and 0.33% (w/w) AgNP to an orthodontic adhesive (Transbond) reduced the adhesive capacity, on the other hand, growth inhibition of *S. mutans* was reported after 48 h [83]. According to Barszczewska-Rybarek & Chladek [84], the higher the concentration of silver nanoparticles in composites containing Bis-GMA/TEGDMA the lower the degree of conversion, which consequently reduces the adhesive capacity.

Titanium dioxide (TiO_2) nanostructures are interesting because of their photocatalytic properties, are non-toxic, inexpensive, and have a high modulus of elasticity (230 GPa) and high refractive index, which allows modulation of the degree of opacity, brightness, and opalescence [85]. Cao et al. [86], evaluated brackets coated with a thin film of TiO_2 nanoparticles with nitrogen and reported antimicrobial properties against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscous*, and *Candida albicans*. Dias et al., [87] studied composite resin modified by TiO_2 and TiO_2/Ag nanoparticles and found that increasing the nanoparticle content reduced bacterial growth. However, high Ag concentrations affect color stability and compromise homogeneous distribution in the composite resin. And yet, Guimarães et al., [85] observed changes in the degree of conversion and Knoop microhardness when TiO_2 nanostructures functionalized with 3-(aminopropyl) triethoxysilane (APTMS) and 3-(trimethoxysil) propyl methacrylate (TSMPM) were incorporated into a resin. Cibim et al., [88] observed that adding 5% TiO_2 to a conventional glass ionomer cement significantly increased Knoop microhardness, however, a limitation was obtaining a homogeneous mixture. Garcia-Contreras et al. [89] reported that low concentrations (3% and 5%) of TiO_2 nanotubes did not interfere with the adhesion of glass ionomer cement to dental tissues. And 5% improved its compressive strength [90].

Nanohydroxyapatite is another agent studied in order to improve mechanical, morphological, antibacterial, and fluoride release properties of materials such as glass ionomer cement. Alatawi et al., [91] observed improved compressive strength and antibacterial effect against *Streptococcus mutans* in addition to increased fluoride ion release. Jardim et al., [92] observed that dental composites with higher content of hydroxyapatite nanoparticles (HApNP) released higher amounts of Ca^{2+} and PO_4^{3-} and the release rate was pH-dependent, i.e., they released higher amounts at pH 4 and 5.5 than at pH 7. Nano hydroxyapatite doped with strontium showed the formation of agglomerations instead of stable individual particles [93]. Sodagar et al. [94], observed that orthodontic adhesive disks containing 5 and 10% silver/hydroxyapatite nanoparticles exhibit antibacterial properties against biofilms, but the same effect was not observed when 1% silver/hydroxyapatite nanoparticles were added to the orthodontic adhesive.

The development of surfaces with bioactive, functionalized coatings, and with controlled release of metallic nanoparticles aims to reduce infection rates. However, concerns over cytotoxicity, bioaccumulation, acquired autoimmunity, and systemic toxicity has gained attention in the same importance status [95]. Wang et al. [96], observed a significant antibacterial effect for *S. aureus* when titanium surfaces were coated with silver nanoparticles, and further, stated that the nanoparticles were safely anchored to the titanium surface in addition to being non-cytotoxic.

Implant bioengineering also studies hybrid strategies that attempt to associate topography with the use of antimicrobial agents. In this sense, different additive manufacturing specimens received the addition of antimicrobial agents, such as silver ions or nanoparticles [97–99], antibiotic drugs [30,100], the addition of copper or silver to the titanium alloy [101,102], ZnO nanoarrays [103], modifications by calcium phosphate incorporated into TiO_2 nanotubes [104] and among others polystyrene and acrylic acid solutions [105]. Sarker et al. [106], fabricated additive manufacturing specimens with tilts and observed a reduction in *S. aureus* biofilm formation, and these results were associated with changes in surface topography, such as wettability and roughness.

Although there are many antibacterial agents applied in order to reduce the biofilm formation, the current context still shows resistant species to antimicrobial therapies, so it is necessary more studies in order to control or reduce pathogenic biofilms looking for new products and techniques. In this sense, the efforts made by materials engineering are essential.

Of the biofilm control strategies that act directly on bacteria, quorum-suppressing enzymes or quorum sensing inhibitors act by inactivating acyl-homoserine lactone molecules (AHLs) which consequently prevents bacteria from synchronizing their virulent behavior [10,62,107]. Some examples of these enzymes are lactonase, acylase, oxidoreductase, and paraoxonase. Some

plant or animal derivatives are also able to degrade AHL signals and lead to quorum-sensing signal disruption [51,62]. And furthermore, one can inhibit quorum sensing mechanisms that are microorganism-specific, such as the QPS of *Pseudomonas aeruginosa*, the competence-stimulating peptide, or the X-inducing peptide of *S. mutans* [51,71], the intercellular adhesive polysaccharide (IAP) produced by *S. epidermidis*, which is essential for cell-to-cell attachment and subsequent biofilm development, and the autoinducer peptide signal (AIP) synthesized and secreted by *S. aureus* [20].

Another effective way to disperse biofilm is to inhibit the signaling pathways of secondary messengers (c-di-GMP, c-di-AMP, and the (p)ppGpp) or reduce the intracellular levels of these messengers [48,107]. This can be achieved by hindering the synthesis of the molecules or by direct degradation/inactivation [10,51]. An example would be the use of small organic molecules that can inactivate messengers such as c-di-GMP, and interfere with biofilm formation in addition to contributing to the destruction of the pre-formed biofilm by inhibiting the synthesis of matrix components [8,51].

Another possible approach is to prevent the matrix from forming. By disintegrating its bonds, it loses its protective effect, leading to biofilm dispersion and the release of planktonic cells. Changes also occur in the gene expression of the bacteria, making them susceptible to antimicrobials [8,10]. Some potential agents are matrix-degrading or biofilm-dispersing enzymes, which may be useful agents for the treatment and prevention of biofilm-related infections in clinical settings [108]. Some examples of EPS matrix-degrading enzymes include deoxyribonucleases (DNase I, purified human DNase1L2, *Bacillus licheniformis* extracellular recombinant NucB, *Staphylococcus aureus* thermonuclease), restriction endonucleases, glycosidic hydrolases, proteases, and dispersin B [10,51,107,108].

Bacteria use, in addition to quorum sensing, a variety of sensory systems to monitor their environment to enable adaptation to stress conditions [48]. And in this sense, different strategies are studied to act on these systems. One example is the enzyme Sortase A, present in the cell wall of Gram-positive bacteria [56], which is responsible for attaching proteins to the envelope surface. Due to the localization of this enzyme, it is studied as a target for anti-virulence drug development [56]. Small molecule inhibitors may be able to block SrtA by inhibiting the incorporation of surface proteins into the staphylococcal envelope. Thus, they may be useful as anti-infectives to prevent *S. aureus* infection without affecting the growth of other bacteria, since SrtA inhibitors may interfere with adhesion and intercellular communication rather than bacterial growth [109]. Another example is proteases, which are degradative enzymes secreted by many bacteria that can interfere with cell-to-cell communication by degrading the competence-stimulating peptide [8].

8. Conclusions and perspectives

The bacterial cell and the medium in which it develops are extremely dynamic, and the modification of one of them can have an impact on the way they interact with each other. If we can understand the forces and physical interactions that govern this adaptation, as well as bacterial attachment, we could find ways to control unwanted adhesion. This would make it possible to alleviate infections associated with biomaterials, as well as to control the pathogenicity of biofilms.

Although antimicrobials continue to be the main treatment option for bacterial infections, increased drug resistance makes new alternatives necessary to treat infections and fight the spread of multi-resistant bacteria. A possible strategy that is being studied to control adhesion and biofilm formation is interference with the chemical signal of the quorum-sensing system. If it is possible to interfere with communication, bacteria will have difficulties adapting to the environment.

The search for understanding the mechanism of bacterial adhesion to different materials used in oral rehabilitation can bring new perspectives of research aiming at modifying the surfaces and constituents of dental materials, from the knowledge of bacteria action mechanisms. It is still uncertain whether a universal nanostructured substrate will be found, but an in-depth understanding of adhesion will allow technological advances in the medical and dental fields, capable of introducing more effective antimicrobial surfaces.

Conflict of Interes

The authors report no conflicts of interest.

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