Hydrodynamics on Microbial Adhesion and Biofilm Formation

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Studying microbial adhesion and biofilm growth is crucial for understanding the physiology of sessile organisms and forming the basis for the development of novel antimicrobial materials. Fluid hydrodynamics is one of the most important factors affecting cell adhesion, as well as biofilm structure and behavior. Therefore, to simulate the relevant biofilms of different fields (environment, industry, and medicine) in the laboratory, it is of utmost importance to select an adequate biofilm platform and be able to operate it at hydrodynamic conditions that are as close as possible to those encountered in a real scenario.

Keywords: biofilm ; microbial adhesion ; hydrodynamics ; shear stress ; shear rate ; computational fluid dynamics ; flow systems

1. What Are Biofilms?

Biofilms are surface-attached communities of microorganisms, establishing three-dimensional structures composed of bacteria surrounded by a self-made matrix [1]. This matrix consists of polysaccharides, proteins, and extracellular DNA and influences biofilm structure and morphology [2]. It is estimated that more than 90% of the bacterial cells in natural environments reside in a biofilm [3], since it gives protection against hostile conditions (pH changes, lack of nutrients, hydrodynamics, and antimicrobial compounds), encourages gene transfer, and facilitates the colonization of niches [4].

The established model for biofilm development includes five steps, starting with the (i) reversible attachment of cells to a preconditioned surface, (ii) production of extracellular polymeric substances (EPS) causing irreversible cell attachment, (iii) early development of biofilm architecture, (iv) biofilm maturation, and (v) cell dispersion from the biofilm into the surrounding environment [5][6]. An immersed substratum is rapidly covered by molecules from the liquid, forming a conditioning film that may change the properties of that surface, making it more or less suitable for bacterial adhesion [7][8]. Then, cell adsorption at the surface occurs, followed by release or reversible adhesion. The physical forces associated with conditioning film formation and reversible adhesion are electrostatic and van der Waals forces, as well as hydrophobic interactions [9]. The next step starts when the cells become irreversibly attached to the surface due to the presence of stronger attractive forces, such as covalent and hydrogen bonds, and cellular surface structures, such as fimbriae and flagella [9]. After maturation, biofilm growth and detachment/sloughing balance each other so that the biomass amount is approximately constant in time.

2. Problems Caused by Biofilms

Biofilm development is a problem faced by the environmental, industrial, and biomedical areas. Regardless of the setting where it takes place, it is well known that biofilm establishment and growth are affected by different factors, such as surface properties, nutrient availability, hydrodynamics, temperature, pH, and microbial cell properties [10]. Among these factors, hydrodynamics will be considered in detail.

In the environment, biofilms particularly affect the efficiency of shipping, aquaculture, and coastal industries [11]. The fouling phenomenon increases the surface roughness of the ship hulls, hence increasing the friction between the fouled hull and the water [12]. This resistance increases fuel consumption and, consequently, the emissions of greenhouse gases to the atmosphere [11][12]. Additional problems related to biofouling in the environment are associated with immersed offshore structures (cages, netting, and pontoons), onshore equipment, and structures such as pumps, pipelines, and filters, due to the high drag and accelerated biocorrosion to which they are exposed [13].

Besides affecting cleaning and disinfection, biofilms formed in industrial facilities can reduce energy transfer in heat exchangers, obstruct fluid flow, and cause localized corrosion attacks [14]. It has been reported that biofilm development in

industries corresponds to approximately 30% of the plant operating costs [15]. In the case of the food industry, biofilms have a detrimental effect not only on the process but also on the final product or consumer. The Center for Disease Control and Prevention (CDC) has estimated that 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths occurred annually in the US due to the dissemination of foodborne pathogens [16].

In the biomedical field, the sessile cells are responsible for infections, as they are usually more resistant to antimicrobial therapy than their planktonic counterparts and less susceptible to host defenses [17]. The National Institutes of Health (NIH) revealed that 65% of all microbial infections are caused by biofilms [18], which can grow in indwelling medical devices and have an estimated direct cost burden of 7 billion EUR in Europe alone [19].

The ubiquitous nature of biofilms and their increasing resistance impose great challenges for the use of conventional antimicrobials and suggest the need for combined or multi-targeted approaches. In this sense, the study of strategies capable of preventing microbial adhesion through the modification of surface properties (for instance, making them antimicrobial and/or antiadhesive) may be one of the simplest and most reasonable ways to inhibit surface colonization and delay biofilm growth [20][21][22][23]. However, few studies have evaluated the effectiveness of these promising surfaces in conditions that mimic real scenarios, particularly regarding hydrodynamics.

3. Impact of Hydrodynamics on Microbial Adhesion and Biofilm Formation

The flow conditions of each system where there is a surface material (natural, industrial, or biomedical) have a very strong influence on the biofilm onset. During initial adhesion, hydrodynamics dictates the rate at which macromolecules (specific for each type of fluid) and microorganisms are delivered to the surface, the time they reside close to the surface, and the shear forces at the surface-fluid interface [24]. According to Katsikogianni and Missirlis [25], there is an optimum flow rate for bacterial adhesion, reflecting the balance between the rate of cell delivery and the force acting on adhered bacteria. Furthermore, the bacteria–substratum interaction determines the shear forces that adhered bacteria will be able to withstand [25].

Besides the relevant role of hydrodynamics on the microbial adhesion step, it is also one of the most important factors in biofilm formation and structure. The fluid surrounding a biofilm is the source for nutrients and vehicle for cell by-product removal [26]. An increase in flow velocity promotes the flux of molecules (nutrients, cells, biocides, antibiotics, metabolites, etc.) by changing their concentrations in the biofilm–fluid interface. Hydrodynamics also regulates the physiological properties of the biofilm by changing the mechanical shear stresses at the interface [24]. Higher shear forces often lead to the formation of thinner, denser, and stronger biofilms [27]. Although higher flow velocities enhance molecular transport by convection, the higher density of biofilms reduces the diffusivity of the molecules inside them [28][29]. Additionally, stronger shear forces can be responsible for higher biofilm sloughing or detachment [27].

Given the importance of shear forces on initial adhesion and biofilm development, it is essential to characterize them. The vast majority of biofilm studies under flow conditions only report the tested flow rate. Nevertheless, the flow rate by itself provides little information about shear forces since it does not take into consideration the geometry of the flow system. Two main parameters should be considered to characterize shear effects: the shear rate and the shear stress. Mathematically, the shear rate is the derivative of the velocity in the perpendicular direction from the wall system [30] and quantifies the frequency at which cells contact the surface. The shear stress in Newtonian fluids is proportional to the shear rate, where fluid viscosity is the constant of proportionality [30], translating the friction from the fluid acting on the adhered cells or the biofilm. Therefore, shear stress is commonly used as a descriptor of the shear forces acting on the biofilm during maturation or detachment.

Computational fluid dynamics (CFD) are commonly used to model biofilm reactors because they enable the estimation of the fluid flow parameters of these systems, such as the shear stress and the shear rate, at relatively low cost and faster, in comparison to experimental techniques [31][32]. CFD requires that the geometry to be analyzed is divided into a finite set of volumes, called cells, forming a computational grid, called mesh. Fluid flows are described by differential equations for the conservation of mass, momentum, and energy; CFD replaces these equations with algebraic equations, which can be numerically solved for each cell, resulting in a flow field [33]. These equations describe how the single operating parameters are related. Although CFD is very useful for understanding biofilm behavior, one must bear in mind that most simulations are performed for clean surfaces. Thus, these simulations are particularly recommended for the study of initial adhesion, early stages of biofilm development (such as those usually investigated in biomedical settings), and surfaces that are frequently cleaned (as is the case with food processing equipment) [34].

4. Platforms for *In Vitro* Adhesion and Biofilm Formation Studies under

Flow Conditions

The most commonly used platforms for the *in vitro* assessment of microbial adhesion and biofilm formation under flow conditions are modified Robbins devices, flow chambers, rotating biofilm devices, microplates, and microfluidic devices. These testing platforms have been used transversally in the environmental, industrial, and medical fields, mainly with the aim of evaluating the effects of different substratum features, microbial strains, and shear forces on adhesion and biofilm formation, due to their ability to control the hydrodynamics (flow rate and/or shear stress or shear rate) and recreate *in vivo* flow conditions. This becomes a critical step in translating research into practical applications.

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