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Toward plasmonic monitoring of surface effects on bacterial quorum-sensing

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ABSTRACT

Biofilm formation is facilitated by cell–cell communication processes known as quorum sensing, which enable collective behavior and metabolic coordination. Surface topography and chemistry play a significant role in bacterial adhesion and biofilm formation, yet methods for monitoring quorum sensing *in situ* suffer limitations. Herein we suggest the use of surface-enhanced Raman scattering to study the effects of surface topography and chemistry on quorum sensing signals involved in biofilm growth.

1. Introduction

Bacteria are capable of growing together in a dense colony that mimics the hierarchy of a larger organism, and a communication system of chemical sensing called quorum-sensing is the means of cooperative growth. Biofilms are of significant interest to medicine due to their prevalence in certain medical conditions and infections, and their ability to protect bacteria, which can thereby become antibiotic resistant. Recent research has shown that the intercellular signals involved in these cues for biofilm formation can be detected by Raman spectroscopy. Furthermore, the use of metal nanoparticles for plasmonic enhancement of the Raman signal allows detection of these chemical signals at levels far below conventional methods. The scope of this review spans the basics of bacterial biofilms on surfaces, the effects of surface topography and surface chemistry on bacterial adhesion and biofilm growth and examples found in nature, as well as various techniques for studying biofilm growth over the last two decades. The concept of topographically-patterned plasmonic substrates for monitoring bacterial growth is put forth as a means to study materials which resist bacterial adhesion and growth and the effects of physical confinement on bacterial signaling.

1.1. Biofilms — bacterial simplicity with tissue-like complexity

Biofilms are a mode of sessile growth common to bacteria and cyanobacteria along with some species of fungi and microalgae, forming characteristic structures which can be resistant to antibiotic and antimicrobial sterilization techniques, as compared to their planktonic growth phases. In the 1970s researchers began to take note that sessile bacteria in the form of biofilms make up a major component of bacterial biomass. Bacterial biofilms consist of heterogeneously organized microcolonies of bacteria on a surface, forming a dense plaque made up of both live and dead bacteria, stuck together by a “glue” of exopolysaccharides which may be self-produced or arise from the lysis of the dead bacteria in the biofilm matrix. An analogy between bacterial biofilms and tissues formed by eukaryotic cells is clear in the higher level of organization and cooperativity that is observed in bacterial biofilms compared to mixed populations of planktonic cells. The physiological cooperativity and protection from environmental changes that are present in the biofilm matrix constitute a system which does indeed resemble eukaryotic tissue. The emergence of biofilms occurs early in the fossil record (*ca.* 3.25 billion years ago), coinciding with the first evidence of the transition from unicellular to multicellular organization [1••]. Fossils of prokaryotic filamentous biofilms and mats formed by cyanobacteria-like organisms marked an evolutionary transformation, which suggests that the formation of biofilms was advantageous for life in the harsh environments of early earth. Additionally, biofilm formation has been observed in a wide variety of organisms such as archaea and eukaryotes including fungi.

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The study of bacterial organization and biofilm formation has become an essential means for explanation of phenomena such as virulence, antibiotic-resistance, long-term colony survival, and surface adhesion [2]. Bacterial biofilms exhibit a variety of interesting adaptations which aid in their survival in adverse environments. Upon detection of environmental stress signals, planktonic bacteria will respond by initiating attachment onto a surface, which is the start to the formation of a biofilm. Research on *Pseudomonas aeruginosa* suggests a five-stage biofilm development sequence, as illustrated in Scheme 1, [3,4]. The first two stages of biofilm growth are associated with loose attachment to a surface, followed by strong adhesion. The next stages involve the clumping of cells into microcolonies and growth into a biofilm structure, which can be flat or mushroom-like (Fig. 1). The shape of the colony is influenced by the growth and rearrangement of cells through pili-mediated gliding, depending on the available nutrients [5,6]. In the final stage of biofilm growth some motile cells are shed from the biofilm, where they are then able to colonize other surfaces in a new location. There are many ways bacteria in a biofilm can detach and continue to spread, depending on the different hydrodynamic and environmental conditions. In static conditions in liquids, clumps of bacteria may be easily dislodged from the biofilm under changes of shear forces in the system. Detachment of bacteria from biofilms was originally considered to be a passive behavior, however it may actually be a means for the organism to strategically engage in colonization of the surrounding environment before the nutrients in the local environment are depleted.

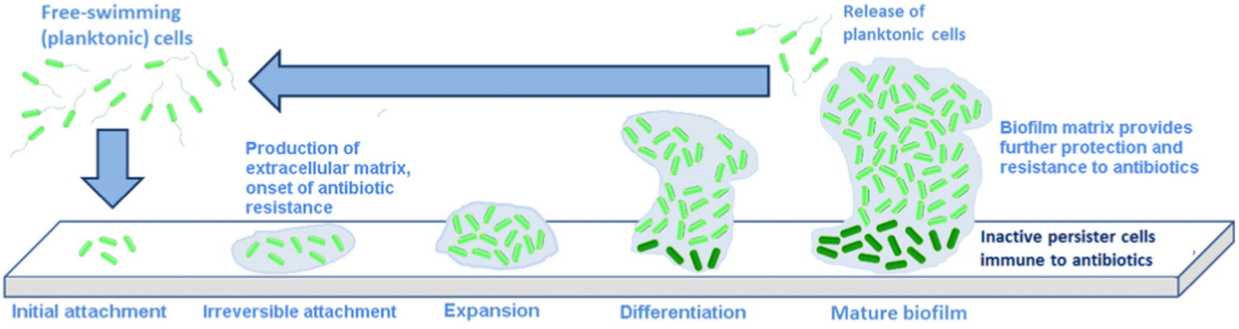
Depending on the bacterial strain, different mechanisms of detachment and reattachment for further colonization have been observed. Three basic dispersal mechanisms have been suggested, and are largely dictated by the motility mechanisms available to the bacteria that make up the biofilm [8]. For example, *P. aeruginosa* – rod-like Gram-negative bacteria – can engage in "swarming" motility, by which certain bacteria in the biofilm differentiate into elongated cells that can navigate the surface of solid substrates. *P. aeruginosa* can also spread along solid surfaces through pili-mediated "twitching" [9]. In the case of the Gram-positive, spherical bacterium *Staphylococcus aureus*, clumps of bacteria can detach and disperse through a liquid medium. These clumps have been observed to have rolling motility on solid surfaces, by which they are able to travel away from the biofilm where they originated and reattach to form a biofilm at a new location. On the other hand, in pathogenic nontuberculous mycobacteria such as *Mycobacterium fortuitum*, detachment of clumps of bacteria and dispersion into the medium can occur in a similar fashion, but the surface motility of these organisms comes from a sliding mechanism. Some selected examples of mixed and monoculture biofilms are shown in Fig. 1.

The different growth states and dispersion of bacteria are dictated not only by nutritional gradients but also by intercellular communication processes known as quorum sensing (QS) [14]. Bacterial biofilms coordinate their metabolism and structural exopolysaccharide production through QS signals, which also induce detachment of bacteria from established biofilms for further propagation [15]. The initial adhesion of planktonic cells to surfaces at the early stages of biofilm formation is affected by stress signals, which are usually part of a quorum sensing system. In QS, bacteria are able to monitor their local environment and population density through the production and detection of small-molecule signals that are secreted by the bacteria [14]. The simultaneous production and detection of these signals results in the coordination of gene expression within a given population of bacteria which are in sufficiently close proximity, leading to large-scale regulation of physiology including production of chemical species and growth state [16]. In addition to regulating the production of chemicals such as virulence factors and antibiotics, the coordination of gene expression that arises from quorum sensing is one of the major influences on the formation of biofilms by bacteria. It also enables the cell–cell communication between bacteria and other cells, with which mixed colonies can survive symbiotically or competitively (Fig. 1a,c) [14].

The molecules responsible for QS vary between species and encompass a wide range of chemical species including nucleic acids, peptides, and various small molecules [17•]. Molecules known as autoinducers are QS signals that can elicit alterations in gene expression at sufficiently high concentrations, and following these changes in expression other molecules are produced leading to terminal QS molecules. In many instances, the terminal QS products are antibiotic, induce gene transfer, or are pathogenic to other species. Examples of autoinducers and terminal QS signals from different organisms are described below.

The most common QS molecules are acyl homoserine lactones (AHL), which are predominant in Gram-negative bacteria, and can occur with numerous sidechains across many species (Fig. 2a) [18•]. Other common signals in Gram-negative bacteria include diketopiperazines, diffusible signal factors, 4-hydroxy-2-alkylquinolines, whereas quorum sensing peptides are largely found in Gram-positive bacteria (Fig. 2b) [19]. In - fungal species such as *Candida albicans*, quorum sensing pathways are not as well understood as in prokaryotes, but the molecule farnesol has been found to be related to QS and has an inhibitory effect on bacteria such as *P. aeruginosa* [20]. In the case of the fungi *Uromyces phaseoli*, methyl 3,4-dimethoxycinnamate was found to inhibit spore-germination in a mechanism that allows dispersal of the spores to take place prior to germination (Fig. 2c) [21]. Another interesting fungal QS system involves the cross-feeding of Trisporic acid precursors between opposite mating types, as neither has the ability to completely synthesize it themselves [21]. The detection of QS signals is generally carried out by genetic modification of the organism to express a fluorescent molecule in the presence of the signal, however several organisms produce terminal QS signals which are colored or fluorescent pigments and can be detected by colorimetry or fluorescence. Surface enhanced Raman scattering (SERS) spectroscopy has recently been proposed a means to detect QS molecules without labels or genetic modification of the organisms studied, as discussed below.

The formation of biofilms is of particular interest due to the environmental problems that they can cause, due to their strong resilience, functional heterogeneity, and complex nature. In healthcare, the



Scheme 1. Stages of biofilm growth.
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