QUESTION 1: What is the life cycle of biofilm and the mechanism of its maturation?

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Response:

A biofilm may be defined as a microbe-derived sessile community characterized by organisms that are attached to a substratum, interface, or each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with respect to growth, gene expression, and protein production The biofilm infection life cycle generally follows the steps of attachment (interaction between bacteria and the implant), accumulation (interactions between bacterial cells), maturation (formation of a viable 3D structure), and dispersion/detachment (release from the biofilm). The life cycle of biofilm is variable depending on the organism involved. There are characteristics in the life cycle of biofilm formation. These include, attachment, proliferation/accumulation/maturation, and dispersal. Biofilm can either be found as adherent to a surface or as floating aggregates.

Level of Evidence: Strong (this is a scientific review)

Delegate Vote: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

Post Meeting Rationale:

To answer this question the authors searched PubMed and Google Scholar between January 1950 – August 2018. Search words included: Biofilms, biofilm formation, biofilm life cycle, staphylococci biofilms, Gram positive organisms, pseudomonas aeruginosa biofilms, antibiotic resistance, prosthetic joint infections (PJIs). Relevant papers based on the above search words were reviewed. Most studies found were animal studies, laboratory studies, in vivo studies and a few clinical studies.

The biofilm life cycle generally follows a consistent series of steps or stages starting with attachment (interaction between bacteria and a surface) followed by accumulation (interactions between bacterial cells), maturation (be phenotype shift to the sessile form as well as modification and maintenance of the 3D structure), and dispersion/detachment (release from the biofilm).[1,2,3] The progression through these stages is mediated by the interplay of a number of microbial, host, and environmental factors which are usually different across microbial species or amongst even strains within species. A rapid progression through theses stages can be seen with virulent, biofilm-forming pathogens in a susceptible host (e.g. a virulent *S. aureus* strain in a host with immunosuppression). In contrast, an infecting microbe with slow growth and low virulence (e.g. *Cutibacterium acnes* – basonym *Propionibacterium acnes*) in a healthy host capable of suppressing biofilm formation can produce an indolent infection with delayed progression. Although many bacterial pathogens are capable of forming biofilms in a range of clinical contexts, *S. aureus* is a common etiological agent associated with periprosthetic joint infection. Therefore, formation of *S. aureus* biofilm is being described in depth.

The initial phase of biofilm formation is characterized by the attachment of planktonic cells to a surface. In a planktonic mode of growth, *S. aureus* up-regulates the expression of key mediators for immune evasion (e.g. Protein A), and the attachment to biotic surfaces. These mediators are a variety of proteins anchored in the cell wall, the largest group of which are termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs).[4] Binding of MSCRAMMs to host components such as fibronectin, fibrinogen, collagen, and cytokeratin are an important first step in the attachment of *S. aureus* to initiate biofilm formation.[5] Attachment to abiotic surfaces is also determined by properties and physicochemical characteristics of the abiotic surface as well as the bacterial surface, with hydrophobic and electrostatic interactions playing a major role.[6]

Following this initial attachment, bacteria proliferate and produce an extracellular matrix (ECM), often referred to as slime or glycocalyx, comprised of proteins (both host derived and bacterial), carbohydrates, and extracellular DNA (eDNA). These serve as a scaffold for maturation and three-dimensional structuring of the biofilm.[7] Ultimately, through coordinated degradation of ECM via proteases, nucleases, delta hemolysin, and other factors (modulins), bacterial cells are released from the biofilm with the potential to seed secondary sites of infection.[8]

The next phase of biofilm formation entails the proliferation and accumulation of attached bacterial cells. During this early phase, intercellular attachment plays a key role in stabilizing the early biofilm before a significant amount of ECM can be produced to protect the attached cells from disruptive forces such as shear.[7] One key contributor to intercellular adhesion is the polysaccharide intercellular adhesin (PIA), first studied in *Staphylococcus epidermidis.* [9] Also known to contribute are the MSCRAMMs (discussed above), and certain cytoplasmic proteins shown to bind to eDNA.

The maturation phase of the biofilm life cycle entails the three-dimensional structuring of biofilms into classic architectural structures (towers and mushroom-like structures) and the development of microcolonies displaying some degree of phenotypic diversity.[6, 7] This complex structuring is coordinated through the balance of adhesive and disruptive factors.[6] Adhesive factors include the ECM components discussed above such as PIA, proteins, and eDNA. Disruptive factors include enzymes that degrade these components such as proteases and nucleases, as well as the surfactant-like molecules, phenol-soluble modulins (PSMs). These disruptive factors allow for the remodeling and maturation of biofilm structures.

The final step of the biofilm life cycle involves the dispersal of cells which have the ability to travel to distal sites to disseminate infection. The mechanism by which *S. aureus* regulates this step, is largely mediated by the accessory gene regulator (*agr*) quorum-sensing system.[10] The *agr* system responds to cell density through the accumulation of signal molecules, allowing for dispersal to occur once a threshold density is reached. The micro-colonies then travel to other regions of the host to attach and promote biofilm formation on virgin areas.

References:

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