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QUESTION 4: Does the timescale of biofilm formation differ between bacterial species? If so, what is the timescale for common causative organisms?

RESPONSE / RECOMMENDATION: Currently, there is no clinical research available to answer whether the timescale in the development of biofilm formation differs between bacterial species. In vitro studies show high variability in biofilm formation based on bacterial strains and conditions. Animal studies have demonstrated rapid (minutes to hours) biofilm formation. The group notes that the timeline of biofilm formation may not correlate with the onset of infection symptoms.

LEVEL OF EVIDENCE: Strong

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

PRE-MEETING RATIONALE

Biofilms are comprised of single or multiple species of microbial aggregates embedded in a self-produced matrix of extracellular polymeric substances. Regardless of the bacterial species, biofilm formation proceeds in known and well-defined steps. The first step or stage, adhesion, begins when bacteria sense and attach to surface of a material. The second stage is accumulation, where bacteria aggregate to form a mature biofilm. The last stage is dispersion or detachment [1]. The duration of each of these steps in biofilm formation varies from nanoseconds to hours to weeks, depending on various factors such as size of inoculum, mechanism of colonization (direct perioperative inoculation, later direct colonization due to break of barrier, bacteremic spread), surface properties of the foreign material, bacterial strain and virulence, bacterial species, host immunity, prior antibiotic usage and environmental factors, etc. [2–10].

For example, *Pseudomonas aeruginosa* (*P. aeruginosa)* contains several genes that are turned on within 15 minutes of its attachment to a surface that can be a starting point of biofilm formation [3]. Kanno et al. developed full thickness wounds on the backs of rats and inoculated them with *P. aeruginosa* carrying the green fluorescent protein gene; they found that biofilms could develop within eight hours [4]. When *Staphylococcus aureus (S. aureus)* was inoculated onto animal wounds, researchers found the development of clusters of cells (characteristic of a biofilm) after 6-24 hours post inoculation [11,12]. Oliveria et al. evaluated the time course evolution of biofilm in mastitis isolates and found no significant difference between *S. aureus* and *Staphylococcus epidermidis*. In their study biofilm forming ability increased with incubation period for both species [5]. Hoffman et al. researched adhesion patterns of single bacterium *Caulobacter crescentus* on a glass surface in a microfluidic device. They showed the importance of pili for hastening bacterial adhesion. In their study, irreversible adhesion events were more frequent in wild-type cells (3.3 events/min) compared to pilus-minus mutant cells (0.2 events/min) [13]. Koseki et al. [6] evaluated the difference in early biofilm formation of polysaccharide intercellular adhesin (PIA)-positive *Staphylococcus epidermidis* on five types of biomaterials and found no significant difference in biofilm coverage rate at two to four hour incubation, but at six hours post incubation cobalt-chromium-molybdenum alloy (Co-Cr-Mo) had a significantly lower biofilm coverage rate than other materials like titanium alloy (Ti-6Al-4V), commercially pure titanium and stainless steel. In this study authors point out a similar degree of smoothness across materials as a reason for no significant difference between them initially (two to four hours). In this study average roughness (Ra) was less than 10 nm [6]. This is corroborated by the previous reports that bacterial adhesion is influenced by the threshold of surface roughness at values more than 200 nm [14,15].

Some evidence suggests that bioactive substances such as hydroxyapatite may be more prone to bacterial adhesion than bioinert metals, such as titanium alloys and stainless steel [7]. Further studies have demonstrated that polymethyl methacrylate (PMMA) is capable of hosting biofilms that can cause acute, chronic and delayed-onset infections [8,9].

Biofilm adherence to biological or synthetic materials and foreign cells and resistance to antimicrobials are poorly understood. As biofilm formation can proceed through different pathways and time ranges, its detection may differ according to the time of observation. Investigational models to determine how environmental factors, such as surface geometry, physical and chemical characteristics, and local blood flow and immune system affect biofilm development on prosthetic joints are essential to further understand various bacterial biofilms and provide insight to therapeutic strategies.

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