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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: 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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QUESTION 5: Do bacteria form biofilm on the surface of cement spacer in a similar fashion to a metallic implant?

RESPONSE: Yes. While the vast majority of studies have been in vitro, there is clinical evidence that majority of bacteria are able to form biofilm on the surface of cement spacer.

LEVEL OF EVIDENCE: Strong

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

PRE-MEETING RATIONALE

The majority of data assessing biofilm growth on polymeric materials and smooth surfaces has been collected from in vitro experiments [1]. As a general outline, microbial adherence to materials occurs in the following order: latex > silicone > PVC > Teflon > polyurethane > stainless steel > titanium [1,2]. This hierarchy of materials to bacterial adherence suggests that biofilms may develop more readily on polymer-based versus metallic material surfaces. Roughness may play a role in this [3]. However, time is also an important factor to consider. Verran et al. showed that *Candida albicans* adhered to a greater degree on roughened surfaces compared to smooth [4]. In their experiment, polymeric samples were incubated for one hour, and then assessed for adhesion profiles. Similar work was performed by Taylor et al. on cobalt-chrome materials with the same conclusion [5]. Although a one-hour incubation period may be beneficial to determine initial adherence profiles, it would be difficult to compare test criteria such as these to clinical scenarios where implanted materials are present for days, weeks, months or years. Wolcott et al. have shown that time may play an important role in biofilm maturation and antibiotic tolerance [6]. Biofilms are well-known to condition surfaces and make them conducive to their growth requirements [3]. Perhaps one of the most well-known examples of this is *Streptococcus mutans*, which conditions the enamel surface that allows adherence for hundreds of other bacterial species [7]. Given enough time, biofilms may flourish on surfaces in many environments and on surfaces that may otherwise be considered less culturable [3,8,9]. In-house experiments that are in process of publication have shown that even amongst the same species, varying strains can differ in rates of biofilm formation on titanium surfaces, but over time degree of biofilm formation is similar in bench-top conditions.

The principles and problem of biofilm formation apply to bone cement and metallic surfaces used in orthopaedic applications. Biofilms have been shown to develop on both material types and adversely affect clinical outcomes [10–13]. A seminal paper published by Gristina et al. provided one of the first indications of biofilm growth on an implanted metallic implant that was found to contribute to biofilm-related infection [14]. More recently, Stoodley et al. directly observed biofilms on antibiotic-loaded bone cement associated with an infected total elbow arthroplasty [12]. McConoughey et al. have also identified bacterial biofilms on implanted components [15]. Shaw et al. observed biofilm, via methylene blue staining, that had developed on a tibial tray and other total joint components during revision surgery [16]. In multiple cases, biofilm has been observed directly on clinical samples. Due to the heterogeneous and at times difficult nature of collecting clinical samples, more highly controlled, albeit confirmatory outcomes of biofilm growth on metallic and cement materials have been obtained from in vitro and in vivo experiments.

Minelli et al. showed the ability of multiple staphylococcal bacterial strains to form biofilm on bone cement samples in all cases [17]. Neut et al. observed that slime-producing *Pseudomonas aeruginosa* can readily form biofilm on cement material, and in the biofilm phenotype it may be more tolerant to antibiotics loaded in cement than planktonic bacteria [18]. Ensing et al. assessed biofilm growth on cement material and the potential of ultrasound to remove its presence [19]. More recently in a study by Ma et al., polymethylmacrylate spacers that were removed at the time of reimplantation following treatment of infected total knee arthroplasty were shown to have high levels of bacterial DNA despite extended exposure to antibiotics [20]. Biofilm formation on metal surfaces is also well-documented [21–24]. Nishitani et al. have also observed growth of biofilms on metallic implants in mice [25]. Williams et al. have shown that over multiple days of growth in a CDC Biofilm Reactor, polymicrobial biofilms of methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis* grow similarly on smooth or rough titanium surfaces [26].

In summary, indications that biofilm forms on bone cement and metallic surfaces in a similar fashion are present from clinical samples as well as in vitro and in vivo animal studies. There are indications that bacterial cells may adhere to and form biofilms more quickly on rough/porous materials, but over time bacteria may condition material surfaces that are smoother in nature such as metal and allow biofilm to form to a similar degree.

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