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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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QUESTION 6: Does *Mycobacterium tuberculosis* (*M. tuberculosis*) form a biofilm on implants?

RESPONSE/RECOMMENDATION: Few data from experimental in vitro and in vivo studies and a limited number of case reports indicate that *M. tuberculosis* has a slow, albeit significant, ability to form biofilm on metal surfaces. The group suggests that management of *M. tuberculosis* implant-related infections should be treated using the same principles as that of other implant-related infections.

LEVEL OF EVIDENCE: Strong

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

PRE-MEETING RATIONALE

Methods

A search of the English language literature on the question published during the period 1966–May 20, 2018 was conducted. The search strategy in PubMed used the terms *M. tuberculosis* and biofilm and identified 177 articles. All articles were reviewed for the response to the question. The vast majority of articles were categorized as basic sciences articles focusing on the components for tubercular biofilm formation in vitro. A systematic review to answer the provided question is not meaningful. Hence, the response of the question is answered as a summary of a narrative review.

Narrative Literature Review and Discussion

It is important to differentiate between *M. tuberculosis* and non-tuberculous mycobacterium. This review focusses only *M. tuberculosis*.

M. Tuberculosis Forms Biofilms

In the laboratory, *M. tuberculosis* shows peculiar aggregated growth, or in other words, can form organized pellicle-like structures [1]. The hallmark of biofilms is the self-production of the extracellular polymeric substance that holds the mycobacterial community together and confers phenotypic heterogeneity to