QUESTION 3: Is the biofilm on orthopedic implant surface permeable to neutrophils and macrophages in vivo? Are these innate immune cells (meaning any macrophages or neutrophils) capable of engulfing and killing bacteria?

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Response:
A mature bacterial biofilm has limited permeability to neutrophils and macrophages. Those that get through are clinically ineffective at eradicating biofilm bacteria. While neutrophils and macrophages are capable of engulfing and killing planktonic bacteria, they are not innately capable of effectively engulfing and killing sessile bacteria in biofilm.

Level of Evidence: Strong

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

Post Meeting Rationale:

The authors completed a systematic review on the peer-reviewed literature identified by a PubMed search performed on February 24, 2018 using the keywords "electron microscopy" and "biofilm" and implant surface. The search identified 148 references from 1991 to 2018. Of these, three references discussed "neutrophil"[1-3] and eight references discussed "macrophage"[1; 3-9]. Another PubMed search with the words "biofilm", "neutrophils" and "phagocytosis" gave 66 references, and a search with the words "biofilm", "macrophages" and "phagocytosis" gave 60 references. All these references were reviewed in order to select those that meet the question criteria. Furthermore, to assess the literature for negative findings, a PubMed search was performed using the keywords "natural history" and "biofilm" and "implant," which identified three references[10-12]. Within this literature, only four publications described EM of explanted infected biofilms.

The ability of these immune cells to penetrate mature bacterial biofilms and phagocytosis the infecting bacteria have mainly been evaluated in cystic fibrosis [1–5], urinary catheter related infection, [6–14] and periprosthetic infection [15].

Neutrophils (PMN) have shown the ability of sticking, but not penetrating, into a mature biofilm and phagocytizing biofilm encased microorganisms [1–5,7,8,11,16–20]. The exopolymeric substances of the biofilm matrix seem to be involved in the formation of neutrophil extracellular traps (NETs) in biofilm of *Streptococcus suis* [16], *Candida albicans* [7] and *C. glabrata* [8]. Data shows that neutrophils can destroy a 2-6 day old *S. aureus* biofilm, but a mature biofilm is capable of resisting penetration by these cells [21].

Alhede M et al, evaluated the role of immune system against biofilm formed by *Pseudomonas aeruginosa*. They demonstrated that both *in vitro* and *in vivo* biofilms of *P. aeruginosa* produce a shield of excreted rhamnolipids, which offers protection from the bactericidal activity of PMNs [22].

Another study showed that PMNs surround biofilm and become activated, but were not able to migrate into the biofilm, probably because of a lack of a chemotactic signal as well as by hindrance of migration into the "slimy" material. Thus, the inability of PMNs to penetrate biofilm results in progression of implant related infections [23].

Macrophages can penetrate into a mature biofilm in a similar way as neutrophils, and phagocytize biofilm encased microorganisms, but not destroy them [6,9,10,15]. Moreover, these sessile phagocytized bacteria can even persist into peri-implant tissue inside macrophage-like cells not only in experimental models, but also in the tissues of patients with intravenous catheters colonized by different bacteria [13,14]. *In vivo* model studies in *S. aureus* prosthetic infection showed that limited bacterial macrophage uptake is due to inflammatory attenuation by *S. aureus* biofilm [10], which favor the transformation from M1 type macrophages presents a high antimicrobial activity to M2 type macrophages with limited antimicrobial activity [10], and the cell death induction though LukAB [24] and Hla production [15]. At the site of staphylococcus biofilm infection, macrophages exhibit: down-regulation of IL-1β, TNFα, CXCL2, and CCL2 expression, reduced bacterial uptake, minimal iNOS expression. Consequently, these inhibited macrophages display low efficiency in killing phagocytized bacteria, and reduced induction of lymphocyte produced interferon-γ. As a result, these scavenging cells appear able to migrate into the biofilm, but cannot clear the site from the pathogen causing the infection as their bactericidal activity appears compromised [23].

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