ICM 2025 Question B17: "Does biofilm form in the synovial fluid?"

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Response/Recommendation: The available data strongly suggests that suspended biofilm aggregates of different *Staphylococcus* species form in synovial fluid *in vitro*. While there are fewer studies dealing with other species and/or *in vivo* biofilm formation, the available evidence strongly suggests that the majority of PJI pathogens can form suspended biofilms in synovial fluid, also *in vivo*.

Level of Evidence: Strong

Delegate Vote: Agree: [% vote], Disagree: [%], Abstain: [%]

Rationale: Central to the pathogenesis of prosthetic joint infections (PJIs) is the formation of microbial biofilms, which are structured communities of microorganisms encased in a self-produced extracellular matrix. Biofilm-associated microorganisms typically exhibit reduced susceptibility to a wide range of antimicrobial agents and are shielded from immune responses. As a consequence, eradication of such biofilms is difficult. Historically, microbiologists and clinicians have focused on surface-attached biofilms (such as biofilms attaching to implant surfaces), but recent work has highlighted that *in vivo* biofilms often occur as non-surface-attached aggregates, for example in the respiratory tract of people with acute and chronic lung infections[1; 2]. To answer this question a comprehensive literature search was conducted using the search words "biofilm", "synovial fluid", "synovia", and "joint fluid" within PubMed and Scopus, which initially identified 98 potentially relevant unique studies, screened by two independent reviewers, of which 42 were included for evaluation.

Whether such non-surface associated/suspended biofilm aggregates also form in synovial fluid has been addressed in a number of *in vitro* studies. However, methodologies and models used frequently differ between studies, making direct comparisons difficult. Aggregation has been studied in diluted and undiluted human, bovine, porcine, and equine synovial fluid[3-6], while several artificial/synthetic synovial fluid media have also been developed to study aggregation [7; 8]. Most studies so far have focused on *Staphylococcus aureus*, where formation of suspended biofilms (aggregation) was observed microscopically for the majority of *S. aureus* strains investigated (both MRSA and MSSA) (e.g.[5; 8-10]). This phenotype was also observed for a range of coagulase-negative staphylococci (including *Staphylococcus epidermidis*, *Staphylococcus lugdunesis* and *Staphylococcus capitis*)[4; 7; 8; 10]. More recently, a wider range of organisms, including Gram-negatives like *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis*, Gram-positives such as *Streptococcus agalactiae, Enterococcus faecalis*, and *Cutibacterium acnes*, as well as various *Candida* spp. have been confirmed to be able to form aggregates in synovial fluid[3; 8; 10-12].

Currently there is only limited *in vivo* data that confirms biofilm formation in the synovial fluid. Confocal microscopy on clinical synovial fluid samples of six patients with a confirmed PJI provides direct evidence for *in vivo* biofilm formation in synovial fluid, as biofilm aggregates could be observed in samples obtained from patients infected with *S. aureus* or *S. lugdunesis* (but not in samples obtained from a single patient infected with *Prevotella bivia*)[13]. Using material recovered from PJI, SEM analysis of implant-attached biofilm and floating aggregate morphology was described[14], although aggregate size was not presented. Indirect evidence for the formation of

suspended *S. aureus* biofilm aggregates in synovial fluid comes from the observation that clinical synovial fluid samples from patients with a confirmed *S. aureus* PJI demonstrate elevated antibody levels to an *S. aureus* biofilm antigen, suggesting that these samples contain an *S. aureus* biofilm[15]. Other indirect evidence comes from the observation that the sensitivity of microbiological detection in clinical synovial fluid samples significantly increased upon pre-treatment with dithiotreitol, which increases detachment of bacteria from biofilm aggregates[16].

Data on antimicrobial susceptibility of synovial fluid aggregates are limited, but do suggest that these suspended biofilm-like aggregates (similar to surface-attached biofilms), exhibit a drastically reduced susceptibility to a wide range of antibiotics, at least *in vitro*[5; 8; 17].

With regard to the mechanisms of biofilm aggregation, experimental data are limited to *S. aureus*. Various studies reported that aggregation of *S. aureus* is mediated by binding to fibrinogen and/or fibronectin[5; 14; 18; 19]. Hyaluronic acid was also noted to contribute to the formation of synovial fluid aggregates of *S. aureus*[6; 14].

Conclusion: All of the available evidence strongly suggests that the majority of PJI pathogens can form suspended biofilms in synovial fluid, albeit that the extent of biofilm formation as well as the biofilm morphology (such as aggregate size) may differ between different microorganisms. The limited data currently available suggests these suspended biofilms demonstrate drastically reduced susceptibility to antimicrobial agents, and as such could influence treatment outcomes.

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