Reply to Perez and Patel

TO THE EDITOR—We read with interest the letter by Perez and Patel, who reported that Staphylococcus epidermidis strains form macroscopic aggregates during growth in synovial fluid that resemble those that we recently observed when Staphylococcus aureus was grown or incubated in synovial fluid [1]. The observations that Perez and Patel made of S. epidermidis are very similar to those we made of S. aureus [1], inasmuch as these authors also found strong bacterial aggregation in synovial fluid and ruled
out that synovial fluid inhibits staphylococcal growth. Perez and Patel reported that the bacteria needed a much longer time to aggregate in their experimental setting as compared to ours (24 hours vs 20 minutes). We concur with their assessment that this longer aggregation time is very likely due to the relatively low concentrations of fibrin in the commercially available synovial fluid from healthy bovine joints that they used, rather than due to categorical differences between the aggregation properties of the 2 species. In our study, we obtained and used human synovial fluid from the joints of patients undergoing surgery, which, as in all traumatized joints, contain high concentrations of fibrin [2]. We believe that this experimental environment, which more closely resembles the circumstances under which human joint infections develop, was an important strength of our study. Notably, our findings indicated that host-derived fibrin is a major component of the bacterial aggregates and showed that the fibrinolytic protease plasmin can be used to destroy the fibrin-containing macroscopic bacterial agglomerations, thereby increasing antibiotic efficacy. These key observations that point to potential novel strategies to combat antibiotic-resistant staphylococcal joint infections [3] would likely not have been achievable with synovial fluid from nontraumatized animal joints, as used by Perez and Patel.

Furthermore, in our study, we extended the mere observation of macroscopic staphylococcal aggregation to a molecular-level analysis of the bacterial factors that this phenotype requires. Using an S. aureus transposon bank, we identified S. aureus fibrinogen-binding and fibronectin-binding proteins to be crucial for aggregation in synovial fluid. Moreover, while those proteins are prerequisites for aggregation, we recently showed that the very low activity of the accessory gene regulator (Agr) system and, consequentially, the low production of the Agr-controlled phenol-soluble modulins are responsible for the exceptional degree of aggregation in synovial fluid (unpublished data). Phenol-soluble modulins are known as biofilm-structuring and biofilm-dispersing molecules [4, 5]; thus, their absence leads to increased biofilm-like aggregation.

Finally, we believe it to be of great importance to extend the initial observations of Perez and Patel to a molecular-level investigation similar to the one we undertook in S. aureus. Given that S. epidermidis also has fibrinogen-binding proteins, such as the intensely studied fibrinogen-binding protein SdrG, and also uses phenol-soluble modulins to structure biofilms in an Agr-controlled manner [6, 7], one would anticipate that the mechanisms underlying S. epidermidis aggregation during joint infection are similar to those we reported in S. aureus. Such similarity, if experimentally established, would greatly facilitate the development of therapeutic strategies aiming simultaneously at both S. aureus and S. epidermidis, the most important causes of prosthetic joint infection.

**Notes**

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**References**


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