Arthroplasty Today 25 (2024) 101314

Contents lists available at ScienceDirect

Arthroplasty Today

journal homepage: http://www.arthroplastytoday.org/

Brief Communication

The Location of Biofilms on Chronic Prosthetic Joint Infections and the Ramifications for Clinical Practice

James B. Doub, MD [a, b, *], David Parmiter, BA [c], Christine A. Brantner, PhD [c], Matthew Moshyedi, BA [d], Meghan Hughes, MD [e], Matthew Kolevar, MD [e], Aaron Johnson, MD [e]

[a] The Doub Laboratory of Translational Bacterial Research, University of Maryland School of Medicine, Baltimore, MD, USA
[b] Institute of Human Virology, Division of Clinical Care and Research, University of Maryland School of Medicine, Baltimore, MD, USA
[c] Electron Microscopy Core Imaging Facility, University of Maryland Baltimore, Baltimore, MD
[d] University of Maryland School of Medicine, Baltimore, MD
[e] Department of Orthopedic Surgery, University of Maryland School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 11 July 2023
Received in revised form
22 November 2023
Accepted 10 December 2023
Available online xxx

Keywords:
Biofilm
Periprosthetic joint infections
Scanning electron microscopy
SEM
Methylene blue

ABSTRACT

Revision surgery is paramount to cure chronic prosthetic joint infections because these infections are associated with biofilms on prosthetics that conventional antibiotics cannot eradicate. However, there is a paucity of research on where in vivo biofilms are located on infected prosthetics. Consequently, the objective of this pilot study was to address this gap in knowledge by staining 5 chronically infected prosthetics, that were removed at the time of revision surgery, with methylene blue. Scanning electron microscopic images were then taken of the methylene blue–stained areas to visualize biofilms. The findings show that all chronically infected prosthetics had biofilms located on the bone–prosthetic interface, yet only 2 had biofilms also located on the prosthetic interface exposed to synovial fluid. Subsequently, this pilot study provides a pathophysiological understanding of why the current treatment paradigm for chronic periprosthetic joint infection requires a revision surgery and not debridement and an implant retention surgery.

The current theory for why debridement and implant retention surgery (DAIR) for chronic periprosthetic joint infection (PJI) is associated with poor outcomes is because the chronicity and thus hardiness of biofilms deposited on prosthetic surfaces are unable to be removed by debridement and antibiotics alone [1,2]. However, it is well recognized that biofilms form rapidly, within 24 hours, and these newly formed biofilms are already highly resistant to antibiotics [3,4]. Therefore, there is a gap in knowledge on why chronic PJI treated with DAIR is associated with poor outcomes. Consequently, we hypothesized that the location of biofilms on chronic PJI is the reason for poor success rates with DAIR rather than temporal duration of biofilms. Therefore, the aim of this pilot study was to assess where in vivo formed biofilms are located on chronically infected prosthetics by staining with methylene blue (MB) and using scanning electron microscopy (SEM).

The study was approved by the University of Maryland, Baltimore Internal Review Board (HP-00099312). From November 1, 2022 through February 2, 2023, the first 5 chronically infected knee PJI that underwent revision surgery were evaluated. Here PJI was defined based on the musculoskeletal infection society definition [5]. In addition, chronic PJI was defined as having symptoms for more than 3 weeks and being more than 3 months since the index arthroplasty. In the operating room, explanted prosthetics were placed in a sterile container filled with 0.1% MB for 2 minutes, which is extrapolated from similar use of MB [6,7]. Then the prosthetics was rinsed in a separate container with 0.9% normal saline for 1 minute, which again was extrapolated from previous studies [6,7]. Images of the stained prosthetics were then obtained with attention to the surfaces that were in contact with the joint space and the prosthetic surfaces that were in contact with the bone. Figure 1a-e shows the tibial components stained with MB for these 5 cases. All 5 of the chronic infections had areas that stained on the bone-prosthetic surface. However, only the 2 staphylococcal PJIs (Fig. 1a and d) had obvious staining on the prosthetic interface

* Corresponding author. Division of Infectious diseases, 725 west Lombard Street, Baltimore, MD 21201.
E-mail address: Jdoub@ihv.umaryland.edu

https://doi.org/10.1016/j.artd.2023.101314

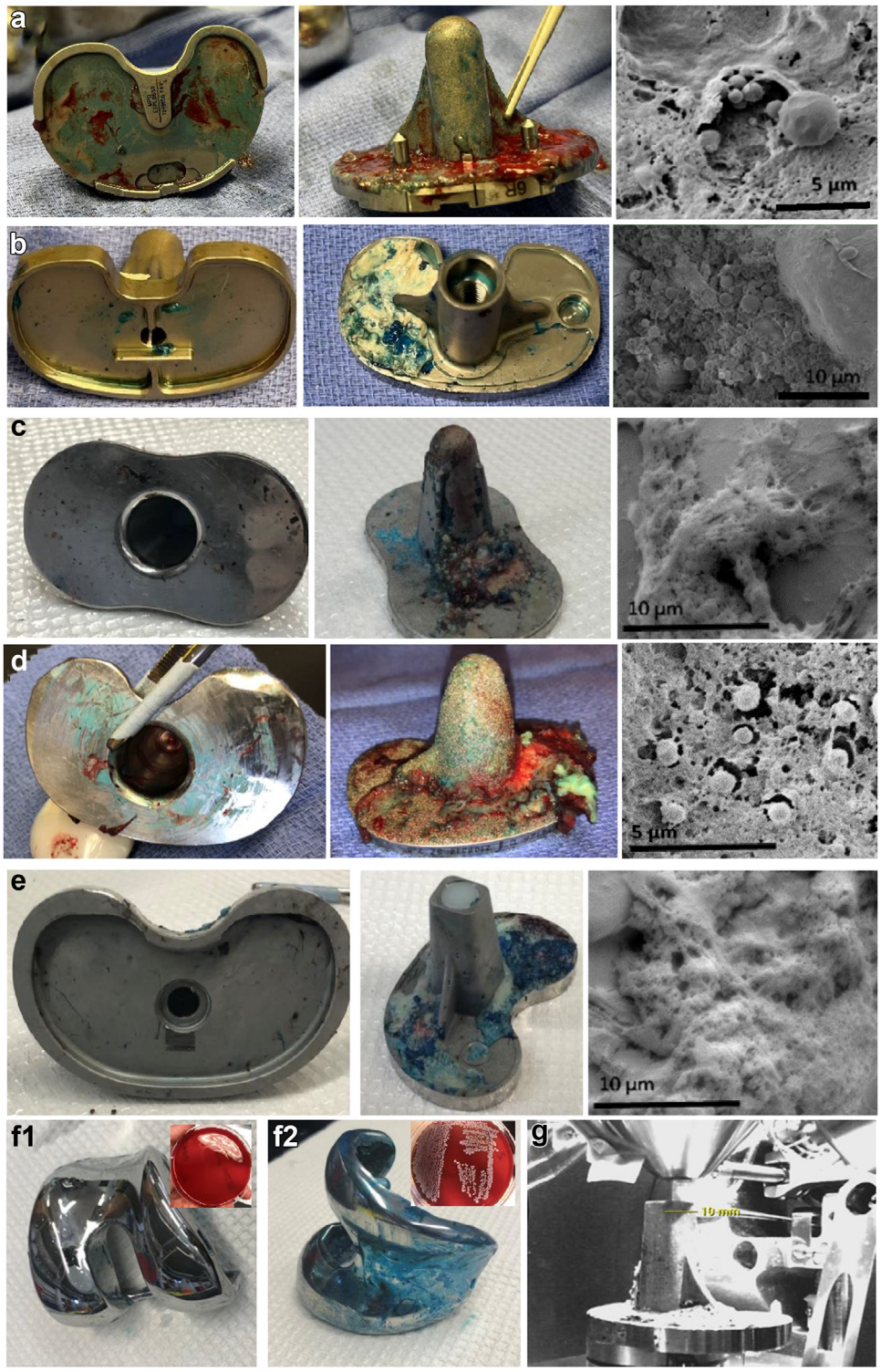

Figure 1. Location of biofilms on explanted tibial components. (a) *Staphylococcus aureus* PJI with biofilms located on both the bone—prosthetic interface and the interface in contact with the joint. *S. aureus* biofilm with bacterial cells seen under biofilm matrix at 24,000× magnification, 2 kV, working distance 5.0 mm; B1, (b) *Candida glabrata* PJI with biofilm located predominately on the bone-prosthetic interface. *C. glabrata* biofilm at 5000× magnification 2 kV, working distance 14.5 mm; (c) culture-negative PJI with biofilms located predominately on the bone-prosthetic interface. Culture-negative biofilm at 10,000× magnification, 2 kV, working distance 6.7 mm; (d) *S. aureus* PJI with biofilms located on both interfaces. *S. aureus* biofilm at 20,000× magnification, 2 kV, working distance 5.0 mm; (e) culture-negative PJI with biofilms located predominately on the bone-prosthetic interface. Culture-negative biofilm at 10,000× magnification, 2 kV, working distance 4.7 mm; (f_1) negative control with sterilized femoral component placed in methylene blue where no staining occurred, and no bacteria were cultured from prosthetic; (f_2) positive control with femoral component placed in broth of *S. aureus* for 24 hours and then stained with methylene blue in which larges areas of biofilm can be observed. In addition, *S. aureus* could be recovered by culturing these areas stained with methylene blue; (g) image showing a tibial component on the SEM stage with limited capability to move the prosthetic under the electron beam.

that is exposed to the joint space itself and polyethylene liner. Furthermore, this study had positive and negative controls. The negative controls included sterile femoral components that had been further sterilized in 200% ethanol for 24 hours and autoclaved. These were then stained with MB in which none stained with MB staining (Fig. 1f$_1$). Positive controls included sterile femoral components that were sterilized in same fashion and then placed in a broth with *Staphylococcus aureus* for 24 hours and then stained with MB (Fig. 1f$_2$).

To ensure these areas were biofilms, we utilized SEM. However, given the dimensional constraints of the SEM (Quanta 200, FEI, Hillsboro, OR), only the explanted tibial components were visualized. The tibial components that had been stained were placed in 2% paraformaldehyde and 2.5% glutaraldehyde overnight. Then the prosthetics were dehydrated in ethanol and hexamethyldisilazane. Components were coated with platinum/palladium, and the areas that had stained with MB were used to direct where to image with SEM. Figure 1a-e shows the SEM images of the areas that stained with MB. All SEM images show microbial biofilms. Moreover, the geometric constraints associated with tibial components in the SEM can be observed in Figure 1g.

To our knowledge, this is the first study to evaluate the location of in vivo–formed biofilms on infected prosthetics. Others have used MB in the operating room to show where infected tissues were present and as a tool for in vitro biofilm research [6,7]. Here with the use of MB and SEM, we show that chronic PJI have biofilms located not only on the surface that is exposed to the joint space but rather predominately on the bone-prosthetic interfaces. This has important clinical ramifications in that it demonstrates that chronic PJI biofilms are typically located in areas that are not accessible to debridement when conducting DAIR surgery. This explains from a pathophysiological standpoint why chronic PJI treated with DAIR are associated with poor outcomes and require a revision surgery to remove these niduses of microbes.

While we utilized similar techniques that other studies have used to mitigate subjectiveness, this was a pilot study, and thus, larger studies are needed to validate the findings shown here [6,7]. As well, this study was able to visualize some biofilm structures, but it is unknown if the fixatives and dehydration processes truncated the biofilms. This is because there is a paucity of research evaluating the proper fixative needed to preserve in vivo–formed biofilms to thereby visualize biofilms structures and the extracellular polymeric substances. It has been shown that the use of paraformaldehyde and glutaraldehyde does not reliably allow for preservation of extracellular polymeric substance structures and that the use of nonaqueous fixatives could be more advantageous in visualizing biofilm structures [8]. Follow-up studies are needed to assess what is the most beneficial fixative to use to visualize biofilms that have been formed in vivo on prosthetic material.

Furthermore, we would have liked to also visualize the femoral components, but the dimensional constraints of the SEM chamber rendered this not feasible. The tibial components could be imaged because they were small enough to allow them to rest on the SEM stage while also allowing for the stage to move in the x and y axes to place areas of interest under the electron beam (Fig. 1g). SEM imaging for larger or uneven prosthetics will require a larger SEM chamber and a method to hold these prosthetics in place while allowing the stage to move. This has important implications for using this technique with other explanted prosthetics, as the size and dimensions of the prosthetics are important to correlate with the size of the SEM chamber. At the present time, it may not be possible to visualize all explanted MB-stained biofilms with standard SEM chambers, and the use of confocal microscopy could be an alternative option for larger prosthetics.

In conclusion, this pilot study shows that chronic PJI have in vivo–formed biofilms routinely located on the bone-prosthetic interface which is not accessible to DAIR surgery. Undoubtedly larger studies will be needed to reinforce the findings shown here, but the findings provide a pathophysiological understanding of why the current treatment paradigm for chronic PJI requires prosthetic component removal. Moreover, this study is immensely valuable because the knowledge gained is vital to those creating novel treatment therapeutics and prevention methods for PJI and other infectious syndromes.

Acknowledgments

The authors appreciate the help of the University of Maryland School of Medicine's and University of Maryland School of Dentistry's Electron Microscopy Core Imaging Facility.

Funding

The authors acknowledge the support of the University of Maryland, Baltimore, Institute for Clinical & Translational Research (ICTR) and the National Center for Advancing Translational Sciences (NCATS) Clinical Translational Science Award (CTSA) grant number 1UL1TR003098.

Conflicts of interest

The authors declare there are no conflicts of interest.

For full disclosure statements refer to https://doi.org/10.1016/j.artd.2023.101314.

Authors' contributions

All authors contributed to investigation, framing the study methodology, and in review and editing of the article. A.J., C.B., and J.D. contributed to conceptualization. A.J., M.M., M.H., D.P., C.B., and J.D. performed data curation. M.M. and J.D. wrote the original draft. M.H., D.P., and J.D. carried out the formal analysis. C.B. and J.D. contributed to supervision. J.D. contributed to funding acquisition, project administration, and in obtaining validation.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the University of Maryland Institutional Review Board (HP-00099312).

Data sharing

Data can be shared upon reasonable request.

References

[1] Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America. Clin Infect Dis 2013;56:e1–25. https://doi.org/10.1093/cid/cis803.

[2] Zimmerli W, Sendi P. Orthopaedic biofilm infections. APMIS 2017;125:353–64. https://doi.org/10.1111/apm.12687.

[3] Schilcher K, Horswill AR. Staphylococcal biofilm development: structure, regulation, and treatment strategies. Microbiol Mol Biol Rev 2020;84:e00026-19. https://doi.org/10.1128/MMBR.00026-19.

[4] Sato T, Uno T, Kawamura M, Fujimura S. In vitro tolerability of biofilm-Forming trimethoprim-/sulfamethoxazole-resistant small colony variants of Staphylococcus aureus against various antimicrobial agents. Microb Drug Resist 2021;27:1282–9. https://doi.org/10.1089/mdr.2020.0379.

[5] Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, et al. The 2018 definition of periprosthetic Hip and knee infection: an evidence-based and validated criteria. J Arthroplasty 2018;33:1309–1314.e2. https://doi.org/10.1016/j.arth.2018.02.078.

[6] Shaw JD, Miller S, Plourde A, Shaw DL, Wustrack R, Hansen EN. Methylene blue-guided debridement as an intraoperative adjunct for the surgical treatment of periprosthetic joint infection. J Arthroplasty 2017;32:3718–23. https://doi.org/10.1016/j.arth.2017.07.019.

[7] Shaw JD, Brodke DS, Williams DL, Ashton NN. Methylene blue is an effective disclosing agent for identifying bacterial biofilms on orthopaedic implants. J Bone Joint Surg Am 2020;102:1784–91. https://doi.org/10.2106/JBJS.20.00091.

[8] Dassanayake RP, Falkenberg SM, Stasko JA, Shircliff AL, Lippolis JD, Briggs RE. Identification of a reliable fixative solution to preserve the complex architecture of bacterial biofilms for scanning electron microscopy evaluation. PLoS One 2020;15:e0233973. https://doi.org/10.1371/journal.pone.0233973.