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Pulse Lavage is Inadequate at Removal of Biofilm from the Surface of Total Knee Arthroplasty Materials

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ABSTRACT

In acute periprosthetic infection, irrigation and debridement with component retention has a high failure rate in some studies. We hypothesize that pulse lavage irrigation is ineffective at removing biofilm from total knee arthroplasty (TKA) components. *Staphylococcus aureus* biofilm mass and location was directly visualized on arthroplasty materials with a photon collection camera and laser scanning confocal microscopy. There was a substantial reduction in biofilm signal intensity, but the reduction was less than a ten-fold decrease. This suggests that irrigation needs to be further improved for the removal of biofilm mass below the necessary bioburden level to prevent recurrence of acute infection in total knee arthroplasty.

Total knee arthroplasty (TKA) is a highly successful and cost effective intervention for controlling pain and improving function in advanced arthritis [1–5]. There has, however, been a steadily increasing volume of revision procedures[6]. Infection has been reported as the most common cause of early revision [1,7], and as one of the most common reasons for long term failure at 15 years [8].

For periprosthetic joint infections identified with in one month of suspected onset, a standard treatment option includes irrigation, debridement, synovectomy, component retention, and exchange of the polyethylene bearing followed by long-term intravenous antibiotics. Failure rates have been reported at 60-80% in several studies [9–18]. A two stage reimplantation carries more risk for the patient, a higher morbidity, longer recovery period, and larger economic burden [19]. Because of these high costs and substantial morbidity, irrigation and debridement of acute periprosthetic joint infections remain a popular surgical option despite its reported low success rate.

The high failure rate of open irrigation debridement may result from the biofilm established shortly after infection [20]. Biofilms include aggregates of organism embedded in a complex extracellular polymeric substance composed of an extracellular polymer of polysaccharides, nucleic acids, and protein [21–23]. The extracellular polymeric substance of the biofilm enhances bacterial adhesion, and shields the bacteria from antibiotics. Additionally, the sessile state of a bacterial biofilm decreases metabolism, further decreasing the efficacy of antibiotics [24]. A key to improving the initial success of the debridement in a TKA infection involves disrupting and dispersing the biofilm, decreased bioburden, and increased efficacy of antibiotics [20].

Open irrigation and debridement is a two-fold strategy of attempting to remove biofilm secondary to shear force during the irrigation followed by eradication of the infection with antibiotics in the post-operative period. The current high failure rate of this approach [9–18] suggests that a substantial volume of biofilm remains on the components following the initial debridement. This is a popular hypothesis in the field; however there is a remarkable paucity of evidence measuring the amount of biofilm debrided from arthroplasty materials using current surgical techniques. A previous study has indirectly measured biofilm debridement on titanium [25]; however, direct biofilm mass has not been directly imaged and quantified following debridement methods. We hypothesize that pulse lavage irrigation is ineffective at removing a substantial portion of the biofilm mass from TKA components. To test this hypothesis, biofilm was

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cultured on the three materials used in TKA, and we directly quantified biofilm debridement before and after pulse lavage irrigation using two separate imaging modalities.

Methods

Biofilm Culture and Debridement

A clinically isolated methicillin sensitive strain of *Staphylococcus aureus* (Xen 29, Caliper, Waltham, MA) transfected with luciferase to allow visualization with a bioluminescent photon collection camera was selected. Cobalt chrome metal, polymethyl methacrylate (PMMA), and polyethylene (ultra-high molecular weight polyethylene) coupons (Fig. 1) were inoculated with *S. aureus* at an absorbance optical density of 0.5 in tryptic soy broth media for 24 hours in an agitating water bath (37 ºC, 30 RPM). Coupons had a surface area of approximately 1 cm^2. For biofilm debridement, each coupon was irrigated with 3 L of normal saline solution using pulse lavage irrigation set at the high setting (Zimmer, Warsaw, IN). The nozzle was kept perpendicular to the surface at a range of approximately 1–3 cm from the coupon surface and moved in a random but equal fashion over a single coupon as determined by a single operator over the entire surface to simulate common conditions in the operating room.

Coupon Fabrication

Careful attention was placed to ensure the surface of the cobalt chrome had a comparable surface smoothness to an unused TKA implant as surface roughness was likely to affect biofilm affinity. After surface grinding, lapping, and polishing, the coupons surface roughness measurements were taken using an Ambios (Santa Cruz, CA) Xi-100 non-contact (interferometer type) profiler with Ambios Image Studio software. The Xi-100 had a maximum vertical resolution of 0.2 nm which was sufficient for accurately assessing orthopaedic joint replacement metallic bearing surfaces average roughness and maximum peak-to-valley distance per American Society for Testing and Materials standard (ASTM) F2083[26].

PMMA coupons were fashioned from medium viscosity bone cement (SmartSet; Depuy Orthopaedics, Warsaw, IN) without the addition of antibiotics in molds to match the dimensions of the cobalt chrome coupons. Ultra-high molecular weight polyethylene patella implants (Sigma oval domed patella single peg; Depuy Orthopaedics, Warsaw, IN) of similar dimensions were used for the polyethylene coupons.

Bioluminescent Imaging

The bioluminescent signal of the luciferase transfected *S. aureus* was imaged with a Xenogen IVIS 100 (Caliper, Waltham, MA) and the

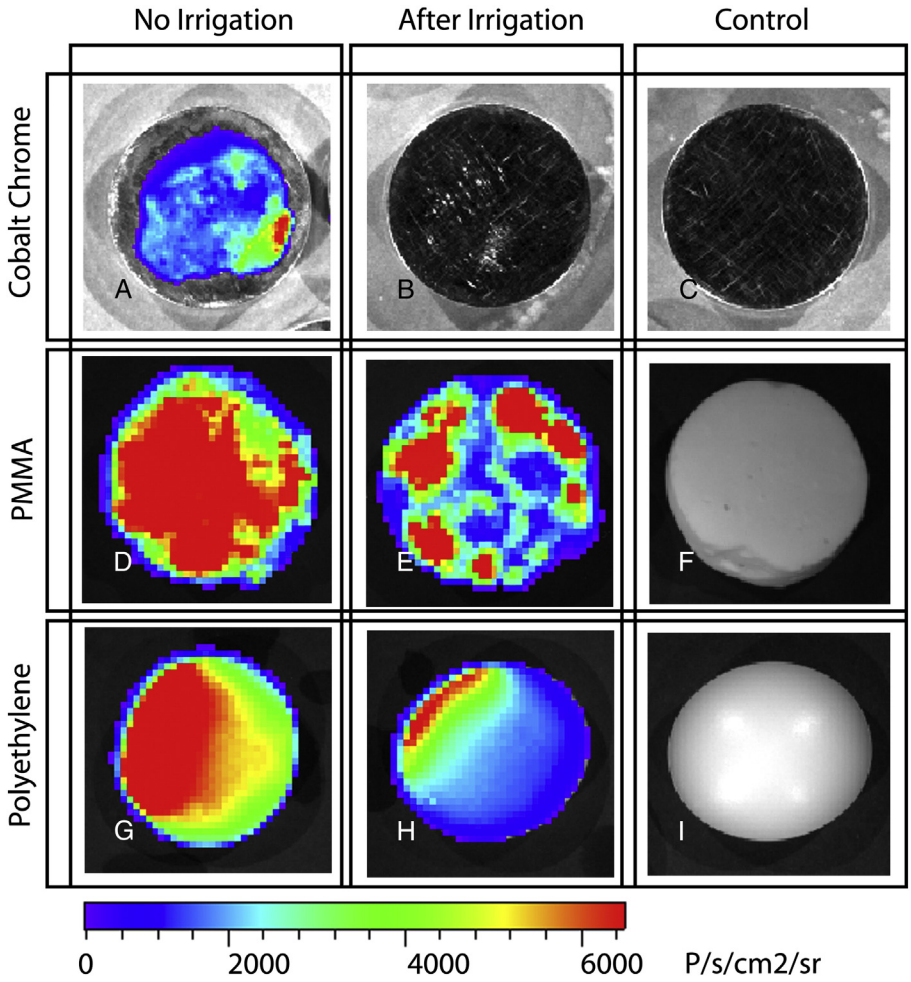

Fig. 1. Biofilm bioluminescent signal remains on TKA components following pulse lavage irrigation. The biofilm mass of *S. aureus* transfected with the luciferase gene can be measured using bioluminesence imaging. (A) A strong biofilm signal was present on the non-irrigated cobalt chrome. (B) Following pulse lavage, the biofilm signal on the cobalt chrome coupons was below the range of the heat-map scale as compared to non-irrigated and irrigated materials. (C) A control cobalt chrome coupon is provided for comparison. (D and E) A strong biofilm signal was present on non-irrigated PMMA and remained after 3 L of pulse lavage irrigation. (F) A control PMMA coupon is provided for comparison. (G and H) A similar pattern was observed with non-irrigated and irrigated polyethylene. (I) A control polyethylene is provided for comparison.

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bioluminescent signal intensity was quantified. After 24 hours of biofilm growth, cobalt chromium, PMMA, and polyethylene coupons were imaged at both 10 and 30 seconds for luminescence signal intensity. Samples were irrigated with pulse lavage as described above and then re-imaged. Control groups were re-measured without irrigation to verify no loss of signal intensity.

Confocal Microscopy

Microscopy was performed using a laser-scanning confocal imaging system (Leica TCS SP2 AOBS; Leica Microsystems Heidelberg GmbH, Heidelberg, Germany). Image stacks were collected using FITC (490 nm excitation and 525 nm emission) and TRITC (557 excitation and 576 nm emission) beam path settings using a 63× objective lens and 0.35 mm step size across the z-axis for a stack of 30 images. Laser power and pin hole settings remained constant between washed and unwashed coupons. Live/Dead stain (0.02 M SYTO 9, 0.06 M propidium iodide; Moleculer Probes, Eugene, OR) was used according to manufacturer instructions with volumes doubled for the Syto 9 staining. After coupons were imaged at three locations, disks were irrigated as described above and then re-stained using identical parameters.

Image Analysis

Cell counts were made using ImageJ (version 1.45j, National Institute of Health; http://rsb.info.nih.gov/ij). Cells were identified by particles 15–100 pixels in area after segmentation with manual threshold and application of watershed filters.

Statistical Analysis

Data are expressed as a mean ± standard error except where noted. All statistical tests were completed using R (R Core Development Team, www.r-project.org). Direct comparisons between two cell populations were made using an unpaired, Student's *t* test. Multiple group comparisons were made using two-way analysis of variance. In both cases, significance levels were determined using the Student-Newman-Keuls pairwise comparison. Statistical significance was determined if $p < 0.05$.

Results

To test the hypothesis that pulse lavage was ineffective at removing biofilm from TKA components, bioluminescence *S. aureus* was used to macroscopically visualize and quantify biofilm debridement (Fig. 1). A large bioluminescent signal could be visualized following pulse lavage on PMMA and polyethylene. The signal on the cobalt coupons was below the visible threshold scale to allow comparison to the other materials, but still did have a signal above control comparisons as discussed below.

The bioluminescent signal of the biofilm can be quantified. The ratio of bioluminescent signal before and after pulse lavage irrigation was measured (Fig. 2). The PMMA and polyethylene coupons had an approximate one order of magnitude (ten-fold) reduction in signal, and had a statistically significant decreased reduction in signal as compared to the cobalt chrome coupons. The polyethylene patella and PMMA coupons had a comparable loss of bioluminescent biofilm signal.

Confocal microscopy was used as an additional method to measure the level of biofilm debrided following pulse lavage irrigation (Fig. 3). Cobalt chrome coupons were selected for confocal microscopy analysis given the low signal intensity following irrigation to image the surface and verify results. A reduction in cell density and viability between non-washed and washed coupons could be visualized for the metallic coupons (Fig. 3A and B, respectively). The biofilm cell density and depth had an approximate 50% reduction after washing (Fig. 3C and D, respectively).

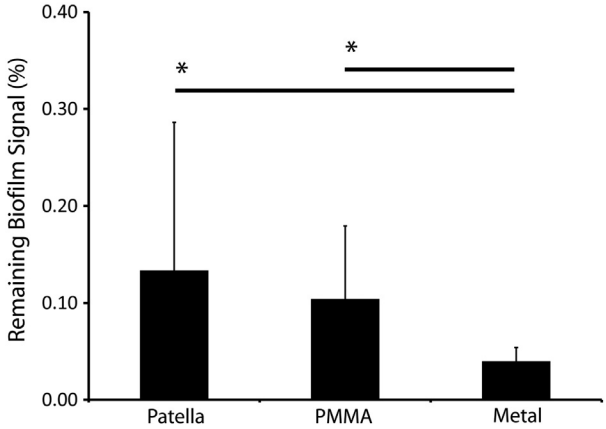

Fig. 2. A persistent mass of biofilm remains after irrigation. The remaining biofilm signal after pulse lavage irrigation was quantified as a percentage of the initial biofilm signal on the coupon before irrigation. A value of one would represent no biofilm mass removal. *$P<.05$.

Discussion

The establishment of a biofilm on TKA components is believed to be responsible for the high failure rate of single stage irrigation and debridement with component retention. The inability of pulse lavage to remove substantial mass of biofilm from TKA components is anecdotally held, but there is a remarkable absence of evidence supporting this hypothesis in the literature. Our results used direct visualization and quantification of the remaining biofilm to demonstrate pulse lavage irrigation debrides a large mass of biofilm from the surface of TKA materials, but also suggest that a substantial mass of biofilm still remains. It also suggests that the surface of highly polished cobalt chrome has a larger volume of biofilm debridement than the surfaces of polyethylene and PMMA.

Similar findings have been supported by other groups. The measured biofilm removal by manually scrapping the biofilm from titanium coupons and quantifying colony forming units is approximately 2 orders of magnitude (100-fold) [25]. This is similar to our results. Limitations in their technique include the lack of accuracy from direct visualization, the absence of sonication using only rough mechanical scraping to remove a portion of the biofilm, and not culturing biofilm on the other main components used in TKA, cobalt chrome, PMMA, and polyethylene. An additional advantage of the bioluminescence imaging includes the macroscopic visualization of biofilm signal levels across the entire coupon surface.

Caution should be used when extrapolating results from an in vitro model to a clinical scenario. Our conditions may not accurately reflect the actual level of clinical debridement and biofilm disruption. The in vitro model may overestimate biofilm debridement compared to a clinical setting by placing the pulse lavage directly over the known location of the biofilm for a prolonged period of time. The previous use of sonication to identify organisms in infected implants indirectly demonstrates the existence of a biofilm on infected components [27,28]. Isolated case reports have imaged biofilm on the surrounding tissue of infected ankle and elbow arthroplasty [29,30], but not directly on the surface of the implants.

Pulse lavage irrigation of TKA components removes a substantial mass of biofilm, and our results support its continued use in the operating room. Our results also suggest that a sufficient volume of biofilm is unlikely to be debrided to consistently eradicate infection.

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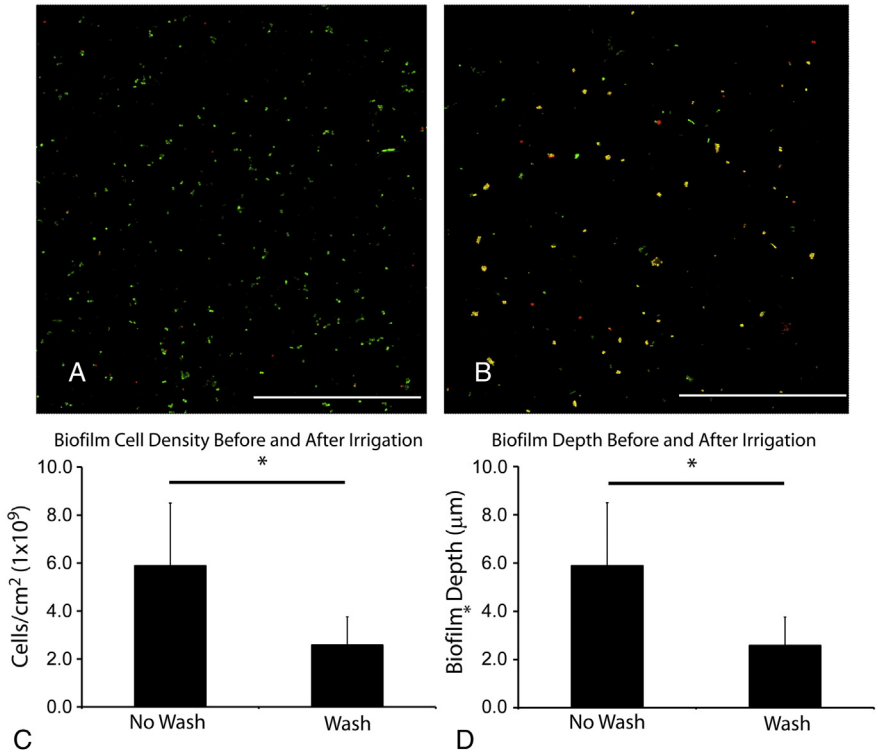

Fig. 3. *S. aureus* biofilm imaged and quantified with confocal microscopy before and after pulse lavage irrigation. (A and B) Laser scanning confocal microscopy was used to image biofilm on cobalt chrome surfaces before and after irrigation with pulse lavage, respectively. A critical mass of biofilm remained after irrigation. Viable bacteria appear green and nonviable bacteria stained either red or yellow (from overlapping red and green signals). Scale bars represent 100 μm. (C) The viable biofilm cell density was quantified before and after irrigation demonstrating a reduction in biofilm mass. (D) Similar results were observed measuring biofilm depth before and after irrigation. *P<.05.

The minimum microbe inoculation density for a TKA to become infected, the approximate biofilm mass of an infected TKA, and the required reduction in mass of biofilm to have a consistent successful single stage treatment of periprosthetic infection are all unknown. We postulate that the bioburden of an infected TKA would need to be reduced by several orders of magnitude to prevent re-infection. This emphasizes the important role of antibiotics in the treatment of periprosthetic joint infection. In current clinical practice, these results support the importance of a meticulous debridement of the synovium and exchange of the modular polyethylene to decrease as much of the bioburden as possible.

We were unable to achieve more than a one order of magnitude (ten-fold) reduction in biofilm mass. This supports the concept that the failure of antibiotic therapy after an open irrigation and debridement involves, in part, an initial inability to sufficiently debride a pre-existing biofilm. Conventional therapy has focused on using antibiotics to kill the remaining microorganisms and has not optimized the debridement of the biofilm. To improve the success of irrigation and debridement with exchange of the modular polyethylene bearing surface in periprosthetic joint infections, new techniques and technologies that increase biofilm debridement need to be developed. New mechanical and chemical methods that respect the soft tissue envelope of the joint capsule and improve biofilm removal could have a valuable clinical impact.

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References

1. Bozic KJ, et al. The epidemiology of revision total knee arthroplasty in the United States. Clin Orthop Relat Res 2009;468(1):45.
2. Quintana JM, et al. Prevalence of knee and hip osteoarthritis and the appropriateness of joint replacement in an older population. Arch Intern Med 2008;168(14):1576.
3. Berger RA, et al. Long-term followup of the Miller-Galante total knee replacement. Clin Orthop Relat Res 2001;388:58.
4. Indelli PF, et al. The Insall-Burstein II prosthesis: a 5- to 9-year follow-up study in osteoarthritic knees. J Arthroplasty 2002;17(5):544.
5. Keating EM, et al. Long-term followup of nonmodular total knee replacements. Clin Orthop Relat Res 2002;404:34.
6. Kurtz S, et al. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007;89(4):780.
7. Fehring TK, et al. Early failures in total knee arthroplasty. Clin Orthop Relat Res 2001;392:315.
8. Vessely MB, et al. The Chitranjan Ranawat Award: Long-term survivorship and failure modes of 1000 cemented condylar total knee arthroplasties. Clin Orthop Relat Res 2006;452:28.
9. Wilson MG, Kelley K, Thornhill TS. Infection as a complication of total knee-replacement arthroplasty. Risk factors and treatment in sixty-seven cases. J Bone Joint Surg Am 1990;72(6):878.
10. Brandt CM, et al. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. Clin Infect Dis 1997;24(5):914.
11. Deirmengian C, et al. Open debridement of acute gram-positive infections after total knee arthroplasty. Clin Orthop Relat Res 2003;416:129.
12. Hartman MB, et al. Periprosthetic knee sepsis. The role of irrigation and debridement. Clin Orthop Relat Res 1991;273:113.
13. Marculescu CE, et al. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis 2006;42(4):471.
14. Mont MA, et al. Multiple irrigation, debridement, and retention of components in infected total knee arthroplasty. J Arthroplasty 1997;12(4):426.
15. Rand JA. Alternatives to reimplantation for salvage of the total knee arthroplasty complicated by infection. J Bone Joint Surg Am 1993;75(2):282.
16. Rasul Jr AT, Tsukayama D, Gustilo RB. Effect of time of onset and depth of infection on the outcome of total knee arthroplasty infections. Clin Orthop Relat Res 1991;273:98.
17. Schoifet SD, Morrey BF. Treatment of infection after total knee arthroplasty by debridement with retention of the components. J Bone Joint Surg Am 1990;72(9):1383.
18. Bradbury T, et al. The fate of acute methicillin-resistant *Staphylococcus aureus* periprosthetic knee infections treated by open debridement and retention of components. J Arthroplasty 2009;24(6 Suppl):101.

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19. Insall JN, Thompson FM, Brause BD. Two-stage reimplantation for the salvage of infected total knee arthroplasty. J Bone Joint Surg Am 1983;65(8):1087.
20. Peel TN, Buising KL, Choong PF. Prosthetic joint infection: challenges of diagnosis and treatment. ANZ J Surg 2011;81(1–2):32.
21. Neut D, et al. The role of small-colony variants in failure to diagnose and treat biofilm infections in orthopedics. Acta Orthop 2007;78(3):299.
22. Hadi R, et al. Biofilm removal by medical device cleaners: comparison of two bioreactor detection assays. J Hosp Infect 2010;74(2):160.
23. Steinberg D, et al. Effects of various antiplaque agents on fructosyltransferase activity in solution and immobilized onto hydroxyapatite. Eur J Oral Sci 2002;110(5):374.
24. Brindle ER, Miller DA, Stewart PS. Hydrodynamic deformation and removal of Staphylococcus epidermidis biofilms treated with urea, chlorhexidine, iron chloride, or DispersinB. Biotechnol Bioeng.
25. Schwechter EM, et al. Optimal irrigation and debridement of infected joint implants: an in vitro methicillin-resistant Staphylococcus aureus biofilm model. J Arthroplasty 2011;26(6 Suppl):109.
26. ASTM, 12 Standard Specification for Knee Replacement Prosthesis, in Standard F2083. 2013, ASTM International: West Conshohocken, PA.
27. Trampuz A, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357(7):654.
28. Nguyen LL, et al. Detecting bacterial colonization of implanted orthopaedic devices by ultrasonication. Clin Orthop Relat Res 2002;403:29.
29. Stoodley P, et al. Characterization of a mixed MRSA/MRSE biofilm in an explanted total ankle arthroplasty. FEMS Immunol Med Microbiol 2011;62(1):66.
30. Stoodley P, et al. Direct demonstration of viable *Staphylococcus aureus* biofilms in an infected total joint arthroplasty. A case report. J Bone Joint Surg Am 2008;90(8):1751.

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