



## 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.

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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.

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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

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 °C, 30 RPM). Coupons had a surface area of approximately 1 cm<sup>2</sup>. 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.

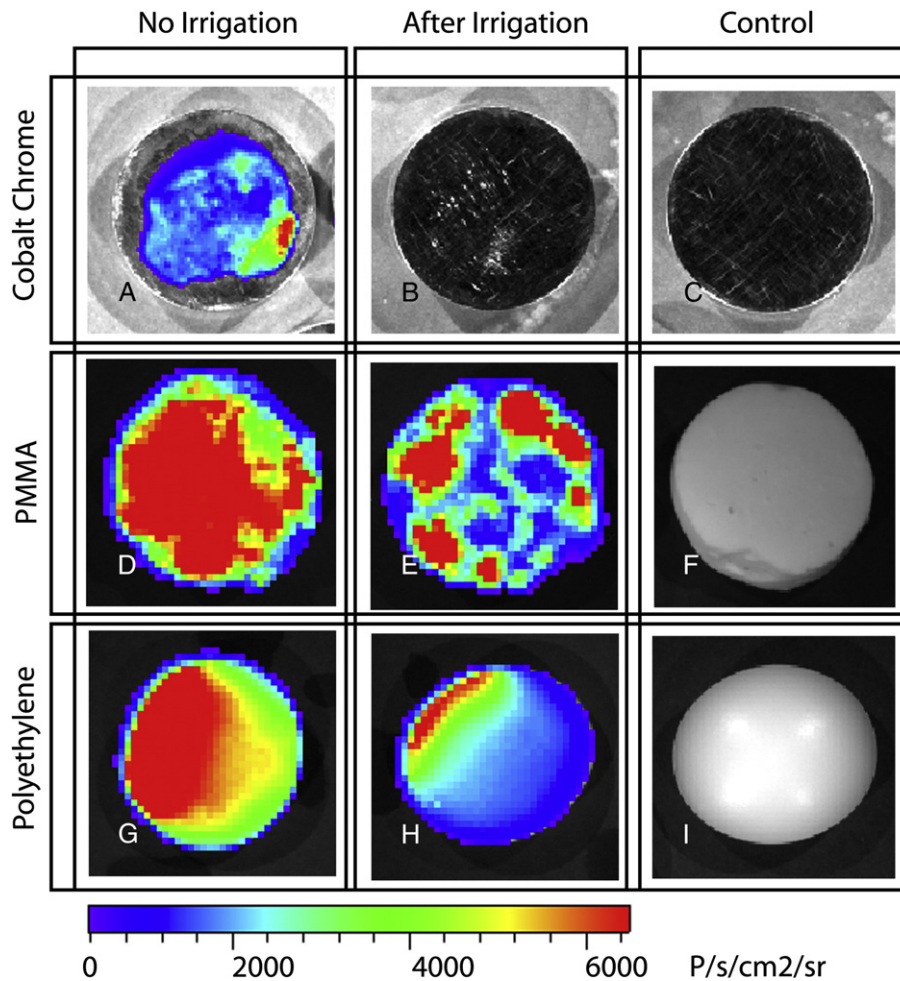
### Coupon Fabrication

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 bioluminescence 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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