Vitamin E Doped Highly Cross-Linked (HXL) UHMWPE Demonstrates Lower S. epidermidis and P. aeruginosa Adherence Compared to HXL Virgin UHMWPE

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Disclosures:

Introduction: Total joint replacement (TJR) therapy has become a routine practice in successful and predictable treatment of multiple joint-related disorders. However, one of the most common post-surgical complications following TJR is infection that causes septic loosening and requires revision surgery. Regardless of the etiology (intra-operative versus post-surgical), biofilm formation on ultra-high molecular weight polyethylene (UHMWPE) enables bacteria (particularly those residing in the deepest layers) to remain protected from the antibiotic treatment. The most common strains observed in TRJ infections include Staphylococcus species and Pseudomonas aeruginosa, both of which are well-known for their abilities to form biofilms [1]. Previous in vitro studies have compared the bacterial adherence on vitamin E containing UHMWPE versus virgin UHMWPE, demonstrating that S. aureus and S. epidermidis have lower adherence rates to vitamin E-containing UHMWPE [2,3]. The objective of our study was to determine if biofilm-producing strains of S. epidermidis and P. aeruginosa exhibited a decreased adherence rate to (1) virgin highly cross-linked UHMWPE, subjected to 100 kGy irradiation and melt-stabilized at 150°C, and (2) highly cross-linked UHMWPE containing vitamin E (approximately 0.8% by weight), diffused after 100 kGy irradiation.

Methods: UHMWPE: Virgin highly cross-linked UHMWPE, subjected to 100 kGy irradiation (PE) and melt-stabilized at 150°C, and highly cross-linked UHMWPE containing vitamin E (approximately 0.8wt%), diffused after 100 kGy irradiation (VE) were provided by Biomet. The samples were 10 mm in diameter and 2 mm in thickness. Two different types, “rough” and “smooth,” of surface roughness were used. The “rough” samples had a Ra of 2.57±0.88 µm, while the “smooth” samples had a Ra of 0.65±0.05 µm. Bacterial strains: Strains used in this study were S. epidermidis (ATCC 35984) and P. aeruginosa (ATCC 10145). Bacterial seeding: N=6 samples were seeded with each type of bacteria for each timepoint. For the first experiment, rough samples were incubated for 24 and 72h. For the second experiment, smooth samples were incubated for 24h only. Briefly, S. epidermidis was cultured in Tryptic Soy Broth (TSB) while P. aeruginosa was cultured in Nutrient Broth (NB) at 37°C for 24 hours. Different bacterial strains were suspended and diluted to a concentration of 10^8 colony forming unit (CFU)/ml. This was determined by colony counting using Tryptic Soy Agar (TSA) and Nutrient Agar (NA) plates. UHMWPE discs were inserted into 48-well plates and 1 ml of diluted bacterial suspension was added to each disc. For samples incubated for 72h, an additional 0.1 ml TSB or NB added to wells after 48 h incubation according to specified bacteria. After incubation, discs were removed from wells and rinsed twice with PBS and inserted into a 15 ml polypropylene tube with 2 ml PBS. For biofilm detachment, a 4-step protocol was used: 1. 30 sec. vortex; 2. sonication at 40 Khz for 7 min.; 3. 30 sec. Vortex; 4. centrifuging at 3000 rpm for 5 min. Pre- and post-biofilm detachment was confirmed using SEM. After biofilm detachment, the PBS bacteria solution inside the polypropylene tubes was used to quantify bacteria using a spectrophotometer. Additionally, serial plate streaking was done either on TSA or NA plates and CFU were quantified. FTIR: To assess possible differences on chemical properties of discs, Fourier transform infrared spectroscopy (FTIR) was used before and after bacterial incubation. Thin sections (150 µm-thick) were analyzed using FTIR after extraction by boiling hexane for 16 hours. An oxidation index was determined by taking ratio of the area of peak at 1740 cm^-1 to 1370 cm^-1 (1330-1390 cm^-1). An average oxidation index was calculated throughout the depth of the samples. Statistical analysis: Groups were compared using an independent samples t-test with post-hoc Bonferroni’s correction for multiple comparisons. Data is reported as means +/- standard deviation; p value < 0.05 was considered significant.

Results: Bacterial strains were able to produce biofilms on the UHMWPE surfaces (Figure 1). On SEM, there were no visual differences in the biofilm structure produced in the vitamin E versus virgin UHMWPE. In addition, the vortexing-sonication-centrifugation protocol successfully removed biofilm from the UHMWPE, as confirmed by SEM imaging. For the first experiment using UHMWPE with the “rough” surface, there was a statistically significant difference in the amount of S. epidermidis adhered to vitamin E UHMWPE versus virgin UHMWPE. Less bacteria were found to adhere to vitamin E UHMWPE both at 24h (7.41x10^7 vs. 1.75x10^8 counts/ml; p<0.01) and 72h (1.54x10^8 vs. 3.86x10^8 counts/ml; p<0.05). The same trend was observed for P. aeruginosa both at 24h (7.67x10^7 vs. 1.67x10^8 counts/ml; p<0.05) and 72h (9.25x10^7 vs. 2.26x10^8 counts/ml; p<0.05) (Figure 2).
For the second experiment using the "smooth" UHMWPE surfaces, there was also a statistically significant difference at 24h for S. epidermidis and P. aeruginosa (1.25x10^8 vs. 5.83x10^7, p<0.01 and 1.24x10^8 vs. 4.14x10^7, p<0.01). There was no difference in the oxidation level between the pre-seeded (0.12) and post-incubation samples at 24 hours and 72 hours (0.10; p=0.008 and 0.10; p=0.009, respectively) as measured by FTIR.

**Discussion:** In this study we have demonstrated a decreased S. epidermidis and P. aeruginosa adhesion to vitamin E-doped highly cross-linked UHMWPE. UHMWPE samples utilized in this study followed the same manufacturing protocol (UHMWPE molding, vitamin E doping and sterilization) as the one used for acetabular and tibial inserts that are in current clinical use. Other studies have also reported a similar trend in regards to bacterial adhesion using non-cross-linked UHMWPE, where vitamin E reduced bacterial adhesion. In the current study, with cross-linked UHMWPE we observed similar bacterial adhesion trends, including P. aeruginosa, which had not been analyzed previously. We investigated smooth and rough surfaces to represent the articular and backside surfaces of a polyethylene component, respectively.

**Significance:** Vitamin E-doped UHMWPE causes a decreased adhesion of S. epidermidis and P. aeruginosa after 24h and the impact is stable over 72h, suggesting that vitamin E containing polyethylene components may be less prone to bacterial adhesion.

**Acknowledgments:**

**Figure 1.** SEM images were obtained to qualitatively assess the biofilm formation of (a) *S. epidermidis*, X5080 magnification, (b) *P. aeruginosa*, X12160 magnification. (Scale bar = 2 μm for both).

**Figure 2.** A statistically significant difference was encountered regarding the amount of *S. epidermidis* and *P. aeruginosa* adhered to VE (vitamin E UHMWPE) versus PE (virgin UHMWPE) at 24 and 72 hours. *p < 0.01* and **p < 0.05**.