INTRODUCTION:
Prosthetic joint implant-associated infections caused by methicillin-resistant Staphylococcus aureus (MRSA) are especially difficult to resolve with antimicrobial therapy due to the organism’s propensity to form biofilms on implant surfaces. Previous in vitro studies of MRSA biofilms on titanium (Ti) or polymethyl methacrylate (PMMA) bone cement were conducted with biofilms grown in nutrient rich bacteriological broth. Nutrients in growth media impact bacterial biofilm formation and maturation[1] and regulate virulence in MRSA [2]. The purpose of this study was to examine the development of MRSA biofilms growing on Ti and PMMA in a medium that would more closely mimic human synovial fluid [3] and to evaluate the susceptibility of these biofilms to vancomycin.

METHODS:
MRSA strain KRH1 was isolated from a septic knee implant (MIC for vancomycin < 1 µg/ml). Ti and PMMA disks were incubated with gentle rocking for 24 hours at 37°C in trypticate soy broth (TSB) or synovial fluid simulant (1% w/v sodium hyaluronate in physiological saline, 25% v/v fetal calf serum, 1 mg/ml glucose; SYN) inoculated with 1 X 10^4 log phase MRSA. After incubation, samples of TSB and SYN (N=4/substrate) were collected, serially diluted, and aliquots plated in triplicate on trypticase soy agar (TSA) plates to determine how well SYN supported growth of MRSA. Disks were rinsed with 20 ml phosphate buffered saline (PBS, pH 7.2) from a syringe and then incubated for a further 24 hours in TSB, SYN, or containing either 10 µg/ml (the recommended trough concentration for treating septic joints) or 100 µg/ml vancomycin (N ≥ 4 disks of each material/treatment). Biofilms were harvested with glass beads in PBS [4], supernatants were serially diluted, and aliquots plated in triplicate on TSA plates. Plates were incubated at 37°C overnight. Results of growth in TSB vs. SYN expressed as mean log_{10} CFU of MRSA /ml ± standard error of the mean. MRSA colonies on the substrates were expressed as the mean log_{10} CFU of MRSA strain KRH1/mm² of Ti or PMMA ± standard error of the mean. Means were compared with a two-tailed unpaired t test with significance set at P ≤ 0.05.

RESULTS:
After 24 hours incubation at 37°C there were no significant differences (P ≥ 0.1) in the numbers of planktonic MRSA in TSB containing Ti (8.1 ± 0.1) or PMMA (7.7 ± 0.2) disks. Similarly, there were no significant differences (P ≥ 0.6) in the numbers of planktonic MRSA in SYN containing Ti (6.4 ± 0.5) or PMMA (6.1 ± 0.5) disks. However, there were significantly fewer (P ≤ 0.01) CFU of planktonic MRSA/ml growing in SYN vs. TSB, regardless of what substrates they contained, indicating that SYN does not support growth of MRSA to the same extent as TSB.

The numbers of MRSA adhering to substrates as a biofilm after 24 or 48 hours incubation are shown in Figure below. The numbers of MRSA adhering to substrates as a biofilm after treatment with either 10 or 100 µg/ml vancomycin in SYN are shown in the Figure below.

DISCUSSION:
In this study, MRSA did not thrive or form robust biofilms in a nutritional environment approximating human synovial fluid, the medium bathing natural and prosthetic joints, when compared to growth and biofilms formation in a rich medium formulated for optimal bacterial growth. The anti-staphylococcal properties of synovial fluid have been recognized [5] but any mechanism for this phenomenon remains unknown. The reduced ability to form biofilms on orthopedic materials in the synovial fluid simulant highlights the potential for the development of new treatment modalities.

REFERENCES: