INTRODUCTION: Implant associated infections contribute to increased patient morbidity and cost. We have previously reported that surface modification with certain hydrophobic polycations, such as N,N-dodecyl,methyl-polyethyleneimine, renders implants bactericidal. Adhesion of serum proteins to the implant and low vascularity in the area of trauma create an ideal environment for bacterial adherence. Within a biofilm, bacteria synthesize an extracellular matrix that protects them from the host’s immune response and systemic antimicrobials. Typical treatments include extensive local tissue debridement, prolonged systemic and targeted local antimicrobial therapy. Frequently the infected device must be removed to fully resolve the problem. Localized antimicrobial delivery systems have been developed in attempt to prevent implant associated infections including poly(methyl methacrylate) cements, biodegradable polymers, and regional limb perfusions. The elution kinetics of antimicrobials from these carrier systems are characterized by an initial supra-therapeutic release that ultimately drops below the minimal inhibitory concentration (MIC). Unfortunately, sub-MIC concentrations favor the emergence of drug-resistant bacterial strains. Quorum sensing and altered gene expression may further contribute to antimicrobial resistance. Importantly, bacterial colonization onto substrates appears to be the critical step in biofilm formation. We have focused our attention on surface modifications that inhibit the adherence of bacteria to implants and thereby prevent the root cause of orthopedic infections. We hypothesized that coating orthopedic fracture plates with certain hydrophobic polycations could favorably influence bone healing in a large animal fracture infection model.

MATERIALS AND METHODS: Twelve mature Polypay Ewes were enrolled in a prospective study using a previously validated long bone infection model. Under general anesthesia a medial approach to the left tibia was used. A unilateral mid-diaphyseal transverse tibial osteotomy was performed. The osteotomy was reduced using a narrow 4.5mm eight hole stainless steel 316L locking compression plate (LCP). Prior to closure a 14g catheter was placed at the osteotomy site and after soft tissue closure, 10⁶ CFUs of Staphylococcus aureus ATCC25923 were inoculated. Six sheep received a HPC coated implant (treatment cohort) and the remaining six animals received a non-coated implant (control cohort). Implants were aseptically coated intraoperative in a three step procedure. Aliquots of LCPs were run in parallel with each surface modification in vitro verification of adequate HPC coating. Radiographs were obtained immediately postoperative and at one month before euthanasia. Radiographs were scored by three blinded reviewers for presence of osteomyelitis and callus morphology. The left hind limb was harvested and aseptically prepared for implant retrieval. A sterile culture was taken before implant removal. Both the overlying soft tissue and bone were scored for presence of infection and bone remodelling by a blinded observer. The implants were removed and sectioned for ex vivo analysis. Select tibiae underwent µ-CT for qualitative 3-D reconstructions. The osteotomy region was harvested and processed for histology and sections were scored by a blinded veterinary pathologist. Plate pieces were processed, sputter-coated and viewed in the SEM at 1500x and 3000x. Statistical analysis was carried out on radiographic, histological and explant scores. A paired t-test was used to form preliminary associations and a statistical significance of p<0.05 was used for all tests. RESULTS: All animals completed the study. Radiographic evaluation revealed significantly greater healing and bony remodelling consistent with normal “fracture healing” in treatment animals compared to controls (p<0.05). Gross evaluation revealed the osteotomy sites in control animals to be grossly unstable with evidence of infection. Overall, the explant scores evaluating the osteotomy site and surrounding soft tissue envelope were significantly lower for the treatment cohort when compared to the controls (p<0.05). The 3-D reconstructed micro-CT images of the control tibiae supported our data from radiographic and gross observations with evidence of a poorly organized, septic callus. Histological evaluation showed evidence of a smooth bridging and remodeling callus in the treatment cohort. In control animals there was evidence of a non-union of the osteotomy with significant abscessation and bacterial colonization present. Treatment animals had a significantly lower histology score when compared to controls (p<0.05) consistent with normal bone healing. SEM visualization of explanted LCPs displayed abundant biofilm formation covering >95% of the plate surface in control plates compared with no bacterial growth on HPC coated implants. DISCUSSION: Radiographic, histological and explant analysis confirmed our hypothesis that HPC coated implants would prevent bacterial implant colonization and result in normal remodeling of the osteotomy site when compared to infected control cohorts. SEM analysis of control surfaces revealed massive biofilm formation while visualization of HPC coating was devoid of bacteria. Major advantages of HPC surface modification are (1) the ease with which the coating can be applied intraoperatively to any geometry implant, (2) death of the bacteria by mechanical disruption of the cell wall is less likely to create multidrugresistant bacteria. The successful protection of implant surfaces from bacterial colonization could serve as a practical approach in reducing implant-associated infections in the orthopedic patient.

Significance: Conferring protection from pathogenic bacteria to an orthopedic implant of industrial size and geometry in vivo is promising for reducing implant-associated infections in the orthopedic patient. To our knowledge, this is the first report demonstrating safety and efficacy of a bactericidal surface modification methodology in a clinically relevant large animal model.

NEW

Significance (1-2 sentences): Please provide context for this study in terms of its significance and clinical relevance in your abstract.