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Confirming Sterility of an Autoclaved Infected Femoral Component for Use in an Articulated Antibiotic Knee Spacer: A Pilot Study

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ABSTRACT

Antibiotic spacer designs have proven effective at eradicating infection during a two-stage revision arthroplasty. Temporary reuse of the steam-sterilized femoral component and a new all poly tibia component has been described as an effective articulating antibiotic spacer, but sterility concerns persist. Six explanted cobalt chrome femurs from patients with grossly infected TKA's and six stock femurs inoculated with different bacterial species were confirmed to be bacteria-free after autoclaving under a standard gravity-displacement cycle. The effect of steam sterilization on cobalt chrome fragments contaminated with MRSA biofilm was analyzed microscopically to quantify remaining biofilm. The autoclave significantly reduced the biofilm burden on the cobalt chrome fragments. This study confirmed sterility of the femur after a standard gravity–displacement cycle (132 °C, 27 PSIG, 10 minutes).

In the setting of chronic prosthetic joint infection (PJI), the preferred method for treatment in the United States is a two-stage exchange arthroplasty with concomitant 6 week course of parenteral antibiotic [1,2,3,4,5-8]. The use of two stage exchange is now advocated for acute PJI where irrigation and debridement alone or one stage exchanges have failed [3]. There is an incredible variety of antibiotic spacer options that have proven to eradicate PJI and considerable debate exists around each method. All antibiotic spacers share common benefits of improving stability, reducing soft tissue contracture, and delivery of high concentrations of local antibiotic to the infected wound bed [2,9,10]. There are generally two categories of spacers: articulating and static. Benefits of the static cement only spacer include technical ease of implantation and low implant cost. Unfortunately, cement only static spacers can migrate and have reports of erosion through the extensor mechanism, anterior soft tissues, and surrounding bone [11]. This complication can be decreased by augmenting the construct with intramedullary pins or nails; however this offsets some of the cost benefit and still requires the patient to be in full extension which can alter patient satisfaction during treatment [11].

Articulating spacers have the benefit of improved patient function, pain, range of motion, ease of second stage dissection, and less incidence of bone loss and patellar baja [12-16]. Some authors also report improved knee PJI eradication rate with articulating spacers compared to static [17]. Obvious concern is the significantly increased cost compared to a cement only spacer whether static or articulated. All cement articulating spacers have the unfortunate complication of cement debris causing synovitis, persistent third body debris at time of the second stage, and increased OR time molding the implant [18].

One method proposed to reduce cost associated with articulating spacers is temporary reuse of the femoral component after steam sterilization in a standard autoclave [10,19-21]. While clinical data exist on the effectiveness of this technique in eradication of knee PJI, opponents to this method have concern of the effectiveness of immediate use steam sterilization of the component and its effect on the glycocalyx. This concern over sterility of the autoclaved femoral component has prevented the articulated antibiotic spacer from widespread adoption. The Center for Disease Control (CDC), association of operating room nurses (AORN), health care institutions, implant companies, and medical consult teams are understandably hesitant to temporarily reuse implants for medical, legal, and financial reasons [22,23]. Guidelines for temporary reuse in the setting of infection have not been addressed in the current CDC recommendations [22-24]. The current medical environment favors attempts to lower cost while maintaining or improving a standard of care. If we can prove that immediate use steam sterilization can render the femoral component sterile, we will secure this

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technique as a safe and cost-effective option to improve patient function during revision for infection.

As a pilot study, we hypothesize that an immediate use standard gravity–displacement cycle can render a contaminated femoral component sterile from bacterial pathogens and eliminate the biofilm.

Materials and Methods

Assessment of Bacterial Contamination of Patient Explants

Six separate patients with documented chronic total knee infection were referred to the clinic and elected to undergo two-stage revision arthroplasty. The six infected cemented femoral implants were removed from the patients in the operating room on separate operative days, packaged in a sterile container and transferred to the University of South Florida Microbiology Lab. The femurs were thoroughly swabbed with sterile swabs, followed by streaking onto blood agar plates. The explanted femurs were then packaged in a sterile container and autoclaved on a standard gravity–displacement vacuum cycle (121 °C, 15 PSIG, 45 minute cycle). Explants were again swabbed, before streaking on blood agar. Bacterial colonies were then allowed to form by incubation at 37 °C overnight.

Identification of Bacteria From Patient Explants

The identification of contaminating bacteria was determined via 16*S* rRNA gene sequencing, as described previously [25]. Briefly, isolated colonies obtained from blood agar plates were grown in trypticase soy broth (TSB) overnight at 37 °C. Genomic DNA was then extracted, and PCR amplification of 16*S* rDNA for each isolate was performed using forward primer 5′-TCC TAC GGG AGG CAG CAG T-3′ and reverse primer 5′-GGA CTA CCA GGG TAT CTA ATC CTG TT-3′. Resulting PCR products were purified using a Qiagen Gel Extraction Kit, according to manufacturer's instructions. Purified PCR products were subjected to Sanger sequencing (MWG Operon). Returned sequences were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/) to determine the identity of each isolate.

Implant In Vitro Test (121 °C, 15 PSIG, 45 minutes)

Six sterile cobalt chrome femur implants (2 cementless, 4 cemented) were separately inoculated with approximately 1×10^6 of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Klebsiella pneumoniae*, or *Acinetobacter baumannii*. These implants were then allowed to dry in a sterile environment for 15 minutes. After this time, three implants were subject to autoclaving on a standard gravity–displacement vacuum cycle (121 °C, 15 PSIG, 45 minutes), while the remaining implants were maintained in a sterile environment at room temperature. After the autoclaved implants had cooled, all devices were separately placed in a sterile, sealed plastic bag containing phosphate buffered saline (PBS). After the implants were subjected to 5 minutes of sonication in a water bath to disperse adhered bacteria, the PBS was serially diluted and plated on trypticase soy agar (TSA). These plates were then incubated overnight at 37 °C, and CFU determined via enumeration. The effect of autoclaving on bacterial viability was determined by comparing treated devices to control samples.

Implant In Vitro Test (132 °C, 27 PSIG, 10 minutes)

The above implant *in vitro* test (121 °C, 15 PSIG, 45 minutes) protocol was performed again on a standard gravity-displacement vacuum cycle (132 °C, 27 PSIG, 10 minutes).

Biofilm In Vitro Test

A biofilm assay was performed as previously described, with minor modifications [26]. Briefly, six cobalt chrome pieces were incubated with 20% human plasma in carbonate buffer overnight at 4 °C. The next day, the cobalt chrome pieces were placed in the wells of a 24-well plate, which were then filled with biofilm media containing around 1×10^6 of *S. aureus*. Biofilms were then allowed to form on cobalt chrome pieces by overnight incubation at 37 °C. After this time, three of the cobalt chrome pieces were autoclaved on a standard gravity–displacement cycle (121 °C, 15 PSIG, 45 minute cycle). Following treatment, all six fragments were thrice washed with PBS, fixed with 100% ethanol, and stained with crystal violet stain. The six cobalt chrome pieces were allowed to dry overnight at room temperature, and then the crystal violet stain was eluted from the cobalt chrome pieces with 100% ethanol. The amount of biofilm present on fragments was then determined by measuring the amount of retained crystal violet via OD_{595} nanometer readings using a BioTek Synergy 2 plate reader.

Scanning Electron Microscope

A biofilm assay was performed as previously described above, with the following modifications. Following overnight incubation with *S. aureus* in biofilm media, fragments were removed and allowed to air dry in a sterile environment for 15 minutes. After this time, 1 fragment was subject to autoclaving on a standard vacuum cycle (121 °C, 15 PSIG, 45 minute cycle). This piece was allowed to cool, and then both were separately fixed in 2.5% glutaraldehyde. Samples were imaged by scanning electron microscopy at the University of South Florida microscopy core facility, as described previously [27]. Images shown herein are representative of more than 5 separate frames per sample.

Results

Assessment of Bacterial Contamination of Patient Explants

Six patients were chosen for the in vivo arm of the study; 5 male 1 female, ages 47–80 (Table 1). All patients were diagnosed with a grossly infected TKA prior, based on labwork, radiographs and physical exam. Every patient had elevated inflammatory markers utilizing CRP and ESR. Furthermore every patient had a large effusion, which was aspirated, prior to any surgery to identify a bacteriological species. All six patients had positive cultures. Two patients (#1 and #2) had draining sinuses and three patients (#2, #3, and #5) had failed prior surgical intervention with a washout and polyethylene change. Five out of the 6 patients had had prior surgeries unrelated to the infection ranging from 2 to 6 procedures.

Agar plates streaked with swabs from the contaminated explanted femoral component (patients #1, #3, #4, and #5) grew multiple varied colonies of *Staphylococcus* species. Bacterial identity was confirmed by comparing results to the pre-operative aspiration which proved to be MRSA in 2 patients #1 and #3 while MSSA was cultured from patients #4 and #5. This was done via 16*S* rRNA gene sequencing, as described previously [25].

Agar plates streaked by swabs from all 6 of the femoral explants after autoclave showed no growth on any plate.

Implant In Vitro Test (121 °C, 15 PSIG, 45 minutes)

The diluted sonicate of the six inoculated femurs that underwent autoclave treatment at 121 °C, 15 PSIG, 45 minutes showed no grow on any agar plate. The diluted sonicate of the six control femurs that did not undergo autoclave treatment after inoculation demonstrated growth of multiple colonies of the original bacteria (Fig. 1A).