SHORT COMMUNICATION

Elution of antibiotics from poly(methyl methacrylate) bone cement after extended implantation does not necessarily clear the infection despite susceptibility of the clinical isolates

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One sentence summary: Manuscript demonstrates that ex situ antibiotic loaded bone cements elicit killing of medically important bacteria after extended implantation times in in vitro testing, the infection did not resolve.

ABSTRACT

Chronic orthopedic infections are commonly caused by bacterial biofilms, which are recalcitrant to antibiotic treatment. In many cases, the revision procedure for periprosthetic joint infection or trauma cases includes the implantation of antibiotic-loaded bone cement to kill infecting bacteria via the elution of a strong local dose of antibiotic(s) at the site. While many studies have addressed the elution kinetics of both non-absorbable and absorbable bone cements both in vitro and in vivo, the potency of ALBC against pathogenic bacteria after extended implantation time is not clear. In this communication, we use two case studies, a Viridans streptococci infected total knee arthroplasty (TKA) and a MRSA-polymicrobial osteomyelitis of a distal tibial traumatic amputation (TA) to demonstrate that an antibiotic-loaded poly(methyl methacrylate) (ALPMMA) coated intermedullary rod implanted for 117 days (TKA) and three ALPMMA suture-strung beads implanted for 210 days (TA) retained killing ability against Pseudomonas aeruginosa and Staphylococcus aureus in vitro, despite different clinical efficacies. The TKA infection resolved and the patient progressed to an uneventful second stage. However, the TA infection only resolved after multiple rounds of debridement, IV vancomycin and removal of the PMMA beads and placement of vancomycin and tobramycin loaded calcium sulfate beads.

Keywords: biofilm; chronic infection; antibiotic-loaded poly(methyl methacrylate); bone cement; MRSA; MSSA; Pseudomonas; elution; zone of inhibition; extended implantation

Antibiotic-loaded bone cement (ALBC) is commonly used for the prevention and treatment of orthopedic infections (Zalavras et al. 2004; Cui et al. 2007). ALBC serves to maintain joint space and/or fill bone voids, but also delivers high doses of antibiotics locally. Numerous studies have investigated the variables that may affect the elution kinetics of ALBC such as the total antibiotic load, type of bone cement, mixing methods, single versus multi-antibiotic loads, surface-to-volume ratio and...
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infection in her left knee. Until this point, the patient was admitted for I&D because of a swollen stump and drainage, and the beads, which had been implanted for 210 days, were removed. The wound underwent two additional I&Ds and one 10 cc pack of Stimulan high purity calcium sulfate bone filler beads mixed with 1000 mg of vancomycin and 400 mg of tobramycin were placed in the wound. Negative pressure wound therapy was applied in an inscissional fashion until the draining ceased.

The patient received IV vancomycin for six weeks and was then switched to Bactrim DS tablets for one year. Six months later, the patient shows no symptoms of infection and is doing well with the prosthesis.

The ALBC specimens were treated similarly in each case. That is, the specimens were collected in the operating room at the time of explantation in sterile plastic buckets and transported to the laboratory within 20 min. Each case specimen was immediately washed thrice with sterile room temperature (RT) phosphate buffered saline (PBS, 1 L total) to remove tissue debris and any transient or loosely adhered bacteria that may have contaminated the specimens during retrieval. For the PJI case, we first assessed the zone of inhibition (ZOI) of broken VT-PMMA pieces on brain heart infusion (BHI) agar plates (100 × 15 mm). We opted to use BHI media to promote and indicate the growth of any bacteria that might already be colonizing the VT-PMMA itself. Two plates were seeded with 100 \( \mu \)L of a 1:100 dilution of overnight grown luciferase-expressing methicillin susceptible \( S. \) \( aureus \) strain SAP229 (MSSA) or \( P. \) \( aeruginosa \) PA01 strain Xen41. Thus, in addition to the typical ZOI assay, which assesses a zone of clearing, we are able to assess antibiotic efficacy via luciferase activity, which is linked to metabolism. Then, an appropriately sized piece of VT-PMMA was placed in the center of the plate. The plates were incubated for 24 h, and we acquired digital camera images of the ZOIs (Figs 1A and B). The estimated radial ZOI in Figs 1A and B were 13.97 (±0.29) and 16.81 (±1.14) mm against MSSA and PA01, respectively. Interestingly, we observed the generation of individual PA01 colonies within the edge of the ZOI (Fig. 1A). This might indicate a propensity for ALPMMA to generate resistant or slow-growing mutants, or persisters (Kirisits et al. 2005; Fauvar et al. 2011). Luciferase imaging of the plates indicated zones of inactivity consistent with the ZOI (data not shown). This result highlights the importance of providing enough drug coverage through localized delivery to avoid surrounding zones where the antibiotic concentration is adequate to kill most bacteria but low enough to allow the development of resistant mutants.

It was possible that the ZOI measurement of the VT-PMMA broken pieces was augmented because the pieces had newly exposed surfaces allowing the release of antibiotic that had yet to elute from the cement in vivo. Therefore, we repeated the ZOI assay with a VT-PMMA-coated femoral rod, which had not been broken during explantation. For the rod, we poured a fresh BHI agar plate (150 × 20 mm) infused with Congo red dye (0.1%, to check for the possibility of contamination). For this assay, Congo
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Figure 1. Zones of inhibition for PJI (1A and B, 117 day implant) and TA case (1C-E, 210 day implant). Antibiotic-loaded PMMA (ALPMMA) after 24 h incubation, respectively. (1A) Two different broken pieces of ALPMMA against MSSA and P. aeruginosa. (1B) ALPMMA coated intermedullary rod against MSSA (left-hand side) and P. aeruginosa (right-hand side). (1C and E) Closeup and full-plate perspective of ALPMMA bead against MSSA. (1D) Luciferase image of ZOI assay depicting a dark zone directly around the ALPMMA bead and intense light production at the ZOI-growth interphase.

red dye was included as a contrast agent between the offwhite cement and growth media to further help indicate if the VT-PMMA specimen was contaminated with any transient/resistant bacteria. In addition, due to the relatively large size and shape of the rod, and the fact that we only had one rod specimen to test, we thought it would be a novel and optimal approach to seed one half of the BHI agar plate with MSSA and one half with P. aeruginosa. We took care to avoid overt mixing of the MSSA and P. aeruginosa during seeding. Figure 1B demonstrates that the unbroken rod also retained killing ability, most notably in the center of the rod (away from the ends), for which we measured a ZOI of 13.68 (±1.03) and 12.13 mm versus PA01 and MSSA, respectively. One end of the rod did develop a crack (bottom of image Fig. 1B) in the VT-PMMA coating and a small increase in ZOI was observed against MSSA, but not P. aeruginosa. Nevertheless, the VT-PMMA continued to elicit killing after 117 days in vivo.

For the TA case, which consisted of three V-PMMA oblong suture-strung beads, we elected to test the ZOI using only 1/3 beads explanted from the patient. The other two beads were used for additional, yet unrelated analyses (data not shown). The VT-PMMA bead was treated similarly to the PJI case, whereas, we tested the ZOI on a large BHI agar plate, but only against MSSA. We opted to leave out Congo red dye because (1) we did not observe growth with our previous test on the VT-PMMA rod (Fig. 1B), and (2) our luciferase-expressing MSSA strain had not previously been optimized during growth on Congo red dye. Figure 1C and E demonstrate a small yet notable ZOI measuring 11.01 (±2.18) mm from the center of the bead. Figure 1D is a luciferase image demonstrating the light production by the MSSA (SAP229) strain. The use of SAP229 for this experiment provided an additional and novel way of determining antibiotic efficacy since luciferase expression is linked to cellular metabolism. Here, we note that the most intense light production occurs just immediately at the ZOI-growth interface. Although this is intriguing, we presume this to be an artifact of increased nutrient availability in the adjacent uninhabited ZOI.

This two-case analysis presents data that suggests that ALBC, in various orthopedic conformations, may retain the ability to kill planktonic bacteria after extended periods of time within the body, particularly at close proximity to the ALBC surface. One study reported that the minimum inhibitory concentration (MIC) drops below therapeutic levels at 14 and 17 days for gentamicin and vancomycin, respectively (Kelm et al. 2006). However, the MIC was assessed in liquid medium where the drug is equally distributed (Kelm et al. 2006). Using the agar diffusion method, rather than assessing elution into a liquid,
we determined that the cements continued to generate MIC concentrations at the cement surface. These discrepancies are reminiscent of Adams and colleagues results citing varying antibiotic concentrations depending on the tissue medium sampled (i.e. serum versus bone) (Adams et al. 1992). The intraoperative cultures obtained for the PJI case at the time of spacer explantation were negative, indicating that the VT-PMMA spacer, and the six weeks of penicillin treatment was successful in eradicating the viridians streptococci infection. For the TA case, however, the intraoperative cultures obtained at the time of VT-PMMA bead implantation were positive for a polymicrobial infection including MRSA and sensitive P. aeruginosa and S. marcescens. Interestingly, the antibiogram reported for the MRSA strain isolated for the TA case consistently showed that the strain was susceptible to vancomycin. Thus, it appears while the Gram-negative bacteria were eradicated by the VT-PMMA beads, I&D and IV vancomycin failed to clear the MRSA in vivo; a scenario for which we propose two explanations. First, it is possible that the MRSA infection was localized elsewhere and/or beyond the efficacious range of the VT-PMMA beads in vivo, and indeed, the observed ZOI for the VT-PMMA bead was relatively small. In another scenario, it is possible that the infecting MRSA existed in vivo in the biofilm state and was recalcitrant to antibiotic (Fux et al. 2003, 2005; McConoughey et al. 2014). Of course, a combination of the two explanations above could have occurred. Additionally, the fact that the infection resolved after the PMMA bead removal suggests that it is possible that biofilm developed on the VT-PMMA itself, as has been shown previously (Stoodley et al. 2008). The PMMA itself then becomes an additional foreign body for attachment of bacteria. Unfortunately, due to limited material we were not able to directly examine the surface of the beads for biofilm. However, the infection meets a number of criteria for a biofilm infection. The infection was chronic, localized and impossible to eradicate with antibiotics in the presence of a persisting foreign body despite demonstrated susceptibility of the cultured pathogen (Parsak and Singh 2003). It is interesting that the infection resolved after the removal of the PMMA beads, which were replaced with absorbable calcium sulfate beads. Absorbable cements negate the issue of presenting a residing foreign body for potential future biofilm formation but cannot be used for mechanical function due their being much weaker than PMMA. (Howlin et al. 2015). For this study, we addressed the killing efficacy of expanded ALBC against planktonic P. aeruginosa and/or S. aureus. We chose these organisms because they represent model organisms for chronic infections, particularly chronic orthopedic infections. We did not address, however, the antimicrobial efficacy of ALPMMA against bacterial biofilm. Because each specimen was unique, it was not possible to retest the specimens against bacterial biofilm and compare the potency of VT-PMMA explants between planktonic versus biofilm bacteria. In the future, it would be useful, and perhaps more relevant, to test ALPMMA explants against preformed biofilms of medically important bacteria. It should be noted that, for this work, our IRB did not permit recovery of clinical cultures; thus, we remained blinded to the clinical culture data until our research was completed, but in the future it would useful to conduct ALBC assays against the clinical isolates in both planktonic and biofilm modes of growth. In closing, this work reiterates the pressing need for research in the area of antimicrobial resistance and biofilms in human medicine, as well as the elution kinetics and efficacy of ALBC under physiological conditions against planktonic and biofilm bacteria alike.

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Conflict of interest. None declared.

REFERENCES


