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Effects of loading concentration, blood and synovial fluid on antibiotic release and anti-biofilm activity of bone cement beads

Devendra H. Dusane [a,*,1], Scott M. Diamond [b,1], Cory S. Knecht [b,1], Nicholas R. Farrar [c,1], Casey W. Peters [d], Robert P. Howlin [e], Matthew C. Swearingen [a], Jason H. Calhoun [f], Roger D. Plaut [g], Tanya M. Nocera [c], Jeffrey F. Granger [h], Paul Stoodley [a,h,i]

[a] Department of Microbial Infection and Immunity, The Ohio State University, Columbus 43210, USA
[b] Department of Medicine, The Ohio State University, Columbus 43210, USA
[c] Department of Biomedical Engineering, The Ohio State University, Columbus 43210, USA
[d] Department of Biochemistry, The Ohio State University, Columbus 43210, USA
[e] Centre for Biological Sciences, Faculty of Natural & Environmental Sciences & Institute for Life Sciences, University of Southampton, Southampton, UK
[f] Department of Muscoskeletal Sciences, Spectrum Health Medical Group, Grand Rapids, USA
[g] Division of Bacterial, Parasitic, and Allergenic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring 20993, USA
[h] Department of Orthopaedics, The Ohio State University, Columbus 43210, USA
[i] National Center for Advanced Tribology at Southampton (nCATS), Engineering and the Environment, University of Southampton SO17 1BJ, UK

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ABSTRACT

Antibiotic loaded cement beads are commonly used for the treatment of biofilm related orthopaedic periprosthetic infections; however the effects of antibiotic loading and exposure of beads to body fluids on release kinetics are unclear. The purpose of this study was to determine the effects of (i) antibiotic loading density (ii) loading amount (iii) material type and (iv) exposure to body fluids (blood or synovial fluid) on release kinetics and efficacy of antibiotics against planktonic and lawn biofilm bacteria. Short-term release into an agar gel was evaluated using a fluorescent tracer (fluorescein) incorporated in the carrier materials calcium sulfate ($CaSO_4$) and poly methyl methacrylate (PMMA). Different fluorescein concentrations in $CaSO_4$ beads were evaluated. Mechanical properties of fluorescein-incorporated beads were analyzed. Efficacy of the antibiotics vancomycin (VAN) or tobramycin (TOB) alone and in combination was evaluated against lawn biofilms of bioluminescent strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Zones of inhibition of cultures (ZOI) were measured visually and using an *in-vivo* imaging system (IVIS). The influence of body fluids on release was assessed using $CaSO_4$ beads that contained fluorescein or antibiotics and were pre-coated with human blood or synovial fluid. The spread from the beads followed a square root of time relationship in all cases. The loading concentration had no influence on short-term fluorescein release and pre-coating of beads with body fluids did not affect short-term release or antibacterial activity. Compared to PMMA, $CaSO_4$ had a more rapid short term rate of elution and activity against planktonic and lawn biofilms. This study highlights the importance of considering antibiotic loading and packing density when investigating the clinical application of bone cements for infection management.

1. Introduction

Orthopaedic periprosthetic joint infections (PJI) are difficult to treat with systemic antibiotic therapy and can lead to severe complications such as removal of the implant with functional loss of the affected body part or life-threatening conditions [1,2]. The extent of infection depends upon several factors, such as health of the patient, length of time of infection and condition of local soft tissues.

Antibiotic-loaded bone cement beads are a commonly-used option functioning to fill the dead space and managing infection for the treatment of PJI [3,4] (Fig. 1). Beads are made directly in the operating room from poly (methyl) methacrylate (PMMA) or mineral based formulations such as calcium sulfate ($CaSO_4$) [4–6].

PMMA was first used in a structural role in order to stabilize the implant or providing a spacer [7], but by adding antibiotics, surgeons have also used it in the form of spacers and beads to control infections. However, PMMA is dense, with compressive strength of >70 MPa, acrylic, and non-resorbing material, which generally must be removed in a second surgical procedure when its function has been fulfilled to avoid becoming a nidus for future infection [8,9]. $CaSO_4$ on the other hand assists

* Corresponding author.
E-mail address: Devendra.Dusane@osumc.edu (D.H. Dusane).
[1] Equal contribution.

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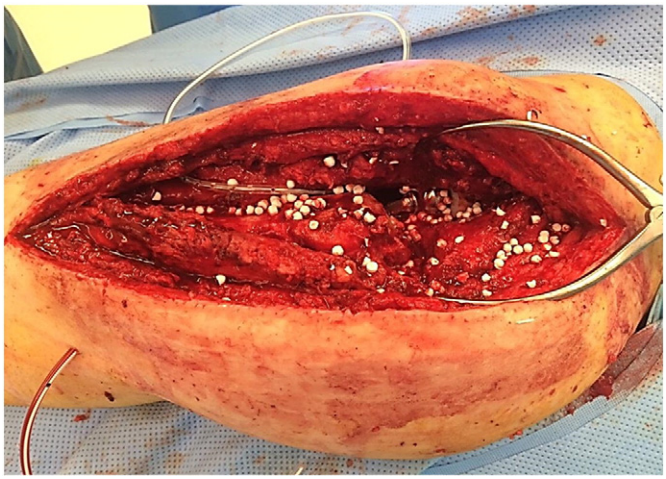

Fig. 1. Surgical site of infection showing antibiotic loaded calcium sulfate ($CaSO_4$) beads.

in the regeneration of bone, but is not utilized for structural integrity as it is a weak inorganic compound [10,11]. High purity synthesized $CaSO_4$ has the advantage of less adverse reactions in patients than with cements purified from mined mineral. It is also biodegradable, so that all antibiotic is released and no material remains and it cures at relatively low temperatures and is therefore compatible with a wide spectrum of antibiotics [12].

Biofilms are communities of microorganisms that colonize surfaces or exist as aggregates and play an important role in the pathogenesis of PJI [13]. In the case of PJI, biofilms are commonly associated with a foreign body such as a prosthetic [5,13]. Bacteria in a biofilm becomes highly tolerant to antibiotics as compared to the planktonic phenotype, therefore localized antibiotics delivery is needed to provide sustained high concentrations of antibiotics, that cannot be achieved systemically [14,15].

However, to achieve these concentrations, whether incorporating the required dose of antibiotics in few beads or distributing the dose in multiple beads to provide better release and efficacy is unclear. In a previous study, we reported that the zone of inhibition of *Pseudomonas aeruginosa* PAO1 from $CaSO_4$ carrier beads loaded with tobramycin (TOB) was similar regardless of whether beads were placed singly or in groups of 2, 3 or 4 (Fig. 2A) [5]. This is important since it shows that in diffusion limited environment, such as might be found in periprosthetic tissue or quiescent fluid, that the packing density of the beads is an important consideration in providing adequate coverage of the infected area. Further, multiple beads placed together also provide a multiplicity of antibiotic at a specific location. Moreover, when the release is diffusion limited for an absorbable material in which all antibiotic will be released it might be expected that the higher bead density would provide longer coverage.

Antibiotic release from loaded bone cement has been earlier reviewed [17,18]. The release of antibiotic from bone cement materials can be affected by factors such as type [19], preparation [20], surface characteristics [21], and porosity of the cement [22], as well as the amount and type of the antibiotic. Highly porous cement may elute more antibiotic and for a longer period of time relative to cement with less porosity [23]. The porous cement material such as $CaSO_4$ is a better antibiotic carrier material than PMMA for local delivery of antibiotics [5]. In a recent study by Chang et al. [24] it was shown that adding antibiotics (such as vancomycin, amphotericin B) powder in distilled water before mixing with bone cement significantly improves the efficiency of antibiotic release than same dose of antibiotic powder [24]. However, the ultimate compressive strength of the beads was significantly reduced in specimens containing liquid antibiotics.

Release of antibiotic from bone cement has previously been reported and measured in phosphate buffered saline (PBS) by estimating the zone of inhibition [25] or by using HPLC [26] or LC-MS [27]. However, the diffusion of antibiotics from a carrier bead into surrounding soft tissue and quiescent joint fluid is a diffusion limited environment and the degree of penetration of an antibiotic into the infected site is an important determinant of therapeutic success [4,28]. We have earlier reported the use of agar diffusion method to analyze the zone of inhibition [5] and extended these observations to study the importance of antibiotic loading density, loading amount, material type and exposure to body fluids on release kinetics and efficacy of antibiotics against planktonic and lawn biofilm bacteria. The agar method allows understanding how antibiotics might spread into the surrounding tissues as we can monitor the elution distance, antibacterial effects and preserve the gradients that are likely to develop *in vivo* in areas that are diffusion limited. The diffusion limited agar technique therefore becomes relevant and clinically important.

To investigate the release kinetics of diffusion from beads into a surrounding gel in real time we used fluorescein dye tracer which has previously been used as a surrogate to study antibiotic release based on its hydrophilic characteristics [16]. In the clinical setting, antibiotic-loaded beads immediately come into contact with the body fluids, such as blood and synovial fluid that may influence the properties and release from the beads. We hypothesized that exposure to these body fluids influences the release kinetics of antibiotic from antibiotic-loaded orthopaedic cements. Calcium is a known clotting factor and it is possible that blood clots that form around the beads may also influence release of antibiotics. This might be disadvantageous (by trapping antibiotics

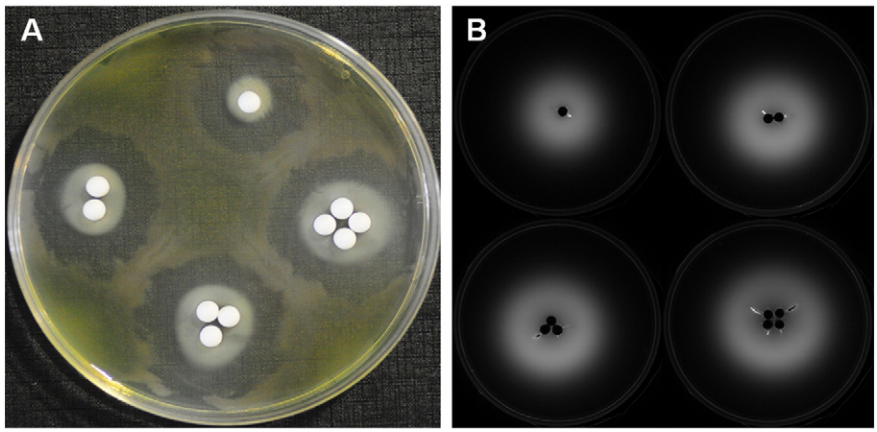

Fig. 2. Influence of single and multiple beads on local high concentration of antibiotic. (A) ZOI against *P. aeruginosa* PAO1 by multiple TOB-loaded $CaSO_4$ beads after 24 h is relatively similar as with single bead [16], the white cloudy area immediately adjacent to the beads within the zone of clearance is mineral precipitation ††. (B) Representative images showing fluorescein elution through multiple beads after 24 h was indifferent than with single fluorescein-loaded $CaSO_4$. ††Adapted from [5].