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HIGH LOCAL DELIVERY SYSTEMS OF ANTIBIOTICS IN THE TREATMENT OF BIOFILM RELATED INFECTIONS

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ABSTRACT

The use of local antibiotic delivery systems is common in the management of biofilm-related infections as they provide high concentrations of local antibiotics while simultaneously avoiding complications from systemic toxicity. Older delivery mechanisms were associated with a high incidence of wound complications (up to 25%) requiring reoperation. The high wound complication rate was thought to be due to impurities from the mined calcium sulfate, hydrophobic behavior, and acidic pH. We are presenting a 100% pure synthetic calcium sulfate hemihydrate (PSCSH) powder mixed with 240 mg liquid tobramycin and 500 mg of vancomycin powder per 10 cc of the hemihydrate for use in revision surgeries for periprosthetic joint infections (PJI). The purified carrier demonstrates superior utility to similar vehicles such as poly-methyl-methacrylate (PMMA) due to the bioabsorbablity which takes 2-3 weeks as demonstrated by disappearance of the hydrated crystal on x-ray. This is also preferable to the 4-6 weeks bioabsorbablity seen in the mined crystal calcium sulfate variants. The physiological pH of the PSCSH and the hydrophilicity demonstrated in serum probably account for the low 4% wound complication rate. The elution of vancomycin and tobramycin was greatest on day 1 compared with those concentrations obtained on days 2, 3, 4, and 5 post-operative while serum concentrations were mostly undetectable. Our findings demonstrate that this PSCSH preparation provides therapeutic delivery of vancomycin and tobramycin locally at log 2-3 above the minimum inhibitory concentration (MIC), while avoiding dangerous serum concentrations.

Keywords: Biofilm; Antibiotics; Infected total joint prosthetics; Calcium sulfate carrier.

INTRODUCTION

Chronic infection presents a serious complication following the surgical implantation of total joint prosthetics. Once a patient becomes contaminated, bacteria adhere to surfaces of implanted components and start forming colonies of bacteria called biofilm [1-4]. Bacteria entering into this sessile form produce through quorum sensing biofilm. This includes the production of a matrix called the glycocalyx. They proliferate up to a point and stop. Through quorum sensing, there is lateral exchange of genetic information between various microorganisms in which interaction is mediated via cell to cell interactions [5,6]. With biofilm development, pathogen genomes are altered through cell-to-cell signaling pathways, becoming phenotypically distinct from planktonic counterparts (singular organisms) [6]. This change allows sessile forms to be recalcitrance to antibody mediated immune regulatory mechanisms and relying more on cell mediated immunity. As the biofilm matures the cell becomes metabolically inactive with the lowest activity seen with pO_2 gradients in the central portion of the glycocalyx. The cells are called persister cells and because they are not dividing, they are not sensitive to antimicrobial agents [7]. At certain times, the mature biofilm releases active planktonic forms of the pathogen which show all the signs of acute infection. Also, there is concern over the management of the subsequent chronic infection because the cell mediated immunity with histiocytes causing local destruction of bones [5,8-10]. Ideally, parenterally administered antibiotics used in treating biofilm-mediated infections should be capable of disrupting the bacteria's structure [5,8,11,12]. However, this therapy has shown to treat planktonic bacterial infections with more success than suppressing biofilm related infections [5,12]. Osteomyelitis is a bone infection

associated with PJIs and is multifactorial in etiology [5]. Then by some mechanism including direct inoculation, hematogenous seeding, or airborne contamination, the bacteria contacts, adheres, and proliferates on dead bone matrix or hardware [5,8].

In response, surgeons employ surgical debridement of biofilm related infection, hardware removal, and the use of local antibiotic carriers as an adjunct to one or two-stage revision protocols for PJIs [12-17]. The local delivery systems in the past have included high concentrations of antibiotics in PMMA in the two-stage treatment of PJIs [13,18-23]. This treatment causes the local release of antibiotic concentrations between 10-20 times above the MIC the first day and maintains above MIC levels for approximately 2 weeks [24]. This discourages the reformation of biofilm-based infections [21]. The current standard for antibiotic therapy in two-stage revisions of PJI is through the use of antibiotic-impregnated poly-methyl-methacrylate (PMMA) spacers [5,17,22-26]. However, PMMA spacers are not bioabsorbable so additional surgery becomes necessary for removal followed by revision to the definitive reconstruction [13,17]. The time between the second operation for definitive reconstruction varies amongst institutions from 2 weeks to 6 months [26-28]. Because the spacers used in the first stage are put in loose, it is associated with pain to the patient, loss of functionality, and arthrofibrosis [29-30]. Moreover, once the surface bleaching of the antibiotic impregnated PMMA is below MIC, bacterial adhesion may occur, possibly leading to secondary infection [19,28-33]. Finally, the use of certain types of antibiotic loaded PMMA may be recovered in the serum [17]. This correlates with the observation of sustained antibiotic serum concentrations that may result in allergic reactions or complications secondary to the antibiotics [17-34].

In this study, we propose pure synthetic calcium sulfate hemihydrate (PSCSH) antibiotic carrier that offers multiple significant advantages over antibiotic loaded PMMA, making it a more favorable option in one-stage procedures. (1) They are bioabsorbable at 2-3 weeks as demonstrated by disappearance of the beads on x-ray. (2) They are good for filling soft tissue dead spaces left after the debridement procedure. (3) The antibiotic loaded beads are hydrophilic and soften when hydrated, therefore they do not scratch the total joints. (4) Mixing with heat sensitive antibiotics remains unproblematic due to minimal heat generation on curing of the antibiotic impregnated PSCSH pellets [35-39].

These advantages make the use of an antibiotic impregnated PSCSH vehicle intriguing for application in several scenarios, including abscess or soft tissue infection treatment, prophylaxis in high risk patients with comorbidities undergoing revision total joints, and for the use with physiologic constructs in one-stage procedures for PJIs as an adjunct to antibiotic loaded PMMA. Preparation of calcium sulfate beads provides a stable platform for the delivery antibiotics; however, problems associated with the use of calcium sulfate involve complications with persistent postoperative wound drainage [40-45].

This study examines clinical elution profiles of a PSCSH preparation in contrast to other less pure forms of calcium sulfate (Osteoset). Thus, we ask the following: (1) Does this calcium sulfate preparation provide local delivery of antibiotics at concentrations exceeding MIC of common infecting pathogens? (2) Will serum concentrations of antibiotics remain at safe concentrations?

MATERIALS & METHODS

For the purposes of this study, we chose Stimulan (Biocomposites, Keele Science Park, Staffordshire, United Kingdom) as the purified synthetic antibiotic loaded calcium sulfate hemihydrate. We selected vancomycin and tobramycin to treat gram-positive and gram-negative bacteria, respectively [46]. PSCSH powder is catalyzed by liquid tobramycin and the vancomycin slows down the set time to make the antibiotic beads.

With higher doses of liquid Tobramycin greater than 240 mg and higher doses of Vancomycin greater than 500 mg, the hardening rate of the pellets were prolonged. In order to prepare the antibiotic loaded PSCSH pellets, 6 ml of liquid tobramycin (240 mg) and a 10 cc pack of Stimulan Rapid Cure powder were combined and mixed with 500 mg of vancomycin powder until it formed a creamy paste (about 2 minutes). The paste was applied to the mold and allowed to harden which took about 10 minutes. 20 cc of pellets were used in each patient regardless of the soft tissue defect caused by the surgical debridement.

In the one stage treatment of PJIs, there is a dirty and clean setup. After the radical debridement and the removal of the prior prosthesis and hardware, the wound is irrigated and all gowns, gloves, and drapes are changed. Separate new instrumentation (clean side) is used for placement of the revision prosthesis and implantation instrumentation. The mixing of antibiotic loaded PSCSH powder is performed on the clean instrument side. Antibiotic impregnated PSCSH pellet preparation and dose were prepared identically for each patient. For surgical placement, the calcium sulfate/antibiotic paste is pressed into a pelletizing mold (supplied with the product) to produce beads of different dimensions. The paste is allowed to set within the mold, and the resulting beads are removed by flexing the mold over a sterile container. The beads remain covered in the container until implantation at the end of the case for management of the resected dead space.

Patients are then fitted with a drain prior to wound closure for collection of the local eluates. In the first five postoperative days, simultaneous serum and drain samples were assayed with Fluorescence Polarization Immunoassay (FPIA) with the Abbott Architect C8000 Chemistry Analyzer to evaluate the eluting characteristics of the Stimulan carrier impregnated with vancomycin and tobramycin at the above-mentioned concentrations. Upon collection and delivery to the lab, protocol provided by the Abbott User Manual was followed [47]. The internal standard was sterile water. The upper and lower limits of quantification were 15 and 5 times the concentration of the internal standard, respectively.

The first fifty patients that consented for the procedure were included and patient data included in Table 1. There was no differentiation based on gender or age. All patients'

glomerular filtration rate (GFR) greater than 60 and had PJIs. Both serum and local drain samples were collected every morning for 5 days at 0600 and taken immediately to the lab for same day analysis. Serum samples were collected in red-top tubes with 3 mL aliquots. The local drain samples were collected in sterile glass tubes with 5 mL aliquots.

RESULTS

We analyzed 50 patients undergoing revision arthroplasty for infected total joints or major multiple revisions. Cases included 33 knees (1 bilateral), 15 hips, 1 elbow, and 1 shoulder from 22 females and 28 males. Average patient age was 61 years (range: 13-82). None of the patients experienced relapse of infection or wound complications secondary to persistent wound drainage. This was compared to a 25% early wound complication rate postoperatively (100 cases) in which Osteoset mined calcium sulfate carrier was used as the carrier of choice to treat PJIs [45]. The Fisher exact statistic was 0.0013 ($p<0.01$).

We evaluated local antibiotic concentrations to determine if the concentrations contained in the eluent exceeded the MIC of common infecting pathogens and concentrations in the serum were also evaluated for all subjects [48-51]. Table 2 lists the results of others that were reported in the literature. Vancomycin is primarily effective against gram-positive cocci. S. aureus and S. epidermidis, both methicillin-susceptible (MSSA & MSSE) or resistant-species (MRSA & MRSE), are typically sensitive to vancomycin with MICs less than 1.5 µg/mL. Most strains of streptococcus are sensitive to vancomycin. Vancomycin is considered bactericidal (MBC/MIC <4 µg/mL) except with enterococci and some tolerant *Staphylococci* (MBC/MIC >32 µg/mL) [49-52].

Mean values obtained from local eluate demonstrated therapeutic concentrations of vancomycin and tobramycin in each of the five postoperative days. Concentrations of both antibiotics peaked on day 1 (Averages: Vancomycin: 297 µg/mL; Tobramycin: 31 µg/mL, & Ranges: Vancomycin: 28.3-736.4; Tobramycin: 6.4-97.2). Table 3 lists the change in mean values of the local eluate from the drains for each of the 5 days for the 50 patients, and Figures 1 and 2 illustrate the trend in mean value reduction over time. For the results of Table 3, assayable values of antibiotics were obtained from local exudate of drain samples. Furthermore, FTIR comparing the Osteoset and PSCSH pellets showed higher degrees of purity with the PSCSH pellets, which is evidenced by the narrower peaks shown in Figure 3.

We evaluated drain and blood serum concentrations of vancomycin and tobramycin on a standard hospital assay for these drugs in the five postoperative days. The assay reported antibiotic concentrations that were detected within specific concentration limits (vancomycin 2 µg/mL to 400 µg/mL, tobramycin 0.5 µg/mL to 20 µg/mL). Additional specific maximum assayable values for each antibiotic were recorded for a selection of the results. The majority of patients exhibited antibiotic blood serum concentrations below the lower concentration limits of the standard assay (vancomycin <2 µg/mL, tobramycin <0.5 µg/mL); However, a few patients exhibited detectable serum concentrations of antibiotics as follows: twelve (of the 50 patients) at day 1, five patients at day 2, and

Table 1: Patient demographics and laboratory data (n=50).

Gender	**22 Females/28 Males**
Age (years)	Range: 13 – 82 Mean: 61
# of Knee PJI* Cases	33
# of Hip PJI* Cases	15
# of Elbow PJI* Cases	1
# of Shoulder PJI* Cases	1
GFR (mL/min/1.73 m^2)	All Patients: >60
*PJI=Periprosthetic Joint Infection	

Table 2: Minimum Inhibitory Concentrations (MIC) for tobramycin and vancomycin reported by others [44-47].

Organism (Tobramycin)	**MIC Range (µg/mL) (Tobramycin)**	**Organism (Vancomycin)**	**MIC Range (µg/mL) (Vancomycin)**
E. faecalis	8-32	*Enterococci*	4.0
E. coli	0.25-1.0	MSSA	<2.0
P. aeruginosa	0.25-1.0	MRSA	<2.0
S. aureus	0.12-1.0	Coagulase-negative *Staphylococci*	4.0
n/a	n/a	*Streptococci* other than *S. pneumonia*	≤1.0

Table 3: Local antibiotic concentrations of vancomycin and tobramycin from the eluate in the drains in our patients.

Variables	**Mean (Range)**	**Mean (Range)**
Post-op Day	Vancomycin (µg/mL)	Tobramycin (µg/mL)
1	297 (28.3-736.4)	31 (6.4-97.2)
2	202 (14.2-466.7)	9.4 (2.4-19.6)
3	156 (5.8-394.5)	6.4 (1.6-19.9)
4	121 (19.5-352.5)	5.3 (1.3-18.6)
5	82 (5.8-190.5)	4.6 (2.2-9.8)

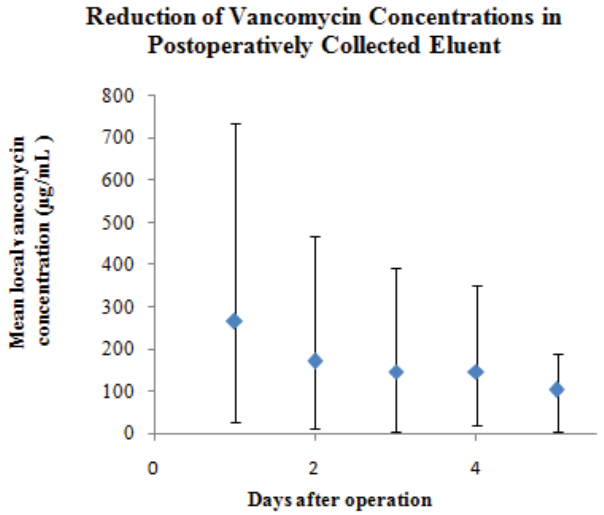

Figure 1: Mean eluent vancomycin concentrations in the five postoperative days. Error bars represent the range of mean local vancomycin concentrations for each day.

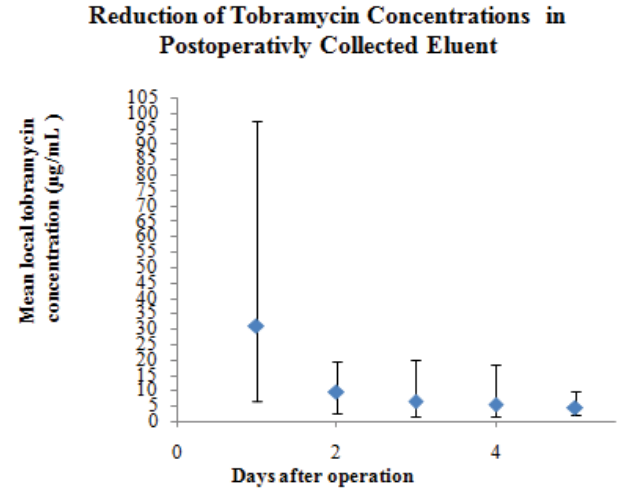

Figure 2: Mean tobramycin concentrations eluent in the five postoperative days. Error bars represent the range of mean local tobramycin concentrations for each day.

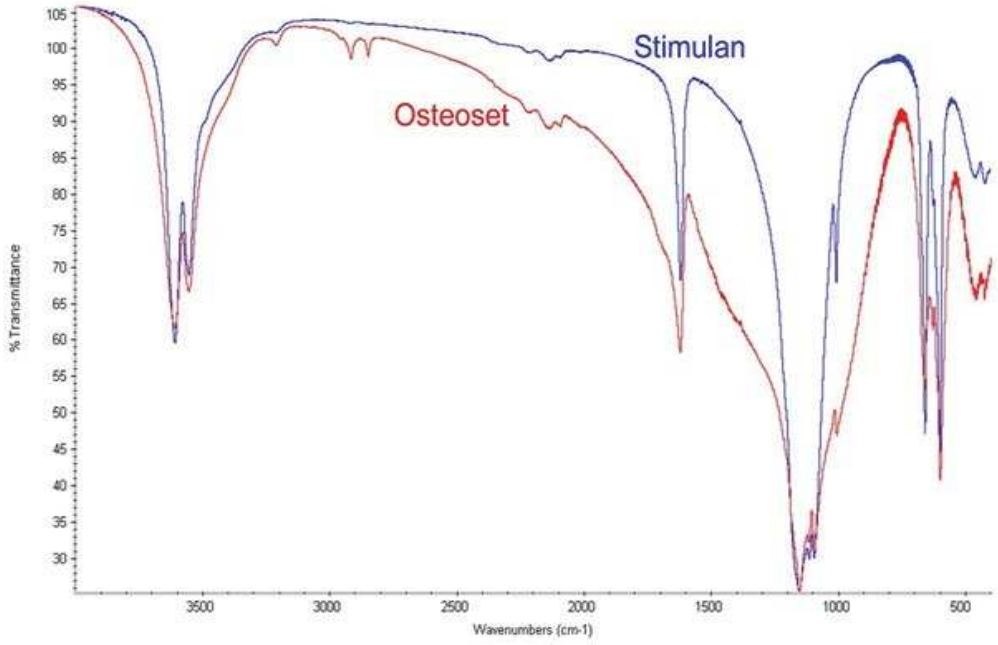

Figure 3: Comparative FTIR analysis of Stimulan & Osteoset pellets. The narrower peaks exhibited by the Stimulan sample indicate higher calcium sulfate purity.

Table 4: Serum concentrations of vancomycin and tobramycin in postoperative days 1-5.

Detectable serum concentrations of vancomycin and tobramycin in patients on postoperative days 1-5(µg/mL)										
Pt. ID # *	**Day 1**		**Day 2**		**Day 3**		**Day 4**		**Day 5**	
	VANC	**TOBRA**	**VANC**	**TOBRA**	**VANC**	**TOBRA**	**VANC**	**TOBRA**	**VANC**	**TOBRA**
04	**2.4**	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
05	<2.0	**0.7**	<2.0	**0.7**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
06	**2.5**	**0.7**	**2.4**	<0.5	**2.0**	<0.5	<2.0	<0.5	<2.0	<0.5
08	**2.6**	**1.8**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
16	**3.1**	**1.1**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
21	**2.2**	**0.9**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
23	**3.7**	**4.1**	**3.3**	**1.5**	**3.0**	<0.5	**2.5**	<0.5	<2.0	<0.5
26	<2.0	**0.6**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
28	<2.0	**2.1**	<2.0	**0.8**	<2.0	**0.6**	<2.0	<0.5	<2.0	<0.5
30	<2.0	**0.7**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
32	<2.0	**1.7**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
35	<2.0	**1.7**	<2.0	**0.7**	<2.0	<0.5	<2.0	<0.5	<2.0	<0.5
Detectable values are highlighted in bold.										

three patients at days 3, and one patient at 4. No patients exhibited detectable serum concentrations on day 5. Table 4 lists the antibiotic serum concentrations for each patient. Note, only patients with detectable serum values were included in Table 4.

DISCUSSION

By controlling the source of infection in PJIs through the disruption of biofilm formation, the surgeon creates an acute wound milieu that helps activate host immune mechanisms for effective wound healing. This control may be accomplished by combining the strict adherence to wound management through radical debridement with the administration of a local antibiotic capable of eluting high concentrations of the antibiotics for eradicating remaining exposed biofilm after the debridement process [52]. Antibiotic-laden PSCSH aid in the management of the dead space after the debridement with high doses of antibiotics and after placement of the definitive prostheses while avoiding complications of toxicity associated with parenteral administration . A mixture of broad-spectrum antibiotics perpetuates eradication of heterogeneous bacterial populations [53]. We prefer vancomycin and tobramycin for this purpose at the concentrations eluded locally to kill biofilm related infections as demonstrated in the center for biofilm engineering drip reactor system [16]. This bead becomes soft after hydration allowing for placement in joints without scratching the articular surfaces and demonstrates complete dissolution within a few weeks [52].

High serum concentrations of vancomycin and tobramycin with these two drugs can result in serious side effects, specifically ototoxicity and nephrotoxicity. The purpose of our study was therefore twofold: To verify that concentrations of vancomycin and tobramycin eluted from calcium sulfate would still be capable at high concentrations of inhibiting bacterial biofilm locally with low serum concentrations avoiding the complications of vancomycin and tobramycin. For reference, previous literature showed peak systemic concentrations of vancomycin after a 20 mg/kg dose to average 75.6 mg/L in a patient population with similar kidney function [54].

Limitations of this study include the ceiling placed on assayable values of antibiotic concentrations (as determined by FPIA analysis), the duration of evaluation and the comparison between different volumes placed into wounds. In spite of the truncated measurements, this study still demonstrates expected local and systemic elution characteristics. With regard to the temporal limitations, we would have preferred continued measurement of the antibiotic concentrations. However, our decision to discontinue after five days was based on concern for contamination from prolonged placement of the drains. Some of the patients in which we observed detectable serum concentrations also had concurrent implantation of PMMA cement impregnated with vancomycin and/or tobramycin as part of the reconstructive surgery. This concurrent implantation of an additional antibiotic delivery system may be a contributory factor to the incidences of detectable serum concentrations. Furthermore, controlling for the patient population may have yielded different results. For example, GFR changes with age and gender, therefore medication levels may be different based on the specific patient. Also, a comparative group with PMMA or a control was not measured. Inclusion of a comparative group would have improved the specificity of the results and provided more evidence for the superiority of the PSCSH beads. However, there is significant evidence of the inefficacy of the PMMA for antibiotic implantation into bone [16-18]. Compared with mined calcium sulfate variants, the carrier we used was hydrophilic, demonstrated bioabsorbablity (2-3 weeks), had physiological pH in a hydrated crystal, and was synthetically pure as seen by Fourier Transform InfraRed (FTIR) analysis [Figure 3]. A commonly encountered problem associated with the less pure forms of calcium sulfate carriers has been persistent postoperative wound drainage and complication rate (25%) [40-45]. We feel this drainage may be due to specific physical properties of mined calcium sulfate preparations, including its hydrophobicity, acidic pH, and impurity due to the presence of trace minerals as seen by FTIR analysis [55].

The clinical data also possessed limitations due to inaccuracy of antibiotic concentrations in eluent as a result of the standard laboratory assay used. The laboratory agency conducting the FPIA analysis established standard

routine reporting of antibiotic concentrations within specific concentration limits (vancomycin 2 mg/mL to 400 mg/mL, tobramycin 0.5 mg/mL to 20 mg/mL). In most assays conducted, maximum assayable limits for vancomycin and tobramycin were reported as >400 µg/mL and >20 µg/mL, respectively. As a result, mean values reported are lower than actual values. Additionally, without the drains, higher concentrations of antibiotics would be expected locally for longer periods of time; however drains are standard of care for patients with severe osteomyelitis. The exact values for antibiotic concentrations above the upper limits were not recorded for all patients. Therefore, the data does not give an accurate evaluation of local antibiotic concentrations in these cases. However, the data does indicate that these reported upper limits were present usually for only 1 to 2 days post-implantation. Thus, the use of antibiotic concentrations from eluent to indicate the diminishing local antibiotic concentrations over the five-day period remains possible. In addition, the blood serum concentrations indicate that these high local concentrations post-implantation do not manifest into high systemic antibiotic concentrations. Moreover, additional laboratory data such as hepatic function, coagulation studies and co-morbidities would have been useful in controlling for any other variables that could confound the systemic antibiotic concentration measurements. However, the data was collected at the time of the initial study and retrieval of the other information is not possible due to the deletion of the hospital records given some patient records eclipsed the eight-year maximum storage rule by the hospital system. Another limitation is that this study did not control for variability of the amount of antibiotic based on the volumetric size of the surgical bed. For example, larger surgical beds with the same amount of pellets as smaller surgical beds will have smaller areas of antibiotic diffusion.

CONCLUSION

Based on our results and clinical experience, the antibiotic loaded pure calcium sulfate hemihydrate demonstrates adequacy as a platform for the local delivery of antibiotics at therapeutic concentrations, as well as a stable vehicle for incorporation of both vancomycin and tobramycin. In each of the 5 postoperative days evaluated, mean local concentrations of antibiotics exceeded values capable of inhibiting common pathogens. We observed no adverse reaction based on the presence of elevated serum concentrations of antibiotics and no occurrence of persistent wound drainage associated with the antibiotic loaded pure calcium sulfate hemihydrate.

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DECLARATIONS

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Conflicts of Interest

Smith and Nephew- Royalties

Waldemark-Link- Consultant Agreement

Ethics Approval

All procedures in this study were in accordance with the 1964 Helsinki declaration (and its amendments), and the details of the Ethics Committee or institutional review board which approved the study.

Informed Consent

At the time of the study, no additional consents were obtained. All consent related to the study was incorporated into the operative consent form.

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