Evaluation of Two Sources of Calcium Sulfate for a Local Drug Delivery System: A Pilot Study

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Abstract

Background Local drug delivery has substantial potential to prevent infections compared with systemic delivery. Although calcium sulfate (CaSO_4) has been studied for local drug delivery and two types are commercially available, it is unknown whether they differentially release antibiotics.

Questions/purposes We determined the differences between two sources of CaSO_4 and the K_2SO_4 catalyst's presence on the degradation, daptomycin elution, and activity against *Staphylococcus aureus*.

Methods We formed pellets from synthetic and naturally sourced (from gypsum) CaSO_4 and loaded with 5% daptomycin and 3% or 0% K_2SO_4 . We used in vitro experiments to determine the daptomycin concentration and degradation profiles over 10 days. Turbidity assays were used to evaluate the activity of the daptomycin eluates against *S. aureus*.

Results All pellets exhibited a bolus release with the highest daptomycin concentration on Day 1 with the sourced CaSO_4 pellets. The synthetic CaSO_4 pellets with 3% K_2SO_4 exhibited a slower drug release compared with the synthetic CaSO_4 pellets with 0% K_2SO_4 , which degraded and eluted daptomycin too quickly to inhibit *S. aureus*. Turbidity assays demonstrated that all CaSO_4 pellets inhibit *S. aureus* for expected lengths of time.

Conclusions Our preliminary in vitro data suggest differences in the degradation, elution, and activity properties between sourced and synthetic CaSO_4 pellets. The addition of K_2SO_4 appeared beneficial when using synthetic CaSO_4 . Synthetic CaSO_4 may be effective when slow degradation and longer elution times are needed.

Clinical Relevance Local delivery of eluted daptomycin can be tailored through material selection and K_2SO_4 addition.

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Introduction

Wound infection and osteomyelitis can be challenging to treat, especially with complex musculoskeletal trauma in civilian and military populations, and can result in multiple surgeries and increased costs [7, 20]. Local drug delivery is emerging as a useful route for treating acute and chronic wound infections. Because drugs are delivered to the entire body in systemic drug delivery, toxicity issues can often arise as well as antibiotic resistance [9]. Organisms that are typically antibiotic-resistant in systemic delivery can be more susceptible to the higher levels of antibiotics provided through local delivery [9]. Additionally, the drug can take a longer time to reach the wound when delivered systemically and some avascular areas of the body are unreachable [9]. In local drug delivery, the wound area is

targeted and the drug does not substantially affect other parts of the body; however, local drug delivery systems do not completely eliminate toxicity issues and antibiotic resistance. The ideal local drug delivery system would be implanted, deliver antibiotics at appropriate levels and lengths of time based on application, and then degrade so there would be no need for a second surgical procedure for removal [9].

Another difficult challenge with complex musculoskeletal trauma is resistant bacteria. Wound infection can be complicated by wound contamination with antibiotic-resistant bacteria, which can increase treatment difficulty. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious concern in modern medicine and serves as a problem in wound treatment. Antibiotic-resistant bacteria have created a need for newer antibiotics as an adjunct for past antibiotic selections such as vancomycin. Daptomycin is a semisynthetic antibiotic with activity against Gram-positive bacteria and has demonstrated activity against MRSA [6, 15]. Daptomycin has been previously researched combined with a calcium sulfate (CaSO_4) carrier, and daptomycin reportedly elutes similarly to tobramycin in vitro [22, 27]. However, like with many antibiotics used for infected wound treatment, bacterial resistance to daptomycin has been recently reported [26].

CaSO_4 has been previously used as a bone void filler and in bone defects is absorbed by the surrounding tissue, possibly accelerating bone healing [21]. CaSO_4 is a biocompatible, biodegradable material with osteoconductive properties that exhibits a bolus, or burst, release followed by an extended drug release from several hours to weeks depending on the formulation [11, 23]. CaSO_4 has been used to deliver vancomycin, gentamycin, tobramycin, lincomycin, cefazolin, teicoplanin, and fucidin [4, 10, 16]. Another, and the most common, material used for local drug delivery is polymethylmethacrylate (PMMA) [5]. Although PMMA implants are not biodegradable, their removal may be necessary at times for planned staged reconstruction or late bead failure [18]. Because CaSO_4 degrades over time, the need for surgical removal is eliminated. Clinical studies and animal models suggest using antibiotic-loaded calcium sulfate pellets minimizes infection [1, 17, 25]. Although there are numerous potential applications in which calcium sulfate is beneficial as a drug delivery material, the mechanical properties of calcium sulfate do make it insufficient for load-bearing applications [8]. Recently, two sources of CaSO_4 that are commercially available have been studied for their use in local antibiotic delivery. To make pellets with daptomycin from one type of CaSO_4 (hemihydrate synthesized from naturally occurring USP grade gypsum), a catalyst, K_2SO_4 , is required [22]. It has been proposed the K_2SO_4 catalyst would not be necessary to form pellets from a synthetic CaSO_4 .

A comparison of antibiotic elution, pellet degradation, and the need for a catalyst of these two CaSO_4 sources has not been investigated.

We asked the following questions: (1a) is the pellet degradation and (1b) daptomycin elution profile different for two different forms of CaSO_4 , naturally sourced from gypsum and synthetic; (2) does the presence of a catalyst, potassium sulfate (K_2SO_4), affect the degradation and the daptomycin elution profile of the CaSO_4 pellets manufactured from two different sources; and (3) is the daptomycin released from both sources of CaSO_4 pellets active against bacteria associated with osteomyelitis, specifically *S. aureus*?

Materials and Methods

To determine whether the pellet degradation, daptomycin elution profile, and activity against *S. aureus* were affected by the type of CaSO_4 or the presence of a catalyst, we prepared the CaSO_4 pellets in three groups, synthetic CaSO_4 and 3% K_2SO_4 , synthetic CaSO_4 and 0% K_2SO_4 , and naturally sourced CaSO_4 with 3% K_2SO_4 (Fig. 1). Four of each type of pellet was placed in each vial and there were four total replications, or vials, for each group in each test. Each pellet group was tested in an elution study,

Fig. 1 A flowchart demonstrates the study design with the three test groups and the key variables from three property characterization tests.

degradation study, and activity testing with the dependent variables being daptomycin concentration, degradation rate, and turbidity, respectively.

The two types of CaSO_4 used in our study were Osteoset (Wright Medical Technology, Arlington, TN), a sterile surgical grade, CaSO_4 α -hemihydrate from naturally sourced US Pharmacopeia grade gypsum, and Stimulan (Biocomposites, Wilmington, NC), a high-purity, completely synthetic sterile CaSO_4 α -hemihydrate. The naturally sourced and synthetic CaSO_4 pellets were both cast with 4.75 g of CaSO_4 , with and without 5 wt%, 0.25 g, of daptomycin (Cubist Pharmaceuticals Inc, Lexington, MA; Lot MCB2007). The 5 wt% was previously determined using prior research on naturally sourced CaSO_4 pellets [22, 27]. Throughout our study, we used aseptic techniques in the fabrication of the pellets. The synthetic CaSO_4 pellets were made with and without 2.5 mL of sterilized 3% K_2SO_4 solution, whereas the naturally sourced CaSO_4 pellets were only made with 2.5 mL of 3% K_2SO_4 solution. Previous research has determined that a concentration of 3% K_2SO_4 is necessary for the naturally sourced CaSO_4 pellets because the pellets will not retain their shape without the catalyst [27]. The 3% K_2SO_4 solution was substituted with 1.7 mL of sterile phosphate-buffered saline for the synthetic CaSO_4 pellets with 0% K_2SO_4 . We carried out all experiments in four replications ($n = 4$). Pellets were cast using 3-mm OSTEOSSET® Resorbable Mini-Bead Kits (Wright Medical Technology; Catalog 8400-0511; Lot LB04-0028-27) and following the mixing method used by Wright Medical Technology described in previous research [22, 27].

We performed the elution statically with four sets of each pellet variation distributed into four steam autoclaved 20-mL glass scintillation vials. The elution was conducted without stirring to maintain the structure of the pellets. We loaded 4 mL of phosphate-buffered saline (PBS) into each vial and placed them in an oven at 37°C. The eluates were mixed and 1-mL samples were taken at Days 1, 2, 4, 6, 8, and 10. The elution studies were stopped at 10 days because the antibiotic levels were below the high-performance liquid chromatography detection limit. After each day's eluate sample was collected, we replaced the remaining liquid with a fresh 4 mL PBS and the vials were returned to the 37°C oven. Eluate samples were immediately frozen in a Fisher Scientific Isotemp freezer at -20°C.

To measure the initial daptomycin concentration loaded in the pellets, samples were made of each pellet variation were constructed and crushed before placing them into a PBS solution. The drug content of the pellets was measured before the elution because previous CaSO_4 and daptomycin pellets had some daptomycin loss from initial loading. The percent daptomycin content loaded into the pellets was

compared with the percent daptomycin released from the elution.

To determine the eluate's antibiotic concentration, we performed high-pressure liquid chromatography (HPLC) testing according to previously used methods [22]. The Varian (Palo Alto, CA) HPLC system was comprised of a ProStar 240 Solvent Delivery, ProStar 410 Autosampler, and ProStar 325 UV-Vis Detector modules. We performed module control and data processing using Varian's Galaxie Chromatography Data System (Version 1.8.508.1). The mobile phase consisted of a HPLC-grade acetonitrile and water (62:38, v/v) solution including 4 mM ammonium dihydrogen phosphate brought to a pH of 3.25 using glacial acetic acid. Separation was accomplished using a Varian Microsorb-MV C8 column, 150-mm length and 4.6-mm inner diameter, with a flow rate of 1 mL/min. Daptomycin was detected at 232 nm with a retention time of 13.8 minutes. We performed the method in a temperature range of $23.3 \pm 1.1^\circ\text{C}$.

We determined the CaSO_4 pellets' degradation by a common weight-based experiment [2, 22, 24]. Initially, the weights of clean 20-mL glass scintillation vials were established and then the weight of each vial with four pellets of an individual variation was measured. We loaded 4 mL of PBS into each vial and stored them at 37°C. At 1-, 2-, 4-, 6-, 8-, and 10-day time points, the PBS was removed and the pellets were allowed to dry at 70°C until a constant vial and pellet weight was obtained. After each time point's sample was collected, we placed 4 mL PBS into the vials and they were returned to 37°C storage. The degradation was determined by the percent of pellets remaining at each time point calculated using the following relationship:

Percent remaining (%)

$$= \frac{(\text{vial and pellets at } \times \text{ hours weight} - \text{vial weight})(\text{mg})}{(\text{vial and initial pellets weight} - \text{vial weight})(\text{mg})} \times 100$$

We determined antibiotic activity against *S. aureus* (Cowan I strain obtained from Dr David Hasty, University of Tennessee Health Science Center) by using antibiotic elution samples in a turbidity assay. In this experiment, solution clarity after sufficient bacterial incubation with antibiotic eluates indicated bacterial inhibition resulting from antibiotic activity. As instructed by the protocol provided by the manufacturer, we supplemented BBL™ Mueller Hinton II Broth (Becton, Dickinson and Co, Sparks, MD; Lot 0071612) with 25 $\mu\text{g/mL}$ Ca^{2+} to achieve a total Ca^{2+} concentration of 50 $\mu\text{g/mL}$. Two hundred microliters of daptomycin eluates were added to the inoculum containing 1.75 mL Mueller Hinton II Broth + Ca^{2+} and 50 μL *S. aureus* in 5-mL polystyrene test tubes. The experimental setup created a 1:10 dilution of all eluates.

Experimental groups, blanks containing neither *S. aureus* nor eluate samples, and positive controls containing *S. aureus* without antibiotic eluates were mixed and incubated at 37°C. After approximately 20 hours of incubation at 37°C, we vortexed the tubes and the absorbance at 530 nm was recorded.

All data are presented as mean $\pm$ SD and met the assumptions of a parametric test. We determined differences in percent degradation and daptomycin concentration between the pellets made from two different types of CaSO₄ and the pellets made with and without K₂SO₄ using repeated measures analysis of variance and Tukey's post hoc analysis using 2007 Microsoft Office Excel (Microsoft Inc, Redmond, WA) and 2004 SigmaStat (San Jose, CA). Differences in degradation were also analyzed with Kaplan-Meier survival analysis with log rank, in which 10% of the original pellet remaining was classified as failure of the pellets. When only 10% of the original pellet remains, the antibiotic concentration in the pellets is typically too low and the pellets are too small to accurately measure.

Results

The degradation studies indicated differences between the sourced and synthetic CaSO₄ pellets with 3% K₂SO₄ without daptomycin (Fig. 2) at Days 2 ($p = 0.002$), 4, 6, and 8 ($p < 0.001$). However, Kaplan-Meier analysis with log rank revealed no overall differences among all three CaSO₄ groups without daptomycin ($p = 0.368$). When 5 wt% daptomycin was incorporated into the pellets (Fig. 3), differences were seen between the sourced CaSO₄ pellets and the synthetic CaSO₄ with 0% K₂SO₄ at Days 1 through 10 ($p < 0.001$). Using Kaplan-Meier analysis with log rank, overall differences were seen between the sourced and synthetic CaSO₄ pellets with 0% K₂SO₄ ($p < 0.001$) and between the synthetic CaSO₄ pellets with 3% and 0% K₂SO₄ ($p < 0.001$). However, no differences were seen between the degradation of the sourced and the synthetic CaSO₄ pellets with 3% K₂SO₄ ($p > 0.05$) from the analysis of variance or Kaplan-Meier survival analysis.

We observed differences in the elution profile of the sourced CaSO₄ pellets and the synthetic CaSO₄ pellets with 3% K₂SO₄ at Day 1 ($p < 0.001$) and Day 2 ($p = 0.002$) (Fig. 4). The sourced CaSO₄ pellets released the highest amount of daptomycin on Day 1 and correspondingly had the highest amount of cumulative daptomycin eluted (Fig. 5), but the synthetic CaSO₄ pellets with 3% K₂SO₄ released the largest amount of antibiotic on Day 2. For evaluation of mass balance in these studies, the average percent daptomycin loaded and released from the three types of pellets was measured. The average percent of daptomycin for the sourced CaSO₄ pellets was highest at

Fig. 2 The graph shows the degradation results of the naturally sourced and synthetic CaSO₄ pellets with 0% daptomycin present. There were differences in average degradation of naturally sourced and synthetic CaSO₄ pellets with 3% K₂SO₄ and naturally sourced and synthetic CaSO₄ pellets with 0% K₂SO₄ at Days 2, 4, 6, and 8. Differences were observed between the synthetic CaSO₄ pellets with 3% and 0% K₂SO₄ at Days 4, 6, and 8. Degradation is reported in wt% of original pellet remaining $\pm$ SD with $n = 4$ (* $p < 0.001$).

Fig. 3 The graph shows the degradation results of the naturally sourced and synthetic CaSO₄ pellets with 5% daptomycin present. We observed differences in average degradation of naturally sourced and synthetic CaSO₄ pellets with 0% K₂SO₄ and synthetic CaSO₄ pellets with 3% and 0% K₂SO₄ at Days 1–10. Degradation is given in wt% of original pellet remaining $\pm$ SD with $n = 4$ (* $p < 0.001$).

loading, at 85.1%, but the percent daptomycin released was highest in the synthetic CaSO₄ pellets with 3% K₂SO₄ at 48.8% (Table 1). The average percent daptomycin was

Fig. 4 The graph shows the elution results from the naturally sourced and synthetic CaSO₄ pellets with 5% daptomycin loaded with mass and concentration of daptomycin released on the primary and secondary y axes, respectively. We found differences in the average mass and concentration of daptomycin released in the eluates between the naturally sourced and synthetic CaSO₄ pellets with 3% and 0% K₂SO₄ at Days 1 and 2. Differences are shown in average mass and concentration of daptomycin released in the eluates between the synthetic CaSO₄ pellets with 3% and 0% K₂SO₄ at Day 2. Mass and concentration are given in micrograms and micrograms per milliliter, respectively, of daptomycin released per pellet $\pm$ SD with $n = 4$ (* $p < 0.001$).

Fig. 5 The graph shows the elution results in the cumulative release form from the naturally sourced and synthetic CaSO₄ pellets, with and without 3% K₂SO₄, with 5% daptomycin loaded. Mass is given in micrograms of daptomycin released per pellet $\pm$ SD with $n = 4$.

lowest, both loaded and released, in the synthetic CaSO₄ pellets with 0% K₂SO₄.

The presence or absence of K₂SO₄ created differences in the degradation and the elution in the synthetic CaSO₄ pellets. The synthetic CaSO₄ pellets with 0% K₂SO₄ and 5 wt% daptomycin degraded much more quickly than both

Table 1. Average percent daptomycin loaded in pellets and released in elution testing

Pellet type	Percent daptomycin loaded	Percent daptomycin released
Sourced CaSO ₄ pellets with 3% K ₂ SO ₄	85.1	42.8
Synthesized CaSO ₄ pellets with 3% K ₂ SO ₄	81.8	48.8
Synthesized CaSO ₄ pellets with 0% K ₂ SO ₄	45.8	39.3

Values are mean ($n = 4$).

Table 2. The average activity for 5% loaded daptomycin (1:10 dilution) against *Staphylococcus aureus*

Pellet type	Time points (days)					
	1	2	4	6	8	10
Sourced CaSO ₄ pellets with 3% K ₂ SO ₄	–	+	–	–	+	+
Synthesized CaSO ₄ pellets with 3% K ₂ SO ₄	–	–	–	–	–	+
Synthesized CaSO ₄ pellets with 0% K ₂ SO ₄	–	–	+	+	+	+

– = inhibition of *S. aureus*; + = growth of *S. aureus* ($n = 4$).

types of pellets with 3% K₂SO₄, and as previously mentioned, differences were seen in degradation between the synthetic CaSO₄ pellets with 0% K₂SO₄ and the other pellets using analysis of variance and Kaplan-Meier analysis. Differences were seen in the elution (Fig. 4) between the synthetic CaSO₄ pellets with 0% K₂SO₄ and 3% K₂SO₄ at Day 2 ($p < 0.001$) and the naturally sourced CaSO₄ pellets with 3% K₂SO₄ at Day 1 ($p < 0.001$) and Day 2 ($p = 0.002$).

The eluates of the sourced CaSO₄ pellets with 3% K₂SO₄ and 5 wt% daptomycin demonstrated sufficient activity against *S. aureus* with no bacterial growth at Days 1, 4, and 6 (Table 2). Eluates from synthesized CaSO₄ pellets with 0% K₂SO₄ were only active against *S. aureus* at Days 1 and 2; however, the eluates from the synthesized CaSO₄ pellets with 3% K₂SO₄ were active against *S. aureus* at all time points except at 10 days.

Discussion

The treatment of osteomyelitis is becoming complicated by the increased emergence of antibiotic-resistant bacteria and increases the need for local drug delivery over systemic delivery. The purpose of our study was to evaluate parameters of a local CaSO₄ drug delivery system, including

Table 3. Comparisons of present study with similar previous research on CaSO₄ and PMMA

Study	Type of material	Antibiotic	Catalyst/additive	Drug release on Day 1	Degradation	Activity testing
Richelsoph et al. [22]	Sourced CaSO ₄	Daptomycin or tobramycin	K ₂ SO ₄	537.3 µg/mL/g daptomycin and 827.6 µg/mL/g tobramycin	Yes: 100% degraded at 6 days	No
Webb et al. [27]	Sourced CaSO ₄	Daptomycin	K ₂ SO ₄	697.4 µg/mL/g for clinical pellets	N/A	Yes: inhibition through 28 days, except Days 10 and 14
Kanellakopoulou et al. [12]	Synthetic CaSO ₄	Daptomycin	None	1320.1 µg/mL	N/A	No
McLaren et al. [19]	PMMA	Daptomycin	None, xylitol or glycine	84.375 µg/mL with xylitol	N/A	No
Lewis and Janna [14]	PMMA	Gentamicin sulfate	None	≈ 10 × 10 ⁻³ g/mL/h for 4.25 wt% loading	N/A	N/A
Lewis et al. [13]	PMMA	Daptomycin	None	< 50 µg/mL/g for 4.5 wt% loading	N/A	Yes: inhibition through 7 days
Parker et al. (current study)	Sourced CaSO ₄	Daptomycin	K ₂ SO ₄	97.48 µg/mL	Yes: 21% of original pellets remaining at 10 days	Yes: inhibition at Days 1, 4, and 6
Parker et al. (current study)	Synthetic CaSO ₄	Daptomycin	K ₂ SO ₄	76.16 µg/mL with 3% K ₂ SO ₄ and 74.19 µg/mL with 0% K ₂ SO ₄	Yes: 30.9% and 2.25% of original pellets with 3% and 0% K ₂ SO ₄ remaining at 10 days	Yes: inhibition at Days 1 and 2 with 0% K ₂ SO ₄ and Days 1–8 with 3% K ₂ SO ₄

PMMA = polymethylmethacrylate; N/A = not applicable.

(1a) to determine if the pellet degradation and (1b) daptomycin elution profile is different for two different commercially available forms of CaSO₄; (2) to examine if the presence of a catalyst, K₂SO₄, affects the degradation and antibiotic elution profile of the CaSO₄ pellets; and (3) to verify the daptomycin released from both types of CaSO₄ pellets is active against *S. aureus*, a bacterium commonly associated with osteomyelitis.

Our study is limited by several factors. First, a common limitation of most in vitro research is the difficulty of translating in vitro studies to in vivo studies [18]. Release characteristics in an in vivo environment, especially associated with a wound, are different than antibiotic release characteristics in vitro, in which a set volume of eluate and PBS is exchanged at certain intervals [18]. This study is not meant to serve as a surgical wound model itself but as a supplement to in vivo studies. Additionally, although we are testing four pellets at one time, elution, degradation, and activity properties of these pellets may be completely different in vivo in larger pellet quantities. Second, we tested activity of the antibiotic to planktonic *S. aureus* and did not test inhibitory activity to bacteria within biofilms, which can be more resistant to antibiotic therapy and typically require additional antibiotic levels required for inhibition of planktonic bacteria [3]. Third, we used only one antibiotic. Increasing the number of evaluated antibiotics could predict if the CaSO₄ type or presence of K₂SO₄ impacts drug elution and pellet degradation in different

ways. Fourth, both elution and degradation are dependent on numerous factors, including physical and chemical properties of materials, elution volumes with and without fluid exchange, and antibiotic interactions and thus we cannot predict elution and degradation performance across all potential applications. Both elution and degradation studies only provide details about the release and degradation characteristics of the local delivery vehicle itself and do not account for affects from a local wound and the interactions among the delivery system, fluid, and drug.

Our preliminary data indicate differences in degradation and elution between both forms of CaSO₄ pellets, naturally sourced and synthetic, that could affect the clinical selection of a local delivery system. Like with previously researched sourced CaSO₄ pellets, all pellets exhibited a bolus release of antibiotics on Day 1 [21, 26]. However, the total percent release of daptomycin from the naturally sourced CaSO₄ pellets was higher and the concentration of daptomycin released on Day 1 was lower than previously reported (Table 3) [22, 27]. The initial daptomycin released from the naturally sourced and synthetic calcium sulfate pellets is closer to initial antibiotics eluted from PMMA beads (Table 3) [13, 14, 19]. An explanation for the higher total release of the antibiotic could be the result of faster elution to measurement activities in comparison to previous studies. Extended elution and storage could impact stability of daptomycin in solution. The lower initial daptomycin concentration could be explained by the

larger size of the previously researched CaSO₄ pellets [21, 26]. Although the synthetic CaSO₄ pellets also exhibited a bolus release, more daptomycin was eluted on Day 2 compared with the naturally sourced CaSO₄ pellets, which could demonstrate slower antibiotic release.

The difference in daptomycin release could be partially explained by the degradation differences between the types of pellets. The synthetic CaSO₄ pellets with 3% K₂SO₄ and 5% daptomycin demonstrated the slowest degradation as compared with the other two types of pellets. Although the naturally sourced CaSO₄ pellets had a faster degradation than the synthetic CaSO₄ pellets with 3% K₂SO₄, the degradation of the naturally sourced CaSO₄ pellets in our study was slower than previously reported [22]. However, the results could have been different because distilled water was used for the degradation solution instead of PBS in the previous research [22]. The presence of daptomycin increased the degradation rate in all three pellet types studied but had the greatest effect on the degradation of the synthetic CaSO₄ pellets with 0% K₂SO₄. An explanation for this difference in degradation that has been previously reported could be that when the K₂SO₄ is absent, the daptomycin prevents the setting reaction of the CaSO₄, which impacts the stability of pellets [21].

Although it has been previously determined that K₂SO₄ is necessary to form pellets of naturally sourced CaSO₄ [22], we were able to form pellets out of synthetic CaSO₄ without the K₂SO₄ catalyst. By not incorporating K₂SO₄ into the pellets, the synthetic CaSO₄ pellets degraded much more rapidly and released less daptomycin than the other pellets with the catalyst present. The daptomycin released from the synthetic CaSO₄ pellets with 0% K₂SO₄ is substantially less than the amount released from the same type of CaSO₄ with 0% K₂SO₄ that was previously reported (Table 3) [12]. However, the local delivery pellets used in these previous research studies were much larger than the pellets in this current research, 160 by 100 mm compared with 3-mm pellets, and Mueller Hinton broth was used as the elution vehicle instead of PBS [12].

Our preliminary data suggest the eluted daptomycin from both naturally sourced and synthetic CaSO₄ pellets, with and without K₂SO₄, is active against *S. aureus*, inhibiting bacterial growth for varying lengths of time. Although the synthetic CaSO₄ pellets with 3% K₂SO₄ demonstrated the highest activity against *S. aureus*, the synthetic pellets with 0% K₂SO₄ provided inhibition of *S. aureus* on Days 1 and 2 only, which can be explained by the low amount of daptomycin eluted from the pellets compared with the pellets with K₂SO₄. The inhibitory activity of the synthetic pellets with 0% K₂SO₄ is much lower than previously reported daptomycin inhibition of *S. aureus* from CaSO₄ and PMMA; however, the bacterial

inhibition of the synthetic CaSO₄ pellets with 3% K₂SO₄ is much higher than the same reported data [12, 26]. The positive growth measured on Day 2 for the naturally sourced CaSO₄ pellets may have been caused by an unknown contaminant because there were similar amounts of daptomycin present in the pellets on Days 4 and 6. Additionally, similar naturally sourced CaSO₄ pellets were previously reported to inhibit *S. aureus* for 10 days without any contamination and inhibited *S. aureus* for 28 days with two cases of contamination [27].

This preliminary research provides researchers and clinicians with additional information on the two commercially available forms of CaSO₄ for a local delivery system for infection prevention or treatment. Our data also show that although K₂SO₄ is not necessary for forming pellets from synthetic CaSO₄, it may be helpful for decreased degradation, an increased daptomycin elution profile, and potentially extended activity against susceptible organisms. Further investigations, including preclinical in vivo studies and biofilm-associated investigations, are needed to determine functional differences and applications of these local drug delivery systems.

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