North American Spine Society Journal (NASSJ) 18 (2024) 100323

Contents lists available at ScienceDirect

North American Spine Society Journal (NASSJ)

journal homepage: www.elsevier.com/locate/xnsj

Basic Science

Synthesis and evaluation of a novel vancomycin-infused, biomimetic bone graft using a rat model of spinal implant-associated infection

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ARTICLE INFO

Keywords:
Animal model
Antibiotic-infused graft
Bone graft
Implant-associated infection
Osteomyelitis
Spinal fusion
Spine infection

ABSTRACT

Background: Postoperative infection is a complication of spinal fusion surgery resulting in increased patient morbidity. Strategies including intraoperative application of powdered vancomycin have been proposed to reduce the incidence of infection; however, such antimicrobial effects are short-lived.

Methods: Instrumentation of the L4–L5 vertebrae was performed mimicking pedicle screw and rod fixation in 30 rats. Titanium instrumentation inoculated with either PBS or 1×10^5 CFU bioluminescent MRSA, along with biomimetic bone grafts infused with varying concentrations of vancomycin and 125 μg of rhBMP-2 (BioMim-rhBMP-2-VCM) were implanted prior to closure. Infection was quantified during the six-week postoperative period using bioluminescent imaging. Arthrodesis was evaluated using micro-CT.

Results: Infected animals receiving a bone graft infused with low-dose (0.18 mg/g) or high-dose vancomycin (0.89 mg/g) both exhibited significantly lower bioluminescent signal over the six-week postoperative period than control animals inoculated with MRSA and implanted with bone grafts lacking vancomycin (p=.019 and p=.007, respectively). Both low and high-dose vancomycin-infused grafts also resulted in a statistically significant reduction in average bioluminescence when compared to control animals (p=.027 and p=.047, respectively), independent of time. MicroCT analysis of animals from each group revealed pseudoarthrosis only in the control group, suggesting a correlation between infection and pseudoarthrosis. MRSA-inoculated control animals also had significantly less bone volume formation on micro-CT than the PBS-inoculated control cohort (p<.001), the MRSA+low-dose vancomycin-infused bone graft cohort (p<.001), and the MRSA+high-dose vancomycin-infused bone graft cohort (p<.001).

Conclusion: BioMim-rhBMP-2-VCM presents a novel tissue engineering approach to simultaneously promoting arthrodesis and antimicrobial prophylaxis in spinal fusion. Despite mixed evidence of potential osteotoxicity of vancomycin reported in literature, BioMim-rhBMP-2-VCM preserved arthrodesis and osteogenesis with increasing vancomycin loading doses due to the graft's osteoinductive composition.

Abbreviations: BMI, Body mass index; CFU, Colony forming units; CT, Computed Tomography; IVIS, In vivo imaging system; MRSA, Methicillin Resistant Staphylococcus aureus; PBS, Phosphate buffered saline; rhBMP-2, Recombinant human bone morphogenetic protein-2; ROI, Region of interest; SIS, Single-photon emission computed tomography; SPECT, Small Intestine Submucosa; SSI, Surgical site infection; TP, Transverse process.

FDA device/drug status: Not applicable.

Author disclosures: ***CJR:*** Nothing to disclose. ***JAT:*** Nothing to disclose. ***TM:*** Nothing to disclose. ***ADD:*** Nothing to disclose. ***APP:*** Nothing to disclose. ***BFJ:*** Nothing to disclose. ***KYZ:*** Nothing to disclose. ***VNH:*** Nothing to disclose. ***JL:*** Nothing to disclose. ***JLL:*** Nothing to disclose. ***EC:*** Nothing to disclose. ***TFW:*** Consulting: NIH/Neurotech (B); Speaking and/or Teaching Arrangements: Augmedics (B); Trips/Travel: Globus (B).

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https://doi.org/10.1016/j.xnsj.2024.100323
Received 11 December 2023; Received in revised form 5 April 2024; Accepted 6 April 2024
Available online 9 April 2024

Background

Postoperative infection is a complication of spinal fusion surgery resulting in increased patient morbidity. The incidence of infection following spinal fusion procedures is estimated to be between 0.7%–2.3% and 2%–20% in cases of non-instrumented and instrumented spine surgery, respectively [1,2]. Postoperative spinal infection is associated with chronic pain, instrumentation failure, pseudoarthrosis, wound dehiscence, and increased cost of care [3]. The increased risk of infection in instrumented spinal fusion surgery is thought to be related to the proclivity of infectious organisms to adhere to metal implants and cause chronic, sustained infection through the formation of biofilms [4,5]. The most common causative organisms seen in cases of surgical site infection (SSI) following spinal fusion are *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), coagulase-negative Staphylococci, Streptococci, and enterococci: all organisms capable of biofilm formation [1,6–8]. Other independent risk factors for infection following instrumented spine surgery include increased patient age, diabetes mellitus, increased body mass index (BMI), smoking, greater number of levels fused, and increased length of the surgery [2,5,9]. Treatment of spinal implant-associated infection often necessitates debridement, washout, and less commonly implant removal conferring an increased risk of morbidity, mortality, length of hospital stay, and increased healthcare costs [10,11]. There is also a lack of consensus regarding the optimal timing for removal of infected instrumentation, either during wound washout or subsequent surgery [11].

Perioperative prophylaxis has been highlighted as a strategy to mitigate the risk of infection in patients undergoing spinal instrumentation. Intravenous first-generation cephalosporins, clindamycin, gentamicin, and tobramycin are used pre- and postoperatively for their broad coverage of gram-positive microorganisms. In addition, local application of vancomycin remains the most prevalent intraoperative antibiotic of choice due to relatively low cost, wide availability, and broad coverage for common agents implicated in spinal surgical site infection including MRSA [2,12–14]. However, multiple systematic reviews exploring the local intraoperative application of vancomycin have demonstrated that the evidence for its prophylactic value in this application is mixed [2,12,15]. Therefore, there exists a need for additional antimicrobial strategies to prevent surgical site and implant associated infection in spinal fusion.

A systematic review of 48 studies investigating the use of bone grafts for local antibiotic delivery to mitigate implant related infection suggested that bone grafts are a suitable carrier for local antibiotics for both prophylaxis and therapeutics [16]. Furthermore, vancomycin-infused bone grafts were not found to significantly alter bone healing [16]. Among included studies, the most common method of antibiotic impregnation was via antibiotic solution or dry powder mixing, the former of which is also utilized during preparation of *BioMim* in the present study. Spine surgery-specific applications of bone grafts for antimicrobial delivery have also been investigated. Shiels et al. demonstrated the use of vancomycin-infused demineralized bone matrix in lumbar fusion procedures; however, the graft was not studied in conjunction with spinal instrumentation [2,17].

This study proposes a novel tissue engineering approach to intraoperative prophylaxis in instrumented spine surgery. We present a vancomycin infused iteration of our previously described recombinant human bone morphogenetic-2 (rhBMP-2) loaded, biomimetic bone graft, *BioMim*, tested in our previously described rat model of spinal implant-associated infection [18,19]. We hypothesize that the infusion of vancomycin and rhBMP-2 in *BioMim* (BioMim-rhBMP-2-VCM) will mitigate infectious colonization of the spinal implant, despite inoculation with MRSA, while also promoting arthrodesis via the prolonged elution of rhBMP-2.

Methods

Experimental design

All experiments were performed following Johns Hopkins Animal Care and Use Committee (Protocol #RA21M151) and Institutional Biosafety Committee (IBC Registration #P2109210101) approved protocols and procedures. Thirty syngeneic female Lewis rats (Charles River Laboratories, Wilmington, MA), 6 to 8 weeks of age, weighing 100 to 150 g, were randomly assigned to six experimental groups. Experimental groups were categorized according to dosage of inoculated agent (phosphate buffered saline [PBS] or 1×10^5 MRSA CFU) and vancomycin infusion within the implanted bone graft: no vancomycin infusion, low-dose vancomycin infusion (0.18 mg/g), or high-dose vancomycin infusion (0.89 mg/g; Table 1). All grafts also contained a 125 μg rhBMP-2 infusion to facilitate arthrodesis.

Culture of bioluminescent MRSA

Inocula of 1×10^5 methicillin-resistant *S. aureus* (MRSA) were prepared as previously described [18]. All work with bacteria was performed in Biosafety Level 2 certified fume hoods using aseptic technique. The bioluminescent USA300 *S. aureus* strain was chosen for all inocula because of its stably integrated *luxABCDE* operon from *Photorhabdus luminescens* that confers bioluminescence with metabolic activity, and there exists evidence in the literature of this strain establishing biofilms on surgical-grade titanium implants [20,21].

Preparation of titanium implant

Titanium implants that were to be inoculated with either PBS or MRSA were prepared as previously described [18]. A surgical-grade titanium wire (22-AWG/.34 mm^2, Grade 1, TEMCo, Fremont, CA) was shaped into a staple-like design to mimic pedicle screws and rod fixation in spinal fusion surgeries. A 7 mm base length was chosen to span the L4–L5 vertebrae, and 3 mm prongs were angled at 35 degrees ventrally to insert into the intended pedicles from the entry point at the base of the transverse processes.

Synthesis of BioMim bone graft

The bone graft used in this experiment is an iteration of our previously described graft, BioMim-PDA [19]. *BioMim* is a porous, homogeneously dispersed solid mixture of pro-regenerative small intestinal submucosa (SIS) as the organic component, and bioactive glass as the inorganic component [19]. Vancomycin (VCM) was infused into the scaffold to convey antimicrobial properties. Briefly, pharmaceutical-grade

Table 1
Experimental grouping

Experimental group	Implant inoculation	Vancomycin infusion	No. animals	Mortality rate within 72 hours of surgery
PBS (Control)+No Vancomycin+125 μg rhBMP-2	1X PBS	None	5	20%
PBS (Control)+Low Dose Vancomycin+125 μg rhBMP-2	1X PBS	0.18 mg/g	5	0%
PBS (Control)+High Dose Vancomycin+125 μg rhBMP-2	1X PBS	0.89 mg/g	5	0%
S. aureus 1×10^5 CFU+No Vancomycin+125 μg rhBMP-2	*S. aureus* 1×10^5 CFU	None	5	20%
S. aureus 1×10^5 CFU+Low Dose Vancomycin+125 μg rhBMP-2	*S. aureus* 1×10^5 CFU	0.18 mg/g	5	0%
S. aureus 1×10^5 CFU+High Dose Vancomycin+125 μg rhBMP-2	*S. aureus* 1×10^5 CFU	0.89 mg/g	5	20%

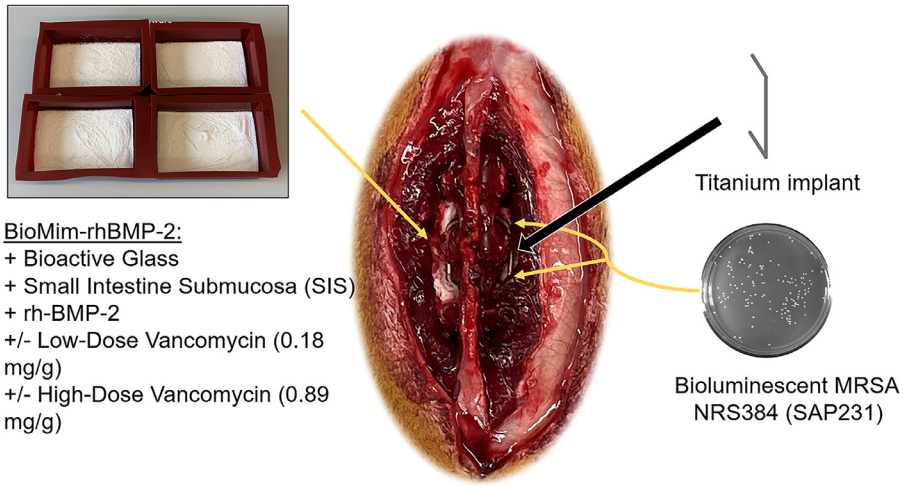

Figure 1. Surgical procedure. Titanium implants inoculated with bioluminescent MRSA and biomimetic bone grafts infused with varying concentrations of vancomycin were introduced at the L4–L5 level in the spines of 30 female Lewis rats. Titanium implants were inserted into lumbar pedicles at the L4–L5 levels, mimicking pedicle screw and rod fixation in spinal fusion procedures and providing an abiotic surface for bacterial growth and biofilm development. The biomimetic bone graft was overlaid between the L4 and L5 transverse processes following decortication in order to promote bony fusion.

SIS was digested using a pepsin-HCl solution. Following neutralization with NaOH, rhBMP-2, 45S5 bioactive glass (inorganic component), and the appropriate vancomycin dose (low dose: 0.18 mg/g; high dose: 0.89 mg/g) were added and homogeneously incorporated while stirring at room temperature.

Because a systematic review of antibiotic-loaded bone grafts found that vancomycin doses greater than 10,000 μg/mL caused significant osteoblastic death while concentrations less than 1,000 μg/mL had no impact on osteoblast survival [16], vancomycin dosage was selected such that one group would receive a low-dose of vancomycin infusion (982 μg/mL) below the 1,000 to 10,000 μg/mL range, while the other group would receive a high-dose (4,920 μg/mL) that is within this range. Organic/inorganic components were introduced at a 1:2 weight/weight ratio as previously described [19]. The mixture was then poured into a 2″ x 3″ mold and lyophilized to form the final biomimetic, rhBMP-2 and vancomycin-infused bone graft, termed BioMim-rhBMP-2-VCM. The graft was then cut into 16 mm x 6 mm pieces for implantation using sterile technique and stored at -20°C until implantation.

Surgery

Posterolateral instrumentation and fusion at the L4–L5 level was performed on thirty female Lewis rats divided into six experimental groups (Section I, Fig. 1). Induction of anesthesia and surgical procedures were performed as previously described [18,22]. The L4 and L5 transverse processes (TPs) were exposed, decorticated using a motorized burr, and then cannulated to create a trajectory through rat pedicles into which the titanium implant could be inserted. A 26-gauge needle was used to verify that the planned pedicle trajectory was without lateral or medial breach. The titanium implant was then inserted, and bone grafts with or without vancomycin were subsequently inserted lateral to the titanium implant along the L4–L5 fusion bed, in accordance with the randomly assigned experimental group (Fig. 1). Prior to closure, rats were taken inside a fume hood, and titanium implants were inoculated with 10 μL of either PBS or 1×10^5 CFU MRSA.

In vivo imaging of bioluminescent MRSA

Establishment of infection over the six-week postoperative period was quantitatively evaluated using the Lumina III IVIS system (PerkinElmer, Hopkinton, MA, USA) as previously described [18]. Postoperative scans were performed immediately after the procedure, on the third postoperative day, and each week following the procedure. Living Image software was used to quantify bioluminescent total flux (photons/second) emitted in the thoracolumbar region in each rat using regions of interest (ROIs) with uniform dimensions.

MicroCT assessment of spinal fusion

Spines were harvested six weeks postoperatively and fixed in 4% paraformaldehyde. Harvested lumbar spines were then imaged using a nano Single-Photon Emission Computed Tomography (SPECT)/Computed Tomography (CT) Small Animal Imaging System (Mediso Medical Imaging Systems). Coronal CT images were evaluated by two authors (C.J.R. and A.D.D) in a blind fashion. Volumetric analysis of the fusion masses in coronal sections was performed using ImageJ Software (US National Institutes of Health) and the Volumest ImageJ Software plugin to quantitatively assess arthrodesis as previously described [23]. Each voxel of the CT reconstruction corresponded to 0.2 mm^3 [23].

Fusion masses on both sides of the spine (e.g., left or right-sided from the TP of L4 to the TP of L5) were also radiographically scored as follows: a score of 0 if no continuous fusion mass was present between the TPs of L4 and L5, a score of 1 if a fusion mass was observed with partial bridging between the TPs of L4 and L5, and a score of 2 if continuous bridging bone from the TPs of L4 to L5 was observed. A modified CT score was then assigned to each harvested spine as an average of the unilateral scores of each individual fusion mass, as previously described [24].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 software (GraphPad, La Jolla, CA, USA). One-way ANOVA with multiple pairwise comparisons was performed for a time-dependent comparison of *in vivo* bioluminescence between groups. Two-way ANOVA with pairwise comparison was performed for a time-independent comparison of the average *in vivo* bioluminescence of the animals in each group. Two-way ANOVA with pairwise comparison was also performed to compare the average fusion mass volumes between animals of each group based on each group's inoculation agent and the vancomycin dose of the implanted bone graft. CT scores of the fusion masses were compared using Kruskal-Wallis testing with multiple comparisons. A p-value of less than .05 was considered statistically significant for all comparisons.

Results

Surgical outcomes

After surgery, rats were closely monitored for the development of neurological deficits as well as changes in general condition. Three of thirty rats died between 48 and 72 hours post-surgery. Of the three non-surviving rats, one was inoculated with PBS and received a bone

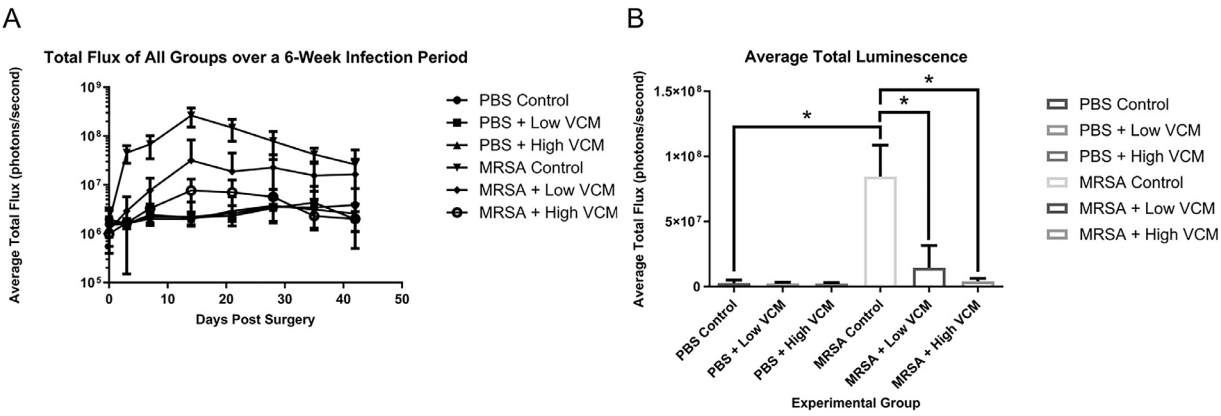

Figure 2. In-vivo bioluminescent flux by experimental group. Error bars are obtained from standard deviations of average luminescence scans per group (n=3 for PBS control, n=5 for the PBS+low vancomycin, n=5 for the PBS+high vancomycin, n=3 for the MRSA CONTROL, n=3 for the MRSA+low vancomycin, and n=2 for the MRSA+high vancomycin). One PBS control animal is omitted from analysis due to contamination. Panel A quantifies bioluminescent flux per IVIS scan over a 6-week infection period for all vancomycin infused groups as compared to control groups which received grafts without vancomycin infusion. Total flux is graphed on a logarithmic scale for ease of comparison between groups. MRSA-infected rats treated with low-dose and high-dose of vancomycin both exhibited significantly lower bioluminescence than MRSA-infected animals over the entire six-week infection period (p=.019 and p=.007, respectively). Panel B depicts the average luminescence value for each rat and the mean+SD of each experimental group, irrespective of time. One-way ANOVA with multiple comparison analysis revealed that MRSA-infected control animals exhibited significantly greater average bioluminescence than their respective PBS-inoculated controls (p=.045), indicating the successful establishment of infection. MRSA-infected animals treated with low-dose vancomycin and MRSA-infected animals treated with high-dose vancomycin both had significantly reduced bioluminescence compared to MRSA-infected control animals (p=.027 and p=.047, respectively), indicating successful antimicrobial prophylaxis. Further, MRSA-infected animals treated with low-dose vancomycin and MRSA-infected animals treated with high-dose vancomycin did not exhibit significantly different bioluminescence than their respective PBS-inoculated treatment groups (p=.514 and p=.669, respectively). *Denotes p<.05.

graft with no vancomycin infused, one was inoculated with 1×10^5 CFU MRSA and received a bone graft with no vancomycin infused, and one was inoculated with 1×10^5 CFU MRSA and received a bone graft with a high dose vancomycin infusion (Table 1). Because these rats could not be fixed in paraformaldehyde in a timely fashion, these samples were excluded from further postmortem analysis. A single animal inoculated with PBS and receiving no vancomycin infusion in its bone graft exhibited contamination in the postoperative period, as defined by the unintentional presence of enhancing bioluminescent MRSA signal in IVIS scans. This sample was removed from postoperative analysis. Of note, among our remaining 26 rat cohort, five animals, one in the MRSA Control group, two in the MRSA+Low VCM group, and two in the MRSA+High VCM group, received inoculations of a non-luminescent strain of MRSA due to error and were excluded from in-vivo imaging analysis only. One sample in the MRSA Control group was unable to be imaged due to sample deterioration and was excluded from postoperative microCT analysis only.

Assessment of infection with in-vivo bioluminescent imaging

Tracking of MRSA bioluminescence *in vivo* revealed both the successful establishment of infection and the antimicrobial activity of the vancomycin-infused bone graft in mitigating infection (Fig. 2A). Animals infected with 1×10^5 CFU of MRSA that received a bone graft lacking vancomycin displayed significantly higher bioluminescence over the six-week infection period than animals that received a PBS inoculation with no vancomycin infusion, indicating successful establishment of infection in our MRSA control group (p=.029). One-way ANOVA of infected groups determined a statistically significant difference in in vivo bioluminescence based on vancomycin dose (p=.007). Both the low-dose vancomycin infused grafts and the high-dose vancomycin infused grafts led to significantly lower bioluminescence over the six-week infection period when compared to MRSA controls using multiple comparison testing with one-way ANOVA (p=.019 and p=.007, respectively).

To conduct a time-independent analysis, the average bioluminescent flux was calculated for each animal and then stratified based on their experimental group (Fig. 2B). Here, we observed that both the MRSA plus low-dose vancomycin graft group and the MRSA plus high-dose vancomycin graft group had significantly lower average total flux than MRSA controls using two-way ANOVA with pairwise comparison (p=.027 and p=.047, respectively). Further, there was no significant difference in the average bioluminescent flux between animals in low and high-dose vancomycin graft groups (p=.587), suggesting little if any added antimicrobial benefit of high-dose vancomycin in reducing average infection on a per-cohort basis in this model.

MicroCT evaluation of bone formation

MicroCT analysis revealed successful arthrodesis in all experimental cohorts except for MRSA control (Fig. 3). At six-weeks postoperatively, the average volumes of fusion masses (mm^3) were significantly higher in PBS control animals when compared to MRSA control animals, both of which received a bone graft without vancomycin (p<.001). MRSA+low-dose vancomycin graft and MRSA+high-dose vancomycin graft groups also exhibited significantly increased volumes of fusion masses when compared to MRSA control (p<.001 for both comparisons; Fig. 3A). Further, when comparing fusion mass volume among the three PBS-inoculated cohorts receiving different doses of vancomycin infusion in their bone graft, two-way ANOVA demonstrated no significant difference in fusion mass volume based on vancomycin dose (p=.991). The average modified CT score at 6 weeks postoperatively, with higher scores indicative of increased continuity of TP-TP fusion masses, indicated that significantly higher CT scores were given to rats in the MRSA+low-dose vancomycin, MRSA+high-dose vancomycin, and PBS control groups when compared to the MRSA control group, with p values of .045, .040, and .015, respectively, using Kruskal-Wallis testing with multiple comparisons (Fig. 3B). Further, Kruskal-Wallis testing of the modified CT scores from the three PBS-inoculated cohorts receiving different doses of vancomycin revealed no significant difference in modified CT score (p=.825).

Representative coronal microCT images of harvested spines from each experimental group demonstrated successful fusion between the TPs of L4 and L5 in all groups except for MRSA control, which depicted pseudoarthrosis and a significant bone defect in the L5 vertebral body due to implant loosening and vertebral osteomyelitis (Fig. 4). Migration

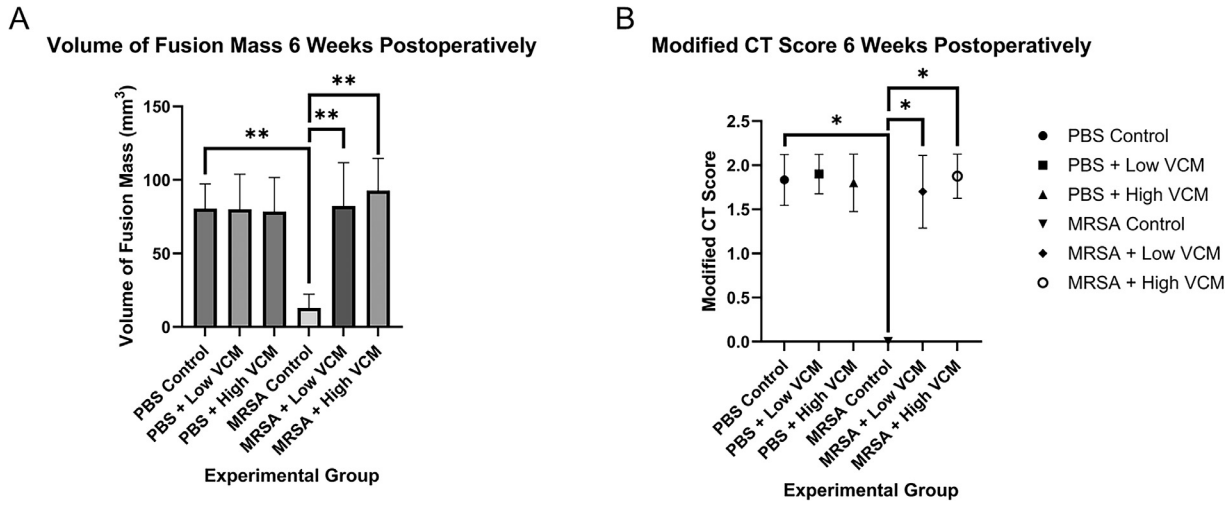

Figure 3. Assessment of TP-TP fusion masses and representative CT score at 6 weeks postoperatively. Error bars are obtained from standard deviations of average fusion volumes per group (n=3 for PBS control, n=5 for the PBS+low vancomycin, n=5 for the PBS+high vancomycin, n=3 for the MRSA control, n=5 for the MRSA+low vancomycin, and n=4 for the MRSA+high vancomycin). Panel A depicts the average fusion mass volume (mm^3) for each group. A significantly increased fusion mass volume is observed in PBS control animals when compared to MRSA control animals, both of which received a bone graft without vancomycin ($p<.001$). MRSA control animals also exhibited a significantly decreased volume of fusion masses when compared to both MRSA+low-dose vancomycin graft ($p<.001$) and MRSA+high-dose vancomycin graft ($p<.001$) groups. Panel B depicts the average modified CT score for each group at 6 weeks postoperatively. Significantly higher CT scores were given to rats in the MRSA+low-dose vancomycin, MRSA+high-dose vancomycin, and PBS control groups when compared to the MRSA control group, with p values of .045, .015, and .040, respectively. *Denotes $p<.05$. **Denotes $p<.01$.

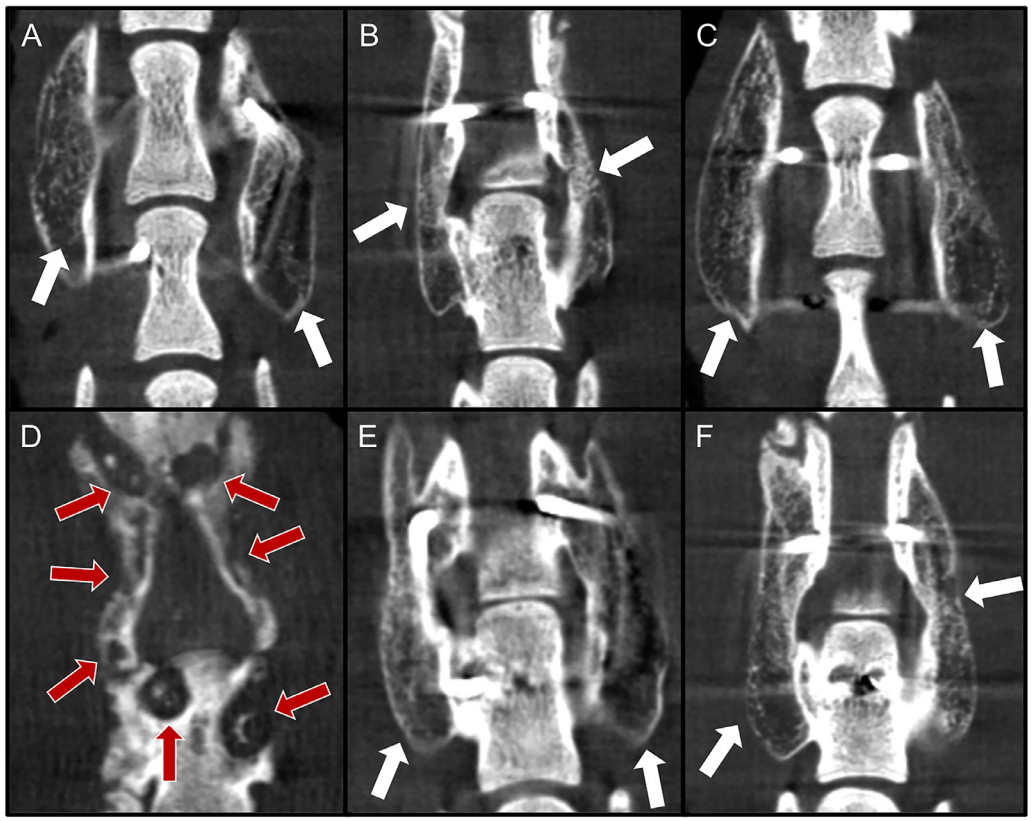

Figure 4. Coronal MicroCT images of spies harvested from representative animals from each group six weeks postoperatively. (A) PBS control, (B) PBS+low vancomycin, (C) PBS+high vancomycin, (D) MRSA Control, (E) MRSA+low vancomycin, and (F) MRSA+high vancomycin. Successful bilateral fusion (white arrows) is noted between transverse processes of the L4 and L5 vertebrae in all groups except (D) MRSA control, representing infected animals which received a graft not infused with vancomycin. (D) MRSA control micro-CT image depicts pseudoarthrosis and a significant bone defect (red arrows) in the L5 vertebral body due to implant loosening and vertebral osteomyelitis.

of the titanium implant was also noted in the remaining three MRSA control spines at 6-weeks postoperatively.

Discussion

Current strategies for prophylaxis of spinal implant-associated infection following instrumented spine surgery vary. In cases of such infection, implant removal may be indicated, which is associated with pseudoarthrosis, spinal instability, loss of normal lumbar lordosis, and infectious seeding of adjacent tissue [25–28]. Infection is also independently associated with increased likelihood of pseudoarthrosis, which may necessitate a revision procedure [29,30]. We utilize a rodent model of chronic spinal-implant associated infection to demonstrate the effect of a vancomycin-infused bone graft, *BioMim*, on both infection prophylaxis and arthrodesis. We demonstrate that (1) the low and high vancomycin doses of BioMim-VCM were equivalently prophylactic for preventing the establishment of infection despite MRSA inoculation, as indicated by bioluminescent imaging; and (2) fusion, as measured by volume of fusion mass and microCT scoring, was not negatively impacted by the presence of vancomycin at dosages utilized in the *BioMim* graft.

To the authors' knowledge, this is the first application of an antimicrobial-infused bone graft to prevent implant-associated infection in a rat model of instrumented spinal fusion. One prior non-instrumented rabbit model of spinal surgical site infection demonstrated the effectiveness of vancomycin-loaded demineralized bone matrix in reducing bacterial burden following infection [17]. However, this study also reported significantly reduced arthrodesis in the vancomycin-loaded cohort and proposed the utility of vancomycin-loaded demineralized bone matrix as an extension of iliac bone crest autograft [17]. Multiple retrospective clinical studies of infection in spinal fusion surgery have also demonstrated the effectiveness of vancomycin impregnation in autologous bone grafts to prevent infection [31,32]. However, the use of antibiotic-loaded bone grafts is currently not standard in spinal or orthopedic applications due to the possible impairment of osteoblast differentiation [33,34].

A systematic review of antibiotic-loaded bone grafts found that vancomycin doses greater than 10,000 μg/mL caused significant osteoblastic death while concentrations less than 1,000 μg/mL had no impact on osteoblast survival. However, evidence of osteotoxicity in studies that used dosages between 1,000 and 10,000 μg/mL was inconclusive [16]. Because the vancomycin concentrations in *BioMim* prior to lyophilization were 982 μg/mL and 4,920 μg/mL for the low-dose and high-dose respectively, evidence in the review for osteotoxicity of our high-dose group is inconclusive. Prior to lyophilization, both infusions surpass the reported vancomycin minimum inhibitory concentration for MRSA of 2 μg/mL [35].

Because of *BioMim's* synthetic nature, the graft can be manipulated with various osteoinductive and/or antimicrobial small molecules, growth factors, or scaffold materials to elicit prophylaxis and promote arthrodesis despite an infectious inoculation. This quality makes BioMim unique from native bone or FDA-approved biomaterials such as Infuse absorbable collagen sponge (ACS). Present clinical use of currently available FDA-approved graft materials involves the topical application or mixing of the antibiotic with the scaffold, and a recent meta-analysis observed application of powdered vancomycin in spine surgery procedures has been suggested to increase rates of pseudarthrosis [36]. Further, the abundance of basic science studies demonstrating the osteotoxic effects of vancomycin in vitro and in vivo would suggest against its use with a cellular bone graft such as autologous bone or allograft harvested bone [16,37,38,17,39]. For example, in a previous study conducted by our laboratory using this posterolateral model of spinal fusion, we observed a vancomycin dose-dependent decrease in bone volume formation and rate of arthrodesis when iliac crest bone graft was mixed with vancomycin prior to implantation [24].

In this study, we used 45S5 bioactive glass for the inorganic scaffold component of the BioMim scaffold and a supplementary pre-loaded infusion of rhBMP-2 and vancomycin, incorporating two substances with osteoinductive activity and one substance with potential osteotoxicity [40–42]. We have previously demonstrated the effectiveness of rhBMP-2 infusion in *BioMim* to promote increased bone formation when compared to Infuse despite the use of lower concentrations of rhBMP-2 [19]. Further, the incorporation of small intestine submucosa as the organic component of our scaffold conveys a pro-regenerative substrate that has been demonstrated in cardiovascular tissue engineering to promote native tissue growth while maintaining reduced antigenicity [43]. Therefore, despite the reported osteotoxic potential for high doses of vancomycin to impede spinal fusion, we observed no statistical difference in rates of arthrodesis or volume of bone formation with increasing doses of vancomycin infusion. In fact, we observed the greatest bone volume formation in our MRSA+high-dose vancomycin cohort, although this result was not statistically significant. Additionally, lyophilized bone grafts, such as *BioMim*, have been reported to be less antigenic and have significantly greater storage shelf lives compared to fresh frozen grafts [44,45]. This lyophilization also conveys a biomimetic porosity to *BioMim*, possibly inducing similar superior osteoinductive capabilities that give cancellous bone an advantage over cortical bone for use in autologous bone grafts [45].

Except for alterations in vancomycin concentrations, the same formulation of *BioMim* was used in all experimental groups. However, we did not observe comparable rates of osteogenesis and arthrodesis in our MRSA control cohort which demonstrated significant establishment of infection *in vivo*. Infection is a potential etiology of pseudarthrosis following spinal fusion [46,47]. A retrospective study by Burkhard et al. investigated 128 cases of revision surgery for pseudoarthrosis and observed an occult infection rate of 10% [47]. We also observed implant loosening or implant migration in all animals in the MRSA control cohort. In clinical cases of spinal fusion, significant evidence exists implicating infection as a contributor to implant failure [48–50]. A retrospective study by Boishardy et al. observed an infection rate of 29.1% in 110 cases of spinal metal explantation following spinal fusion surgery [30]. Implant migration was also proposed to be a consequence of pseudoarthrosis [51]. Therefore, it is difficult to conclude whether reduced arthrodesis in our MRSA control group is due to infection or implant migration as a consequence of infection. However, this trend does highlight the importance of effective prophylaxis to promote successful arthrodesis, as all animals outside of the MRSA control cohort exhibited significantly greater fusion volume and rates of arthrodesis without implant loosening or migration.

While the present study does suggest antimicrobial and osteogenic potential of *BioMim* in posterolateral spinal fusion, it does have several limitations. First, the use of rhBMP-2 in spine surgery remains controversial due to potential complications including ectopic bone growth, implant displacement, infection, radiculitis, and retrograde ejaculation [52–56]. Our study is also limited by a small sample size of five animals per group, which was further reduced by three mortalities excluded from all postoperative analysis and five erroneous inoculations with a non-bioluminescent strain of MRSA that were excluded from *in vivo* bioluminescent analysis. Therefore, the level of evidence that this study conveys is limited, particularly concerning the antimicrobial utility of BioMim because of the smaller sample size for in vivo bioluminescent analysis. Further, while we acknowledge procedural invasiveness as a risk of mortality, the procedure was tested extensively with microCT verification of implant placement prior to our experiment and in our previously published model to reduce this risk [18]. We also observed contamination in 1/15 or 6.67% of our PBS-inoculated animals, which is consistent with the 0.7-20% infection rate observed in adult posterior spinal fusion procedures [9]. This contaminated animal was part of the PBS-inoculated cohort lacking vancomycin infusion within the bone graft, and open-air exposure to bacteria in the same hood may have contributed to this contamination, comparable to cases of contamination in the operating room [57].

There are also limitations inherent to the use of bioluminescent imaging to quantify infectious colonization *in vivo*. While this method was used in our previous model as well as other models of spinal implant-associated infection [18,58,59], MRSA biofilm formation may reduce the metabolic activity of embedded bacterial colonies, consequently reducing bioluminescence. We acknowledge additional limitations to the resolution of bioluminescent imaging of low-density bacterial colonies less than 500 CFU [60]. We also acknowledge that the use of BioMim as a delivery vector for vancomycin and rhBMP-2 in this study introduces an additional variable that is less clinically translatable than the choice of an FDA-approved bone graft such as Infuse or native bone. Future work includes further optimization of *BioMim* for clinical translation, including potential incorporation of anti-biofilm agents.

Conclusion

We demonstrate the prophylactic efficacy of BioMim-rhBMP-2-VCM, a vancomycin-and rhBMP-2-infused bone graft, in a rat model of instrumented spinal fusion. MRSA-infected cohorts receiving bone grafts with low or high-dose vancomycin infusions exhibited significantly lower total bioluminescent flux throughout the entire six-week study (p=.019 and p=.007, respectively) and demonstrated significantly lower average bioluminescent signal per animal than MRSA control animals receiving grafts without vancomycin (p=.027 and p=.047, respectively). However, we acknowledge that the small sample size of two and three animals in the MRSA+High VCM group and MRSA+Low VCM group, respectively, for in vivo bioluminescent imaging analysis specifically, limits the level of evidence provided by this study outcome.

Despite the reported osteotoxicity of vancomycin, both qualitative scoring of arthrodesis (p=.825) and bone volume quantification (p=.991) were not found to be statistically different when comparing PBS-inoculated cohorts. Further, MRSA+low-dose vancomycin graft and MRSA+high-dose vancomycin graft cohorts were both found to have statistically greater qualitative arthrodesis scoring (p=.045 and p=.015, respectively) and bone volume formation (p<.001 for both cohorts) than the MRSA control cohort receiving grafts without vancomycin, indicating a correlation between effective infection prophylaxis and arthrodesis. Further study of the antimicrobial application of *BioMim*, particularly its effectiveness against other infectious agents, may aid in the clinical translation of this material and inform future testing of novel antimicrobial agents.

Summary

BioMim-rhBMP-2-VCM effectively mitigated implant-associated infection while promoting arthrodesis in a rat model of instrumented lumbar fusion.

Funding disclosures

Dr. Cottrill reported grants from the National Institute on Aging and National Institute of General Medical Sciences during the conduct of the study and a patent pending for intellectual property related to biological scaffolds. Dr. Witham reported grants from the Gordon and Marilyn Macklin Foundation, a philanthropic grant from Jay Scaramucci during the conduct of the study, and a patent pending for intellectual property related to biological scaffolds.

Declarations of competing interests

One or more of the authors declare financial or professional relationships on ICMJE-NASSJ disclosure forms.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.xnsj.2024.100323.

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