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# The Effects of Antibiotic-Impregnated Spacers on Bone Healing in an Animal Model of the Induced Membrane Technique

## Healing of a Critical-Size Femoral Defect in a Rat Model

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**Background:** Surgeons performing the induced membrane technique (IMT) often incorporate antibiotics into the spacer at the first stage of the surgical procedure to prevent or treat infection. However, the effect of antibiotic use on subsequent bone healing is not clear. This study aimed to investigate if antibiotic-impregnated spacers impact subsequent bone healing in a rat model of the IMT.

**Methods:** Inbred male rats (Fischer 344) were randomly divided into 3 groups according to the antibiotic dose in the spacer: (1) control (no antibiotics), (2) low-dose (1.2 g tobramycin and 1.0 g vancomycin per 40 g of polymethylmethacrylate [PMMA]), and (3) high-dose (3.6 g tobramycin and 3.0 g vancomycin per 40 g of PMMA). We created a 5-mm segmental defect in the right femoral diaphysis. The bone was stabilized with a plate and screws, and the assigned spacer was inserted into the defect. Four weeks later, the spacer was removed and bone graft was placed within the defect. Radiographs made 12 weeks after grafting were scored according to union status and degree of bone healing. Micro-computed tomographic (CT) analysis and biomechanical testing were also performed at 12 weeks.

**Results:** Full radiographic union was achieved in 10 (83%) of 12 control animals, 13 (100%) of 13 low-dose animals, and 8 (62%) of 13 high-dose animals (high-dose compared with low-dose: risk ratio, 11.0; $p = 0.039$). The control group demonstrated higher bone volume compared with the high-dose group (mean difference, 9.0 $mm^3$; $p = 0.039$), and there was a trend toward higher bone volume in the low-dose group compared with the high-dose group (mean difference, 8.1 $mm^3$; $p = 0.06$). The biomechanical results demonstrated that maximum stiffness was significantly higher in the low-dose group compared with the high-dose group (mean difference, 14.1 N*mm/degree; $p = 0.009$).

**Conclusions:** Our results demonstrated that low doses of antibiotics in PMMA spacers used for the IMT did not impair bone healing. However, high doses of antibiotics demonstrated inferior bone healing.

**Clinical Relevance:** The addition of high-dose antibiotics to the PMMA spacers used for the IMT may result in impaired bone healing and should be used with caution.

The induced membrane technique (IMT), also known as the Masquelet technique, has become an increasingly popular treatment option for managing segmental defects and nonunion[1]. The IMT is a 2-stage surgical technique. In the first stage, the bone is debrided of devitalized tissues and stabilized, and a polymethylmethacrylate (PMMA) spacer is inserted into the bone defect. The PMMA spacer triggers a foreign-body immune response, resulting in the formation of a periosteum-like membrane around the spacer. In the second stage, the spacer is removed through an incision in the induced membrane, and the defect is filled with autologous cancellous bone graft, followed by membrane closure[2].

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Although not described in the original reports of the technique, surgeons now frequently incorporate antibiotics into the PMMA spacer to prevent or treat infection[3,4]. No clinical studies have evaluated the impact of antibiotic use on subsequent outcomes of the IMT. Preclinical studies have demonstrated that antibiotic use in the PMMA spacers is effective at eradicating infection and impacts various membrane properties differently, depending on the type of antibiotic used[5,6]. However, to our knowledge, the ultimate impact of these changes in membrane properties on subsequent bone healing has not been reported. Overall, there has been a paucity of studies directly evaluating the effects of antibiotic-impregnated spacers on bone healing.

Accordingly, there is a clear need to understand if the common clinical use of antibiotic-impregnated spacers as part of the IMT impacts the critically important goal of bone healing. This study aimed to evaluate the effect of adding antibiotics to the PMMA spacer, in both low and high doses, on bone healing in a small-animal model of the IMT. We hypothesized that antibiotics would not demonstrate a negative effect on bone healing when used in IMT spacers.

## Materials and Methods

### *Animals*

Male Fischer 344 inbred rats (250 to 300 g; Charles River Laboratories) were used in order to allow for inter-animal graft transfer. All procedures were approved by the St. Michael's Hospital Animal Care Committee. Rats were randomly divided into 3 groups, each receiving a distinct PMMA spacer in the first stage of the IMT: (1) PMMA alone (control group; n = 12), (2) PMMA + 1.2 g of tobramycin and 1.0 g of vancomycin per 40 g of PMMA (low-dose group; n = 13), or (3) PMMA + 3.6 g of tobramycin and 3.0 g of vancomycin per 40 g of PMMA (high-dose group; n = 13). The low and high doses of antibiotic were selected to represent the lower and higher ends of the spectrum of antibiotic use in the IMT, based on a systematic review of the clinical literature on the IMT that we conducted[4] and on consultation with experts in orthopaedic trauma.

### *Surgical Intervention*

#### Stage 1

The surgical procedures were performed as previously described[7]. Briefly, the animals were anesthetized with 2.5% isoflurane (1 L/min), then given sustained-release buprenorphine subcutaneously and intramuscular cefazolin. The right femur was exposed through a lateral approach and a 5-hole mini-plate (2.5 cm; Synthes) was fixed onto the femur. A 5-mm segmental defect was then created in the mid-diaphysis at the central, empty screw hole.

A PMMA spacer (Simplex P; Stryker) with no, low-dose, or high-dose antibiotics (according to the group assignment) was then placed and molded within the defect. The PMMA spacers that included antibiotics were mixed by the surgeon, using predetermined amounts of powdered antibiotics to achieve the antibiotic concentrations described above. The incision was closed in a standard fashion, and the animals were allowed to return to full weight-bearing immediately.

#### Stage 2

Donor Fischer 344 inbred rats were anesthetized and then killed via cervical dislocation. Morselized corticocancellous bone was isolated from the proximal and distal femora, proximal tibiae, and thoracolumbar vertebral bodies of the donor rat using aseptic technique. One donor provided bone graft for 3 to 4 recipient rats, and bone graft from all sources was pooled such that all recipient animals received identical grafts. The time from euthanasia of the donor animal to graft implantation was limited to a maximum of 150 minutes. A Fischer 344 inbred rat was used to simulate the use of autogenous bone graft, which the vast majority of clinicians use when employing the IMT.

Four weeks after stage 1, radiographs were made to confirm that the implant was stable and the PMMA spacer remained in position. Following anesthesia, the defect site was exposed with a small incision through the previous surgical site. The induced membrane was identified visually and confirmed to be present in all cases. It was then incised longitudinally, and the spacer was removed with maintenance of the plate and screw fixation. Subsequently, a standardized weight of bone graft (80 mg) was placed within the defect, and the membrane was sutured closed to contain the graft.

### *Radiographic Assessment*

Following the second surgical procedure, all animals underwent standardized anteroposterior radiographs every 2 weeks until the study end point at 12 weeks after stage 2. The radiographs were evaluated independently by 2 orthopaedic trauma surgeons in a blinded fashion. The radiographs were classified as showing full union, partial union, or nonunion. A modified version of the Lane and Sandhu scoring system[8] was also used to score defect healing at 12 weeks (Table I). Our modifications were intended to give more weight to the union status at the proximal and distal ends of the defect, an important consideration for bone healing in the setting of a segmental bone defect[7].

### *Study End Point*

At 12 weeks following stage 2, all animals were anesthetized with isoflurane (2.5%) and were killed via an intracardiac injection of T-61 (Merck Animal Health).

### *Micro-Computed Tomography (CT) Analysis*

Micro-CT (SkyScan 1174; Bruker) was used to quantify bone volume, bone volume/total volume, and bone mineral density. The full details of our micro-CT protocol are provided in the Appendix.

### *Biomechanical Analysis*

Following micro-CT, operatively treated femora were tested under a torsional load using an MTS Bionix 858 (MTS Systems), and the maximum torque, maximum stiffness, and yield point were calculated. The full details of our biomechanical testing protocol are provided in the Appendix.

### *Statistical Analysis*

An a priori power calculation was performed using G*Power software (Heinrich Heine University). Our study was powered

**TABLE I Modified Lane and Sandhu Scoring System***

| | |
|---|---|
| Degree of bone formation | |
| None | 0 |
| 25% of defect | 1 |
| 50% of defect | 2 |
| 75% of defect | 3 |
| 100% of defect | 4 |
| Union status: proximal | |
| Nonunion | 0 |
| Partial union | 2 |
| Full union | 4 |
| Union status: distal | |
| Nonunion | 0 |
| Partial union | 2 |
| Full union | 4 |
| Bone remodeling | |
| No sign of remodeling | 0 |
| Evidence of medullary canalization | 2 |
| Evidence of cortical remodeling | 4 |
| Maximum total score | 16 |

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to detect a 25% between-group difference in maximum torque, our primary outcome, corresponding to an effect size of 0.7. Based on previous biomechanical results in a similar bone defect model, the sample size required to achieve 90% study power with a 2-sided, type-1 error rate of 5% was 10 rats per group (30 rats total)[9]. Accounting for a possible surgical or biomechanical error rate of 20%, the total sample size was increased to 36 rats.

Statistical analysis was performed using GraphPad Prism 9 (Dotmatics) and SAS (version 9.4; SAS Institute). Between-group differences in maximum torque were our primary outcome. Significance was set at $p \leq 0.05$. Full details of the statistical methods and tests used for each outcome are available in the Appendix.

## Results

### *Radiographic Results*

Radiographs confirmed that PMMA spacers were optimally placed in all animals and did not migrate after 4 weeks, and that bone graft was contained within the membrane during the second-stage surgical procedure. At 12 weeks after grafting, the majority of animals in each group reached full union (Table II). Complete union was achieved in 83% (10 of 12) of the animals in the control group, 100% (13 of 13) in the low-dose group, and 62% (8 of 13) in the high-dose group. The rate of union was significantly higher in the low-dose group compared with the high-dose group (risk ratio for not achieving union, 11.0 [95% confidence interval (CI), 0.67 to 180.66]; p = 0.039). Representative radiographs are shown in Figure 1.

The 12-week radiographs were also scored using a modified 16-point version of the Lane and Sandhu scoring system. The low-dose antibiotic group demonstrated higher scores than the control and high-dose groups in all categories (Table III); however, no differences between groups reached significance.

### *Micro-CT Results*

Quantitative micro-CT analysis was performed on all specimens at 12 weeks (Fig. 2, Table IV). The control group demonstrated higher bone volume compared with the high-dose group (mean difference, 9.0 $mm^3$ [95% CI, 0.4 to 17.6 $mm^3$]; p = 0.039) (Fig. 2-A). There was a trend toward higher bone volume in the low-dose group compared with the high-dose group (mean difference, 8.1 $mm^3$ [95% CI, −0.3 to 16.5 $mm^3$]; p = 0.06). Bone volume was similar between the low-dose and control groups (mean difference, 0.9 $mm^3$ [95% CI, −7.7 to 9.5 $mm^3$]; p = 0.96). There were no significant differences between groups with regard to bone volume/total volume or bone mineral density (Figs. 2-B and 2-C).

### *Biomechanical Results*

Three femora (2 in the high-dose group and 1 in the low-dose group) broke during manipulation or preparation for testing and were therefore excluded from biomechanical analysis. In general, the control and low-dose groups demonstrated superior biomechanical results, with the low-dose group performing the best. Maximum stiffness was significantly higher in the low-dose group compared with the high-dose group (median difference, 14.1 N*mm/degree [95% CI, 1.8 to 26.5 N × mm/degree]; p = 0.009) (Fig. 3-A, Table V). None of the other differences observed were significant, although the high-dose antibiotic group demonstrated consistently inferior biomechanical properties compared with the other 2 groups (Figs. 3-B and 3-C, Table V). The differences observed in the biomechanical strength of the healing in the low-dose group were notable, as the average maximum stiffness, maximum torque, and yield point were approximately double those observed in the high-dose group. The differences were primarily driven by the high number of zero values recorded in the high-dose group (6 of 11 specimens) compared with the low-dose group (1 of 12 specimens) or the control group (2 of 12 specimens), which occurs when there is an absence of clinical union in the

**TABLE II Union Status at 12 Weeks After Bone Grafting**

| | Control (N = 12) | Low-Dose (N = 13) | High-Dose (N = 13) |
|---|---|---|---|
| Full union | 10 (83%) | 13 (100%) | 8 (62%) |
| Partial union | 0 (0%) | 0 (0%) | 5 (39%) |
| Nonunion | 2 (17%) | 0 (0%) | 0 (0%) |

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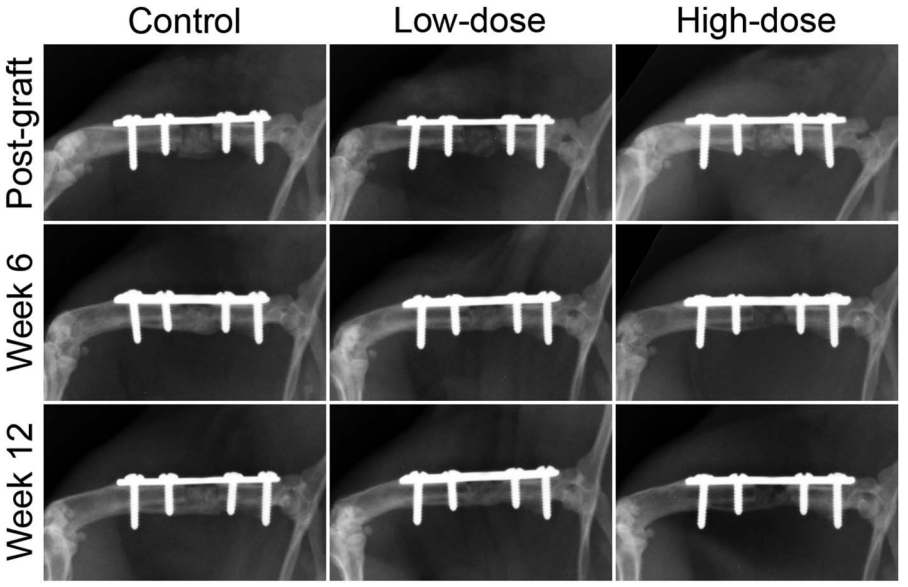


Fig. 1

Representative radiographs of the operatively treated femur made immediately after grafting and at 6 weeks and 12 weeks after grafting in animals from the control group, the low-dose group, and the high-dose group.

specimen once the plate is removed, such that it cannot be subjected to measurable torsion.

## Discussion

The IMT is increasingly being used to manage large bone defects and nonunion. Although many surgeons routinely perform the IMT with antibiotic-impregnated spacers, few studies have examined the effects of antibiotics with respect to this technique. Therefore, we aimed to investigate the impact of vancomycin and tobramycin-impregnated PMMA spacers, at both low and high doses, on the ultimate goal of bone healing in a preclinical model. Our results suggest that, at low doses, antibiotic-impregnated spacers do not negatively affect bone healing; however, at high doses, they may have a negative impact.

Based on radiographic assessment, 39% (5 of 13) of the animals in the high-dose group did not achieve full union, compared with 17% (2 of 12) in the control group, and none (0 of 13) in the low-dose group. The rate of union was significantly higher in the low-dose group compared with the high-dose group (risk ratio for not achieving union, 11.0 [95% CI, 0.67 to 180.66]; p = 0.039). Other outcomes, namely bone volume, maximum stiffness, maximum torque, and yield point, were also the lowest in the high-dose group. Notably, the average biomechanical strength of healing in the high-dose antibiotic group was approximately half of the values observed in the low-dose group, suggesting a clinically important reduction in healing strength. Although significant reductions were only observed in bone volume and maximum stiffness, it is important to note that spacers impregnated with high doses of antibiotics demonstrated inferior bone healing consistently across the majority of outcomes measured, suggesting that high-dose antibiotics should be employed with caution. This is particularly important given that even higher antibiotic doses than those used in our high-dose group are sometimes used clinically as part of the IMT[3]. We speculate that a negative impact on bone healing may be even more apparent at higher doses.

**TABLE III Modified Lane and Sandhu Radiographic Scores at 12 Weeks After Bone Grafting**

| | Control* (N = 12) | Low-Dose* (N = 13) | High-Dose* (N = 13) | F Statistic† | P Value† |
|---|---|---|---|---|---|
| Bone formation (out of 4) | 3.50 ± 0.77 | 3.77 ± 0.44 | 3.50 ± 0.61 | 0.82 | 0.45 |
| Union status: proximal (out of 4) | 3.25 ± 1.36 | 4.00 ± 0.00 | 3.08 ± 1.19 | 2.93 | 0.07 |
| Union status: distal (out of 4) | 3.17 ± 1.11 | 3.92 ± 0.28 | 3.15 ±1.46 | 2.16 | 0.13 |
| Bone remodeling (out of 4) | 0.25 ± 0.45 | 0.38 ± 0.65 | 0.15 ±0.38 | 0.68 | 0.51 |
| Total score (out of 16) | 10.17 ± 3.26 | 12.08 ± 0.91 | 9.88 ± 3.02 | 2.71 | 0.08 |

*The scores are given as the mean and the standard deviation. †Test statistics (F-values) and p values produced by omnibus 1-way analysis of variance tests.

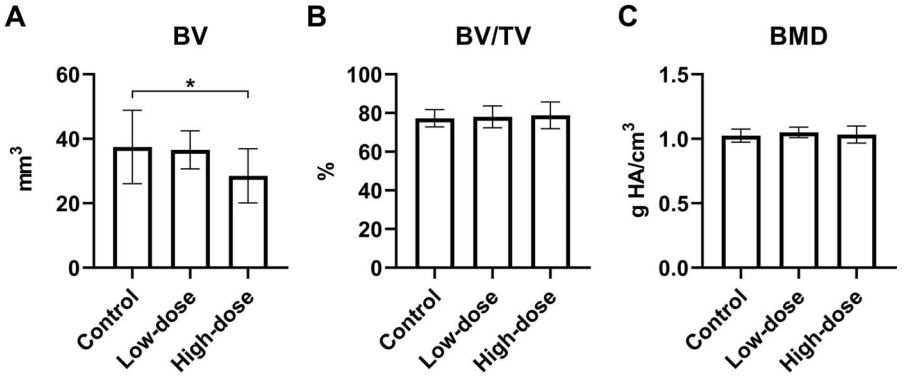


Fig. 2

Bone volume (BV) (**Fig. 2-A**), bone volume ratio (BV/total volume [TV]) (**Fig. 2-B**), and bone mineral density (BMD) (**Fig. 2-C**) quantified by micro-CT analysis of operatively treated femora harvested 12 weeks after bone grafting. The data are presented as the mean and the standard deviation. The asterisk denotes a significant difference ($p \leq 0.05$). There were 12 rats in the control group, 13 rats in the low-dose group, and 13 rats in the high-dose group. HA = hydroxyapatite.

Local delivery of antibiotics, such as through the implantation of antibiotic-impregnated PMMA, allows substantially higher concentrations to reach the surgical site than through systemic delivery[10]. In addition, incorporating both tobramycin and vancomycin in PMMA has been shown to result in synergistic elution due to the increased porosity of the resulting material[11]. Thus, the application of combined antibiotics in the context of the IMT is attractive, given that this technique is commonly used in the setting of known or suspected infection (e.g., infected nonunion) or in the setting of contamination (e.g., high-energy open fracture with bone loss).

In the context of the IMT, high doses of antibiotics eluting from a spacer may impair bone healing by negatively affecting the local environment at the graft-host interface and the induced membrane. Antibiotics can be potentially deleterious to osteoblast survival, migration, and proliferation[12,13]. One study comparing several antibiotic-impregnated spacers in a rat model showed that characteristics of the induced membrane, such as thickness, elastin content, and vascularization, can be influenced differently depending on the antibiotics used[6]. Those induced membranes were significantly thicker after 6 weeks when using spacers impregnated with gentamycin or gentamycin and vancomycin instead of gentamycin and clindamycin. However, whether these differences observed in the membrane affect bone healing remains unknown. Thus, several potential mechanisms exist whereby high doses of antibiotics in the spacer may impair bone healing and explain our results. In a recent systematic review, antibiotic-impregnated spacers were used in 75% of the 48 clinical IMT studies included[4], underscoring the relevance of directly examining the effects of antibiotic-impregnated spacers on healing outcomes.

Based on our findings, low doses of antibiotics included in the spacer do not seem to be deleterious to bone healing; this approach may therefore be beneficial. The low-dose group achieved the highest rate of full union and the best radiographic and biomechanical results. Low doses of antibiotics delivered locally may help to prevent infection following contamination from open fractures or during the surgical procedure, while still allowing favorable bone-healing conditions. Shiels et al. created a drill hole in the femoral condyle of rats and filled it with a gel consisting of demineralized bone graft and bioactive glass[14]. The reconstituted bone void filler contained either no antibiotic or tobramycin at low or high doses (4% or 8%). The healing observed with the filler was not affected by the lower antibiotic dose but it was affected by the higher dose, yet both concentrations allowed for sustained antibiotic elution above the

**TABLE IV Micro-CT Analysis Results***

| | Control† (N = 12) | Low-Dose† (N = 13) | High-Dose† (N = 13) | F Statistic‡ | P Value‡ |
|---|---|---|---|---|---|
| Bone volume *(mm³)* | 37.5 ± 11.4 | 36.6 ± 5.9 | 28.5 ± 8.4 | 4.08 | 0.026§ |
| Bone volume/total volume *(%)* | 77.2 ± 4.5 | 78.0 ± 5.7 | 78.8 ± 6.9 | 0.22 | 0.80 |
| BMD *(g HA/cm³)* | 1.02 ± 0.05 | 1.05 ± 0.04 | 1.03 ± 0.07 | 0.76 | 0.47 |

*BMD = bone mineral density, and HA = hydroxyapatite. †The values are given as the mean and the standard deviation. ‡Test statistics (F-values) and p values were produced using 1-way analysis of variance. §Pairwise comparisons with Tukey adjustment demonstrated a significant difference between control and high-dose groups (mean difference, 9.0 $mm^3$ [95% CI, 0.4 to 17.6 $mm^3$]; p = 0.039). No significant differences were found between low-dose and control (mean difference, 0.9 $mm^3$ [95% CI, −7.7 to 9.5 $mm^3$]; p = 0.96) or between high-dose and low-dose (mean difference, 8.1 $mm^3$ [95% CI, −0.3 to 16.5 $mm^3$]; p = 0.06).

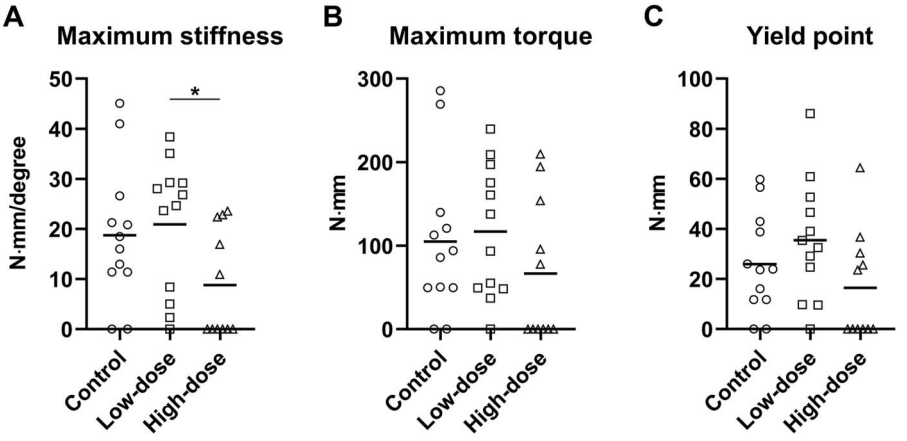


Fig. 3

Maximum stiffness (**Fig. 3-A**), maximum torque (**Fig. 3-B**), and yield point (**Fig. 3-C**) measured by biomechanical testing of operatively treated femora harvested 12 weeks after bone grafting. Each symbol represents an individual result. The bar indicates the mean of the group. The asterisk (*) denotes a significant difference ($p \leq 0.05$). There were 12 rats in the control group, 12 rats in the low-dose group, and 11 rats in the high-dose group.

minimum inhibitory concentration for *Staphylococcus aureus*. Similar to our findings, these results suggest that the local delivery of low-dose antibiotics can be effective in preventing or treating infection without compromising bone healing. In addition, Fung et al., in their systematic review of the clinical literature on the IMT, noted that their analysis demonstrated a trend toward inclusion of antibiotics in the cement spacer having a protective effect against the need for additional procedures[4]. In that review, the specific dose and combination of antibiotics used were noted to be variable; however, these results could be viewed as consistent with our finding that antibiotics at the appropriate dose may be beneficial to the IMT. The mechanism by which low doses of antibiotics in the spacer could improve bone healing remains unclear at this point. Potential mechanisms include protection against infection or biofilm formation and positive impacts on membrane properties, such as thickness or osteogenic potential. Further research is needed to clarify this.

This study had some limitations. Our defect model mimicked aseptic nonunion, and thus the conclusions may inform the routine use of antibiotic-impregnated spacers for infection prophylaxis. Because the IMT is often used in a contaminated defect or infected nonunion, we recognize that competing interests may exist between infection management and bone healing. Although higher antibiotic concentrations loaded in the spacer may be beneficial for treating and preventing infection, our findings suggest that an excessive dose should be avoided. However, given the wide range of doses found in the clinical literature, additional research is necessary to define optimal antibiotic concentrations in various clinical contexts.

Other limitations should be considered when interpreting our results. We chose to combine vancomycin and tobramycin in our spacers, aiming to mimic clinical practice. Combining these antibiotics can increase their elution rates, as discussed previously, but it also prevented us from determining the effect of each antibiotic separately. Moreover, we used a single time point between the first-stage and second-stage surgical procedures. This time point was selected on the basis of preclinical studies suggesting that the properties of the induced membrane are the most favorable for bone grafting after 4 weeks[15,16]. This also represented the median duration of spacer implantation in rat studies on the IMT[17]. We did not characterize the induced

**TABLE V Biomechanical Results**

| | Control* (N = 12) | Low-Dose* (N = 12) | High-Dose* (N = 11) | Chi-Square† | P Value† |
|---|---|---|---|---|---|
| Maximum stiffness *(N*mm/degree)* | 18.5 (11.4 to 26.6) | 25.7 (6.7 to 29.2) | 1.3 (0.8 to 22.4) | 8.68 | 0.013‡ |
| Maximum torque *(N*mm)* | 94.1 (49.6 to 139.7) | 115.6 (50.3 to 192.1) | 27.0 (18.0 to 153.9) | 5.03 | 0.08 |
| Yield point *(N*mm)* | 23.9 (16.1 to 43.0) | 34.0 (25.8 to 49.6) | 10.7 (7.6 to 30.4) | 3.04 | 0.22 |

*The values are given as the median, with the interquartile range in parentheses. †Test statistics (chi-square values) and p values were produced using Kruskal-Wallis tests. ‡Pairwise comparisons demonstrated a significant difference between low-dose and high-dose groups (median difference, 14.1 N × mm/degree [95% CI, 1.8 to 26.5 N × mm/degree]; Dwass-Steel-Critchlow-Fligner (DSCF) test statistic = 4.18; p = 0.009). No significant differences were found between low-dose and control (median difference, 0.69 N × mm/degree [95% CI, −12.9 to 14.2 N × mm/degree]; DSCF test statistic = 0.87; p = 0.81) or between high-dose and control (median difference, 9.8 N × mm/degree [95% CI, −0.9 to 20.5 N × mm/degree]; DSCF test statistic = 2.74; p = 0.13).

membrane to determine if there were morphological or histological differences between our groups that correlated with our bone-healing outcomes. In general, such correlations still remain to be investigated thoroughly, both clinically and in animal models[17,18].

In addition, we only observed radiographic healing up until 12 weeks after stage 2 (bone grafting), and it remains possible that further bone healing would have been observed in the high-dose group at later time points. Thus, further investigation is required to determine if the differences that we observed were a true impairment in bone healing rather than simply a delay in bone healing caused by higher doses of antibiotics. Finally, the loss of 3 specimens prior to biomechanical testing was a limitation. Nevertheless, the study remained adequately powered, as we had accounted for a 20% specimen loss in our a priori sample size calculation and the groups remained relatively well balanced.

In conclusion, the objective of this study was to evaluate whether antibiotic-impregnated spacers, as part of the IMT, affect bone healing in a preclinical model. On the basis of our results, we conclude that low-dose vancomycin and tobramycin-impregnated spacers do not have a negative impact on bone healing and may even improve it. However, at a high antibiotic dose, they may be deleterious to healing. Our findings suggest that low-dose antibiotics can be added to the PMMA spacers used for the IMT without concerns that this will negatively impact subsequent bone healing. The use of high-dose antibiotic spacers should be approached with caution, as this may negatively impact subsequent bone healing. The clinical importance of these findings is that our results support the continued use of antibiotics in the cement spacer as part of the IMT, provided that low doses of antibiotics are used. Further investigation is necessary to confirm these results with different antibiotics, with different timings of the second-stage surgical procedure, and across the broad range of clinical conditions for which the IMT is used.

## Appendix

eA Supporting material provided by the authors is posted with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJSOA/A745). ■

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