# Local Antibiotic Delivery by a Bioabsorbable Gel Is Superior to PMMA Bead Depot in Reducing Infection in an Open Fracture Model

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**Objectives:** Local delivery allows a high concentration of antibiotics to be achieved in the wound while avoiding the side effects and cost of systemic administration. Beads molded from polymethylmethacrylate cement are commonly used for local antibiotic delivery but are not ideal. The purpose of this study was to determine whether a bioabsorbable gel delivering vancomycin and gentamicin is more effective in reducing infection than beads delivering vancomycin and tobramycin.

**Methods:** This study used a segmental defect rat model contaminated with *Staphylococcus aureus* and treated with clinically relevant local antibiotic doses, delivered by gel or beads. In the gel group, 1 mL of gel containing gentamicin and vancomycin was spread throughout the wound. In the bead group, four 3-mm beads containing tobramycin and vancomycin were placed in the wound, 2 in the defect and 2 in the adjacent tissue envelope, there was also a control group that received no antibiotic treatment. After 14 days, bone and hardware was harvested for separate microbiological analysis.

**Results:** There was a significantly lower infection rate in groups treated with antibiotics delivered by gel compared with those treated with either antibiotic beads or no antibiotics at all ($P < 0.001$). Quantitative cultures also demonstrate significantly less bacteria in the wounds treated with the gel than in the control or bead groups ($P \leq 0.004$).

**Conclusions:** These results suggest that antibiotic delivery by a gel is superior to beads. The authors propose that antibiotic depot by polymethylmethacrylate antibiotic beads is less effective because this method has to rely on diffusion of the antibiotic from the high concentration close to the beads to all regions of the wound.

**Key Words:** open fracture, wounds and injuries, infection, antibiotics, local antibiotics, antibiotic beads, bacteria

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## INTRODUCTION

Infections commonly complicate open fractures in civilian and combat injuries despite the ubiquitous and often prolonged use of systemic antibiotics.[1,2] Local delivery of antibiotics has long been used as a method of increasing their concentration near bacterial contamination while keeping the systemic levels low.[3–5] Probably the most commonly clinically used delivery vehicle for local antibiotics in clinical use is polymethylmethacrylate (PMMA) blended with antibiotics and molded into beads.[6–8] There are, however, disadvantages associated with PMMA beads as a local antibiotic delivery vehicle. They are bulky, complicating wound closure, and are not bioabsorbable, necessitating surgical removal and are at risk for bacterial colonization.[9] Also, as an antibiotic depot, there is a reliance on diffusion of the antibiotic from high concentrations close to the beads to the rest of the wound.

This study compares the ability of a novel local antibiotic delivery vehicle, a phospholipid gel containing gentamicin and vancomycin that can be spread over the entire wound, with the existing clinical standard of antibiotic-PMMA beads loaded with vancomycin and tobramycin to prevent infection in an animal model of highly contaminated open fracture.

## MATERIALS AND METHODS

### In Vitro Study

The in vitro release kinetics of the antibiotic gel (n = 4) was evaluated at days 1, 2, 5, 7, and 14. The same bead preparation described below for the in vivo evaluation was used. Four beads or 0.5 mL of gel was placed in 10 mL of phosphate-buffered saline (pH = 7.4). Samples were vortexed vigorously and allowed to stand static at room temperature (25°C) until the next collection. On reaching each time point, 1 mL of the eluent was drawn off and frozen (−20°C) for testing of antibiotic levels on the Dimension Xpand Plus Integrated Chemistry System (Siemens; Malvern, PA). After 1 mL of eluent was drawn off and saved, the remaining ~9 mL of eluent was carefully drawn off (as to not draw off any of the

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nonsoluble phospholipid gel for the gel samples) and disposed off. Ten milliliters of fresh phosphate-buffered saline was added to the elution sample, vortexed vigorously, and allowed to stand static until next time point.

## Study Groups

There were 4 study groups each containing 10 animals, detailed in Table 1.

## Procedure

This study was conducted under a protocol in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care of Use of Laboratory Animals.[10,11] Briefly, a previously described[4,5] contaminated open femoral defect was created using the following technique: Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) with a mean weight of 372 g (350–400 g) were anesthetized with isoflurane and prepared for surgery; their right femoral shafts were exposed and stabilized with a bespoke polyoxymethylene plate, secured with 6 threaded K-wires. A 6-mm defect was then created in the mid-shaft with a reciculating saw, cooled with saline (Fig. 1). The defect was contaminated with 30 mg of sterile bovine collagen soaked with $1 \times 10^5$ colony-forming units (CFUs) of *Staphylococcus aureus* in 0.5 mL of saline. The Xen 36 strain of *S. aureus* used derived from ATCC 49525 originally from a septic human patient (Caliper LifeSciences, CA) and used by other investigators to model musculoskeletal infection.[12]

The wounds were closed in layers, and the animals recovered. Six hours after the initial "injury," the animals were reanesthetized, their wounds were opened and debrided with careful removal of all foreign material and nonviable tissue followed by irrigation with 60 mL of sterile saline delivered at low pressure. Six hours was selected as the point for debridement and antibiotic therapy because treatment at this time has been shown to reduce the bacteria but not completely eradicate them from the wound.[13] The wounds of the control group were closed in layered fashion, and the animals were recovered, allowed full mobility, food, and water. In the other groups, the appropriate local antibiotic treatment (described in Table 1) were placed in the wounds, and the wounds were closed, and animals recovered in similar fashion.

Two weeks after the injury, the animals were killed. The femur and hardware were stripped of soft tissue and separated. The bone tissue was snap frozen in liquid nitrogen and crushed. Bone and implant samples were then sent separately for standard quantitative microbiological analysis. Briefly, crushed bone samples were homogenized with 10 mL saline in an agitator, similarly, implant specimens were rinsed with 10 mL of saline in an agitator; then, aliquots from individual specimens were sequentially diluted and spread onto tryptic-soy-agar plates. After overnight incubation at 37°C, bacterial colonies were counted; the threshold of detectability was 30 CFU/g for bone specimens and 30 CFU/mL for implant specimens. A photon count camera (Xenogen IVIS imaging system 100) was used to verify that the bacteria being measured were descended from the original inoculated strain.

**TABLE 1.** Study Groups Detailing Different Treatments and Quantity of Antibiotic Received by Each Group

| | |
|---|---|
| Control | No treatment, wound closed after irrigation |
| Antibiotic-PMMA beads | Four 3-mm PMMA beads containing 2.5 mg vancomycin and 2.9 mg tobramycin were placed into the wound after irrigation and before closure |
| Beads/gel | Two 3-mm antibiotic beads containing 1.25 mg vancomycin and 1.5 mg tobramycin and 0.5 mL of antibiotic gel containing 8.5 mg vancomycin and 8.5 mg gentamicin was placed into the wound after irrigation and before closure |
| Antibiotic gel | 1 mL of phospholipid gel containing 19 mg vancomycin and 17 mg gentamicin was placed into the wound after irrigation and before closure |

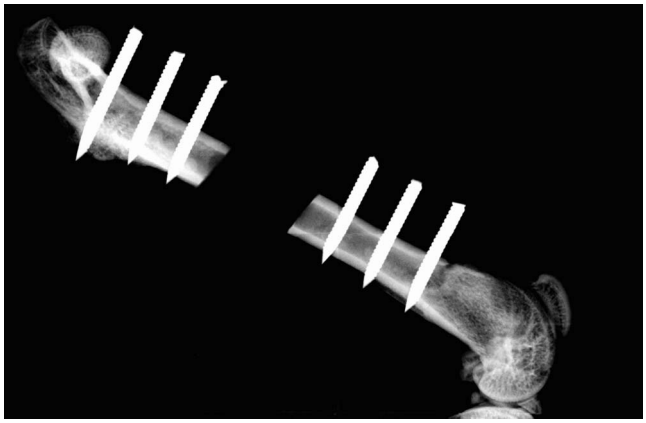


**FIGURE 1.** Micro x-ray image showing 6-mm defect in rat femur stabilized by a radiolucent polyoxymethylene plate secured with 6 threaded 0.9-mm K-wires.

## Antibiotic Gel

The gel tested is a sterile bioabsorbable phospholipid gel, designated DFA-02 containing 1.88% vancomycin and 1.68% gentamicin by weight (provided by Dr Reddy's Laboratories, Inc, Bridgewater, NJ). To prepare the phospholipid gel, formulation water was added to gentamicin sulfate and vancomycin hydrochloride to allow complete dissolution of gentamicin sulfate and vancomycin hydrochloride. Then, lecithin (Phospholipon 90 G; Phospholipid, GmbH, Cologne, Germany) and sesame oil was added, followed by high shear mixing at 5000 rpm for 60 minutes to obtain a uniform primary emulsion. Then, the pH of the primary emulsion was adjusted to 3–4pH by adding 1 N HCL. The primary emulsion was placed in a microfluidizer (Microfluidics, Inc, Newton, MA) to produce a monophasic solution. The monophasic solution was lyophilized to remove water and obtain a dry paste. The dry paste was mixed with dehydrated alcohol (6% wt/wt) and heated to form a viscous clear gel. The clear gel was filter-sterilized by passing the entire mass through a 0.22-μm sterilizing filter (Sartorius Stedim, Inc, Bohemia, NY). One microliter of this gel was placed into the wound after irrigation and before wound closure. Care was taken to ensure that the gel covered the entire surface of the wound.

## Antibiotic-Polymethylmethacrylate Beads

Antibiotic-PMMA beads were manufactured under sterile conditions using Palacos R (Zimmer, Dover, OH) arthroplasty

cement. Forty grams of Polymethylmethacrylate (PMMA) copolymer powder was blended with 2.0 g of vancomycin (Sigma-Aldrich, St Louis, MO) and 2.4 g of tobramycin sulphate (Sigma-Aldrich), and then mixed with 20 mL of MMA monomer liquid. A 3-mm mold was then used to create beads weighing approximately 20 mg and containing 3.1% vancomycin and 3.7% tobramycin sulfate by weight. Four beads were placed in the wound, 2 in the bone defect, and 2 in the surrounding soft tissues; this was the number of beads that reasonably "fit" into the wound. This delivered a dose of 2.5 mg of vancomycin and 2.9 mg of tobramycin.

## Outcome Measures

The outcome measures were the presence and amount of bacteria in the femur or attached to the implants (polyoxymethylene plate and K-wires). Animal weights were also recorded as part of basic animal husbandry.

## Statistics

Statistical analysis was performed using SAS software (SAS Institute Inc, NC). For analysis of bacteria quantification, undetectable samples were regarded as zero; the log mean of the bone and implant values of the different groups were compared separately with Mann–Whitney analysis. For direct comparison between study groups regarding the dichotomous presence or absence of bacteria on bone or implant samples, Fisher test was used. The threshold for significance was set at 0.05.

# RESULTS

## In Vitro Study

There was a detectible amount of both antibiotics in the saline surrounding the antibiotic gel sample at the end of the 14-day study as shown in Table 2. However, concentrations of both antibiotics dropped rapidly within the first 48 hours.

## Animal Study

Bacteria were recovered in all wounds in the control (no antibiotics received) and antibiotic-PMMA bead group. The group treated with antibiotic gel had significantly fewer animals with bacteria ($P \leq 0.004$) detectable in their wounds than these 2 groups, only half of the bone samples and 30% of the implant specimens had detectable bacteria (Fig. 2). Antibiotic gel was significantly superior to antibiotic-PMMA beads at reducing bacteria on both the bone ($P = 0.001$) and the implant samples ($P = 0.004$) (Table 3). Interestingly, there was no difference in the amount of bacteria in the wounds of those treated with antibiotic-PMMA beads and those animals in the control group that received no treatment, with respect to either bone ($P = 0.07$) or implant samples ($P = 0.82$) (Fig. 3). The addition of antibiotic-PMMA beads to antibiotic gel did not reduce the bacteria when compared with gel alone ($P > 0.3$). There was significant weight loss among animals in the groups not treated with antibiotic gel, as shown in Table 4.

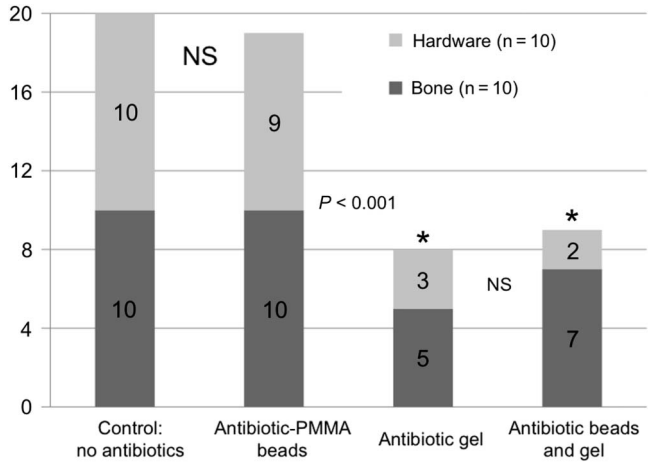



**FIGURE 2.** Proportion of 20 samples from each treatment group with detectible bacteria at 14 days. Statistical differences by Fisher exact test are shown. *The antibiotic gel and antibiotic beads and gel group had a lower portion of the samples with bacteria that were recoverable than the other control and beads groups ($P \leq 0.0004$). NS, no significant difference between adjacent groups.

# DISCUSSION

The results of this study demonstrate that local delivery of antibiotic by the bioabsorbable gel achieving complete wound coverage was more effective in reducing bacteria within a contaminated rat defect than the commonly used antibiotic-PMMA bead depot.

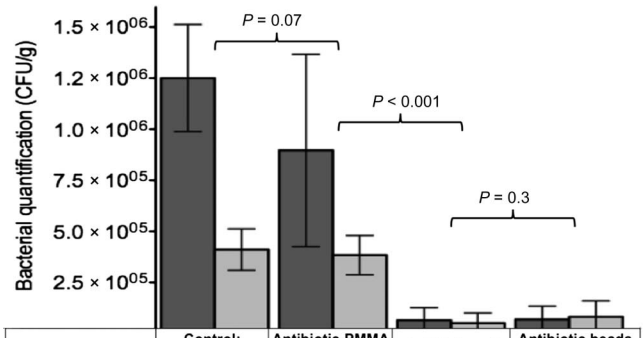
Direct application of antimicrobial drugs into open fracture wounds is not a novel concept. In 1939, Jenson et al[14] presented their experience of treating open fractures with sulfanilamide powder poured directly into the wound before

**TABLE 2.** In Vitro Concentrations (in μg/mL) of Vancomycin and Gentamycin Retrieved From Serial Dilution of 0.5 mL of Antibiotic Gel in 10 mL of Phosphate Buffered Saline Over Time

| | Day 1 | Day 2 | Day 5 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Vancomycin | 776.2 | 90.5 | 11.7 | 2.5 | 1.5 |
| Gentamicin | 778.7 | 88.1 | 11.4 | 2.1 | 1.4 |

**TABLE 3.** *P* for Comparison of Groups by Quantitative Cultures of Recovered Bacteria From Bone and Implant Samples (Mann–Whitney Test) and Presence or Absence of Bacteria in Samples (Fisher Test)

| Comparison | | Mann–Whitney Test | | |
|---|---|---|---|---|
| Group1 | Group2 | Bone | Implant | Fisher Test |
| Gel | Beads | 0.001 | 0.004 | <0.001 |
| Gel | Beads/gel | 0.310 | 0.820 | 1.00 |
| Gel | Control | <0.001 | <0.001 | <0.001 |
| Beads | Beads/gel | 0.001 | 0.007 | 0.001 |
| Beads | Control | 0.070 | 0.820 | 1.0000 |
| Beads/gel | Control | <0.001 | 0.002 | <0.001 |




| | Control: No antibiotics | Antibiotic-PMMA beads | Antibiotic gel | Antibiotic beads and gel |
|---|---|---|---|---|
| Bone mean | $1.25 \times 10^6$ | $8.96 \times 10^5$ | $6.53 \times 10^4$ | $6.90 \times 10^4$ |
| Bone SEM | $2.62 \times 10^5$ | $4.70 \times 10^5$ | $6.19 \times 10^4$ | $6.53 \times 10^4$ |
| Implant mean | $4.11 \times 10^5$ | $3.84 \times 10^5$ | $5.19 \times 10^4$ | $8.20 \times 10^4$ |
| Implant SEM | $1.01 \times 10^5$ | $9.62 \times 10^4$ | $4.92 \times 10^4$ | $7.77 \times 10^4$ |

**FIGURE 3.** Mean quantity of bacteria recovered from bone and implants from different treatment groups. Error bars show standard error of the mean (SEM). Statistical differences between quantities of bacteria recovered from bone samples calculated by Mann–Whitney analysis are shown.

closure and credited introduction of this technique with a reduction in their infection rate from 30% to 5%. The attraction of local delivery is that high concentrations of antibiotic can be achieved in the wound even in avascular areas, without the cost or toxic effects associated with systemically administered antibiotics.

Buchholz et al in Germany first developed the use of PMMA cement blended with antibiotics to treat infected joint prosthesis.[15,16] This approach was then adapted to treat chronic osteomyelitis by Klemm[3] who published his experiences in 1974. However, the only prospective randomized trial of antibiotic-PMMA beads in osteomyelitis did not find that they were superior in isolation or with systemic antibiotics compared with systemic antibiotics alone in the treatment of osteomyelitis.[17]

That being said, there is some evidence that antibiotic depot is effective. In a 1990 case series, Henry et al[18] described the prophylactic use of antibiotic-PMMA beads in addition to systemic antibiotics to reduce the infection rate in open fractures. There were significantly less infections in the GA II and III fractures treated with antibiotic-PMMA beads and systemic antibiotics compared with those who just received systemic antibiotics. In 1995, Ostermann et al[5] published a similar comparison of 240 patients with open limb fractures who received IV antibiotics and 845 patients who received both IV antibiotics and antibiotic-PMMA beads. He found an infection rate of 12% in the IV only group compared with 3.7% in the IV/antibiotic-PMMA bead group ($P$ = 0.001). It should be noted that neither of these studies randomized the treatment groups. Moehring et al[8] published the results of a prospective randomized trial designed to compare antibiotic-PMMA beads with IV antibiotics in the prevention of infection in open fractures. This study described a trend toward superiority of antibiotic beads but it did not reach significance in the 67 patients studied. Interestingly, animal studies have also failed to convincingly establish a significant benefit of augmenting systemic antibiotics with antibiotic-PMMA beads in musculoskeletal infection.[19,20]

Preformed antibiotic-PMMA beads are not marketed in the United States as they are in Europe, but United States surgeons frequently manufacture beads intraoperatively from PMMA cement and antibiotic powder.[7] Despite their widespread use, there is recognition that PMMA beads do not represent the ideal delivery vehicle for local antibiotics. They are bulky and not bioabsorbable, which potentially complicates wound closure and necessitates subsequent removal.[7,9] This prevents their use during definitive closure. In complex high-energy wounds, there is concern that the antibiotic eluting from the discrete depots of a PMMA bead will not diffuse sufficiently to reach all the recesses of a wound, an effect exacerbated by the concurrent use of negative pressure wound therapy,[21] which is well supported as a beneficial technique to reduce infection in open fractures.[22] There is also concern that self-manufactured PMMA beads have a varied and unpredictable antibiotic elution rate.[23] The commercially available antibiotic-PMMA beads Septopal (Biomet, Bridgend, United Kingdom) contains gentamicin alone, however, when antibiotic-PMMA beads are manufactured de novo in the operating room, they are frequently formulated with both aminoglycoside and vancomycin to ensure coverage of both gram-positive and gram-negative organisms.[7] This study mimicked this clinical practice and used both aminoglycoside and vancomycin.

Other local antibiotic delivery vehicles have been used; gentamicin-impregnated collagen sponges have been tested as a bioabsorble vehicle. However, in a nonorthopaedic recent clinical Randomised Controlled Trial of CollaRx (Innocoll, Gallowston, Ireland), the sponge group had a higher rate of surgical site infection (30% vs. 20%, $P$ = 0.01). It was speculated that the antibiotics eluted faster than the sponge degraded, leaving foreign material in the wounds without antibiotics.[24]

Recent development work has focused on other absorbable antibiotic vehicles including a range of synthetic bone grafts impregnated with antibiotics.[9] Osteoset "T" (Wright Medical, Arlington, TN) are calcium sulphate pellets with 10% tobramycin by weight, has been used clinically to treat ostomyelitis with positive results[25] and has found to be as effective at treating osteomyelitis as antibiotic delivery through PMMA beads, with a requirement for less surgery.[26] Other investigators have examined gel-based vehicles for

**TABLE 4.** Animal Husbandry Data Showing Mean Animal Weights in Different Treatment Groups With SEM and $P$ Calculated by Mann–Whitney Analysis

| Group | Mean Initial Weight (SEM) | Mean Final Weight (SEM) | % Change | $P$ |
|---|---|---|---|---|
| Control | 371 (3) | 361 (5) | −2.9 | 0.0276 |
| Antibiotic-PMMA beads | 373 (4) | 359 (5) | −3.6 | 0.0258 |
| Beads/gel | 373 (5) | 380 (3) | −3.3 | 0.6152 |
| Antibiotic gel | 371 (3) | 382 (10) | +2.6 | 0.0765 |
| $P$ | 0.9751 | 0.0081 | | |

SEM, standard error of the mean.

delivering antibiotics in preclinical in vitro models of orthopaedic infection,[27] but, no clinical trials of a gel capable of being spread throughout a wound have yet been reported.

The ideal release profile for a local antibiotic delivery vehicle used to prevent infection in open fractures is not known. It is speculated that eluted local antibiotics should quickly rise above the minimum inhibitory concentration of relevant bacteria, be sustained above this level for several days, then rapidly drop to avoid bacteria being exposed to subinhibitory antibiotic concentration, promoting resistance.[28] It is entirely possible that the ideal release profile of local antibiotic vehicles used for treating established osteomyelitis will be different and may require a more sustained release.

The results of the in vitro study show that high initial concentrations of both vancomycin and gentamicin slowly decline over several days. Similarly, in vivo results from an unpublished study from a rabbit model have shown that the gel under evaluation maintains persistent local tissue levels of both antibiotics greater than the minimum inhibitory concentration of susceptible organisms over 14 days as shown in Table 5 (unpublished data, Dr. Reddy's Laboratories, Study MPI 1115-016). This release profile is thought to be important because it allows the local antibiotic levels to rapidly exceed the minimum inhibitory concentrations and minimum biofilm-eradicating concentrations of the bacteria within the wound. The sustained release is believed to help eradicate any persistors, which are quiescent cells within a biofilm that are not as susceptible to antibiotics.[29]

The model used in this study mimics clinical practice but lacks some features of the reality of clinical open fractures, for example, the lack of soft tissue damage, a surgically created defect rather than fracture, a single surgical treatment, and immediate primary closure among other aspects. It is possible that the observed difference between groups is because of the alternative aminoglycosides used. It was decided to use tobramycin in the bead preparation as this study was testing against the current clinical standard. Even though the initial work in Europe on PMMA delivery of antibiotics in osteomyelitis involved gentamicin,[3] when this work was translated to infection prevention in open fractures in the United States, tobramycin rather than gentamicin was used[30] and continues being the commonly used formulation as until recently, this was the only aminoglycoside available in the United States in powdered form.[7,31] This difference between the preparations was accepted in this study because it represented the clinical standard, and because the efficacy of gentamicin and tobramycin against gram-positive bacteria in general is very similar[32] and with respect to the strain of *S. aureus* used in this study, that is, it is similarly sensitive to both aminoglycoside antibiotics as shown in Figure 4.

A single end point of 2 weeks was chosen even to minimize the number of animals used in this study; based on previously published work using this model, results at 2 and 4 weeks are similar.[13,33] If bacteria persist on bone and implants for 2 weeks, they are likely to have formed a biofilm. Therefore, if the bacteria have not been killed within 14 days, it is unlikely that further exposure to the lessening concentrations of antibiotics released by local antibiotic vehicles will eradicate them in the future.

A group was treated with both gel and beads as it was speculated that because antibiotic-PMMA cement is used by surgeons as a spacer to maintain soft tissues and eliminate dead space, there might be future utility in evaluating the compatibility of simultaneous antibiotic delivery by both gel and cement. These results suggest that this can be done, but that there are no additive antimicrobial effects to this approach.

The differences in effect of the gel and beads is marked and maybe because of the active amount of antibiotics released from these vehicles. A study that examined beads made using the same technique as this study found that only 20% of the total antibiotics was released within 60 days, but

**TABLE 5.** Unpublished Data From a Rabbit Model Showing the Tissue Concentration of Antibiotics (in μg/g Tissue) Eluted From 2 mL Antibiotic Gel Product (DFA-02) Under Test in This Study, Equivalent to a Dose of 11.5 mg/kg of Gentamicin and 12.6 mg/kg of Vancomycin.

| | Gentamicin | Vancomycin |
|---|---|---|
| Predose | 0 | 0 |
| 12 h | 590 | 702 |
| 24 h (1 d) | 106 | 83.2 |
| 72 h (3 d) | 240 | 256 |
| 168 h (7 d) | 72.9 | 73 |
| 240 h (10 d) | 12.8 | 19.3 |
| 336 h (14 d) | 7.4 | 39 |

There were 5 animals study and muscle and fascia tissue samples were taken from the edges of wounds treated with the antibiotic gel (Dr Reddy's Laboratories, Study MPI 1115-016).

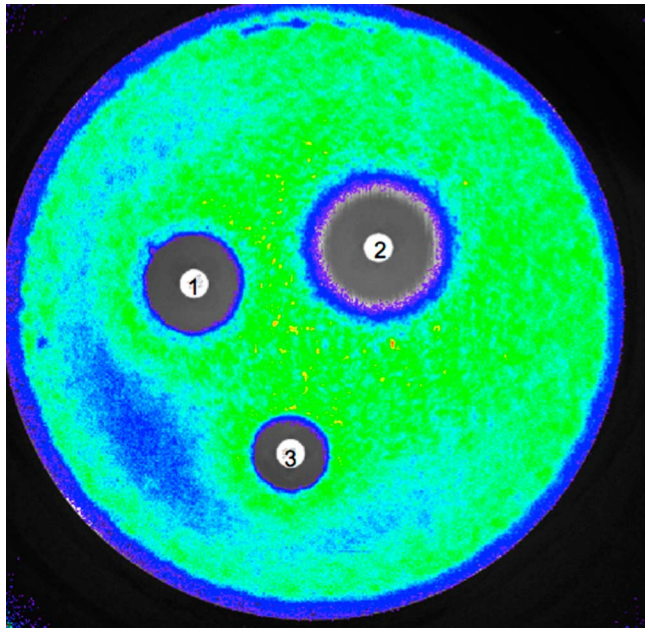



**FIGURE 4.** A limited spectrum photograph of a Kirby–Bauer sensitivity test of the photon-emitting strain of *S. aureus* used in this study. Disc 1, torbramycin; disc 2, gentamicin; and disc 3, vancomycin. The zones of inhibition of tobramycin and gentamicin are comparable. **Editor's note:** A color image accompanies the online version of this article.

half of this was released in the first day.[33] Whereas, a bioabsorbable vehicle, such as the gel in the study, will obviously release 100% of the carried antibiotics as it is degraded. The maximum antibiotic content of these beads is limited by the dilution of the MMA copolymer powder negatively affecting cement integrity.[34] In practice, this confers a further advantage on the gel vehicle in that it enables greater quantities of antibiotic to be delivered, as shown in this study, where there was much higher dosage of antibiotics in the animals treated with the gel. It is also possible that the superior performance of the gel is because of the improved distribution of antibiotic throughout the wound, rather than in discrete "pockets" around the beads. This is because of the need for the active drug to elute and diffuse throughout the wound bed, compared with the gels' immediate drug delivery to the entire wound contact area. The authors believe that this bioabsorbable gel shows promise as a local antibiotic delivery vehicle capable of preventing infection in open fractures and is worth further evaluation.

## REFERENCES


1. Harris AM, Althausen PL, Kellam JF, et al. Complications following limb-threatening lower extremity trauma. *J Orthop Trauma.* 2009;23:1–6.
2. Johnson EN, Burns TC, Hayda RA, et al. Infectious complications of open type III tibial fractures among combat casualties. *Clin Infect Dis.* 2007;45:409–415.
3. Klemm KW. Antibiotic bead chains. *Clin Orthop Relat Res.* 1993;63–76.
4. Henry SL, Ostermann PA, Seligson D. The prophylactic use of antibiotic impregnated beads in open fractures. *J Trauma.* 1990;30:1231–1238.
5. Ostermann PA, Seligson D, Henry SL. Local antibiotic therapy for severe open fractures. A review of 1085 consecutive cases. *J Bone Joint Surg Br.* 1995;77:93–97.
6. Keating JF, Blachut PA, O'Brien PJ, et al. Reamed nailing of open tibial fractures: does the antibiotic bead pouch reduce the deep infection rate? *J Orthop Trauma.* 1996;10:298–303.
7. Zalavras CG, Patzakis MJ, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. *Clin Orthop Relat Res.* 2004;86–93.
8. Moehring HD, Gravel C, Chapman MW, et al. Comparison of antibiotic beads and intravenous antibiotics in open fractures. *Clin Orthop Relat Res.* 2000;254–261.
9. El-Husseiny M, Patel S, MacFarlane RJ, et al. Biodegradable antibiotic delivery systems. *J Bone Joint Surg Br.* 2011;93:151–157.
10. Chen X, Kidder LS, Lew WD. Osteogenic protein-1 induced bone formation in an infected segmental defect in the rat femur. *J Orthop Res.* 2002;20:142–150.
11. Penn-Barwell JG, Murray CK, Wenke JC. Early initial antibiotics and debridement independently reduce infection in an open fracture model. *J Bone Joint Surg (Br).* 2012;94B:107–112.
12. Pribaz JR, Bernthal NM, Billi F, et al. Mouse model of chronic post-arthroplasty infection: noninvasive in vivo bioluminescence imaging to monitor bacterial burden for long-term study. *J Orthop Res.* 2012;30:335–340.
13. Brown KV, Walker JA, Cortez DS, et al. Earlier debridement and antibiotic administration decrease infection. *J Surg Orthop Adv.* 2010;19:18–22.
14. Jenson NK, Johnsrud LW, Nelson MC. The local implantation of sulfanilamide in compound fractures. *Surgery.* 1939;6:1–12.
15. Buchholz HW, Elson RA, Engelbrecht E, et al. Management of deep infection of total hip replacement. *J Bone Joint Surg Br.* 1981;63-B:342–353.
16. Hedstrom SA, Lidgren L, Torholm C, et al. Antibiotic containing bone cement beads in the treatment of deep muscle and skeletal infections. *Acta Orthop Scand.* 1980;51:863–869.
17. Blaha JD, Calhoun JH, Nelson CL, et al. Comparison of the clinical efficacy and tolerance of gentamicin PMMA beads on surgical wire versus combined and systemic therapy for osteomyelitis. *Clin Orthop Relat Res.* 1993;8–12.
18. Henry SL, Hood GA, Seligson D. Long-term implantation of gentamicin-polymethylmethacrylate antibiotic beads. *Clin Orthop Relat Res.* 1993; 47–53.
19. Mendel V, Simanowski HJ, Scholz HC, et al. Therapy with gentamicin-PMMA beads, gentamicin-collagen sponge, and cefazolin for experimental osteomyelitis due to Staphylococcus aureus in rats. *Arch Orthop Trauma Surg.* 2005;125:363–368.
20. Evans RP, Nelson CL. Gentamicin-impregnated polymethylmethacrylate beads compared with systemic antibiotic therapy in the treatment of chronic osteomyelitis. *Clin Orthop Relat Res.* 1993;37–42.
21. Stinner DJ, Hsu JR, Wenke JC. Negative pressure wound therapy reduces the effectiveness of traditional local antibiotic depot in a large complex musculoskeletal wound model. *J Orthop Trauma.* 2012;26: 512–518.
22. Stannard JP, Volgas DA, Stewart R, et al. Negative pressure wound therapy after severe open fractures: a prospective randomized study. *J Orthop Trauma.* 2009;23:552–557.
23. Nelson CL, Griffin FM, Harrison BH, et al. In vitro elution characteristics of commercially and noncommercially prepared antibiotic PMMA beads. *Clin Orthop Relat Res.* 1992;303–309.
24. Bennett-Guerrero E, Pappas TN, Koltun WA, et al. Gentamicin-collagen sponge for infection prophylaxis in colorectal surgery. *N Engl J Med.* 2010;363:1038–1049.
25. Chang W, Colangeli M, Colangeli S, et al. Adult osteomyelitis: debridement versus debridement plus Osteoset T pellets. *Acta Orthop Belg.* 2007;73:238–243.
26. McKee MD, Li-Bland EA, Wild LM, et al. A prospective, randomized clinical trial comparing an antibiotic-impregnated bioabsorbable bone substitute with standard antibiotic-impregnated cement beads in the treatment of chronic osteomyelitis and infected nonunion. *J Orthop Trauma.* 2010;24:483–490.
27. Hou T, Xu J, Li Q, et al. In vitro evaluation of a fibrin gel antibiotic delivery system containing mesenchymal stem cells and vancomycin alginate beads for treating bone infections and facilitating bone formation. *Tissue Eng Part A.* 2008;14:1173–1182.
28. Stallmann HP, Faber C, Bronckers AL, et al. In vitro gentamicin release from commercially available calcium-phosphate bone substitutes influence of carrier type on duration of the release profile. *BMC Musculoskelet Disord.* 2006;7:18.
29. Heijink A, Yaszemski MJ, Patel R, et al. Local antibiotic delivery with OsteoSet, DBX, and Collagraft. *Clin Orthop Relat Res.* 2006;451:29–33.
30. Ostermann PA, Henry SL, Seligson D. The role of local antibiotic therapy in the management of compound fractures. *Clin Orthop Relat Res.* 1993;102–111.
31. Nelson CL, Evans RP, Blaha JD, et al. A comparison of gentamicin-impregnated polymethylmethacrylate bead implantation to conventional parenteral antibiotic therapy in infected total hip and knee arthroplasty. *Clin Orthop Relat Res.* 1993;96–101.
32. Joint Formulary Committee (Great Britain). *British National Formulary (BNF 63).* London, United Kingdom, BMJ Group; 2012. v.
33. Li B, Brown KV, Wenke JC, et al. Sustained release of vancomycin from polyurethane scaffolds inhibits infection of bone wounds in a rat femoral segmental defect model. *J Control Release.* 2010;145:221–230.
34. Lautenschlager EP, Jacobs JJ, Marshall GW, et al. Mechanical properties of bone cements containing large doses of antibiotic powders. *J Biomed Mater Res.* 1976;10:929–938.