

In Vitro Evaluation of Antibiotic Diffusion from Antibiotic-Impregnated Biodegradable Beads and Polymethylmethacrylate Beads

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Antibiotic-impregnated beads are used in the dead bone space following debridement surgery to deliver local, high concentrations of antibiotics. Polymethylmethacrylate (PMMA), 2,000-molecular-weight (MW) polylactic acid (PLA), poly(DL-lactide)-coglycolide (PL:CG; 90:10, 80:20, and 70:30), and the combination 2,000-MW PLA-70:30 PL:CG were individually mixed with clindamycin, tobramycin, or vancomycin. Beads were placed in 1 ml of phosphate-buffered saline (PBS) and incubated at 37°C. The PBS was changed daily, and the removed PBS samples were stored at -70°C until the antibiotic concentration in each sample was determined by microbiological disk diffusion assay. Nondissolving PMMA beads with tobramycin and clindamycin had concentrations well above breakpoint sensitivity concentrations (i.e., the antibiotic concentrations at the transition point between bacterial killing and resistance to the antibiotic) for more than 90 days, but vancomycin concentrations dropped by day 12. All PLA, PL:CG, and the 2,000-MW PLA-70:30 PL:CG biodegradable beads release high concentrations of all the antibiotics in vitro for the period of time needed to treat bone infections (i.e., 4 to 8 weeks). Antibiotic-loaded PLA and PL:CG beads have the advantage of better antibiotic elution and the ability to biodegrade (thereby averting the need for secondary surgery for bead removal) compared to the PMMA beads presently used in the clinical setting.

Adult chronic osteomyelitis is a difficult infection to treat and eradicate. Long-term parenteral antibiotics with multiple surgical debridements are often required for effective therapy (2, 9, 10). Following debridement surgery, the dead space created by osteomyelitic tissue removal must be managed. One management method utilizes surgical implantation of antibiotic-impregnated polymethylmethacrylate (PMMA) beads into the dead space for local delivery of antibiotics (2, 3). The disadvantages associated with using the PMMA beads include a necessary secondary surgery to remove the beads (1) and a less than optimal antibiotic elution profile since only 50% of the antibiotic is eluted from the bead by four weeks (12). Biodegradable beads would avoid the extra surgery necessary to remove the beads and allow all of the antibiotic to be released from the bead.

Recent research at other laboratories has explored the use of antibiotic-impregnated biodegradable beads for potential use in the local delivery of debrided osteomyelitic bone. Zhang et al. demonstrated that, in vitro, gentamicin-high-molecular-weight (high-MW) biodegradable poly(D,L-lactide) cylinders provided a small initial burst followed by a gradual and sustained release of gentamicin (13). While this group did not test the eluted antibiotic against known osteomyelitic organisms, the detected gentamicin concentrations were sufficiently above minimum bactericidal concentrations for these pathogens. Another laboratory (Shinto et al.) described, in vitro, the ability of gentamicin-impregnated calcium hydroxyapatite biodegradable beads to deliver five times the MIC for *Staphylococcus*

species for at least 12 weeks (7). Lastly, Garvin et al. illustrated that polyglycolide beads loaded with gentamicin resulted in the effective treatment of tibial *Staphylococcus aureus* osteomyelitis in a canine model (5). These studies did not, however, investigate other antibiotics commonly used in clinically managing bone dead space following debridement surgery. Also, previous investigators have not used different combinations of bead material in order to observe the effect of altered bead dissolving rates on antibiotic elution. Therefore, the purpose of this study was to investigate, in vitro, the elution of clindamycin, tobramycin, and vancomycin antibiotics from beads composed of PMMA, biodegradable PLA, varied ratios of poly-DL-lactide to coglycolide (PL:CG), and a combination of PLA and PL:CG.

MATERIALS AND METHODS

The six types of bead materials used in this study included nonbiodegradable PMMA (Howmedica Inc., Houston, Tex.), biodegradable PLA (Polysciences Inc., Warrington, Pa.) with an MW of 2,000, varied ratios of biodegradable PL:CG (Polysciences Inc.) (90:10, 80:20, and 70:30), and a combination of PLA and the 70:30 ratio of PL:CG. In order to account for the differences in clinical dosages for each of the antibiotics tested, ratios (in grams of antibiotic per gram of bead material) of 1:6.6, 1:4.1, and 1:10.0, were used for clindamycin, tobramycin, and vancomycin, respectively, per manufacturer's directions.

PMMA and each antibiotic (at its respective concentration) were mixed, and monomethyl-methacrylate was added and stirred according to the directions of the manufacturer (Howmedica Inc., Rutherford, N.J.). The biodegradable materials of 2,000-MW PLA; 90:10, 80:20, and 70:30 PL:CG; and the combination of PLA and the 70:30 ratio of PL:CG were each mixed with antibiotic (at its respective concentration). Methylene chloride was added and stirred according to the manufacturer's directions (Polysciences Inc.). Eight-millimeter PMMA, PLA, PL:CG, and PLA-PL:CG beads were constructed, dried overnight, sterilized with gamma irradiation for 3 days, and weighed. Bead mass ranged from 0.35 to 0.40 g.

One bead of each antibiotic bead combination was placed in 1 ml of phosphate-buffered saline (PBS) (pH 7.2) and incubated at 37°C for 24 h (8, 11). A volume of 1 ml was chosen in order to approximate the volume of serum that would surround the bead when multiple beads are packed into the dead space of

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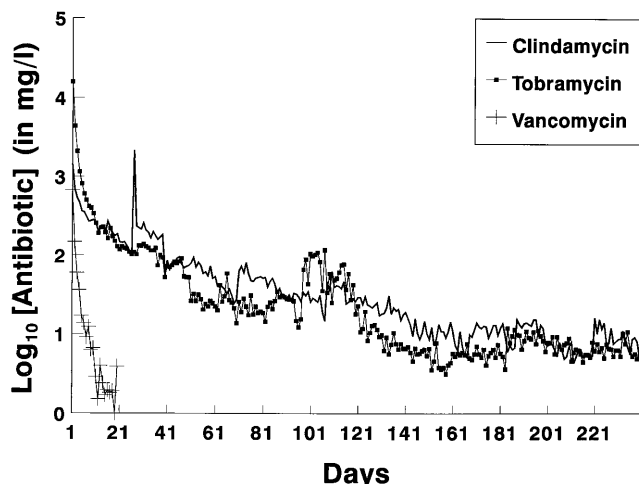


FIG. 1. In vitro elution of clindamycin, tobramycin, and vancomycin from PMMA beads. The breakpoint sensitivity limits for the antibiotics are 1.0, 4.0, and 5.0 mg/liter, respectively.

bone following debridement surgery. The beads were removed, shaken free of excess PBS, transferred to fresh 1-ml aliquots of PBS every 24 h, and incubated. The samples of removed PBS were stored at -70°C until a microbiological disc diffusion assay could be performed (6).

Disc diffusion assays were performed to determine antibiotic concentrations in the samples. For tobramycin and vancomycin, 0.1 ml of a *Bacillus subtilis* spore suspension (Difco, Detroit, Mich.) was added per 100 ml of Antibiotic Agar Medium One (Difco). Five milliliters of this seeded agar was aseptically pipetted into petri dishes. Standard twofold serial dilutions were made in PBS for tobramycin and vancomycin, producing standard concentrations ranging from 10,000 to 0.1 mg/liter. Twenty microliters of each in vitro sample and standard concentration was added to each of four sterile, blank, 6-mm-diameter Bacto concentration disks (Difco), and these were placed on the seeded plates. The plates were incubated overnight at 37°C . The diameter of the zone of inhibition for each standard and in vitro PBS sample was measured. The unknown concentrations for the in vitro samples were determined by comparing their respective zone size means to the standards.

The bioassay for clindamycin differed slightly from those for tobramycin and vancomycin. To each 18 ml of sterile liquefied antibiotic Medium One (Difco), 0.4 ml of an overnight culture of *Sarcina lutea* (ATCC 9341) was added. Five milliliters of this seeded agar was aseptically pipetted into sterile petri dishes to form the seeded agar plates. The remainder of the assay was performed as described for tobramycin and vancomycin.

The antibiotic concentrations at the transition point between bacterial killing and resistance to the antibiotic (the breakpoint sensitivity limit) for the three antibiotics were determined by utilizing tube dilution sensitivities (6). All studies were performed in quadruplicate. Statistical comparison of dissolution rates of the various biodegradable bead types was accomplished with Student's *t* test.

RESULTS

The clindamycin, tobramycin, and vancomycin breakpoint sensitivity limits were 1.0, 4.0, and 5.0 mg/liter, respectively. The elution of each of the three antibiotics from PMMA beads is shown in Fig. 1. On day 1 vancomycin, tobramycin, and clindamycin demonstrated high concentrations. Clindamycin

TABLE 1. Time of in vitro PLA, PL:CG, and PL:CG-PLA bead dissolution

Antibiotic	Days to dissolution				
	2,000-MW PLA	PL:CG			2,000-MW PLA-PL:CG (70:30)
		90:10	80:20	70:30	
Clindamycin	120	100	48	37	39
Tobramycin	150	80	80	38	39
Vancomycin	180	120	41	36	40

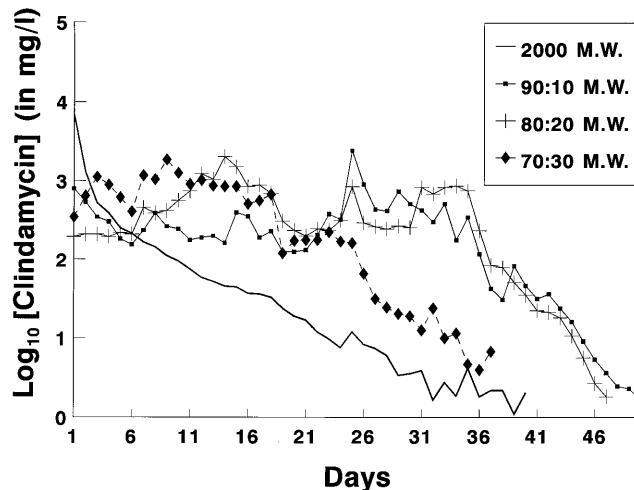


FIG. 2. Clindamycin release from 2,000-MW PLA, 90:10 PL:CG, 80:20 PL:CG, and 70:30 PL:CG beads. The breakpoint sensitivity limit for clindamycin is 1.0 mg/liter.

and tobramycin concentrations remained above their respective breakpoint sensitivities through 220 days. However, vancomycin concentrations dropped to below breakpoint sensitivity by day 12.

The results with clindamycin PLA and PL:CG beads are shown in Table 1 and Fig. 2. The clindamycin 2,000-MW PLA remained above breakpoint sensitivity until day 38 but did not dissolve until 120 days. The 90:10 PL:CG beads released clindamycin through 48 days and dissolved by 100 days. The 80:20 PL:CG beads remained above breakpoint until they dissolved at 48 days, whereas the 70:30 PL:CG beads remained above breakpoint until they dissolved at 37 days.

The results with tobramycin PLA and PL:CG beads are shown in Table 1 and Fig. 3. The tobramycin concentrations of the 2,000-MW PLA dropped rapidly but remained above breakpoint sensitivity for 42 days. The tobramycin 2,000-MW PLA dissolved at 150 days. The 90:10 PL:CG beads remained above breakpoint for 65 days before dissolving at 80 days. The 80:20 PL:CG beads released adequate concentrations through

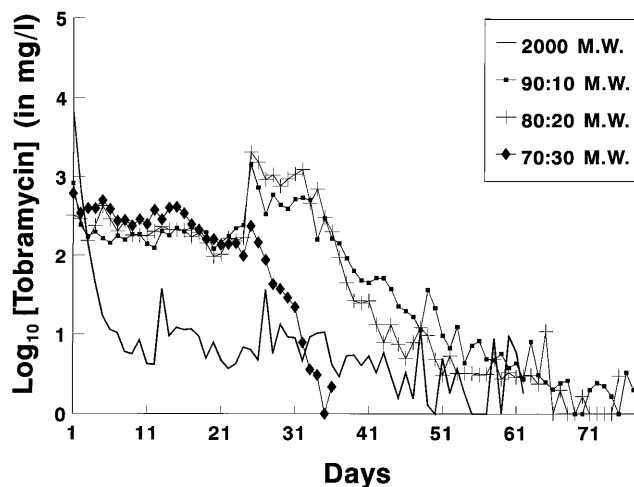


FIG. 3. Tobramycin release from 2,000-MW PLA, 90:10 PL:CG, 80:20 PL:CG, and 70:30 PL:CG beads. The breakpoint sensitivity limit for tobramycin is 4.0 mg/liter.

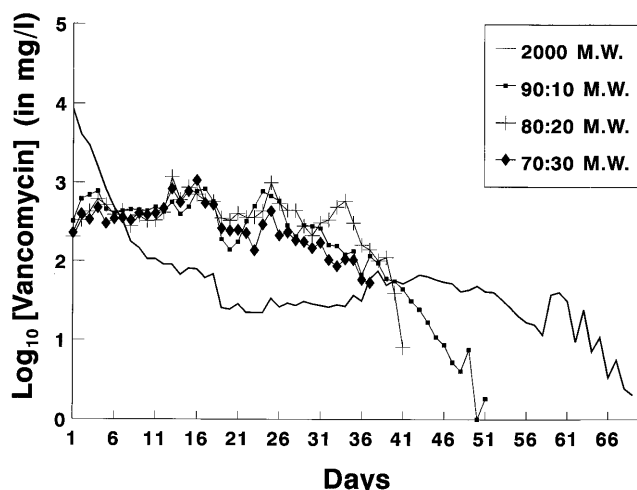


FIG. 4. Vancomycin release from 2,000-MW PLA, 90:10 PL:CG, 80:20 PL:CG, and 70:30 PL:CG beads. The breakpoint sensitivity limit for vancomycin is 5.0 mg/liter.

day 51 before dissolving by day 80. The 70:30 PL:CG beads released adequate concentrations through day 36 before dissolving at day 38.

The results with vancomycin PLA and PL:CG beads are shown in Table 1 and Fig. 4. Vancomycin concentrations for the 2,000-MW PLA beads stayed high through 68 days, and the beads dissolved at day 180. Beads made of 90:10 PL:CG remained above the breakpoint sensitivity through day 49, dissolving on day 120. The 80:20 PL:CG beads released vancomycin concentrations above the breakpoint sensitivity until they dissolved on day 41, while the 70:30 PL:CG beads released vancomycin concentration above the breakpoint sensitivity through day 36 before dissolving. Of the biodegradable beads, the 2,000-MW PLA took the longest time to dissolve, followed by 90:10, 80:20, and finally, 70:30 PL:CG beads ($P < 0.05$).

Table 1 and Fig. 5 show the results for the 2,000-MW PLA-70:30 PL:CG beads. Antibiotic concentrations for clindamycin, tobramycin, and vancomycin remained above the breakpoint

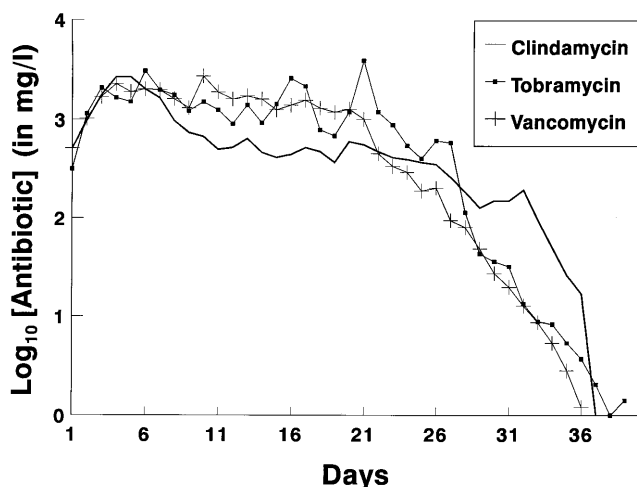


FIG. 5. In vitro release of clindamycin, tobramycin, and vancomycin from 2,000-MW PLA and 70:30 PL:CG beads. The breakpoint sensitivities are 1.0, 4.0, and 5.0 mg/liter, respectively.

sensitivity until dissolution at 39, 39, and 40 days, respectively. Clindamycin maintained good concentrations through day 37, tobramycin through day 36, and vancomycin through day 35.

DISCUSSION

Local antibiotic delivery systems have improved the management of complex wounds in musculoskeletal surgery. When a thorough debridement is augmented with high sustained local antibiotic concentration, the extension of bone and soft tissue contamination to a regional infection may be prevented (1). Antibiotic PMMA beads are often used to sterilize and temporarily maintain dead space following debridement surgery (1-3). The beads are surgically implanted in the debrided bone and covered with soft tissue. Serum, inflammatory fluid, and antibiotic collects in the space (termed a seroma) around the beads. The PMMA beads are left in place for 3 to 4 weeks and are then surgically removed (1, 2, 4).

The antibiotic used in the beads should provide seroma concentrations above the breakpoint sensitivities for 3 to 4 weeks, have adequate granulation tissue and bone concentrations in a clinical setting, and not produce toxic serum drug concentrations. Currently, antibiotics used for bead delivery must be in powdered form and are selected to correspond to the sensitivities of the wound pathogens. At the University of Texas Medical Branch, the most commonly used antibiotic-impregnated beads are clindamycin, tobramycin, and vancomycin. The disadvantages associated with using the PMMA beads include a necessary secondary surgery to remove the beads (1) and a less than optimal antibiotic elution profile (i.e., only 50% of the antibiotic is eluted from the bead by 4 weeks). In this study, tobramycin and clindamycin PMMA beads maintained adequate in vitro antibiotic concentrations through week 4. However, the vancomycin PMMA bead concentrations fell rapidly and failed to elute vancomycin after day 12.

Biodegradable beads have the advantage of releasing all the bead antibiotic and not requiring an additional surgery for bead removal. In this study clindamycin, tobramycin, and vancomycin PLA, PL:CG, and PLA-PL:CG beads demonstrated antibiotic concentrations on the 1st day from 300 to 10,000 mg/liter, and all biodegradable beads dissolved between 36 and 120 days. In vitro, 2,000-MW PLA, PL:CG, and PLA-PL:CG beads released concentrations of clindamycin, tobramycin, and vancomycin above breakpoint sensitivity for a period of at least 30 days. In contrast to PMMA beads, which released adequate vancomycin concentrations for only 12 days, PLA, PL:CG, and PLA-PL:CG beads released vancomycin effectively for 32 (PLA-PL:CG) to 70 (2,000-MW PLA) days.

Low-molecular-weight PLA, PL:CG, and PLA-PL:CG beads have the advantage of averting the need for surgical bead removal, and avoiding a second surgery decreases anesthesia risk and nosocomial infection rates associated with surgery. Further in vitro and in vivo research, already in progress at University of Texas Medical Branch, will help determine more predictable antibiotic release and degradation rates for PLA, PL:CG, and PLA-PL:CG beads and determine if the beads produce toxic serum drug concentrations. Antibiotic-impregnated biodegradable beads have a potential role in the prevention and management of musculoskeletal infections.

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REFERENCES

1. **Calhoun, J. H., and J. T. Mader.** 1977. Antibiotic beads in the management of surgical infections. *Am. J. Surg.* **157**:443-449.
2. **Ciorny, G., and J. T. Mader.** 1984. Adult chronic osteomyelitis. *Orthopedics* **7**:1557-1564.
3. **Ciorny, G., J. T. Mader, and J. J. Pennick.** 1985. A clinical staging system for adult osteomyelitis. *Contemp. Orthop.* **10**:17-37.
4. **Elson, R. A., A. E. Jephcott, D. B. McGeachie, and D. Vereitas.** 1977. Antibiotic-loaded acrylic cement. *J. Bone Joint Surg.* **59B**:200-205.
5. **Garvin, K. L., J. A. Miyano, D. Robinson, D. Giger, J. Novak, and S. Radio.** 1994. Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis: a canine model. *J. Bone Joint Surg.* **76(A)**:1500-1506.
6. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, p. 895. Document M7-T2, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Shinto, Y., A. Uchida, F. Korkusaz, N. Araki, and K. Ono.** 1992. Calcium hydroxyapatite ceramic used as a drug delivery system for antibiotics. *J. Bone Joint Surg.* **74(B)**:600-604.
8. **Wahlig, H., E. Dingeldein, R. Bergmann, and K. Reuss.** 1978. The release of gentamicin from polymethylmethacrylate beads: an experimental and pharmacokinetic study. *J. Bone. Joint Surg.* **60(B)**:270.
9. **Waldvogel, F. A., G. Medoff, and M. M. Swartz.** 1970. Osteomyelitis: a review of clinical features, therapeutic considerations, and unusual aspects. *N. Engl. J. Med.* **282**:198-206, 260-266, 316-322.
10. **Waldvogel, F. A., and H. Vasey.** 1980. Osteomyelitis: the past decade. *N. Engl. J. Med.* **303**:360-370.
11. **Welch, A. B.** 1978. Antibiotics in acrylic bone cement. In vitro studies. *J. Biomed. Mater. Res.* **12**:679.
12. **Wilson, K. J., G. Ciorny, K. R. Adams, and J. T. Mader.** 1988. Comparative evaluation of the diffusion of tobramycin and cefotaxime out of antibiotic-impregnated polymethylmethacrylate beads. *J. Orthop. Res.* **6**:279-286.
13. **Zhang, X., U. P. Wyss, D. Pichora, and M. P. Goosen.** 1994. Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties. *J. Pharm. Pharmacol.* **46**:718-724.