# Increasing the Elution of Vancomycin from High-Dose Antibiotic-Loaded Bone Cement: A Novel Preparation Technique

Tanay J. Amin, MD, Jeffrey W. Lamping, MS, Kelly J. Hendricks, MD, and Terence E. McIff, PhD

*Investigation performed at the Department of Orthopedic Surgery, University of Kansas Medical Center, Kansas City, Kansas*

**Background:** Antibiotic bone cement is commonly used in staged revision arthroplasty as well as the treatment of open fractures. Multiple factors affect antibiotic elution from bone cement. This study was performed to investigate the effect of two variables, the quantity of liquid monomer and the timing of antibiotic addition, on the ultimate elution of antibiotic from bone cement.

**Methods:** Vancomycin-loaded Simplex P and SmartSet MV bone cement was prepared with three different methods: a common surgical technique, a mixing technique that doubled the amount of liquid monomer, and a novel technique that delayed antibiotic addition until after thirty seconds of polymerization. Cylinders of a standardized size were created from each preparation. The elution profiles of five cylinders from each preparation were measured over six weeks with use of high-performance liquid chromatography. Cylinders were tested in compression to quantify strength.

**Results:** Delayed antibiotic addition resulted in significantly greater cumulative elution over six weeks ($p < 0.0001$), with minimal reduction in strength, compared with the other groups. Doubling the liquid monomer significantly reduced cumulative elution over six weeks compared with either of the other techniques ($p < 0.0001$). Vancomycin elution from Simplex P was 52% greater and vancomycin elution from SmartSet MV was 25% greater in the delayed-antibiotic-addition groups than it was in the corresponding standard surgical technique groups. The majority of the antibiotic was released over the first week in all groups.

**Conclusions:** High-dose-antibiotic bone cement prepared with delayed antibiotic addition increased vancomycin elution compared with the standard surgical preparation. Incorporating additional liquid monomer decreased vancomycin elution from high-dose-antibiotic cement. We recommend preparing high-dose-antibiotic bone cement with the delayed-antibiotic-addition technique and not incorporating additional liquid monomer.

**Clinical Relevance:** Both the relative volume of liquid monomer and the timing of antibiotic addition have substantial effects on the elution of antibiotic from bone cement.

Bone cement provides a medium for efficient delivery of antibiotics to eradicate and prevent local infection. The use of antibiotic cement includes spacer treatment in staged revision arthroplasty and void-filling struts or bead pouches in the treatment of open fractures[1-4]. Although advanced aseptic technique has significantly reduced infection rates

**Disclosure:** None of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of any aspect of this work. None of the authors, or their institution(s), have had any financial relationship, in the thirty-six months prior to submission of this work, with any entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. Also, no author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

JBJS | *Express*

This article was chosen to appear electronically on September 26, 2012, in advance of publication in a regularly scheduled issue.

**A commentary by Clifford B. Jones, MD,** is linked to the online version of this article at jbjs.org.

associated with primary surgical procedures, surgeons commonly use antibiotic-impregnated cement as an adjunct prophylactic in primary total joint arthroplasty[5-7]. Various antibiotics, including gentamicin, tobramycin, and vancomycin, have been added to bone cement either by the manufacturer or on site by the surgeon[4,8-10]. Antibiotic selection has a significant impact on eradication of infection, especially in the setting of biofilm formation[11]. Vancomycin, in particular, has demonstrated a much-needed effectiveness against methicillin-resistant *Staphylococcus aureus* and is currently recommended as an antimicrobial prophylaxis in arthroplasty when such resistant organisms are of specific concern[10].

Numerous investigators have evaluated the effect of variations in preparation of antibiotic cement on elution and mechanical strength. The different variables tested include the mixing technique[12-14], initial antibiotic loading[15-17], and use of inert additives intended to increase strength or elution rates[18,19].

Although numerous antibiotics have been incorporated within bone cement, the quantity and formulation of an added antibiotic have an important effect on a cement's mechanical properties that should be considered[15,16,20,21]. Investigators have reported many different techniques for creating antibiotic spacers, incorporating anywhere from 8 to 12 g of antibiotic per 40-g pack of cement[22,23]. International industrial standards state that bone cement used for definitive fixation must have an ultimate compressive strength of at least 70 MPa (International Organization for Standardization [ISO] 5833-2). This standard was established to reduce the likelihood of revision arthroplasties due to premature bone-cement breakdown. One study demonstrated that, depending on the formulation of cement used, approximately 6 g of vancomycin may be added while still maintaining the industry standard of 70 MPa for compressive strength[12].

Numerous reports suggest that vacuum-mixed cement often provides a stronger construct than hand-mixed cement and reduces the number of microscopic pores[8,16,24,25]. Comparisons between vacuum and hand-mixed cement have demonstrated significant differences in porosity[24]. Although a reduction in porosity may yield cement with greater strength, less porosity is believed to decrease total antibiotic elution and to slow elution rates[8,13,14,26,27].

In an effort to increase the workability of bone cement, surgeons adding powder antibiotic are tempted to add more monomer during the mixing process. Adding more monomer, however, has been shown to have unintended consequences. Previous studies have demonstrated conflicting results with regard to the effect of changes in the monomer-to-powder ratio on cement strength. Haas et al. demonstrated no effect[28] whereas Belkoff et al. found decreased strength with an increased liquid monomer-to-powder copolymer ratio[29]. In addition to its effect on strength, alteration of the initial polymerization process and viscosity may influence antibiotic incorporation and delivery. A study by Rasyid et al.[19] demonstrated how the reduction of monomer could increase the elution of gentamicin from bone cement. An understanding of the effects of increasing the monomer on the elution properties of bone cement is also needed.

The numerous studies on various bone-cement preparations have not addressed the effects of altering the timing of antibiotic addition to the cement. The technique of adding antibiotic powder to the cement mixture after initiating the polymerization process is a novel approach to antibiotic incorporation. Initiating polymerization prior to antibiotic incorporation may provide a means for increasing cement strength and antibiotic elution.

In this study, we evaluated the effects of two variations in the mixing technique on the elution and mechanical properties of antibiotic-loaded bone cement. The first variation was an increase in the ratio of liquid monomer to powdered polymer. The second variation was a delay in the addition of antibiotic until after initiation of the polymerization process. We evaluated both characteristics by measuring ultimate compression strength and elution of antibiotic.

## Materials and Methods

### *Cylinder Preparation*

Two types of bone cement were used in this study: Simplex P (Stryker Orthopedics, Limerick, Ireland) and SmartSet MV (DePuy, Blackpool, England). Both types of cement were provided to us sterilized and prepackaged. Three 40-g batches of each cement were used. Five grams of vancomycin hydrochloride powder (Hospira, Lake Forest, Illinois) was added to each 40-g batch of cement. The cement and vancomycin mixtures were prepared with three different techniques, as described below. In each case, the vancomycin was broken up into a uniform powder with use of a small metal spatula before it was mixed with cement. Mixing was done with a commercially available manual cement mixer (Zimmer, Warsaw, Indiana) under vacuum at 66°F to 67°F (18.9°C to 19.4°C). The same mixer was used for each batch, but it was thoroughly cleaned and sterilized with alcohol prior to each use.

The first preparation technique (referred to as the "standard surgical technique") involved mixing the cement powder (40 g) with the vancomycin powder (5 g) by hand for thirty seconds. Next, 20 mL of liquid monomer (the manufacturers' recommended amount for normal cement preparation) was added and was mixed for sixty seconds in a 30-kPa vacuum environment.

The second preparation technique (referred to as the "double-liquid-monomer technique") again involved mixing the cement powder (40 g) with the vancomycin powder (5 g) by hand for thirty seconds. Next, 40 mL of liquid monomer (double the manufacturers' recommended dose of 20 mL) was added and was mixed for sixty seconds under a 30-kPa vacuum.

The third preparation (referred to as the "delayed antibiotic technique") consisted of first mixing the cement powder (40 g) with 20 mL of liquid monomer under a 30-kPa vacuum for thirty seconds. The vacuum was then removed and 5 g of vancomycin was added, followed by an additional thirty seconds of vacuum mixing at 30 kPa.

Test specimens in the shape of small cylinders were formed for compression testing and elution measurement with use of a stainless-steel mold as specified in ASTM standard F451-99a[30]. The mold was cleaned and sterilized by autoclaving. Each of the previously mentioned cement preparations was injected with a 60-mL syringe into an array of holes, 6.0 mm in diameter and 12.7 mm deep, in the mold. Immediately after cement injection, the molds and samples were stored for one hour in an incubator at 37°C. The cylinders were then tapped out of their molds and inspected for major defects or cracks. Exclusion of cylinders was based on ASTM standards[30]. The cylinders suitable for testing were ground to a 12-mm length to meet the ASTM standard.

Fifteen cylinders from each preparation were randomly selected for compression testing. Five of these cylinders were incubated at 37°C for twenty-four hours in a dry test tube and then allowed to cool to room temperature

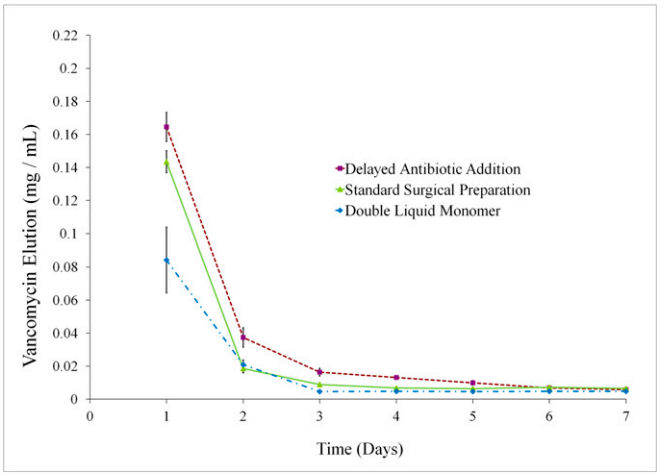


Fig. 1

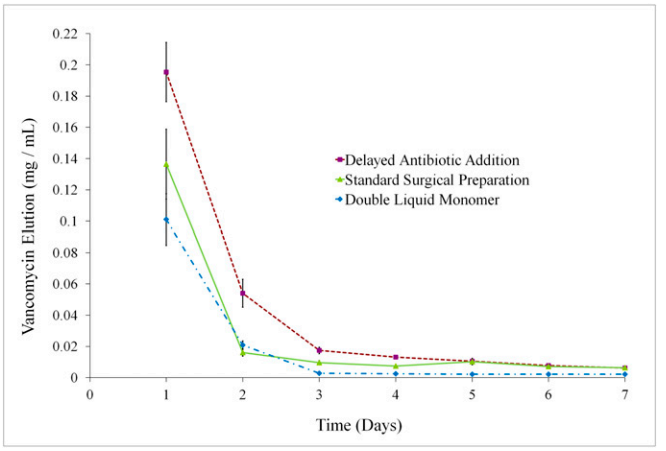


Fig. 2

**Fig. 1** Daily elution of vancomycin from SmartSet MV cement over one week. The error bars represent ±1 SD (standard deviation). **Fig. 2** Daily elution of vancomycin from Simplex P cement over one week. The error bars represent ±1 SD.

before compression testing. Five cylinders were placed in a solution of phosphate-buffered saline solution (PBS) (wet environment) for six weeks at 37°C. The remaining five cylinders were placed in an empty test tube (dry environment) for six weeks at 37°C.

### *Elution Testing*

Five cylinders from each preparation were placed in individual test tubes and immersed in 5 mL of PBS for six weeks. The cylinders were incubated in a 37°C chamber during elution testing. Samples were collected from each test tube at one, two, three, four, five, six, and seven days and at two, three, four, five, and six weeks. At the time of sample collection, each cylinder was rinsed and placed in 5 mL of fresh PBS. Collected samples were analyzed for vancomycin concentration with use of high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) with a C18 column, ultraviolet detection at 214 nm, and a mobile phase consisting of acetonitrile/ammonium acetate at a flow rate of 1 mL/min.

### *Determination of Compression Strength*

Following incubation, compression testing of each cylinder was carried out on an 858 Mini Bionix II materials testing machine (MTS Systems, Eden Prairie, Minnesota) at a crosshead speed of 22 mm/min (ASTM specifications[30]). The ultimate compressive strength was defined as the peak stress applied to the specimen before failure. The ultimate compressive strengths were compared with the ISO standard of 70 MPa (ISO 5833-2).

### *Statistical Analysis*

The effects of the mixing technique (standard surgical preparation, double liquid monomer, and delayed antibiotic addition) on total elution and elution rates were analyzed with use of repeated-measures analysis of variance (STATISTICA software; StatSoft, Tulsa, Oklahoma). Post hoc multiple comparisons of the ultimate compressive strength of the bone cement were performed with use of the Tukey honestly significant difference (HSD) test. Significance was set at $p < 0.05$.

### *Source of Funding*

Internal funding was received for this study from the Marc A. and Elinor J. Asher Orthopedic Research Endowment at the University of Kansas Medical Center.

## Results

The majority of antibiotic elution took place over the first seven days (Figs. 1 and 2). Comparisons of total elution over the first seven days revealed significantly higher rates of vancomycin release from the cement cylinders prepared with the delayed antibiotic technique than from the cylinders prepared with the standard surgical technique ($p < 0.0001$). Doubling the amount of liquid monomer reduced the total vancomycin elution over seven days compared with that from the cylinders prepared with the standard surgical technique ($p < 0.0001$). These differences were likewise significant when cumulative elution was compared over the entire six weeks of measurements ($p < 0.0001$). Total vancomycin elution from Simplex P over six weeks was 52% greater in the delayed antibiotic group than in the standard surgical technique group (Fig. 3). Compared with the standard surgical technique, the use of double liquid monomer led to a 33% decrease in vancomycin elution from Simplex P over six weeks (Fig. 3). There

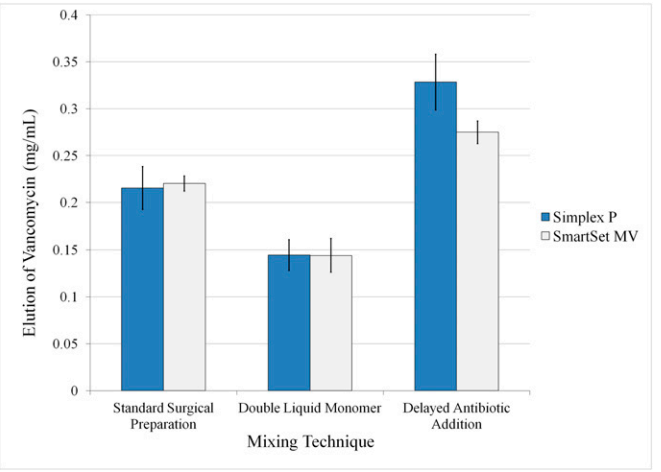


Fig. 3

Cumulative elution of vancomycin over a six-week period from both SmartSet MV and Simplex P cement mixed with 5 g of vancomycin with use of three different mixing techniques. The error bars represent ±1 SD.

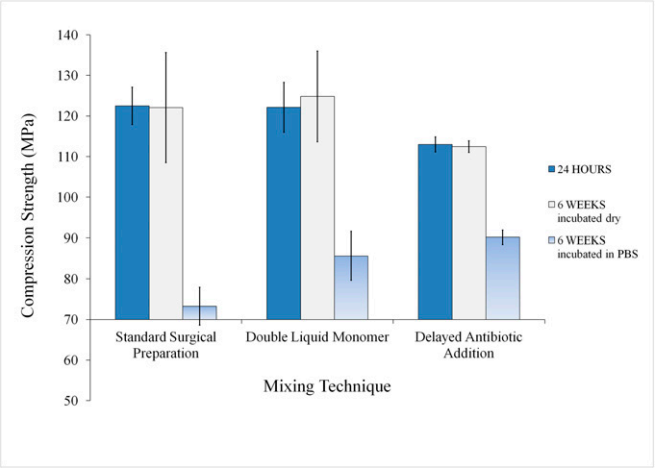


Fig. 4

Compression strength of SmartSet MV cement. Incubated dry = group incubated at 37°C in the absence of PBS solution, and incubated in PBS = group incubated in PBS at 37°C. The horizontal axis crosses at 70 MPa, the ISO standard. The error bars represent ±1 SD.

were similar differences in vancomycin elution from the SmartSet MV preparations over six weeks. Delayed antibiotic addition led to a 25% increase in elution over that associated with the standard surgical preparation, whereas incorporation of double liquid monomer resulted in 35% less elution than that associated with the standard antibiotic technique (Fig. 3). Elution over the last five weeks of measurement did not differ significantly between the standard and delayed antibiotic techniques. The double-liquid-monomer technique, however, still resulted in substantially and significantly lower elution (30% lower for SmartSet MV and 51% lower for Simplex P) over the last five weeks compared with that associated with the standard surgical technique ($p < 0.0001$).

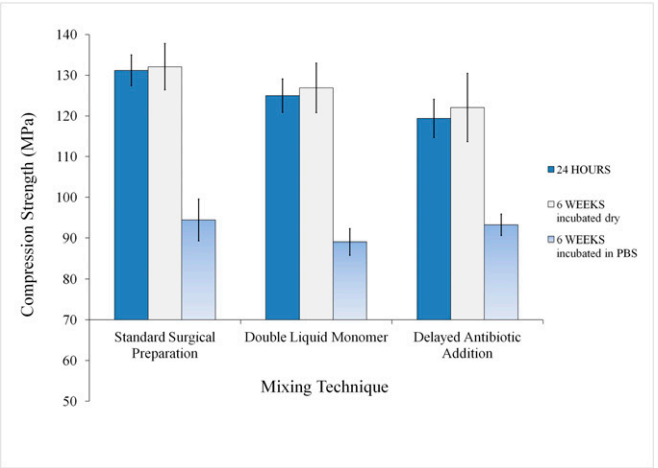


Fig. 5

Compression strength of Simplex P cement. Incubated dry = group incubated at 37°C in the absence of PBS solution, and incubated in PBS = group incubated in PBS at 37°C. The horizontal axis crosses at 70 MPa, the ISO standard. The error bars represent ±1 SD.

Compression testing proved that all three mixing techniques provided high-dose antibiotic-loaded cement within industrial standards, measuring above the recommended 70 MPa at twenty-four hours (Figs. 4 and 5). Statistical analysis did not reveal a significant difference in strength between cylinders tested at twenty-four hours and those tested after incubation in a dry environment for six weeks ($p > 0.95$). Comparison of the compression strength of the cylinders tested at twenty-four hours with that of the cylinders tested after six weeks of elution demonstrated a significant decrease in strength ($p < 0.001$). We observed this decrease in strength in all three cement-preparation groups. All post-elution compression testing showed average cement strength remaining above 70 MPa. However, the SmartSet MV cement prepared with the standard surgical technique demonstrated compression strength with a 95% confidence interval that fell below the 70-MPa recommendation. Furthermore, strength after six weeks of elution in the SmartSet MV standard surgical preparation group was significantly lower than that in either the delayed antibiotic group or the double-liquid-monomer group ($p < 0.01$).

Cumulative vancomycin elution over the entire six-week period ranged from 3.1% to 5.2% of the total added vancomycin, depending on the type of cement and the preparation technique. In other words, approximately 95% to 97% of the vancomycin remained in the cement at the end of the six-week period. The majority of this elution took place over the first week. Roughly 90% (90% to 93%) of the vancomycin elution over the entire six-week period occurred in the first week; only 10% eluted over the remaining five weeks of measurement. Elution profiles illustrate leveling of vancomycin release at approximately one week (Figs. 1 and 2). Detectable, but much lower, levels of vancomycin continued to elute for at least four weeks.

## Discussion

Delayed antibiotic addition yielded both greater and faster vancomycin release from our cement cylinders. This technique produced higher levels of antibiotic elution over the first week and then amounts equivalent to those produced by the standard technique for the remaining measurement period. The mechanism, however, is not clear from our study. There are multiple theories regarding the mechanism of antibiotic elution from cement: diffusion through a solid matrix, diffusion through cement voids, and a surface phenomenon[8]. The primary factor influencing elution is thought to be porosity[8]. The superior elution achieved by the delayed technique may have been due to decreased interference by the vancomycin with the initial polymerization process. The delayed addition of antibiotic may have also increased the porosity of the cement as a whole.

Altering the polymerization process and mechanical properties of bone cement by varying the liquid monomer-to-powder ratio ultimately affects elution of any antibiotic added to the bone cement. In our study, altering the polymerization process by increasing the ratio of liquid monomer to powder significantly decreased the elution of antibiotic from the bone cement. The cement prepared with double liquid monomer

eluted a significantly lower amount of antibiotic than did both the standard surgical and the delayed antibiotic preparations. This decrease in elution may be attributable to less total cement porosity and increased entrapment of antibiotic within the cement matrix during polymerization. Less porosity would allow less antibiotic to be exposed to infiltrating fluids, resulting in lower total antibiotic elution.

It has previously been shown that greater porosity not only increases elution but also decreases bone cement strength[16]. Although, at twenty-four hours, the ultimate compressive strength of the cement prepared with the delayed antibiotic technique was lower than that of the cement prepared with the standard technique, it was less than 10% lower at that time and was equal to or greater than that in the standard technique group after six weeks of elution. The resulting reduction in strength may have been mitigated by the undisturbed, although short, period of polymerization prior to the addition of the antibiotic.

Belkoff et al. understood that surgeons often varied the initial ratio of liquid monomer to powder to alter working time[29]. Their study demonstrated that increasing the liquid monomer-to-powder ratio decreased the compressive strength of bone cement. Haas et al. observed that a decreased liquid monomer-to-powder ratio decreased working time but an increased ratio did not decrease strength[28]. Both groups of authors used Simplex P bone cement. The difference between the results of the two experiments may be attributed to Belkoff et al. having studied cement mixtures with higher ratios of liquid monomer to powder, up to double the manufacturer's specified amount of monomer. Our study, however, did not show that doubling the liquid monomer had any substantial effect on the ultimate compressive strength. Belkoff et al. and Haas et al. did not add antibiotic to their preparations so it is likely that the incorporation of vancomycin in our preparations limited the reduction in strength caused by the additional monomer, at least at the ratio of 1 g:1 mL used in our study.

The ultimate compressive strength following only one day of curing in our study was somewhat higher than that typically reported in the literature[16,20,21]. In our study, curing (incubation) was done under dry conditions at 37°C, whereas most reported values have been measured following one day of curing at 23°C (ASTM standard F451[30]). The curing temperature of 37°C is more representative of in vivo temperatures. Haas et al.[28] clearly showed how this difference in temperature would affect the material properties of curing cement, especially over the first twenty-four hours. When cement is cured at 37°C, little change in material behavior or strength is expected between twenty-four hours and six weeks (i.e., after full curing). Cement that is initially cured at 23°C will not yet have achieved its final material properties, including ultimate compressive strength, at twenty-four hours.

Compression strength testing demonstrated that all three preparation techniques were acceptable according to industrial standards. However, after six weeks of elution, SmartSet MV prepared with the standard surgical technique demonstrated reduced strength with a confidence interval that fell slightly below the industrial standard of 70 MPa. Pelletier et al. found that the compressive strength of bone cement prepared with 6 g of antibiotic decreased to below or near 70 MPa after four weeks of elution[20]. Our study confirms their finding of a decrease in compression strength of high-dose antibiotic-loaded bone cement after elution for multiple weeks. Our results also support the use of the delayed antibiotic technique, which yielded acceptable industrial strength of both brands of cement after six weeks of antibiotic elution. When placing an antibiotic spacer created with the standard surgical technique, surgeons should consider the amount of partial weight-bearing allowed. This weight-bearing limit becomes more important with time as antibiotic elution continues and the mechanical strength of the cement diminishes.

Although we found that the rate and amount of vancomycin elution varied across mixing techniques, investigation of the clinical impact of these findings on eradication of infection was not within the scope of this study. No assay of antimicrobial effectiveness was undertaken. Previous authors, such as Squire et al.[31], have reported using such assays, and finding in vitro bacterial growth inhibition that was in line with the quantitative elution results reported in our study. In our study, the delayed antibiotic technique effectively increased the total vancomycin elution, but this effect was primarily limited to the first few days. By day six, no appreciable difference in elution could be detected among the various techniques. The new technique did not substantially increase the duration of effective antibiotic delivery, but it did increase the total amount delivered. Squire et al. found that in vitro antibacterial properties of premixed bone cements were greatly reduced after the first four days. This implies that other additives or components might be required if antibiotic delivery is needed over a more extended period.

Identifying the optimal timing of the antibiotic addition should be further pursued. Different types of cement and antibiotics respond variably and sometimes unpredictably to alterations in the mixing technique[16]. Adding the antibiotic too early may reduce the total elution, whereas adding it too late may create large defects in the cement, potentially weakening the final material. The optimal time and method of delaying addition of the antibiotic may depend on the cure rate of the particular cement being used.

Only two commercial brands of cement were used in this study. Similar results were obtained with both brands, implying that the results are not unique to a particular commercial cement. However, we examined the effect of the mixing technique on the properties of high-dose-antibiotic cement; we did not evaluate any low-dose combinations. The cement was not premixed by the manufacturer but mixed with vancomycin on site to model the mixing of the antibiotic into the cement at the time of surgery. Studies have demonstrated that increasing the amount of antibiotic added to bone cement affects mechanical properties[16,20,21]. Therefore, the inherent differences between high and low-dose-antibiotic cement may yield different outcomes with regard to mixing technique. The interpretation of our results should therefore be limited to high-dose-antibiotic cements mixed in the operating room.

This study clearly demonstrates the advantages of delaying the addition of antibiotics until after the initiation of polymerization when preparing high-dose antibiotic-loaded bone cement. Adding antibiotic after the initiation of the polymerization process created higher elution while still maintaining mechanical strength that met industrial standards in both the Simplex P and the SmartSet MV group even after six weeks of elution. Furthermore, doubling the amount of liquid monomer led to a significantly lower elution profile compared with the other two preparations. Therefore, to achieve higher antibiotic elution levels, we propose preparation of high-dose-antibiotic bone cements with use of the delayed antibiotic technique. We do not recommend incorporating additional liquid monomer when preparing antibiotic cements. ■

Tanay J. Amin, MD
Jeffrey W. Lamping, MS
Kelly J. Hendricks, MD
Terence E. McIff, PhD
Department of Orthopedic Surgery,
University of Kansas Medical Center,
3901 Rainbow Boulevard, M.S. 3037,
Kansas City, KS 66160.
E-mail address for T.E. McIff: tmciff@kumc.edu

## References

**1.** Anagnostakos K, Wilmes P, Schmitt E, Kelm J. Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers in vivo. Acta Orthop. 2009 Apr;80(2):193-7.

**2.** Jaeblon T. Polymethylmethacrylate: properties and contemporary uses in orthopaedics. J Am Acad Orthop Surg. 2010 May;18(5):297-305.

**3.** Hendriks JG, van Horn JR, van der Mei HC, Busscher HJ. Backgrounds of antibiotic-loaded bone cement and prosthesis-related infection. Biomaterials. 2004 Feb;25(3):545-56.

**4.** Jiranek WA, Hanssen AD, Greenwald AS. Antibiotic-loaded bone cement for infection prophylaxis in total joint replacement. J Bone Joint Surg Am. 2006 Nov; 88(11):2487-500.

**5.** Joseph TN, Chen AL, Di Cesare PE. Use of antibiotic-impregnated cement in total joint arthroplasty. J Am Acad Orthop Surg. 2003 Jan-Feb;11(1): 38-47.

**6.** Dunbar MJ. Antibiotic bone cements: their use in routine primary total joint arthroplasty is justified. Orthopedics. 2009 Sep;32(9). pii: orthosupersite.com/view.asp?rID=42849. doi: 10.3928/01477447-20090728-20.

**7.** Namba RS, Chen Y, Paxton EW, Slipchenko T, Fithian DC. Outcomes of routine use of antibiotic-loaded cement in primary total knee arthroplasty. J Arthroplasty. 2009 Sep;24(6 Suppl):44-7. Epub 2009 Jul 4.

**8.** Anagnostakos K, Kelm J. Enhancement of antibiotic elution from acrylic bone cement. J Biomed Mater Res B Appl Biomater. 2009 Jul;90(1):467-75.

**9.** Lewis G. Properties of antibiotic-loaded acrylic bone cements for use in cemented arthroplasties: a state-of-the-art review. J Biomed Mater Res B Appl Biomater. 2009 May;89(2):558-74.

**10.** Meehan J, Jamali AA, Nguyen H. Prophylactic antibiotics in hip and knee arthroplasty. J Bone Joint Surg Am. 2009 Oct;91(10):2480-90.

**11.** Fernández-Hidalgo N, Gavaldà J, Almirante B, Martín MT, Onrubia PL, Gomis X, Pahissa A. Evaluation of linezolid, vancomycin, gentamicin and ciprofloxacin in a rabbit model of antibiotic-lock technique for Staphylococcus aureus catheter-related infection. J Antimicrob Chemother. 2010 Mar;65(3):525-30. Epub 2010 Jan 18.

**12.** McLaren AC, Nugent M, Economopoulos K, Kaul H, Vernon BL, McLemore R. Hand-mixed and premixed antibiotic-loaded bone cement have similar homogeneity. Clin Orthop Relat Res. 2009 Jul;467(7):1693-8. Epub 2009 Apr 24.

**13.** Shiramizu K, Lovric V, Leung A, Walsh WR. How do porosity-inducing techniques affect antibiotic elution from bone cement? An in vitro comparison between hydrogen peroxide and a mechanical mixer. J Orthop Traumatol. 2008 Mar;9(1):17-22. Epub 2008 Mar 13.

**14.** Neut D, van de Belt H, van Horn JR, van der Mei HC, Busscher HJ. The effect of mixing on gentamicin release from polymethylmethacrylate bone cements. Acta Orthop Scand. 2003 Dec;74(6):670-6.

**15.** Bridgens J, Davies S, Tilley L, Norman P, Stockley I. Orthopaedic bone cement: do we know what we are using? J Bone Joint Surg Br. 2008 May;90(5): 643-7.

**16.** Brock HS, Moodie PG, Hendricks KJ, McIff TE. Compression strength and porosity of single-antibiotic cement vacuum-mixed with vancomycin. J Arthroplasty. 2010 Sep;25(6):990-7. Epub 2009 Aug 12.

**17.** Lewis G, Janna S. Estimation of the optimum loading of an antibiotic powder in an acrylic bone cement: gentamicin sulfate in SmartSet HV. Acta Orthop. 2006 Aug;77(4):622-7.

**18.** Nugent M, McLaren A, Vernon B, McLemore R. Strength of antimicrobial bone cement decreases with increased poragen fraction. Clin Orthop Relat Res. 2010 Aug;468(8):2101-6.

**19.** Rasyid HN, van der Mei HC, Frijlink HW, Soegijoko S, van Horn JR, Busscher HJ, Neut D. Concepts for increasing gentamicin release from handmade bone cement beads. Acta Orthop. 2009 Oct;80(5):508-13.

**20.** Pelletier MH, Malisano L, Smitham PJ, Okamoto K, Walsh WR. The compressive properties of bone cements containing large doses of antibiotics. J Arthroplasty. 2009 Apr;24(3):454-60. Epub 2008 Apr 3.

**21.** Lautenschlager EP, Jacobs JJ, Marshall GW, Meyer PR Jr. Mechanical properties of bone cements containing large doses of antibiotic powders. J Biomed Mater Res. 1976 Nov;10(6):929-38.

**22.** Hsieh PH, Chen LH, Chen CH, Lee MS, Yang WE, Shih CH. Two-stage revision hip arthroplasty for infection with a custom-made, antibiotic-loaded, cement prosthesis as an interim spacer. J Trauma. 2004 Jun;56(6):1247-52.

**23.** Peng KT, Hsu WH, Hsu RW. Improved antibiotic impregnated cement prosthesis for treating deep hip infection a novel design using hip compression screw. J Arthroplasty 2010; 25(8):1304-1306

**24.** Smeds S, Goertzen D, Ivarsson I. Influence of temperature and vacuum mixing on bone cement properties. Clin Orthop Relat Res. 1997 Jan;(334):326-34.

**25.** Askew MJ, Kufel MF, Fleissner PR Jr, Gradisar IA Jr, Salstrom SJ, Tan JS. Effect of vacuum mixing on the mechanical properties of antibiotic-impregnated polymethylmethacrylate bone cement. J Biomed Mater Res. 1990 May;24(5):573-80.

**26.** McLaren AC, Nelson CL, McLaren SG, Wassell DL. Phenolphthalein used to assess permeability of antibiotic-laden polymethylmethacrylate: a pilot study. Clin Orthop Relat Res. 2005 Oct;439:48-51.

**27.** Kuechle DK, Landon GC, Musher DM, Noble PC. Elution of vancomycin, daptomycin, and amikacin from acrylic bone cement. Clin Orthop Relat Res. 1991 Mar;(264):302-8.

**28.** Haas SS, Brauer GM, Dickson G. A characterization of polymethylmethacrylate bone cement. J Bone Joint Surg Am. 1975 Apr;57(3):380-91.

**29.** Belkoff SM, Sanders JC, Jasper LE. The effect of the monomer-to-powder ratio on the material properties of acrylic bone cement. J Biomed Mater Res. 2002; 63(4):396-9.

**30.** Annual book of ASTM standards. West Conshohocken, PA: ASTM International; 2008.

**31.** Squire MW, Ludwig BJ, Thompson JR, Jagodzinski J, Hall D, Andes D. Premixed antibiotic bone cement: an in vitro comparison of antimicrobial efficacy. J Arthroplasty. 2008 Sep;23(6 Suppl 1):110-4. Epub 2008 Jul 9.