An Approach for Determining Antibiotic Loading for a Physician-directed Antibiotic-loaded PMMA Bone Cement Formulation

Gladius Lewis PhD, Jennifer L. Brooks BS,
Harry S. Courtney PhD, Yuan Li PhD,
Warren O. Haggard PhD

Published online: 27 February 2010
© The Association of Bone and Joint Surgeons® 2010

Abstract

Background When a physician-directed antibiotic-loaded polymethylmethacrylate (PMMA) bone cement (ALBC) formulation is used in total hip arthroplasties (THAs) and total knee arthroplasties (TKAs), current practice in the United States involves arbitrary choice of the antibiotic loading (herein defined as the ratio of the mass of the antibiotic added to the mass of the cement powder). We suggest there is a need to develop a rational method for determining this loading.

Questions/purposes We propose a new method for determining the antibiotic loading to use when preparing a physician-directed ALBC formulation and illustrate this method using three in vitro properties of an ALBC in which the antibiotic was daptomycin.

The institution of one or more of the authors (WOH, GL) has received funding from Cubist Pharmaceuticals, Inc (Lexington, MA). Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

This work was performed at The University of Memphis and Veterans Affairs Medical Center.

G. Lewis, Y. Li
Department of Mechanical Engineering, The University
of Memphis, Memphis, TN, USA

J. L. Brooks (✉), W. O. Haggard
Department of Biomedical Engineering, The University
of Memphis, Memphis, TN, USA
e-mail: jbrook12@gmail.com

H. S. Courtney
Veterans Affairs Medical Center and Department of Medicine,
University of Tennessee Health Science Center, Memphis,
TN, USA

Materials and Methods Daptomycin was blended with the powder of the cement using a mechanical mixer. We performed fatigue, elution, and activity tests on three sets of specimens having daptomycin loadings of 2.25, 4.50, and 11.00 wt/wt%. Correlational analyses of the results of these tests were used in conjunction with stated constraints and a nonlinear optimization method to determine the daptomycin loading to use.

Results With an increase in daptomycin loading, the estimated mean fatigue limit of the cement decreased, the estimated elution rate of the antibiotic increased, and the percentage inhibition of staphylococcal growth by the eluate remained unchanged at 100%. For a daptomycin-loaded PMMA bone cement we computed the optimum amount of daptomycin to mechanically blend with 40 g of cement powder is 1.36 g.

Conclusions We suggest an approach that may be used to determine the amount of antibiotic to blend with the powder of a PMMA bone cement when preparing a physician-directed ALBC formulation, and highlighted the attractions and limitations of this approach.

Clinical Relevance When a physician-directed ALBC formulation is selected for use in a TKA or THA, the approach we detail may be employed to determine the antibiotic loading to use rather than the empirical approach that is taken in current clinical practice.

Introduction

Antibiotic-loaded polymethylmethacrylate (PMMA) bone cements (ALBCs) are widely used as a prophylactic [6, 12] or therapeutic [6, 34] modality for prosthetic joint infection (PJI) in TKA and THA. Based on the method used to blend the antibiotic and the cement powder, ALBCs may be

classified into two variants: commercially available and physician-directed. With commercially available brands, a proprietary method is used for the blending (herein referred to as a premixed brand). A number of such brands are approved for both primary and revision TKA and THA. In the United States, physician-directed formulations are commonly used for “high-risk” primary THAs and TKAs and either a premixed or a physician-directed formulation (the second variant) for revision THAs and TKAs [12]. For the latter, the blended mixture is manually prepared in the operating room by mixing in an open bowl, using a stirring spatula or tongue depressor, or mixing in a mechanical mixer just before the prosthesis is implanted [12]. Regardless of the method of blending, there is limited rationale for the antibiotic loading to use, with this loading being defined herein as the ratio of mass of the antibiotic added to the mass of the cement powder. When a physician-directed formulation is used, this loading is at the discretion of the surgeon.

The literature on rational methods for determining the antibiotic loading to use when preparing a physician-directed ALBC formulation is very limited. In fact, only Lewis and Janna [20] have presented such methods but that work has two limitations. First, the activity of the eluate against susceptible microorganism(s) implicated in PJI was not included in the collection of cement properties

determined. Second, one of the properties determined was fatigue life of the cement specimens at a stress of ± 15 MPa, a level much higher than has been estimated to be experienced in the cement zone in a cemented total joint arthroplasty (a maximum of 11 MPa [14]). There is scope for further work to develop rational methods for determining this loading.

Therefore we present a new method for determining this loading, involving experimentally determining various cement properties, each as a function of antibiotic loading; performing correlational analyses of these results; and applying a nonlinear minimization method. We then illustrate the use of this method by utilizing three in vitro properties of a daptomycin-loaded PMMA bone cement.

Materials and Methods

The study design comprised five parts (Fig. 1): (1) We fabricated four groups of specimens, three having different daptomycin loadings (Sets A, B, and C) and one not loaded with the antibiotic (control; Set D); (2) We performed fatigue tests on specimens in the four study sets, determined the profile of the elution of the antibiotic from the specimens in Sets A, B, and C into PBS at 37°C, and determined the activity of the eluted daptomycin from

Fig. 1 A flowchart shows the study design. The key features of the three cement property characterization tests conducted are shown. Study sets included: A, 2.25 wt/wt% daptomycin; B, 4.50 wt/wt% daptomycin; C, 11.00 wt/wt% daptomycin; and D, no daptomycin (control set).

specimens in Sets A, B, and C against *S. aureus* on specimens in Sets A, B, and C; (3) We used the results of the tests on the specimens in Sets A, B, and C to determine the following properties for each of the cements: estimated mean fatigue limit, estimated elution rate of the daptomycin, and percentage inhibition provided by the eluate; (4) Using the values of these cement properties (dependent variables) and the corresponding antibiotic loading (independent variable), we obtained three objective functions, with each function being the best-fit relationship between a dependent variable and the independent variable; (5) We then used these objective functions, together with specified values of the lower and upper bounds on each of the dependent variables, and a nonlinear optimization method (contained in a computer software package) to determine the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement formulation.

The cement used was a commercially available brand (Orthoset® 1; Wright Medical Technology, Arlington, TN) that is used in approximately 12% of THAs and TKAs [2], and the antibiotic used was daptomycin (Cubicin®; Cubist Pharmaceuticals, Lexington, MA), a member of a class of antibiotics called lipopeptides [4, 8, 23, 29, 30].

Before the preparation of the test specimens, the daptomycin was stored at $4^\circ \pm 1^\circ\text{C}$, while the powder and liquid monomer of the bone cement were stored in ambient laboratory conditions (temperature and relative humidity = $21^\circ \pm 1^\circ\text{C}$ and $57\% \pm 2\%$, respectively). A mechanical mixer (OmoMix®; Tecres SpA, Verona, Italy) was used to blend the daptomycin with the bone cement powder, with antibiotic loadings of 2.25 wt/wt% (0.9 g daptomycin + 40 g cement powder; Set A), 4.50 wt/wt% (1.8 g daptomycin + 40 g cement powder; Set B), and 11.00 wt/wt% (4.4 g daptomycin + 40 g cement powder; Set C). For the control set (Set D), no daptomycin was blended with the cement powder. For each of the sets, the daptomycin-cement powder blend and the liquid monomer were mixed using a commercially available mixer (MixeVac® II High Vacuum System; Stryker Instruments, Kalamazoo, MI; evacuation pressure: 74 ± 0.1 kPa) [9]. The curing cement dough was injected into a four-celled silicone mold with the internal configuration and dimensions of a solid cylindrical dog bone, with the dimensions as specified in ASTM F2118-03 [1] (Fig. 2).

For each of the four study sets, the protocol used for the acceptance of the fabricated specimens for testing is detailed in ASTM F2118-03 [1]. This ASTM standard involves visual and radiographic examinations of the test dog-bone specimens for flaws on and in the specimens. The rejection ratio (number of specimens rejected/total number of specimens fabricated) was $23\% \pm 2\%$. From among

Fig. 2 A fully dimensioned drawing shows the specimen used in the fatigue tests. All dimensions are in millimeters. Specimen configuration and dimensions conform to those stipulated in ASTM F2118-03 [1], which was the standard used for the determination of the estimated mean fatigue limits of the cements.

these accepted specimens, manual randomization was used to select the specimens for the fatigue tests. Before a test, the specimens were immersed in PBS at $37 \pm 1^\circ\text{C}$ for 7 days.

Uniaxial tension-compression constant-frequency fatigue tests were conducted on the specimens following the protocols detailed in ASTM F2118-03 [1], with the exception that, for each study set, we did not test a minimum of 15 specimens at each loading level used. Rather, for each study set, we tested eight specimens at a loading corresponding to a stress (S) of ± 20.0 MPa, 15 specimens at ± 15.0 MPa, six specimens at ± 12.5 MPa, and three specimens at ± 10.0 MPa. The number of specimens tested at each stress level was similar to those used in studies on fatigue tests of ALBC specimens, at comparable stresses, reported in the literature [5, 7, 20, 21, 25]. The tests were conducted in ambient laboratory air at a frequency of 2 Hz. Each test was conducted until either the specimen fractured after a certain number of loading cycles (N_f) or run-out occurred. We used a run-out of 1.557 million cycles, as did other workers who used ASTM F2118-03 [16]. The fatigue limit of each cement was estimated through fitting the Olgive equation to the S -log N_f results. This equation is given by Krause et al. [15] as

$$S = a + \left\{ (b - a) / \left[1 + (\log N_f / c)^d \right] \right\}, \quad (1)$$

where a (the lower asymptote to the S -log N_f curve) denotes the fatigue limit of the cement, and b , c , and d are material constants.

Estimates of a , b , c , and d (and the associated 95% confidence limits for each) were obtained using a nonlinear regression method (the Marquardt-Levenberg method [3]), contained in a commercially available curve-fitting software package (Matlab® 7.1; The MathWorks, Natick, MA). In our analysis to obtain the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement, we used the mean value of a , which we designated the estimated mean fatigue limit.

For the elution tests on a given study set, three dog bone specimens (Fig. 2) were manually randomly selected from the collection of those determined acceptable for fatigue testing but were not used. The sample size used for each study group ($n = 3$) is within the range used in some elution studies reported in the literature [13, 32]. Each specimen was weighed using an analytic balance (Model A-160; Fisher Scientific, Inc, Fairlawn, NJ). The specimens were then placed in a 15-mL centrifuge tube containing 10 mL $1 \times$ PBS. The tube was then placed in an incubator at 37°C . At Days 1, 2, 5, 7, 10, 14, 21, and 28, the tube was shaken and four 1-mL aliquots were taken, placed in microcentrifuge tubes, and stored in a freezer (at -80°C) until testing. At each time point, the other 6 mL PBS were discarded and a fresh 10 mL PBS was added. The eluate volume was chosen such that low concentrations could still be measured through days 7–21, while allowing the test specimen to be completely submerged. The daptomycin concentration in each aliquot was measured using a high-performance liquid chromatography unit (Varian Prostar; Varian, Inc, Palo Alto, CA). Further details of the protocol used for the determination of the concentration of the eluted daptomycin are the same as given by Richelsoph et al. [26]. The concentration of daptomycin in the assay was computed as the mass of daptomycin in the eluate normalized by the mass of the specimen and the volume of the aliquots used. The estimated rate of elution of the daptomycin was computed by the following method. The best-fit relationship was derived between the daptomycin concentration and t ($t = 1, 2, \dots, 28$ days) (Marquardt-Levenberg method; Matlab[®] 7.1), and then the estimated elution rate was computed as equal to the slope of the daptomycin concentration-versus- t plot at $t = T$, with T being the time midpoint between $t = 1$ day and the time at which the daptomycin concentration-versus- t plot begins to flatten out.

After the conclusion of the elution tests on a given day, a series of turbidity assays of the eluate was prepared for testing of antibacterial activity. Mixtures of 1.75 mL Mueller Hinton II (MHII) Broth (BD Biosciences, Inc, Franklin Lakes, NJ) supplemented with $25 \mu\text{g/mL}$ CaCl_2 (MHII + Ca) were aliquoted into 4-mL polystyrene tubes and then $200 \mu\text{L}$ of the eluates were added followed by $25 \mu\text{L}$ *S. aureus* Cowan I (approximately 10^4 colony forming units) grown overnight in MHII + Ca at 37°C . A positive control consisted of adding $200 \mu\text{L}$ $1 \times$ PBS to the tubes instead of the eluate. For each test, the blank solution, which was used to zero the spectrophotometer, consisted of 1.75 mL MHII + $200 \mu\text{L}$ $1 \times$ PBS. The tubes were vortexed and then incubated overnight at 37°C , after which the absorbances of the solutions in the tube, at a wavelength of 530 nm (A530), were read using a UV spectrophotometer

(Spectronic 20 Genesys; Spectronic, Greenvale, NY). For Sets A, B, and C, these tests were conducted on eluates collected after Days 1, 5, and 7, while, for Set D, the tests were conducted on solution taken after Day 1. The percentage of inhibition of staphylococcal growth by the eluates (% inhibition) was calculated using the formula: % inhibition = $100[1 - (\text{A530 of eluate}/\text{A530 of positive control})]$. Further details of the activity determination protocol are the same as given by Webb et al. [33].

A commercially available curve-fitting software package (Matlab[®] 7.1) was used to perform regression analyses on the whole collection of (1) estimated mean fatigue limit-versus-daptomycin loading results; (2) estimated elution rate-versus-daptomycin loading results; and (3) % inhibition-versus-daptomycin loading results to obtain the best-fit empirical relationships in each of these three cases. These best-fit relationships are referred to as the objective functions. A programming method for solving nonlinear constrained optimization problems [27] (contained in a commercially available software package [IMSL[®] Fortran Numerical Library Version 6.0]) was used to determine the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement formulation. This loading was the output from the program when the input comprised (1) the three objective functions; (2) the lower and upper bounds on the daptomycin loading (2 wt/wt% and 12 wt/wt%), on the estimated mean fatigue limit (3 MPa and 13 MPa), on the estimated elution rate (3 and $300 \mu\text{g/mL/g}$), and on the % inhibition (0% and 100%). The bounds on the daptomycin loading are consistent with the lowest and highest loadings reported in the literature on the influence of antibiotic loading on properties of PMMA bone cements [5, 7, 13, 19–21, 25]. The bounds on the estimated mean fatigue limit represent the lowest and highest values of the 95% confidence limits on the fatigue limit of plain PMMA bone cements [22]. The bounds on the estimated elution rate are within the range of rates reported in the literature for the elution of gentamicin, tobramycin, and vancomycin from specimens fabricated hand- and vacuum-mixed laboratory-prepared “low-dose” ALBC formulations (herein defined as those with antibiotic loading $< 5 \text{ wt/wt\%}$) [13, 20, 32]. The bounds on the % inhibition are the lowest and highest physically possible values.

Results

With an average increase of 325% in the daptomycin loading, the estimated mean fatigue limit, as obtained from the fatigue test results (Figs. 3, 4), decreased by an average of 29% (Table 1).

Fig. 3A–D Graphs show the results of the fatigue tests at (A) ± 20.0 MPa, (B) ± 15.0 MPa, (C) ± 12.5 MPa, and (D) ± 10.0 MPa. The patterns in the variation of the fatigue test results with daptomycin

loading at ± 20.0 MPa, ± 15.0 MPa, and ± 12.5 MPa are the same but different from the one at ± 10.0 MPa.

Fig. 4 The graph shows the fatigue results (solid diamonds) and the fit of the Olgive equation (Equation 1) to these results in Set B. The continuous curve is the mean fit, and the dotted curves are the 95% confidence limits of the fit. The estimated mean fatigue limit is the stress corresponding to the asymptote to the lower horizontal section of the continuous curve (in this case, 6.1 MPa).

Table 1. Summary of the values of estimated mean of the fatigue limit, estimated elution rate, and % inhibition at 24 h for study sets A, B, and C

Study set	Daptomycin loading (wt/wt%)	Estimated mean of fatigue limit (MPa)	Estimated elution rate ($\mu\text{g/mL/day}$)	% inhibition at 24 hours (%)
A	2.25	6.5	4.71	100
B	4.50	6.1	9.21	100
C	11.00	4.6	29.42	100

For specimens in each of the study sets A, B, and C, (1) there was an initial burst release of daptomycin in the first day, followed by a slower release through Day 7; and (2) after Day 7, the release was negligible ($< 1 \mu\text{g/mL/g}$) (Fig. 5). This pattern of elution is typical for “low-dose” and “medium-dose” (antibiotic loading between 5 wt/wt% and 10 wt/wt%) ALBC formulations [7, 10, 24].

The best-fit relationships between the concentration of the eluted daptomycin (CON) and t (over the time range $1 \text{ day} \leq t \leq 7 \text{ days}$) (Fig. 5A) were determined to be

$$\text{Set A: CON} = 1.62t^2 - 16.04t + 35.98; \text{adjusted } r^2 = 0.69 \quad (2)$$

$$\text{Set B: CON} = 2.68t^2 - 27.99t + 67.6; \text{adjusted } r^2 = 0.82 \quad (3)$$

$$\text{Set C: CON} = 5.91t^2 - 70.77t + 207.10; \text{adjusted } r^2 = 0.72 \quad (4)$$

For each of the study sets A, B, and C, T = approximately 3.5 days (Fig. 5A); thus, the estimated elution rate was computed as the slope of each of the above expressions (Equations 2, 3, and 4) at

$t = 3.5 \text{ days}$. With a mean increase in the daptomycin loading of between four- and fivefold, the estimated elution rate increased by an average of 525% (Table 1).

For each of the study sets A, B, and C, daptomycin was completely active against *S. aureus* (% inhibition = 100) through Day 7, even at concentrations as low as $0.04 \mu\text{g/mL/g}$ (Table 1). The eluate from the control cement (Set D) showed no activity (% inhibition = 0).

The three objective functions, as derived from the results (Table 1), were as follows:

$$\begin{aligned} \text{Estimated mean fatigue limit} \\ = 7.04 - 0.22(\text{daptomycin loading}) \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Estimated elution rate} \\ = 0.087(\text{daptomycin loading})^2 \\ + 1.716(\text{daptomycin loading}) \end{aligned} \quad (6)$$

Fig. 5A–B Graphs show profiles of release of the daptomycin from the bone cement specimens: (A) current amount and (B) cumulative amount. For each of these profiles, the pattern is the same for all the daptomycin loadings; namely, initial rapid release of the antibiotic followed by a much slower release.

$$\% \text{ inhibition (in \%)} = 100 \left[1 - e^{(-2.09 \times \text{daptomycin loading})} \right] \quad (7)$$

Hence, the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement was determined to be 3.40 wt/wt%.

Discussion

Physician-directed formulations of ALBCs are used in TKAs and THAs, especially for revision cases. In these formulations, the antibiotic and the cement powder are blended in the operating room using, for example, a stirring spatula or a mechanical mixer. With the current practice, there is no rationale for the amount of antibiotic used. We therefore presented a new method for estimating the antibiotic loading to use in these cases and illustrated its use with respect to a daptomycin-loaded PMMA bone cement.

There are a number of limitations to our study. First, in the fatigue tests, nonalgorithmic (manual) randomization was used to select the specimens from among those deemed acceptable. Ideally, an algorithmic method, such as random number generation, could have been used. Second, the number of specimens used in three of the stress levels in the fatigue tests (at 10.0 MPa, 12.5 MPa, and 20.0 MPa) may be considered small. However, while an increase in the total number of specimens used in each study set (from the current 32) would increase the goodness-of-fit of Equation 1 to the S-log Nf results, the impact of this increase on the estimated mean fatigue limit obtained is expected to be small. Third, the numbers of specimens used in the elution tests and in the activity tests were not determined using a sample size power analysis. This was not performed because, for each of these tests, analysis of the results using inferential statistical methods was not part of the study design. Fourth, the method used for estimating the daptomycin elution rate was empirical. However, this method is deemed plausible given the fact that the estimates obtained are within the range of rates of elution of other antibiotics (gentamicin, tobramycin, and

vancomycin) from specimens of other “low-dose” and “medium-dose” ALBCs specimens, obtained using other computation methods [13, 20, 32]. Fifth, the determination of the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement formulation was based on only three in vitro properties of the cement. Nonetheless, these properties are relevant to the in vivo performance of cemented total joint arthroplasties. It has been postulated that fatigue of one or more of the cement zones (implant-cement interface, cement mantle, and cement-bone interface) is a key factor adversely affecting the in vivo longevity of a cemented arthroplasty [17, 18]. We recognize, however, that there are other possible limiting phenomena for cemented arthroplasties, such as aseptic loosening caused by particle-induced osteolysis [11]. Our decision to obtain estimates of the fatigue limits of the cements was driven by the fact that the potential for fatigue fracture of a component/structure is high when the applied stress exceeds the fatigue limit or, in some cases, the endurance strength of the material [28]. At the minimum, the efficacy of an ALBC is dependent on the rate of elution of the antibiotic and the activity of the eluate against microorganisms implicated in PJI, including those resistant to methicillin, vancomycin, and linezolid [8, 31]. We do not believe the study limitations jeopardize our findings for our particular choices of conditions in that we consider the value we obtained for the daptomycin loading to use when preparing a physician-directed daptomycin-loaded PMMA bone cement formulation (3.40 wt/wt%) reasonable given the antibiotic loading in premixed ALBC brands; for example, 2.50 wt/wt% tobramycin sulfate in Commercial Simplex with Tobramycin® (Stryker-Howmedica-Osteonics, Mahwah, NJ), 4.22 wt/wt% gentamicin sulfate in SmartSet GHV® (DePuy, Warsaw, IN), and 4.00 wt/wt% gentamicin sulfate + 3.00 wt/wt% clindamycin hydrochloride in Copal® (Merck, Darmstadt, Germany).

Lewis and Janna [20] earlier suggested an approach for determining the antibiotic loading to use when preparing a physician-directed ALBC formulation. In the Lewis and Janna study [20], fatigue performance was obtained as fatigue life at one stress level (± 15 MPa), whereas, in the present work (Table 2), a broader index of fatigue

Table 2. Summary of some features of Lewis and Janna [20] study and the present study

Feature	Lewis and Janna study	Present study
Cement	SmartSet® HV	Orthoset® 1
Antibiotic	Gentamicin	Daptomycin
Antibiotic loadings (wt/wt%)	2.25, 4.25, 11.50	2.25, 4.50, 11.00
Antibiotic blending method	Hand mixing	Commercially available mechanical mixer
Properties determined	Fatigue life*; elution rate	Fatigue limit; elution rate; eluate activity against <i>S aureus</i>

* Under uniaxial tension-compression loading corresponding to a stress of ± 15 MPa; loading frequency = 2 Hz.

performance was obtained, namely, fatigue limit (estimated from fatigue life results at ± 20.0 MPa, ± 15.0 MPa, ± 12.5 MPa, and ± 10.0 MPa). A further difference between these two studies is in the approaches used for determining the antibiotic loading to use. Lewis and Janna [20] used two methods. The first, a “mathematical method,” involved determining the value of the antibiotic loading to use as the loading at which the percentage decrease in the logarithm of the fatigue life at ± 15 MPa (designated $\ln N_f$) with increase in antibiotic loading equals the percentage increase in $\ln$ (elution rate) with increase in antibiotic loading. The second, an “empirical method,” involved defining a range of blended antibiotic-cement powder mixtures bounded on one side by a value of the antibiotic loading that “leads to an unacceptably low fatigue life” (herein designated “antibiotic loading 1”) and on the other side by a value of the antibiotic loading that “leads to an unacceptably low elution rate” (herein designated “antibiotic loading 2”). Lewis and Janna [20] discussed the drawbacks of and challenges in using these methods; for example, (1) the antibiotic loading obtained using the mathematical method is very sensitive to the best-fit relationship between $\ln N_f$ and the antibiotic loading used, and (2) when the empirical method is used, antibiotic loading 1 and antibiotic loading 2 are selected on a subjective basis. The method used in the present study avoided these issues in that it utilized only the results obtained from the experimental tests and well-established principles from the field of optimization theory.

We call to the reader’s attention two important points about issues. First, for the fatigue testing, we used various features of ASTM F2118-03, a standard applicable to fatigue testing of specimens of plain PMMA bone cement. Thus, there is opportunity to develop a standard for fatigue testing of ALBC specimens, in which, among other things, the conditions to which the specimens are exposed before testing (the medium and time in that medium) and during the test (that is, the medium) will be stipulated. In this new standard, method(s) to be used to correct the test results for change of cement fatigue property over the duration of the test should be described. Second, we believe the potential for undesirable reactions in the periprosthetic tissues (eg, rhabdomyolysis) when the antibiotic loading to be used in preparing a physician-direct ALBC formulation is employed should be investigated.

We have presented a new approach for determining the antibiotic loading to use when preparing a physician-directed ALBC formulation. This involves the use of results from in vitro tests on ALBC specimens and a nonlinear optimization method. Based on the results of the variation of three properties of daptomycin-loaded PMMA bone cement (estimated mean fatigue limit, estimated rate of elution of daptomycin into $1 \times$ PBS, and activity of the

eluted daptomycin against *S aureus*) with daptomycin loading, we used this approach to determine the daptomycin loading to use as 3.40 wt/wt% (or 1.36 g daptomycin mixed with 40.0 g cement powder). When a physician-directed ALBC formulation is selected for use in a TKA or THA, we offer the approach presented in this study as a way by which the antibiotic loading to use may be determined as an alternative to the empirical approach that is in current clinical use.

Acknowledgments We thank Cubist Pharmaceuticals and Wright Medical Technology for generous donation of the daptomycin and the bone cement, respectively, used in this investigation and Dr. Teong Tan for help with the optimization work.

References

1. American Society for Testing and Materials (ASTM). Standard F 2118-03: Standard test method for constant amplitude of force controlled fatigue testing of acrylic bone cement materials. In: *Annual Book of ASTM Standards 2008*, Vol 13.01. West Conshohocken, PA: ASTM International; 2008:1136–1143.
2. Australian Orthopaedics Association. National Joint Replacement Registry. Annual Report. Adelaide, Australia: AOA; 2009:169–177.
3. Bates DM, Watts DG. *Nonlinear Regression and Its Applications*. New York, NY: Wiley; 1988.
4. Burdette SD. Daptomycin for methicillin-resistant *Staphylococcus aureus* infections of the spine. *Spine J*. 2009;9:e5–e8.
5. Davis JP, Harris WH. Effect of hand mixing tobramycin on fatigue strength of Simplex P. *J Biomed Mater Res*. 1991;25:1409–1414.
6. Diefenbeck M, Muckley T, Hofmann GO. Prophylaxis and treatment of implant-related infections by local application of antibiotics. *Injury*. 2006;37:S95–S104.
7. Dunne N, Hill J, McAfee P, Todd K, Kirkpatrick R, Tunney M, Patrick S. In vitro study of the efficacy of acrylic bone cement loaded with supplementary amounts of gentamicin: effects on mechanical properties, antibiotic release, and biofilm formation. *Acta Orthop*. 2007;78:774–785.
8. Falagas ME, Giannopoulou KP, Ntziora F, Papagelopoulos PJ. Daptomycin for treatment of patients with bone and joint infections: a systematic review of the clinical evidence. *Int J Antimicrob Agents*. 2007;30:202–209.
9. He S, Scott C, Higham P. Mixing of acrylic bone cement: effect of oxygen on setting properties. *Biomaterials*. 2003;30:202–209.
10. Hendriks JGE, Neut D, Hazenberg JG, Verkerke GJ, van Horn JR, van der Mei HC, Busscher HJ. The influence of cyclic loading on gentamicin release from acrylic bone cements. *J Biomech*. 2005;38:953–957.
11. Jasty M, Moloney WJ, Bragdon CR, O’Connor D, Haire T, Harris WH. The initiation of failure in cemented femoral components of hip arthroplasties. *J Bone Joint Surg*. 1991;73:551–558.
12. Jiranek WA, Hanssen AD, Greenwald AS. Antibiotic-loaded bone cement for infection prophylaxis in total joint replacement. *J Bone Joint Surg Am*. 2006;88:2487–2500.
13. Klekamp J, Dawson JM, Haas DW, DeBoer D, Christie M. The use of vancomycin and tobramycin in acrylic bone cement: biomechanical effects and elution kinetics for use in joint arthroplasty. *J Arthroplasty*. 1999;14:339–346.

14. Krause W, Mathis RS. Fatigue properties of acrylic bone cement: a review. *J Biomed Mater Res.* 1988;22(3 Suppl):37–53.
15. Krause W, Mathis RS, Grimes LW. Fatigue properties of acrylic bone cement: S-N, P-N, and P-S-N data. *J Biomed Mater Res.* 1988;22(A2 Suppl):221–244.
16. Kurtz SM, Villarraga ML, Zhao K, Edidin AA. Static and fatigue mechanical behavior of bone cement with elevated barium sulfate content for treatment of vertebral compression fractures. *Biomaterials.* 2005;26:3699–3712.
17. Lewis G. Properties of acrylic bone cement: state-of-the-art review. *J Biomed Mater Res.* 1997;38:155–182.
18. Lewis G. Fatigue testing and performance of acrylic bone cement: state-of-the-art review. *J Biomed Mater Res.* 2003;66:457–486.
19. Lewis G, Bhattaram A. Influence of a pre-blended antibiotic (gentamicin sulphate powder) on various mechanical, thermal, and physical properties of three acrylic bone cements. *J Appl Biomater.* 2006;20:377–408.
20. Lewis G, Janna S. Estimation of the optimum loading of an antibiotic powder in an acrylic bone cement. *Acta Orthop.* 2006;77:622–627.
21. Lewis G, Janna S, Bhattaram A. Influence of the method of blending an antibiotic powder with an acrylic bone cement powder on physical, mechanical, and thermal properties of the cured cement. *Biomaterials.* 2005;26:4317–4325.
22. Lewis G, Li Y. Dependence of in vitro fatigue properties of PMMA bone cement on the polydispersity index of its powder. *J Mech Behav Biomed Mater.* 2009;3:94–101.
23. Livermore DM. Future directions with daptomycin. *J Antimicrob Chemother.* 2008;62(Suppl 3):iii41–iii49.
24. Merkhani IK, Hasenwinkel JM, Gilbert JL. Gentamicin release from two-solution and powder-liquid poly(methyl methacrylate)-based bone cements by using novel pH method. *J Biomed Mater Res.* 2004;69A:577–583.
25. Persson C, Baleani M, Guandalini L, Tigani D, Viceconti M. Mechanical effects of the use of vancomycin and meropenem in acrylic bone cement. *Acta Orthop.* 2006;77:617–621.
26. Richelsoph KC, Webb ND, Haggard WO. Elution behavior of daptomycin-loaded calcium sulfate pellets: a preliminary study. *Clin Orthop Relat Res.* 2007;461:68–73.
27. Schittkowski K. NLPQL: a Fortran subroutine solving constrained nonlinear programming problems. *Annals Oper Res.* 1986;5:485–500.
28. Shackelford JF. *Introduction to Materials Science for Engineers.* 6th ed. Upper Saddle River, NJ: Pearson Prentice Hall; 2005.
29. Steenbergen JN, Alder J, Thorne GM, Tally FP. Daptomycin: a lipopeptide antibiotic for the treatment of serious gram-positive infections. *J Antimicrob Chemother.* 2005;55:283–288.
30. Thorne G, Alder J. Daptomycin: a novel lipopeptide antibiotic. *Clin Microbiol News.* 2002;24:33–40.
31. Trampuz A, Widmer AF. Infections associated with orthopedic implants. *Curr Opin Infect Dis.* 2006;19:349–356.
32. van de Belt H, Neut D, Schenk W, van Horn JR, van der Mei HC, Busscher HJ. Gentamicin release from polymethylmethacrylate bone cements and Staphylococcus aureus biofilm formation. *Acta Orthop Scand.* 2000;71:625–629.
33. Webb ND, McCanless JD, Courtney HS, Bumgardner JD, Haggard WO. Daptomycin eluted from calcium sulfate appears effective against Staphylococcus. *Clin Orthop Relat Res.* 2008;466:1383–1387.
34. Youngman JR, Ridgway GL, Haddad FS. Antibiotic-loaded cement in revision joint replacement. *Hosp Med.* 2003;64:613–616.