Surfactant-stabilized Emulsion Increases Gentamicin Elution From Bone Cement

Ryan B. Miller MD, Alex C. McLaren MD,
Christine M. Leon MS, Brent L. Vernon PhD,
Ryan McLemore PhD

Published online: 9 June 2011
© The Association of Bone and Joint Surgeons® 2011

Abstract

Background Liquid antimicrobial use for antimicrobial-loaded bone cement is limited because of decreased strength and small volume that can be loaded. Emulsifying the liquid antimicrobial into the monomer may address both issues.

Questions/purposes We determined the effect of using a surfactant-stabilized emulsion on antimicrobial release, compressive strength, and porosity.

Methods We made 144 standardized test cylinders from emulsified antimicrobial-loaded bone cement (three batches, 72 cylinders) and control antimicrobial-loaded bone cement made with antimicrobial powder (three batches, 72 cylinders). For each formulation, five specimens per batch ($n = 15$) were eluted in infinite sink conditions over 30 days for gentamicin delivery; five specimens per batch were axially compressed to failure after elution of 0, 1, and 30 days ($n = 45$); and two noneluted specimens and two gentamicin delivery specimens from each batch ($n = 12$) were examined under scanning electron microscopy for porosity. Antimicrobial release and compressive

strength were compared across cement type and time using repeated-measures ANOVA.

Results Emulsified antimicrobial-loaded bone cement released four times more antimicrobial than control. Compressive strength of emulsified antimicrobial-loaded bone cement was less than control before elution (58.1 versus 81.3 MPa) but did not decrease over time in elution. Compressive strength of control antimicrobial-loaded bone cement decreased over 30 days in elution (81.3 versus 73.9 MPa) but remained stronger than emulsified antimicrobial-loaded bone cement. Porosity was homogeneous, with pores ranging around 50 μm .

Conclusions Emulsified antimicrobial-loaded bone cement has homogeneous porosity with increased drug release but a large loss of strength.

Clinical Relevance Liquid antimicrobials are released from emulsified antimicrobial-loaded bone cement, but increased strength is needed before this method can be used for implant fixation.

Introduction

Formulation of antimicrobial-loaded bone cement (ALBC) has been limited to antimicrobial powders because liquid antimicrobials cause greater loss in mechanical strength than antimicrobial powders [4, 8] by as much as $\frac{1}{3}$ [6] to $\frac{1}{2}$ [14]. Young's modulus is also decreased more [9]. Antibacterials are generally soluble in water but not in methyl methacrylate (MMA) monomer. Water and MMA are highly immiscible. When aqueous antimicrobials are mixed with MMA, they stay in distinct layers and the antimicrobial solution separates during polymerization. In preliminary laboratory testing (ACM, RM), 1 mL water was almost completely entrapped during polymerization;

One or more of the authors (ACM) have received funding from the Herbert J. Lewis Fund at Orthopaedic Research and Education Foundation (Rosemont, IL).
This work was performed at Arizona State University.

R. B. Miller, A. C. McLaren, R. McLemore (✉)
Banner Orthopaedic Residency, Banner Good Samaritan
Medical Center, 901 E Willetta Street, Phoenix, AZ 85006, USA
e-mail: ryan.mclemore@bannerhealth.com

A. C. McLaren, C. M. Leon, B. L. Vernon, R. McLemore
Center for Interventional Biomaterials, School of Biological
and Health Systems Engineering, Arizona State University,
Tempe, AZ, USA

however, more than 60% separated when 12 mL was added per batch. One milliliter of liquid gentamicin loads only 40 to 60 mg into the cement and 12 mL loads less than 500 mg, below the 1000-mg load typically used in low-dose ALBC. Clinical levels in drain fluid from ALBC made with liquid gentamicin are reported to be more than 10 times the usual minimum inhibitory concentration (MIC) on the first day, but those data are confounded by the concurrent addition of vancomycin powder [5, 14] and the levels are still 10 times less than the 100× to 1000× MIC needed to kill bacteria in biofilm. Higher antimicrobial delivery from ALBC formulated with aqueous antimicrobials is desirable. The concentration of liquid antimicrobials cannot be increased by the orthopaedic surgeon. Volume is limited by how much can be entrapped in the cement. Forming an emulsion of the liquids may increase the volume that can be loaded and may increase porosity.

Emulsions are formed when two immiscible liquids are vigorously agitated, breaking up one liquid into droplets that are suspended throughout the other liquid. Highly immiscible liquids such as water and MMA typically form unstable emulsions that separate rapidly. Surfactants are substances with affinity for both liquids that stabilize emulsions, slowing separation. A surfactant-stabilized emulsion of aqueous antimicrobial and monomer [7] remains emulsified long enough for polymerization to

occur, leaving the antimicrobial droplets distributed throughout the ALBC. This “emulsified ALBC” can be loaded with antimicrobial liquid to a volume fraction of 10% or higher, consistent with the volume fraction of high-dose ALBC formulated with particulate porogens.

In this study, we investigated the effects that emulsifying the liquid antimicrobial into MMA had on the antimicrobial delivery from ALBC and the mechanical properties and porosity of ALBC. We therefore asked (1) whether the emulsified ALBC would increase antimicrobial delivery, (2) how much loss in mechanical strength would occur, and (3) whether the porosity created in the emulsified ALBC was homogeneous as is typical for emulsions.

Materials and Methods

This experiment measured gentamicin release and compressive strength over time in elution for two ALBC formulations, one made with antimicrobial powder mixed into the polymer powder (control) and one made with a stabilized emulsion of liquid antimicrobial in MMA, to evaluate the effect of the emulsion formulation method (Fig. 1). We made 144 standardized test cylinders from emulsified ALBC (three batches, 72 cylinders) and control

Fig. 1 This flowchart outlines the experimental design for the comparison of emulsified and nonemulsified (control) ALBC.

ALBC (three batches, 72 cylinders). For each formulation, five specimens per batch ($n = 15$) were eluted in infinite sink conditions over 30 days for gentamicin delivery and five specimens per batch were axially compressed to failure after elution of 0, 1, and 30 days ($n = 45$) (total for each formulation: 20 per batch $\times$ three batches = 60). Two noneluted specimens and two specimens eluted for 30 days from each batch (total for each formulation: four per batch $\times$ three batches = 12) were examined under scanning electron microscopy (SEM) for porosity. Differences in gentamicin release and compressive strength over time in elution between the ALBC groups were analyzed using repeated-measures ANOVA.

Emulsified ALBC was made using a stabilized liquid gentamicin in MMA emulsion. Eight milliliters of monomer (Simplex[®] P), 1 g gentamicin sulfate powder (Fujian Fukang Pharmaceutical Co, Fuzhou, China) dissolved in 1 mL deionized water, and 1 g Pluronic[®] F127 surfactant (BASF Corp, Florham Park, NJ) were vortexed at speed 10 on a VWR minivortexer (VWR, Radnor, PA) for 15 seconds. The emulsion was then added to 20 g poly(methyl methacrylate) (PMMA) powder (Simplex[®] P) and mixed by hand stirring in an open bowl without vacuum. The relative amounts of the MMA, gentamicin solution, and surfactant in emulsion were 8:1:1, determined by using the volume and dose of liquid gentamicin that corresponded with low-dose ALBC and empirically adjusting the surfactant to achieve an emulsion that would not separate for more than the 12-minute set time of Simplex[®] P cement.

Control ALBC was made using Simplex[®] P bone cement in the proportions 1 g gentamicin sulfate powder, 20 g PMMA powder, and 10 mL liquid MMA monomer. The antimicrobial powder was mixed homogeneously into the PMMA powder in a mechanical mixing bowl. The liquid monomer was then added for polymerization, stirring by hand in an open bowl without vacuum.

Once in the dough phase, the ALBC was introduced into a Teflon[®] mold forming cylinders 12 mm long by 6 mm in diameter (ASTM F451-08) [1]. After polymerization, the ends of the cylinders were machined in the mold to make the ends flat and normal to the cylinder axis for mechanical testing and accurate length. All cylinders were transilluminated under $\times 5$ magnification; any cylinder with visible defects (surface irregularities, holes, or voids greater than 0.5 mm) was discarded. All mixes were made in half-batches to minimize waste. The protocol was repeated for three batches of each formulation.

Antimicrobial release was measured by elution in deionized water at 37°C without shaking. Five cylinders from three batches ($n = 15$) for each ALBC formulation were individually placed in 5 mL deionized water completely covering the cylinder in a glass scintillation vial. Total eluant exchange was carried out on Days 1, 7, 15, and

30, maintaining infinite sink conditions. Based on the known stability of gentamicin [10], eluate samples were stored at 4°C until assay. Samples were mixed by agitation before assay. The concentration of gentamicin in the eluate from each exchange was determined by disc-diffusion bioassay. Agar plates were inoculated with *Kocuria rhizophila* Strain 9341 (American Type Culture Collection, Manassas, VA) and incubated for 18 hours at 30°C. The zone of inhibition diameters were measured at 48 hours. The gentamicin concentration in each sample was determined using calibration curves from known gentamicin concentrations (6.25, 12.5, 50, 100, 400, and 800 µg/mL) on every plate. The Pluronic[®] F127 surfactant dissolved in deionized water did not produce inhibition zones at 14 wt% Pluronic[®] F127 and 86 wt% water. Each sample of eluate was assayed in triplicate and averaged to determine the sample value. The cumulative released gentamicin (Mt) was calculated by summing the recovered mass from each time period.

The compressive strength was measured under axial load to failure. Five cylinders from three batches for each ALBC formulation were mechanically tested at three time points, one before elution (Day 0) and two after elution (Days 1 and 30) ($n = 45$). Elution for the cylinders undergoing mechanical testing was carried out with groups of five cylinders in 25 mL deionized water at 37°C for 1 or 30 days. Infinite sink conditions were maintained similar to the elution for drug release using 5 mL water for each cylinder, and total eluant exchange was carried out on Days 1, 7, 15, and 30. The test cylinders were towel dried and stored in sealed glass scintillation vials at room temperature until testing. All test cylinders underwent axial load to failure in compression at 24.0 mm per minute (ASTM F451-08) under dry conditions at room temperature using an MTS Syntech 1/S mechanical testing machine (MTS, Eden Prairie, MN). Load displacement data were analyzed using a custom MATLAB[®] (The MathWorks Inc, Natick, MA) algorithm to establish the compressive strength in accordance with ASTM Standard F451-08.

Four cylinders from each batch of ALBC were studied with SEM to visualize their porosity, two before and two after elution for 30 days. Samples were mounted on stainless steel stands using double-sided carbon tape. After mounting, samples were coated with gold through plasma exposure for 3 minutes at 180 V in a vacuum deposition system (Desk V Series; Denton Vacuum, Moorestown, NJ) to reduce surface charging common with polymers under SEM visualization. The topography of the ALBC surface was imaged using an environmental SEM (FEI XL30 EFSEM; FEI/Phillips, Hillsboro, OR).

We used repeated-measures ANOVA to determine whether gentamicin release and compressive strength differed between the two formulations and over time.

Statistics were performed using MINITAB® (Minitab Inc, State College, PA).

Results

No separation of liquid antimicrobial was seen during polymerization of the emulsified ALBC. Gentamicin release was greater ($p < 0.001$) from the emulsified ALBC than from the control ALBC at all time periods (Fig. 2). After 7 days and 30 days of elution, the mean cumulative mass released was 648 and 1985 μg , respectively, for control ALBC and 3330 and 7953 μg , respectively, for emulsified ALBC. Antimicrobial release was four times greater from the emulsified ALBC than from the control ALBC at 30 days of elution.

The compressive strength of emulsified ALBC was lower ($p < 0.001$) than that of control ALBC (Fig. 3) but did not progressively decrease over time in elution ($p = 0.418$). The compressive strength of control ALBC decreased over time ($p < 0.001$). Before elution and after 30 days of elution, the mean compressive strength was 58.1 and 54.5 MPa, respectively, for the emulsified ALBC and 81.3 and 73.9 MPa, respectively, for control ALBC.

Before elution, we observed extensive interconnecting porosity with emulsified ALBC on SEM (Fig. 4). Compared to the control ALBC, in which pores were not observed at $\times 250$ (Fig. 4A), there were many large pores approximately 50 μm in diameter observed in the emulsified ALBC at $\times 250$ (Fig. 4B). At higher magnification, $\times 2500$, there were smaller interconnected pores less than 1 μm in diameter in the emulsified ALBC (Fig. 4C). The surfaces of the emulsified ALBC did not change in

appearance on SEM after 30 days of elution. The control ALBC had very limited areas that had visible pores.

Discussion

Use of liquid antimicrobials to formulate ALBC is limited by the volume that can be loaded into ALBC and loss in mechanical strength. We determined whether ALBC made by emulsifying liquid antimicrobial in the monomer would increase antimicrobial delivery, decrease the loss in mechanical strength, and appear homogeneously porous on SEM by comparing elution, compressive strength and SEM appearance of emulsified ALBC and ALBC formulated with antimicrobial powder.

There are several limitations of our study. First, we studied only one antimicrobial dose (1 g in 1 mL) with a volume fraction of about 3 vol%. This antimicrobial dose is consistent with low-dose commercially available powder ALBC but more than double the load used by other authors [5, 6, 14]. High-dose emulsified ALBC needs to be evaluated. Second, we studied only one volume (1 mL) of liquid antimicrobial. In pilot experiments, stabilized emulsions up to 20 mL antimicrobial liquid did not separate (ACM, RM). Antimicrobial release and mechanical performance of formulations made with higher volumes need to be evaluated. Third, we tested only compressive strength of the ALBC. Creep or fatigue testing would be necessary to more fully characterize the mechanical properties of emulsified ALBC. Emulsified ALBC is too weak for implant fixation (ISO 5833 standard = 70 MPa) but likely strong enough for applications such as beads and spacers. Fourth, we used only Simplex® P cement. The

Fig. 2 A bar graph shows cumulative mass released of gentamicin from control and emulsified ALBC at 1, 7, 15, and 30 days. The data are reported as a mean $\pm$ SD of five cylinders for three batches each. Gray bars represent control ALBC made with antimicrobial powder mixed in the polymer powder; white bars represent emulsified ALBC made with surfactant-stabilized liquid antimicrobial-in-MMA emulsion. Antimicrobial release was greater ($p < 0.001$) from the emulsified ALBC than from the control ALBC at all time periods.

Fig. 3 A bar graph shows compressive strength of control and emulsified ALBC before elution (Day 0) and after elution (Days 1 and 30). The data are reported as a mean $\pm$ SD for three batches of five cylinders each. Gray bars represent control ALBC made with antimicrobial powder mixed in the polymer powder; white bars represent emulsified ALBC made with surfactant-stabilized liquid

antimicrobial-in-MMA emulsion. Compressive strength of emulsified ALBC over all time points was lower ($p < 0.001$) than that of control ALBC but did not progressively decrease over time ($p = 0.418$). Compressive strength of control ALBC over all time points decreased over time ($p < 0.001$). The horizontal line at 70 MPa corresponds to the ISO 5833 standard.

Fig. 4A–C (A) Scanning electron micrographs at $\times 250$ of control ALBC show few pores, while scanning electron micrographs of emulsified ALBC (B) at $\times 250$ show the presence of many 50- to

100- μ m-diameter pores (arrow) and (C) at $\times 2500$ show many 5- to 10- μ m-diameter interconnected pores (arrow).

volume of liquid antimicrobials that acrylic bone cement can entrap depends on viscosity and gelation rate. Considerable separation occurs during the low-viscosity phase. In our experience, more viscous cements (Palacos[®] R; Biomet Inc, Warsaw, IN) entrap more of the liquid. The volume that separates from Simplex[®] P is almost double the volume that separates from Palacos[®] R. No liquid antimicrobial separates during polymerization of either cement when a stable emulsion is used. Fifth, we studied only one surfactant. Pluronic[®] F127 is a US Food and Drug Administration-approved nonionic surfactant/excipient used in inhalation, oral, and ophthalmic preparations with published safety data [3]. Injectable formulations have been investigated in animals [2, 12, 13]. Pluronic[®] F127 has high affinity for both water and MMA. Other

surfactants would likely stabilize the emulsion with different droplet sizes and have different interactions with the MMA, leading to different release and mechanical properties. Other surfactants need to be studied. Sixth, we mixed the ALBC by hand without vacuum, as is common for surgeon-directed mixing of ALBC. This could account for some of the batch-to-batch variation seen in our data. Finally, we did not conduct a power analysis, as this was a pilot study with no prior data to determine effect size.

Other authors report data from ALBC made with liquid antimicrobial [4, 5, 9, 14]; however, only Seldes [14] and Hsieh et al. [6] report release data. Seldes [14] did not describe his methods or analysis in sufficient detail to compare his data with other studies. Release data from Hsieh et al. [6] appear similar to ours. Our emulsified

Table 1. Comparison of our study to other relevant studies in the literature

Study	Elution	Compressive strength	Porosity
Ger et al. [4] (1977)		Cements mixed with liquid antibiotics have worse compressive strength than those with powder	
Lautenschlager et al. [8] (1976)		Cements mixed with liquid antibiotics have worse bulk mechanical properties	
Leone et al. [9] (2007)		Liquid antimicrobials decrease Young's modulus of cement more than powders	
Hsieh et al. [6] (2009)	Low-dose cement with liquid antimicrobial delivers more antimicrobial than powder mix, similar to our results	Liquid antimicrobials decrease compressive strength by $\frac{1}{3}$	Observed porosity was irregular and much larger than we observed
Seldes [14] (2005)	Liquid antimicrobial improves release from ALBC; comparison is difficult because of methodology	Liquid antimicrobials decrease compressive strength by $\frac{1}{2}$	
Miller et al. (2011)	Emulsified cement provides increased elution relative to poragen	Emulsified cement is less strong than hand-mixed low-dose cement, but not $2\times$	Emulsified cement has antimicrobial pores not visible on cement with powdered antimicrobial

ALBC = antimicrobial-loaded bone cement.

formulation released 7.95 mg by 30 days from a gentamicin load of 36 mg per cylinder (22%). The nonemulsified formulation studied by Hsieh et al. [6] released 2.2 mg by 35 days (26% of 8.6 mg). However, it is impossible to know how much antimicrobial was contained in the ALBC in these studies. Both reported liquid gentamicin is miscible with the MMA and there was no change in the gross appearance or consistency of the cement, with no discussion of separation of the liquid gentamicin during polymerization. We are unable to mix 12 mL liquid antimicrobial into PMMA without separation of the liquid clinically or in the laboratory. Solubility of MMA in water is extremely low, 1.5 g in 100 g water [11]. Our experience is consistent with these known physical properties. Without being emulsified, essentially all the liquid antimicrobial remains in a separate layer, and when 12 mL liquid antimicrobial is used, an important portion of the liquid antimicrobial separates during polymerization, more so for Simplex® P than Palacos® R. Even if some of the antimicrobial separated during polymerization, sufficient liquid gentamicin was retained in the ALBC formulated by Hsieh et al. [6] to deliver drug and weaken the cement, and drug delivery was found clinically [5]. We studied only surfactant-stabilized emulsified preparations because of the unknown amount of retained antimicrobial when the liquid antimicrobial is simply hand-mixed in the monomer. Our control was low-dose ALBC made with antimicrobial powder. Comparisons of reported results with relevant literature are shown (Table 1).

All ALBC formulations made with liquid antimicrobial had a large decrease in compressive strength. The hand-mixed formulations decreased in compressive strength more than the emulsified ALBC. The pre-elution compressive strength of emulsified ALBC was decreased by 28%, whereas the preelution compressive strength seen in controls made with powder antimicrobial was not decreased. Hsieh et al. [6] reported a 37% decrease in compressive strength with liquid antimicrobial; however, they used a loading rate that is $\frac{1}{4}$ of the standardized rate (ASTM F451-08), making direct comparison unreliable. Seldes [14] reported a 50% decrease in compressive strength for ALBC formulated with liquid gentamicin; however, he used a loading rate of less than $\frac{1}{20}$ of the standardized rate and the compressive strength of his control cement with no antimicrobials was 64 MPa (less than the ISO 5833 standard of 70 MPa), making comparison with these data unreliable. Neither Hsieh et al. [6] nor Seldes [14] studied compressive strength over time in elution. Unlike control ALBC made with antimicrobial powder, the compressive strength of the emulsified ALBC in our compression testing did not decrease with time in elution. The ability of the emulsified ALBC to maintain its mechanical properties during elution is a novel and interesting finding that requires further investigation to determine its importance.

The porosity of our emulsified ALBC was about 3 vol%, with spherical interconnecting voids ranging from less than

1 μm to greater than 50 μm in diameter, whereas the porosity from nonemulsified ALBC reported by Hsieh et al. [6] was 16.8% by surface area, with irregular-shaped voids ranging from less than 10 μm to more than 500 μm . Seldes [14] did not report the ALBC porosity.

In conclusion, the use of a surfactant-stabilized emulsion for the formulation of ALBC allows liquid antimicrobial to be loaded into ALBC without separation during polymerization. Antimicrobial release is similar to formulations using antimicrobial powder. The compressive strength is lower than recommended for implant fixation, but the loss is less than occurs with simple mixing of liquid antimicrobial into the cement.

Acknowledgments We gratefully acknowledge the use of facilities within the LeRoy Eyring Center for Solid State Science at Arizona State University and the Mechanical Testing Laboratory of the Mechanical and Aerospace Department at Arizona State University.

References

1. American Society for Testing and Materials. *Standard Specifications for Acrylic Bone Cement*. ASTM F451-08. West Conshohocken, PA: ASTM; 2008.
2. Barichello JM, Morishita M, Takayama K, Nagai T. Absorption of insulin from Pluronic F-127 gels following subcutaneous administration in rats. *Int J Pharm*. 1999;184:189–198.
3. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. *Pharm Res*. 2006;23:2709–2728.
4. Ger E, Dall D, Miles T, Forder A. Bone cement and antibiotics. *S Afr Med J*. 1977;51:276–279.
5. Hsieh PH, Huang KC, Tai CL. Liquid gentamicin in bone cement spacers: in vivo antibiotic release and systemic safety in two-stage revision of infected hip arthroplasty. *J Trauma*. 2009;66:804–808.
6. Hsieh PH, Tai CL, Lee PC, Chang YH. Liquid gentamicin and vancomycin in bone cement: a potentially more cost-effective regimen. *J Arthroplasty*. 2009;24:125–130.
7. Kabalnov A, Weers J, Arlauskas R, Tarara T. Phospholipids as emulsion stabilizers. 1. Interfacial tensions. *Langmuir*. 1995;11:2966–2974.
8. Lautenschlager EP, Marshall GW, Marks KE, Schwartz J, Nelson CL. Mechanical strength of acrylic bone cements impregnated with antibiotics. *J Biomed Mater Res*. 1976;10:837–845.
9. Leone J, Johnson A, Ziada S, Hashemi A, Adili A, de Beer J. Biaxial flexural modulus of antibiotic-impregnated orthopedic bone cement. *J Biomed Mater Res Part B Appl Biomater*. 2007;83:97–104.
10. 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;467:1693–1698.
11. Methyl methacrylate monomer, 18036, Material Safety Data Sheet. Available at: http://www.tedpella.com/msds_html/18036msd.htm. Accessed May 20, 2011.
12. Pec EA, Wout ZG, Johnston TP. Biological activity of urease formulated in poloxamer 407 after intraperitoneal injection in the rat. *J Pharm Sci*. 1992;81:626–630.
13. Saim AB, Cao Y, Weng Y, Chang CN, Vacanti MA, Vacanti CA, Eavey RD. Engineering autogenous cartilage in the shape of a helix using an injectable hydrogel scaffold. *Laryngoscope*. 2000;110(10 Pt 1):1694–1697.
14. Seldes RM. Liquid gentamicin in bone cement: a laboratory study of a potentially more cost-effective cement spacer. *J Bone Joint Surg Am*. 2005;87:268–272.