Characterization of Daptomycin-loaded Antibiotic Cement

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abstract

Antibiotics are commonly mixed with polymethylmethacrylate (PMMA) cement to suppress severe periprosthetic infections associated with total joint arthroplasty. The relationship between antibiotic concentration and the resulting elution kinetics remains unclear. The purpose of this study was to characterize the release of daptomycin from PMMA cement and the subsequent effects on mechanical properties.

Varying concentrations of daptomycin and tobramycin were vacuum mixed in commercially available PMMA and subjected to an in vitro elution period. High-performance liquid chromatography was used to quantify the concentration of the amount of daptomycin eluted at predetermined time points. Samples were subjected to compressive loading to analyze the effect of antibiotic concentration on cement mechanical properties. Daptomycin elution increased when initial tobramycin concentration was increased. Furthermore, the addition of antibiotics increased the compressive strength of the cement in the postelution period. The binary addition of tobramycin with daptomycin antibiotics modifies the elution and mechanical properties of PMMA bone cement. Based on the findings of our study, 2 g of daptomycin and 3.6 g of tobramycin per 40-g packet of cement should be used to promote daptomycin elution without sacrificing PMMA mechanical properties.

Figure: Cumulative release of daptomycin for groups containing 1.0 g (A) and 2.0 g (B) of daptomycin mixed with varying amounts of tobramycin throughout 96 hours of elution.

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evere periprosthetic infection remains one of the most devastating complications of total joint arthroplasty, with a nearly three-fold increase in length of stay and hospital costs for patients with infection after total hip arthroplasty. A 2-stage revision procedure is often the treatment choice for deep periprosthetic infections, in which polymethylmethacrylate (PMMA) cement is commonly mixed with ≥1 antibiotics at varying concentrations and ratios, resulting in the release of the antimicrobial drugs in periprosthetic tissue. This has several advantages compared with oral antibiotics, including localized delivery and the ability to use higher concentrations with minimal or negligible risk of systemic toxicity or complications.

Gram-positive cocci, such as Staphylococcus aureus and Staphylococcus epidermidis, are the most common organisms found in deep periprosthetic infections. It has recently been reported that infectious organisms are developing resistance to commonly used antibiotic agents like gentamicin, tobramycin, and vancomycin. These organisms, such as methicillin-resistant S. aureus (MRSA) and vancomycin-resistant Enterococcus, require the use of novel antibiotics and longer periods of treatment for infectious suppression. Daptomycin has become a viable antimicrobial agent with in vitro and in vivo activity against these resistive organisms. Studies have investigated the role of daptomycin as an alternative to more traditional antibiotics, such as vancomycin, tobramycin, and gentamicin, in the appropriate setting of orthopedic infections.

In addition to the use of novel antimicrobial agents, the use of articulating spacers has increased when treating periprosthetic joint infections. With the use of articulating spacers, patients maintain increased mobility and prolonged resection times, allowing for increased periods of antibiotic elution. An articulating spacer also provides an easier transition to a permanent prosthesis at revision reimplantation. Meek et al reported reasonable function and satisfaction scores after 2-stage revision procedures attributed to the articulating spacer that permitted increased motion during the early postoperative period.

Although increased concentrations of antibiotics have been shown to increase total antibiotic elution, cement with high doses of antibiotics has been shown to have inferior mechanical properties. Characterization of the effect of varying antibiotic concentrations on the mechanical properties of PMMA cement remains important as the use of articulating spacers for prolonged resection periods becomes more popular. As the use of daptomycin becomes available in more locations as a viable treatment option for deep periprosthetic infection, it is important to appropriately select the correct concentration of antibiotics, promoting increased elution without sacrificing the mechanical integrity of PMMA cement.

Currently, no published data exist that fully characterize the effect of varying antibiotic concentrations on daptomycin release from PMMA cement. It is also unknown how the elution of antibiotics influences the mechanical properties of PMMA cement. The purpose of our study was therefore two-fold: (1) to characterize the in vitro elution of daptomycin from PMMA bone cement and (2) to characterize the PMMA mechanical properties of eluted and noneluted cement samples as a function of initial antibiotic concentration.

**Materials and Methods**

**Antibiotic-loaded Cement Preparation**

Predetermined amounts of lyophilized daptomycin (molecular weight = 1620.67 g/mol) (Cubist Pharmaceuticals Inc, Lexington, Massachusetts) and lyophilized tobramycin (molecular weight = 1425.45 g/mol) (Abraxis Pharmaceuticals, East Schaumberg, Illinois) were mixed with 40-g packets of Simplex P SpeedSet bone cement (Stryker Orthopaedics, Mahwah, New Jersey) in a commercially available vacuum mixing system (High Vacuum Bio-Prep Total Joint Kit; Stryker Orthopaedics). Antibiotics were combined in a binary fashion with PMMA cement. For the first series, 1.0 g of daptomycin was combined with 0.0, 1.2, 2.4, and 3.6 g of tobramycin, and 2.0 g of daptomycin was combined with 0.0, 1.2, 2.4, and 3.6 g of tobramycin. A control group contained 0 g of either antibiotic.

Before the curing process was completed, antibiotic-loaded PMMA was dispensed into plastic molds and allowed to polymerize in ambient conditions for at least 60 minutes. This resulted in the formation of cylindrical samples with a diameter and length of 15 mm and 20 mm, respectively. Any excess cement was removed by a scalpel, and the samples were stored at 2°C to 4°C in a humidity-controlled environment until further analysis.

**Elution Study**

Antibiotic elution studies were performed for each treatment group. Six cylindrical specimens from each antibiotic group were immersed in individual borosilicate beakers containing 50 mL of 0.1 M phosphate buffered saline. Beakers were sealed with parafilm to prevent evaporation and were mechanically rocked for a total of 96 hours. At predetermined time points (6, 12, 24, 48, 72, and 96 hours), 3 mL of the phosphate buffered saline was removed for quantification of daptomycin antibiotic released into the solution. An equal volume of phosphate buffered saline was replaced after each sample was taken. All elution periods were performed at a temperature of 37°C and a pH of 7.4.

Daptomycin concentrations during the elution period were quantified by high-performance liquid chromatography based on a method previously established. A Prominence high-performance liquid chromatography system (Shimadzu, Columbia, Maryland) was used for analysis. Briefly, the mobile phase consisted of 38% pure acetonitrile with 62% aqueous...
solution containing 0.45% (w:v) ammonium dihydrogen phosphate (NH₄H₂PO₄). A C8 column (3.9×150 mm, 5 µm pore size) at a flow rate of 0.5 mL per minute at 35°C was used. The ultraviolet/visible spectroscopic detector was set at a 254 nm wavelength, which was the optimum response. For each eluted sample, a 10-µL aliquot was analyzed for daptomycin concentration.

Mechanical properties were characterized by the use of a compression testing protocol previously developed. A total of 10 PMMA cylinder samples from each antibiotic test group were subjected to compressive testing. For each antibiotic test group, 5 specimens were tested that had previously undergone elution testing, and 5 specimens were tested in the non-eluted state. Specimens were loaded in compression axially at a rate of 1.0 mm per minute on an 858 Mini-Bionix II materials load frame (MTS Systems Corp, Eden Prairie, Minnesota). Data were continuously collected throughout each compression test via product software. Stress vs strain curves were constructed from this data for each specimen. The established curves were used to determine the ultimate compressive strength, 0.2% offset yield strength, and compressive modulus for each antibiotic test group.

**Statistical Analysis**

Antibiotic concentrations were evaluated by a 1-way analysis of variance (ANOVA) test with a post-hoc Bonferroni correction. Significance was set at P<.05. Mean antibiotic concentration at each time interval was compared between test groups. This process was repeated for groups with 1 and 2 g of daptomycin, respectively. For mechanical strength, a 1-way ANOVA was used for noneluted and eluted groups comparing mechanical properties against control samples. Statistics were performed with Sigmaplot version 11.0 software (Systat Software, Inc, Richmond, California).

**RESULTS**

**Elution of Daptomycin**

The release of daptomycin peaked within the first 12 hours of immersion in the phosphate buffered saline solution. Between 12 and 24 hours, the release of daptomycin decreased. After 24 hours, daptomycin release is described as either steadily or slowly decreasing. This release profile remained similar between samples, regardless of the initial concentrations.

In addition, daptomycin release was similar between all groups within the first 6 hours of elution. After this period, the total amount of daptomycin eluted varied with tobramycin content. The total amount of daptomycin eluted was greatest when 2.4 or 3.6 g of tobramycin was added for all test groups (Figure 1).

The release of daptomycin from PMMA was modulated by the addition of tobramycin during cement preparation. Groups containing 1.0 g of daptomycin showed higher release when 2.4 or 3.6 g of tobramycin were added compared with groups with 0 g (P=.118 and P=.009, respectively, at 96 hours) or 1.2 g (P=.602 and P=.016, respectively, at 96 hours) of tobramycin. Similarly, groups with 2.0 g of daptomycin showed higher cumulative release when 2.4 or 3.6 g of tobramycin were added compared with groups with 0 g (P=.006 and P=.330, respectively, at 96 hours) or 1.2 g (P<.001 and P=.016, respectively, at 96 hours) of tobramycin were added. Although not statistically significant at all time points, these general release profiles were similar throughout the elution period.

For samples containing 1 g of daptomycin, antibiotic release increased 198%, from 2.7 to 8.1 mg at 96 hours, between groups containing 0 g of tobramycin and 3.6 g of tobramycin, respectively. Similarly, samples containing 2 g of daptomycin increased 126%, from 10.4 to 23.4 mg, comparing 0 and 2.4 g of tobramycin, respectively.
Mechanical Properties

The stress-strain curves developed from the collected data were used to calculate values for ultimate compressive strength, compressive yield strength, and compressive modulus for each test group. These properties were identified for control samples (prepared PMMA samples with no antibiotics) and then compared with the results from the antibiotic test groups. Two samples were mechanically tested for the eluted group with 2.0 g of daptomycin. Control samples had an ultimate compressive strength for noneluted and eluted samples of 91.6 ± 1.8 and 92.9 ± 2.9 MPa, respectively. Figure 2 shows ultimate compressive strength between the control group and test groups for noneluted and eluted samples.

All groups displayed an increase in ultimate compressive strength after samples were subjected to periods of elution, except for the group with 2.0 g of daptomycin. This increase of mean ultimate compressive strength is 9.8% ± 5.5% across all other test groups. Generally, samples with 1 g of daptomycin were insensitive to changes in ultimate compressive strength with the addition of any concentration of tobramycin. Samples with 2 g of daptomycin exhibited declining ultimate compressive strength with the addition of tobramycin, except with samples initially containing 3.6 g of tobramycin. Compared with control samples, noneluted samples exhibited a statistically significant decrease in ultimate compressive strength in 5 groups. Groups with no significant difference had antibiotic combinations of 1.0 g of daptomycin with 2.4 and 3.6 g of tobramycin and 2.0 g of daptomycin with 3.6 g of tobramycin. Conversely, 1 eluted group displayed a statistically significant difference in ultimate compressive strength and contained 2.0 g of daptomycin (P = .004).

The same relationship between noneluted and eluted samples with respect to ultimate compressive strength was observed for yield strength. Cement yield strength was increased for samples that underwent elution, except for the group with 2.0 g of daptomycin. The increase in mean yield strength was 12.8% ± 4.6% for all other test groups. The group with 2.0 g of daptomycin and 2.4 g of tobramycin was the only group to display a statistically significant decrease in yield strength compared with control samples (P < .001).

Samples with the addition of antibiotics showed an increase in compressive modulus after elution compared with samples that did not undergo elution testing. The compressive modulus increased 11.9% ± 6.7% between noneluted and eluted samples for all test groups. No statistically significant difference was noted compared with control values. Mechanical properties for all test groups are displayed in the Table.

**Figure 2:** Ultimate compressive strength of all test groups for noneluted and eluted samples as a function of initial antibiotic concentrations. Abbreviations: Abx, antibiotics; dapto, daptomycin; tobra, tobramycin; UCS, ultimate compressive strength.

**Discussion**

Antibiotic release from PMMA cement is described as an initial burst followed by a sustained low release over an extended period of time. This profile is maintained in relation to elution exhibited in our study. The initial burst was experienced between 6 and 12 hours. This mechanism of elution is described as an initial release due to surface contact with fluid followed by penetration of liquid into voids and cracks in the cement, causing sustained antibiotic release. The addition of multiple antibiotics may increase the porosity and prevalence of cracks throughout the cement, leading to a synergistic effect in antibiotic elution. Our study demonstrated that daptomycin release can be increased with the initial addition of tobramycin. A clear division in release can be distinguished between samples with 0 and 1.2 g of tobramycin and 2.4 and 3.6 g of tobramycin in groups containing 1 and 2 g of daptomycin. The use of commercially available vacuum mixing systems has been shown...
### Mechanical Properties of Test Groups for Noneluted and Eluted Samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Compressive modulus</th>
<th>Compressive strength</th>
<th>Yield strength</th>
<th>Yield modulus</th>
<th>Elute modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.9 ± 3.3</td>
<td>102.1 ± 28.2</td>
<td>148.4 ± 33.6</td>
<td>0.5 ± 0.13</td>
<td>10.0 ± 2.5</td>
</tr>
<tr>
<td>0 g Tobramycin</td>
<td>19.5 ± 4.2</td>
<td>106.7 ± 34.2</td>
<td>155.4 ± 40.4</td>
<td>0.6 ± 0.12</td>
<td>11.0 ± 2.6</td>
</tr>
<tr>
<td>2.4 g Tobramycin</td>
<td>20.1 ± 4.5</td>
<td>110.2 ± 37.8</td>
<td>162.0 ± 45.1</td>
<td>0.7 ± 0.14</td>
<td>12.0 ± 2.9</td>
</tr>
<tr>
<td>4.8 g Tobramycin</td>
<td>20.6 ± 4.8</td>
<td>113.8 ± 39.4</td>
<td>168.6 ± 49.2</td>
<td>0.8 ± 0.16</td>
<td>13.0 ± 3.1</td>
</tr>
<tr>
<td>0 g Daptomycin</td>
<td>19.2 ± 4.1</td>
<td>100.5 ± 32.1</td>
<td>147.2 ± 38.8</td>
<td>0.5 ± 0.13</td>
<td>10.5 ± 2.4</td>
</tr>
<tr>
<td>2.4 g Daptomycin</td>
<td>19.8 ± 4.3</td>
<td>104.1 ± 35.7</td>
<td>153.8 ± 41.9</td>
<td>0.6 ± 0.14</td>
<td>11.5 ± 2.8</td>
</tr>
<tr>
<td>4.8 g Daptomycin</td>
<td>20.4 ± 4.6</td>
<td>108.9 ± 37.3</td>
<td>160.4 ± 44.5</td>
<td>0.7 ± 0.16</td>
<td>12.5 ± 3.1</td>
</tr>
<tr>
<td>0 g Tobramycin + 4.8 g Daptomycin</td>
<td>20.9 ± 4.9</td>
<td>112.5 ± 39.9</td>
<td>167.0 ± 48.7</td>
<td>0.8 ± 0.17</td>
<td>13.5 ± 3.4</td>
</tr>
</tbody>
</table>

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**Table 1:** Compressive and yield strength values for test groups. Values are mean ± standard deviation.
The full extent of this process remains largely unknown, but our results suggest that some type of interaction between antibiotics and cement may play a role in the posthardening process of the cement. The molecular relationship and any subsequent interactions should be investigated further to fully understand the elution process and resulting mechanical strength.

A limitation of our study was that vacuum mixing cement may have limited overall elution of antibiotics. However, our data showed increasing amounts of daptomycin elution by the addition of tobramycin. Further work is underway to investigate the relationship between daptomycin release between vacuum-mixed and hand-mixed cement.

The bactericidal properties of eluted daptomycin were not characterized in our study. However, studies have previously established the antimicrobial potential of eluted daptomycin. Specifically, Kuechle et al. reported sufficient daptomycin release from PMMA disks in vitro while sustaining antimicrobial properties, and Hall et al. reported the in vitro release of daptomycin from PMMA beads with little to no negative effects on antimicrobial properties. Webb et al. reported the antimicrobial potential of eluted daptomycin, suggesting retained antimicrobial efficacy to 28 days.

Compared with vancomycin elution from PMMA cement, daptomycin elution was reported at a similar rate to vancomycin elution from PMMA cement. Daptomycin has the benefits of patient tolerance, low potential for adverse events, and low risk of spontaneous resistance while showing activity in treatment of gram-positive bone and joint infections, including cases of MRSA and vancomycin-resistant Enterococcus. Systemic complications of daptomycin are minimal in this application due to the localized delivery. Although several benefits promote the use of daptomycin in situations of antibiotic resistance, concern is warranted with increased cost and limited availability. Surgeons should be aware of all factors before developing the patient-specific clinical strategy.

Although no fatigue testing was performed in our study, ultimate compressive strength is an important mechanical property for characterization of cement–antibiotic mixtures and understanding the full impact of antibiotic concentration. Further investigation is underway to determine the effects of cyclic compression on antibiotic release.

CONCLUSION

Daptomycin elution from PMMA bone cement can be modified by the addition of varying concentrations of tobramycin. Increasing concentrations of antibiotics added during cement preparation may also result in a change of mechanical properties. Concern exists about increasing amounts of antibiotics negatively affecting mechanical properties of cement, although all samples tested in our study remained above the ASTM recommended minimum. The elution period of antibiotics also influences the compressive strength and requires consideration when selecting antibiotic concentrations. Based on our findings, 2 g of daptomycin and 3.6 g of tobramycin per 40-g packet of cement should be used to promote daptomycin elution without sacrificing PMMA mechanical properties. Surgeons should ultimately make antibiotic selections on a patient-specific basis, keeping in mind the relationship between type of PMMA cement, cement preparation, antibiotic release, and mechanical strength.

REFERENCES


