Effect of Methotrexate on the Mechanical Properties and Microstructure of Calcium Phosphate Cement

GUANGJUN LIAO, MD; DONGXIU SUN, BM; JIAN HAN, MM; JIANGWEI TAN, PHD

Abstract

Calcium phosphate cement (CPC) is widely used as an antitumor bone-filling material. Methotrexate (MTX) is recognized as an effective chemotherapy medicine. The current study examined the effects of MTX on the mechanical properties and microstructure of CPC. Methotrexate-loaded CPC at mass ratios of 0%, 0.1%, 0.2%, and 0.5% were designated as groups A, B, C, and D, respectively, and were pressed into precast cylindrical molds. Solidification time, axial compressive strength, transverse compressive strength, and rotational tensile strength were measured, and scanning electron microscopy images were captured before and after MTX-CPC microstructure changes occurred. Average initial and final setting times increased gradually with increasing drug concentration, but this increase was not significant among the groups. Average axial transverse compressive strength and rotational tensile strength of groups B and C were not significantly different from those of group A (P > 0.05); however, there was a significant difference in these properties between groups A and D (P < 0.05). Scanning electron microscopy observations showed a porous crystalline structure. The addition of MTX to CPC does not significantly affect the basic crystal structure and setting time of CPC. Adding MTX at mass ratios of 0.1% and 0.2% to CPC does not lead to a significant difference in mechanical strength and can therefore be applied in clinical practice. This study may shed some light on the future application of MTX-loaded CPC in the treatment of bone defects after tumor excision.

The authors are from the Department of Orthopedic Surgery (GL, JH, JT) and the Department of Operating Room (DS), Yantaishan Hospital, Yantai, China.

Dr Liao and Ms Sun contributed equally to this work and should be considered as equal first authors. The authors have no relevant financial relationships to disclose.

Correspondence should be addressed to: Jian Han, MM, Department of Orthopedic Surgery, Yantaishan Hospital, No. 91 Jiefang Rd, Yantai 264000, China (greatham07@sohu.com).

Received: January 5, 2014; Accepted: February 20, 2014; Posted: October 8, 2014. doi: 10.3928/01477447-20140924-58
The reconstruction of bone defects is an important topic in orthopedics. In recent decades, many scholars have sought to find suitable bone-filling materials that overcome the shortcomings of poor material plasticity, material incapacity for temporary shaping or curing, and insufficient mechanical strength in the early phases of repair. Furthermore, because some artificial materials cannot be degraded and others degrade into reactive metabolites that cause inflammation, it is urgent that satisfactory filling materials be found.

Calcium phosphate cement (CPC) has been shown to be a good material choice for filling bone defects. It is composed of both solid and liquid phases and has outstanding characteristics, such as temporary shaping and curing, a high degree of biocompatibility, and degradation and osteogenic activity. Unsolidified CPC is arbitrarily shaped according to its container; its shaping process does not produce heat, and it is non-toxic. Clinical applications of CPC, such as repairing bone defects arising from the treatment of bone tumors, have achieved good results. Furthermore, CPC can be used as a pharmaceutical carrier that slowly releases drugs into tissues to maintain a high local concentration. Drugs used with CPC can be roughly divided into 3 categories: (1) antineoplastic agents (eg, 6-mercaptopurine and cisplatin); (2) antibiotics (eg, gentamicin sulfate, indomethacin, cephradine, and aspirin); and (3) biologically active substances (eg, bone morphogenetic protein, insulin, and growth hormone). Because of its ability to serve as a carrier, chemotherapy medicine–loaded CPC can be used as a bone-filling material to release drugs to local tissues and maintain a high drug concentration to kill any remaining tumor cells after bone tumor excision.

Methotrexate (MTX) is recognized as an effective chemotherapy medicine and has been used to treat cancer, autoimmune disease, and ectopic pregnancy. To date, few studies have been conducted on the addition of MTX to CPC. Yang et al observed the release kinetics of MTX-loaded CPC implanted in vivo and histologically investigated its resorption and osteogenesis and found that MTX-CPC has good biodegradability and osteoconductivity. Li et al investigated the resorption of in vivo MTX-loaded CPC implants and their effects on osteogenesis and found that MTX-loaded CPC is an excellent material for filling bone defects and can be used to prepare effective drug-delivery systems to achieve local control of invasive bone tumors. Yang et al investigated the setting times, mechanical properties, microstructures, and in vitro MTX release kinetics of MTX-loaded CPC specimens and concluded that MTX-loaded CPC may be a potentially effective therapy for bone tumors in humans. Considering its potential for use in a wide range of applications, MTX-loaded CPC is worthy of thorough investigation.

For a more thorough analysis of MTX-loaded CPC, different concentrations of MTX were loaded into CPC in the current study. The setting time, biomechanical properties, and microstructure of each sample were examined to elucidate the drug-loading capabilities of CPC with the aim of developing an antitumor bone-filling material.

**Materials and Methods**

Methotrexate was provided by Zhejiang Cyclones Pharmaceutical Co, Ltd, Hangzhou, China; CPC was purchased from Shanghai Respond Biological Material Co, Ltd, Shanghai, China; and a CSS-2202 electronic universal testing machine was provided by Changchun Experimental Institutes, Changchun, China.

**Sample Preparation**

According to the literature, the concentrations of antibiotics and other chemotherapeutics added to CPC are normally between 0% and 2%, most of which are between 0% and 1%. Therefore, the concentrations of MTX added to CPC in this study were randomly selected between 0% and 1%. The MTX and solid-phase CPC powder were mixed at room temperature (18°C) in mass ratios of 1:1000 (0.1%), 2:1000 (0.2%), and 5:1000 (0.5%) to form pastes. The pastes were pressed into prefabricated cylindrical molds 1.2 cm in height and 0.8 cm in diameter. A control sample with no MTX was similarly prepared. The obtained CPC samples with 0%, 0.1%, 0.2%, and 0.5% MTX constituted groups A (control), B, C, and D, respectively.

**Determination of Setting Time**

The setting times of the CPC samples were determined using the Gillmore needle method. The large cross-sectional area light needle (diameter, 2.13 mm; weight, 113.4 g) was used to determine the initial setting time (It), and the smaller cross-sectional area heavy needle (diameter, 1.06 mm; weight, 453.6 g) was used to determine the final setting time (Ft). The needles determine the setting time by applying pressure to the surface of the samples using their weight alone.

**Biomechanical Testing**

The CSS-2202 electronic universal testing machine was used to determine the axial compressive strength, transverse compressive strength, and rotation tensile strength of the samples. Axial compressive strength and transverse compressive strength represent 2 aspects of endurance of direct strength, whereas tensile strength represents endurance of rotary strength. Each strength test used 5 samples, and 15 samples from each group were tested. The result of each strength test was calculated according to the formula C=(4P×9.8)/(πxD²), where P is the maximum load that the test sample can withstand (N), D is the test sample diameter (mm), and C is the desired strength (MPa).

**Scanning Electron Microscopy**

**Observation of Microstructure**

After cleaning, a sample from each group was fixed, dehydrated, and car-
bon treated for good conductivity. High-resolution measurements of the microstructure and porosity were taken using a scanning electron microscope with a mercury porosimeter. The porosity was calculated based on the following formula: porosity=(1-apparent density/density of the material)×100%.

**Statistical Analysis**

The It, Ft, axial compressive strength, transverse compressive strength, and rotational tensile strength data obtained from these tests were processed using SPSS version 13.0 statistical software (SPSS, Inc, Chicago, Illinois) and analyzed using Pearson’s chi-square test, with a P value less than .05 indicating a significant difference.

**RESULTS**

**Setting Time**

The setting time of the 4 groups was determined using Gillmore’s method, and the results are summarized in Table 1. Analysis of variance showed that the setting times of groups B, C, and D were longer than that of group A (the control), but none of the differences was statistically significant (P>.05).

**Biomechanical Test Results**

The axial compressive strength, transverse compressive strength, and rotational tensile strength of the 4 groups were determined with the CSS-2202 electronic universal testing machine, and the results are summarized in Table 2. The ultimate axial compressive strength, transverse compressive strength, and tensile strength decreased with increasing MTX concentration. Compared with group A, groups B and C did not show significant differences in any of the strength tests (P>.05), whereas significant differences were observed for group D (P<.05). Furthermore, as shown in Table 2, group D had a much more sudden decrease in tensile strength than in the other 2 strengths compared with group A. These findings indicate that with the increase in the concentration of MTX, the ultimate strength decreases and the time of endurance decreases. After rupture of the mixture, the strength of group A decreases suddenly, whereas the strength of group D decreases gradually.

**Microscopy and Porosity**

The microstructure and porosity of each group were observed using scanning elec-

---

**Table 1**

<table>
<thead>
<tr>
<th>Setting Time</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>22.1±1.03</td>
<td>22.5±1.07</td>
<td>23.5±1.04</td>
<td>24.7±0.89</td>
</tr>
<tr>
<td>Final</td>
<td>40.5±1.04</td>
<td>40.8±1.07</td>
<td>41.4±0.98</td>
<td>42.3±1.05</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

**Table 2**

<table>
<thead>
<tr>
<th>Strength</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial compressive</td>
<td>39.99±2.52</td>
<td>34.53±2.15</td>
<td>33.94±1.89</td>
<td>32.38±2.34</td>
</tr>
<tr>
<td>Transverse compressive</td>
<td>35.43±2.45</td>
<td>33.23±2.05</td>
<td>32.45±1.67</td>
<td>30.81±2.56</td>
</tr>
<tr>
<td>Rotation tensile</td>
<td>12.32±1.53</td>
<td>10.73±1.25</td>
<td>9.65±1.18</td>
<td>6.63±1.08</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

---

**Figure:** Scanning electron microscope photographs of the microstructure and porosity of the samples from group A (0% methotrexate) (A), group B (0.1% methotrexate) (B), group C (0.2 % methotrexate) (C), and group D (0.5% methotrexate) (D). With the increase in the concentration of methotrexate, the porosity of the samples decreased gradually, although the crystal structure remained unchanged.
electron microscopy, and the results are shown in the Figure. The surface of group A was composed of rough coral-like crystals; the majority of the crystals were fused into a plate-like structure; and many irregular pore diameters were present, with a total porosity of 34.8%. The crystal structure of groups B, C, and D did not change. Methotrexate particles were visibly adhered to the surface of the CPC. The surfaces of these groups had pine needle–like formations. The porosities of groups B, C, and D were 33.4%, 32.5%, and 32.1%, respectively.

**DISCUSSION**

In this study, MTX-loaded CPCs were thoroughly analyzed by examining the setting time, biomechanical properties, and microstructure of each sample after adding different concentrations of MTX to CPC.

The shaping and application of CPC must be performed before the initial setting time because after the CPC’s initial setting time, these procedures cannot be performed without additional factors. While curing, the structure, size, and shape of the body of the CPC restoration must be effectively supported until the final setting time. Studies have shown that antibiotic loads within a certain range of concentrations do not affect the inherent setting time of CPC. In this study, the setting times for groups A, B, C, and D were determined using Gillmore’s method. The results showed that although the It and Ft of groups B, C, and D increased as the concentration of MTX increased, no significant differences were observed when compared with those of group A (P>.05). Otuka et al conduct ed radiographic diffraction analyses of water-soluble drug–loaded CPC and found that the drug did not affect the curing process. The current study’s results are consistent with those in the literature and indicate that the addition of MTX has no significant effect on the solidification time of CPC.

Ginebra et al investigated clinical applications of the standard compression strength of CPC: The clinical criterion for axial and transverse compressive strengths is 30 MPa or more. In the current study, the maximum compressive strengths of groups A, B, C, and D met the clinical criteria, and the rotational tensile strength was greater than that of cancellous bone (approximately 2.1 MPa). The strength of group D decreased significantly compared with group A but still met the above criteria. The compressive strength of CPC is between that of cortical bone and cancellous bone in vitro. Yaszemski also reported a human long bone longitudinal compressive strength between 131 and 224 MPa and a transverse compressive strength between 106 and 133 MPa. Yaszemski also reported that long bones are only exposed to lower compressive strengths in vivo; it is not possible to achieve their longitudinal or transverse compressive strengths. Therefore, CPC is not suitable for repairing long bone defects. However, the compressive strength of CPC after curing is greater than that of cancellous bone (approximately 30 MPa); therefore, it is sufficient to support nonload-bearing bones. Calcium phosphate cement could thus be used to repair and reconstruct defects in nonload-bearing bone. In the current study, as the drug load increased, the compressive strength of the CPC decreased. The strengths of the 0.5% MTX group and the control group were significantly different, with the former reaching the lower limit of the standards proposed by Ginebra et al. Thus, practical applications using loaded CPC should limit the MTX content.

In the current study, the microstructure and porosity of the samples were observed using scanning electron microscopy. The microstructural observations can provide information regarding the distribution of the drug within the CPC, and the calculation of porosity can provide a foundation for further studies of drug release. In this study, high-resolution scanning electron microscopy showed the formation of rough coral-like crystals, fusion of the crystals into a plate-like structure, and many irregular pore diameters in group A. The crystal structures of groups B, C, and D remained unchanged. However, as the drug concentration increased, the pore diameter became gradually smaller and more uniform; MTX particles were visibly adhered to the CPC surface and pores.

This study has some limitations. First, it was not an in vivo study and may not completely reflect the microenvironment in vivo. Therefore, animal experiments are needed to investigate whether the results obtained in this study will be different in vivo. Second, this study did not continuously determine the bionomics of the samples from 0% to 0.5%. Therefore, the maximal load of MTX in CPC that satisfies the standards of clinical application was not determined. Larger numbers of samples and samples containing a range of different concentrations are needed for future studies. Third, this study did not investigate the drug release of the samples. To better predict the value of MTX-loaded CPC in clinical practice, studies on drug release should be performed.

**CONCLUSION**

Loading CPC with MTX does not significantly affect the basic crystal structure and setting time of CPC. The addition of MTX at mass ratios of 0.1% and 0.2% does not cause significant differences in the mechanical strength of CPC and may therefore be applicable in clinical practice. This study may shed some light on the future application of MTX-loaded CPC in the treatment of bone defects after tumor excision.

**REFERENCES**


