Long-term evaluation of a calcium phosphate bone cement with carboxymethyl cellulose in a vertebral defect model

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Abstract: We investigated histological and compressive properties of a calcium phosphate bone cement (BoneSource® (CPC); Stryker Orthopaedics, Mahwah, New Jersey) plus carboxymethyl cellulose (CMC) using a sheep vertebral bone void model. Bone voids were surgically created in L3 and L5 in each of 40 sheep, and the voids were filled with the cement. Histological and radiographic evaluations were performed on one vertebral body from each animal at either: 0, 3, 6, 12, 24, or 36 months after surgery; mechanical testing was performed on operated and non-operated vertebral bodies from 35 sheep. Undecalcified sections were digitized, and the area of the original defect, new bone formation, empty space, fibrous tissue, and residual cement were quantified with histomorphometry. Decalcified sections were evaluated qualitatively. The cement was biocompatible, extremely osteoconductive and underwent steady resorption and replacement by bone and bone marrow. Histomorphometry showed variations in the rate of cement remodeling among animals in each time group, but on average, at 36 months the original defect area was occupied by ~14% bone, 82% cement, and 4% bone marrow. Even in animals that had greater resorption of cement, there was good bone ingrowth with no fibrous tissue. Compressive testing did not reveal a significant difference in the mechanical properties between vertebral bodies augmented with cement and non-augmented controls, irrespective of the postoperative time. BoneSource mixed with CMC had adequate osteoconductivity, biocompatibility, and adequate compressive strength. There was variability among animals, but histology suggests that considerable cement was still present in most samples after 36 months. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 88A: 880–888, 2009

Key words: calcium phosphate cement; vertebral augmentation; mechanical properties; spinal surgery; histology

INTRODUCTION

Vertebral augmentation procedures such as vertebroplasty or kyphoplasty are used to treat selected types of vertebral compression fractures including those caused by osteoporosis, osteolytic metastasis, and myeloma.1–5 Vertebral augmentation is generally performed by placing cannulae into the vertebral body under fluoroscopic guidance and then injecting a cement, usually polymethylmethacrylate (PMMA), to stabilize the fracture. PMMA is easier to mix and deliver, and has a more convenient setting time than some preparations of calcium phosphate cements (CPCs), and the use of PMMA has been associated with excellent clinical results.1,4,5 However, PMMA is not resorbable and hardens exothermally.6,7 Although complications are unusual, PMMA can be associated with spinal cord or nerve compression.8–10 It would also be desirable for an injectable cement to be osteoconductive and to slowly remodel into normal bone. Thus, cements which have the advantage of biocompatibility, biodegradation, bioactivity, or osteoconductivity containing little or no monomer and limited or no exotherm have been proposed.11–16 One such CPC is BoneSource® (Stryker Orthopaedics, Mahwah, New Jersey). BoneSource17–19 gradually cures with essentially no heat emission into a calcium...
phosphate with low crystalline order similar to bone mineral. An injectable material similar to BoneSource was recently used in 30 clinical cases of vertebroplasty, and resulted in pain relief with no side effects.20 However, only a few studies21,22 have reported both histologic features and compressive strength of a CPC used in a vertebral augmentation model in vivo. In these studies, CPCs were biocompatible and osteoconductive, and mechanical properties were considered adequate for vertebral augmentation.

Carboxymethyl cellulose (CMC) is a water-soluble, nontoxic polymer that has been widely used as a pharmaceutical additive. Studies in rats and rabbits have shown that CMC is biocompatible and has no adverse effects on bone formation.23,24 One of the properties of CMC is its ability to form viscous solutions, thereby potentially improved handling properties of other materials.18 CMC was added to the BoneSource cement in this study to form a gel, which provided lubrication to facilitate injection for vertebral augmentation. The purpose of this study was to evaluate the long term histological and mechanical properties of BoneSource plus CMC in a sheep vertebral defect model.

MATERIALS AND METHODS

Test article

BoneSource is a self-setting CPC consisting of an equimolar mixture of tetracalcium phosphate and anhydrous dicalcium phosphate. When mixed with an aqueous solution, the two calcium phosphates dissolve and a hydroxyapatite phase is precipitated. In this study the aqueous solution used for mixing was 0.25M sodium phosphate. The commercially produced CPC had a diametral tensile strength of 12.1 MPA and a set time of 8.5 min. The CMC used in the study was a sterile implant grade.

Overview of study design

Forty skeletally mature (60–80 kg) sheep (Rambouillet X Columbian ewes), were used in this IACUC-approved study. Bone voids were surgically created at L3 and L5 in each spine as described below, and the voids were filled with BoneSource plus CMC. The animals were randomly assigned to one of six time groups and sacrificed at either 0, 3, 6, 12, 24, or 36 months (n = 6 for the 0, 3, 6, and 12 month groups; n = 8 for the 24 and 36 month groups) postoperatively in accordance with their group assignment. Most of the histology and mechanical testing specimens were retrieved from the L3 and L5 levels. The remaining levels were used as untreated controls.

Surgery

Wool was removed from the left lumbar area, and the sheep were positioned in a right lateral recumbent position on the operating table under general anesthesia. The dorsal and dorsolateral lumbar area was prepared for aseptic surgery with multiple scrubs of povidone-iodine alternated with isopropyl alcohol. The area was draped, and a ventrolateral retroperitoneal approach through the oblique abdominal muscles to the plane ventral to the transverse processes was made. The intersegmental vessels at L4–L5 were ligated or secured. The ventral spinal muscles were cleared from the lateral vertebral body. An 8-mm drill hole was made in the lateral cortex of L3 and L5. The drill bit had a stop guard, which permitted the drill to create no more than a 1 cm-deep hole. This created a defect of known volume. Five grams of BoneSource were dry mixed with 100 mg of CMC and subsequently mixed with 1.5 ml of 0.25M sodium phosphate solution. The paste was loaded in a 5-cc syringe and injected into each of the vertebral bodies pre-assigned to receive treatment. Routine closure of external abdominal muscular fascia, subcutaneous tissue and skin was performed.

One animal died 5 days post-operatively. The cause of death was determined to be unrelated to the implantation material. That sample was subsequently replaced by another animal.

CT analysis

Computed tomography scans of the vertebral bodies were performed in vivo on all animals ~1 month after surgery to verify the location of the cement.

Histologic analysis

Forty vertebral bodies underwent histological evaluation at 0, 3, 6, 12, 24, or 36 months. Each vertebral body was radiographed, mainly to determine the approximate location of the injected cement. A band saw was used to cut transverse sections, parallel to the end plate, through each vertebral body.

One representative segment from each specimen was fixed in 70% ethanol, dehydrated slowly without decalcification, and embedded in Spurr’s plastic (Polysciences, Warrington, PA). After the plastic had polymerized, it was further sectioned with a diamond saw, and then hand sanded to a final thickness of ~35 μm. The sections were mounted on Plexiglas and stained with Giemsa. Adjacent sections were decalcified, dehydrated in a graded series of alcohols, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. This provided both thin, decalcified sections and thicker, undecalcified sections for evaluation.

Microscope slides were reviewed with special reference to evidence of foreign body reaction, inflammatory reaction, rate of resorption, and bone remodeling.

Histomorphometry

Large field-of-view (FOV) images of undecalcified sections stained with Giemsa were acquired on an Olympus wide-field microscope using Image-Pro 4.5.1/Scope-Pro software (Media Cybernetics, Silver Springs, MD), and a
CoolSNAP cf (Photometrics, Tucson, AZ) Color CCD Camera. Post-acquisition, images were electronically stitched together to generate a single large FOV image using a montaging macro written for Image-Pro. Quantitative histomorphometric analyses on large FOV images were also performed using Image Pro, measuring the total area of the original defect, new bone formation, empty space or fibrous tissue, and residual cement. These were expressed as a percentage of total defect area localized at the respective treatment sites. The cortical bone area was quantified separately from the defect area in the cancellous bone.

Compressive testing

Three vertebral bodies from each sheep—the augmented level and two adjacent levels—were harvested. The adjacent levels were not treated and served as internal controls. Three vertebral bodies were preserved frozen and allowed to thaw at room temperature 24 h prior to specimen testing. For the 36-month group, only the treated vertebral bodies were measured. Once thawed, each vertebral body was cleaned of its intervertebral disc, and each endplate was potted in acrylic cement (Fastray, Bosworth, Skokie, IL). Each potted vertebral body was placed between platens on a servo-hydraulic materials testing machine (MTS, Eden Prairie, MN) and compressed axially along the central axis of the spine at a rate of 5 mm/min. The resulting load versus displacement data were recorded at 10 Hz. The compressive stiffness and strength of the control vertebral bodies and those augmented with cement were measured.

Statistical analysis

The results of the histomorphometric analyses are expressed as relative percent of the total original defect area (mean ± standard deviation). The differences among each postoperative time were compared using the one-factor analysis of variance with the post hoc Tukey-Kramer test for multiple post hoc group comparisons. Significant differences between the cortex area and the cancellous area were determined with the use of the Mann-Whitney U test.

The mechanical differences between treatment and control groups were evaluated using a multiple linear regression model clustering the data on individual sheep, as one treated vertebral body and two control vertebral bodies came from the same animal. The strength and stiffness data for the vertebral bodies in the 36-month group were compared with the treated levels of the other time groups using a simple linear regression. For both statistical tests, \( p \) values less than 0.05 were considered statistically significant.

RESULTS

Surgical

Surgeries were uneventful with no postoperative complications. This formulation of BoneSource was easy to handle during surgery and could be rapidly injected into the vertebral body defects. All animals recovered from the surgeries without complications related to the injection of BoneSource and maintained full ambulatory status throughout the study.

Radiographic evaluation

With the number of samples available, there appeared to be no significant postoperative time effect. There were no areas of radiolucency, and no changes related to trabecular pattern or bone apposition at the BoneSource - bone interface. No undesirable bone displacement was detected radiologically (Fig. 1).

CT evaluation

The postoperative CT demonstrated adequately positioned and sized segmental defects in all sheep. The scans revealed no cement penetration of the spinal canal.

Histologic analysis

Figure 2 shows macrophotographs of undecalcified sections for each duration. A macrophotograph at time 0 shows the cement filling the cylindrical defect [Fig. 2(a)]. Portions of the cement showed extensive bone apposition at 3 months [Fig. 2(b)]. The cortex healed with new bone more rapidly than the area of cancellous bone occupied by cement [Fig. 2(c)]. Photographs at subsequent times show gradual replacement of cement by Haversian systems of bone (Fig. 2). Although lamellar bone had penetrated throughout the cement at 36 months, the original size and shape of the defect is still evident, and residual cement is easily recognized after 36 months [Fig. 2(f)].

Figure 3 shows the representative photographs of undecalcified sections for each duration. Bone apposition was established by 3 months and continued through 36 months. No fibrous tissue, foreign body, or inflammation reactions were present at the cement-bone interface throughout the 36 months duration of this study. The surface of the cement was irregular [e.g., Fig. 3(a)], and in areas, cement dissolution appeared to start at the surface and extend into the cement, possibly favoring penetration into the cement along cracks. Remodeling led to Haversian systems of new bone, which were demarcated from the cement by reversal lines that indicate the maximum depth of osteoclastic resorption prior to new bone formation [Fig. 3(b–d)].

The cement dissolved during processing of decalcified sections, but these thin sections show areas of
obvious bone formation in the cement (Fig. 4). Bone and probable fibrin illustrate the porosity of the cement. Osteoblasts, osteoclasts, and new bone formation in the cement are also evident in the decalcified sections.

**Histomorphometry**

Although there were differences in the amount of new bone and residual cement among different animals at the same time interval, statistical analysis...
shows a gradual increase in bone and decrease in residual cement in the medullary region of the vertebral body \( (p < 0.05) \) (Fig. 5). The rate at which BoneSource turned over into new bone in the cancellous area was \( 14.4\% \pm 5.8\% \) at 36 months. Residual cement occupied an average of \( 81.6\% \pm 7.3\% \) of the cancellous bone area at 36 months. The cement remodeled significantly more rapidly in the cortex than the cancellous area from 3 months to 36 months \( (p < 0.05) \).

**Figure 3.** Representative photographs of undecalcified sections of BoneSource with CMC. (a) 3, (b) 6, (c) 12, and (d) 24 months. The edge of the original defect is easily recognized in the specimen from 3 months (a), and new bone has filled gaps between the edge of the defect and the CPC. The photographs from 6, 12, and 24 months (b–d) show Haversian systems of lamellar bone gradually replacing CPC without fibrous tissue or inflammation. Reversal lines demarcate the edges of previous remodeling systems that have resulted in apposition of lamellar bone to the cement surface. (original magnification \( 100\times \)).

**Figure 4.** Representative photographs of decalcified sections of BoneSource with CMC at 36 months. The CPC has dissolved during specimen decalcification, resulting in an apparent space (CPC). Haversian systems of lamellar bone containing a central vessel and circumferential lamellae are evident, deep within the injected cement. The “scalloped surfaces” at the boundaries between bone and CPC almost certainly represent sites of previous osteoclastic resorption of cement, and/or remodeling of adjacent bone [original magnification (a) \( 100\times \), (b) \( 200\times \)].
Compressive testing

There were no significant differences in stiffness between the vertebral body levels treated with BoneSource and the adjacent control levels (Table I). There were also no significant differences in peak load strength between the vertebral body levels treated with BoneSource and the adjacent control levels (Table II). There was no significant effect of time on either strength or stiffness, and there was no significant interaction between vertebral level and time for either strength or stiffness.

**Figure 5.** Histomorphometric analysis. Histomorphometry results showing the fractional biopsy area occupied by bone in the medulla (a) and cortex (b), and by cement in the medulla (c), and cortex (d) at each time interval. Values are reported as mean and SD for each duration. * $p < 0.05$, by one-way analysis of variance with the post hoc Tukey–Kramer test.

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>n</th>
<th>Level Above</th>
<th>BoneSource</th>
<th>Level Below</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>5544 ± 1721</td>
<td>5811 ± 1719</td>
<td>4718 ± 1564</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5522 ± 1743</td>
<td>3437 ± 1021</td>
<td>3367 ± 812</td>
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<tr>
<td>6</td>
<td>6</td>
<td>4928 ± 1258</td>
<td>4002 ± 1694</td>
<td>5108 ± 1617</td>
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<tr>
<td>12</td>
<td>6</td>
<td>5445 ± 1988</td>
<td>7064 ± 1754</td>
<td>5506 ± 2131</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>4667 ± 2107</td>
<td>4858 ± 1679</td>
<td>5024 ± 3421</td>
</tr>
<tr>
<td>36</td>
<td>5</td>
<td>NA</td>
<td>4831 ± 2005</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are reported as mean and SD N/mm. The strength and stiffness data for the vertebral bodies in the 36-month group were compared with the treated levels of the other time groups using a simple linear regression. There were no significant differences for each stiffness property.

**TABLE II**

Summary of Peak Load Properties Measured for the Vertebral Body Levels Treated with BoneSource and the Adjacent Control Levels

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>n</th>
<th>Level Above</th>
<th>BoneSource</th>
<th>Level Below</th>
</tr>
</thead>
<tbody>
<tr>
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<td>9741 ± 1588</td>
<td>9895 ± 1686</td>
<td>10,570 ± 2154</td>
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<tr>
<td>3</td>
<td>5</td>
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<td>10,808 ± 2649</td>
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</tr>
<tr>
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<td>6</td>
<td>9804 ± 1013</td>
<td>9155 ± 1901</td>
<td>8750 ± 1339</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>11,633 ± 1116</td>
<td>11,537 ± 2947</td>
<td>12,730 ± 2831</td>
</tr>
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<td>7</td>
<td>8972 ± 3027</td>
<td>9126 ± 2454</td>
<td>8279 ± 3778</td>
</tr>
<tr>
<td>36</td>
<td>5</td>
<td>NA</td>
<td>12,585 ± 4341</td>
<td>NA</td>
</tr>
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</table>

Values are reported as mean and SD. The strength and stiffness data for the vertebral bodies in the 36-month group were compared with the treated levels of the other time groups using a simple linear regression. There were no significant differences for each peak load property.
strength or stiffness (Tables I and II). Thus, destructive compressive testing of individual ovine vertebral bodies showed that there were no differences in the mechanical integrity of the treated sheep vertebral bodies between the vertebral body levels treated with BoneSource and the adjacent control levels in any time group. Among other things, this shows that as the cement is slowly resorbed or dissolved by osteoclasts, the simultaneous bone ingrowth essentially creates a cement/bone composite without any reduction in compressive strength.

**DISCUSSION**

Frankenberg et al.\(^1,16\) evaluated the repair of a metaphyseal defect after treatment with FractureGrout\(^1\), injectable calcium-phosphate cement (an early formulation of Norian SRS\(^1\), Synthes, Paola, PA). They placed either the cement or allograft bone in proximal tibial metaphyseal and distal femoral metaphyseal defects in 72 dogs and performed histological and mechanical (torsional) examination from 24 h to 78 weeks postoperatively. The resulting bone-cement composite underwent gradual remodeling over time in a pattern that was qualitatively similar to the remodeling of normal cortical and cancellous bone, and was similar to the mechanical and histological properties demonstrated in our study. In their study, mechanical testing showed that by eight weeks the tibiae that had been treated with cement had reached nearly 100% of the torsional strength of the control tibiae. Like the study by Frankenburg et al.,\(^1,16\) we also noted that cement was remodeled more rapidly in the cortex than in the medulla. They suggested that the process of cement remodeling is influenced by mechanical, geometric, and physiological factors. If an injected cement were to dissolve more rapidly than it was replaced by bone, then mechanical failure may result, but in our current study as well as the study by Frankenberg et al.,\(^1,16\) bone ingrowth formed a bone/cement composite that maintained compressive strength during the remodeling process.

Some prior studies suggest that mechanical weakness is the main disadvantage of CPC, limiting wider clinical applications.\(^25-27\) Other studies demonstrated improved mechanical properties in fractured vertebrae after injection of CPC in a cadaver model.\(^13,14,28\) For example, Bai et al.\(^14\) evaluated the effects of a biodegradable CPC (α-BSM, ETEX, Cambridge, MA) on the fixation strength and bending rigidity of vertebral body screws in the osteoporotic spine. Using a formulation of BoneSource identical to that used in our study, Lim et al.\(^13\) demonstrated that BoneSource (Howmedica Osteonics, Rutherford, NJ) could be injected easily into the thoracolumbar vertebral body and improved the strength of a fractured vertebral body to at least the level of its intact strength. Both of the groups\(^13,14\) concluded that these CPCs are potential alternatives to the use of PMMA cement.

BoneSource and other CPCs have been approved for use in contained skeletal defects in the United States, and have been studied in applications in which the cements augment metallic hardware for fixation of fractures loaded primarily in compression, osteoporosis, and filling contained skeletal and cranial defects.\(^12,25,26,29-33\) Nakano et al.\(^20\) reported 30 patients with osteoporotic vertebral compression fractures treated with one type of CPC (Biopex; Mitsubishi Materials, Tokyo, Japan). The authors compared the CPC-assisted vertebroplasty group with a conservative treatment group at 12 months. All of the outcome measures, including visual analog scale score of back pain, analgesic requirements, and the radiographically documented rate of vertebral body kyphosis were improved in the vertebroplasty group. No patients showed symptomatic neurological abnormality, although there was a small amount of CPC leakage into the spinal canal in six (20%) of the 30 patients. The authors concluded that CPC-assisted vertebroplasty provides better clinical and radiological results than conservative treatment for primary osteoporotic vertebral compression fractures. Libicher et al.\(^24\) treated 20 postmenopausal female patients with 46 vertebral compression fractures, utilizing CPC (Calcibon, Biomet Merck, Darmstadt, Germany) \((n = 28)\) or PMMA \((n = 18)\) for vertebral stabilization and evaluated the radiological changes at the bone–cement interface of CPC and PMMA 12 months after kyphoplasty. CPC implants showed a significant decrease of the border-voxel density, which was defined as a parameter of cement resorption at the interface, suggested surface resorption of the CPC compared with PMMA. The height restoration and the clinical outcome including incident vertebral fracture rates did not differ based on the cement composition. Moreover, there were no perioperative complications and there was no significant extravasation of cement material into the spinal canal or paravertebral soft tissue perioperatively or during the 12-month follow-up period. Several other studies have described the use of injectable CPCs for augmenting poor quality cancellous bone. For example, Mattsson et al.\(^35\) reported a multicenter, prospective study in which Norian SRS (Synthes, Paola PA) was used in combination with a sliding screw device for treating 112 unstable trochanteric fractures. The results showed improved clinical, radiographic, and functional outcome for the cemented group compared to fractures treated with the sliding screw alone. Dickson et al.\(^36\) performed a randomized, prospective, multicenter
study comparing the use of BoneSource cement with autogenous bone graft for filling metaphyseal bone defects secondary to trauma. Long bone fractures were treated with open reduction and internal fixation, and the results showed that BoneSource cement was at least as good as autograft for maintenance of reduction, fracture healing, and clinical function.

Our results showed variability among different animals in each group, but we were still able to demonstrate a tendency of increasing bone and decreasing residual cement with time. The results also suggest that the rates of changes in new bone and resident cement may not have been linear with time. Cement resorption was evident in the vicinity of osteoclasts and often was accompanied by new bone formation that had a qualitative pattern similar to normal bone remodeling. Vascular ingrowth into the cement was evident, and there was no fibrous tissue.

One limitation to the use of sheep for evaluation of vertebroplasty is the higher bone density of normal sheep compared with humans. Moreover, vertebrae from animals have thicker cortices than human vertebrae, so that the maximum load prior to fracture may mask the changes in cancellous bone of the vertebrae. The strength of sheep vertebral bodies is nearly an order of magnitude greater than the strength of osteoporotic human vertebral bodies. Although the absolute magnitude of stiffness and peak load that might be achieved in osteoporotic vertebral bodies cannot be extrapolated from our results, osteoporotic cadaver vertebral bodies augmented with BoneSource approached the strength and stiffness of native vertebral bodies and were similar to vertebral bodies augmented with PMMA.

In addition, the amount of cement injected (and the proportion of vertebral body occupied by cement) in these animals was less than is usually achieved during augmentation of osteoporotic human vertebrae, suggesting that our model has important limitations with respect to evaluating the expected changes in compressive properties imparted by the cement. On the other hand, the sheep model used in the present study has been used in prior studies and allowed good evaluation of biologic properties.

Finally, we found that the handling properties of the cement, which include its injectability, capacity for nonexothermic setting, and relatively rapid hardening time, are desirable attributes for selected orthopedic applications. Although care must be taken when interpreting the current information for human clinical use, the compressive strength of this formulation of BoneSource is thought to be adequate for human vertebral augmentation.

CONCLUSION

BoneSource mixed with CMC had excellent osteoconductivity, biocompatibility, and adequate compressive strength. There was variability among animals, but histology suggests that considerable cement was still present in most samples after 36 months.

The authors acknowledge the contribution of Brian Edwards to the design and initiation of this study. His death in July 2003, prevented him from seeing the results of his efforts and participating in the writing of this manuscript.

References


