Clinical Evaluation of Mineralized Collagen as a Bone Graft Substitute for Anterior Cervical Intersomatic Fusion

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Although autograft is a routine procedure in anterior interbody fusion of the cervical spine, donor site morbidity is a significant problem. Many bone grafting materials developed such as allograft bone, demineralized bone matrix (DBM), and synthetic implants were used to repair bony defects; however, each has its specific disadvantage. Therefore, alternative materials for autogenous bone in grafting are needed. Ninety-one patients with symptomatic cervical disc diseases were randomly allocated for either autologous iliac crest bone graft (AIG) or mineralized collagen (MC). Clinical and radiological comparisons were performed for the two groups. Patients were followed-up routinely in the outpatient clinic, where clinical and radiological evaluations were taken. The mean operation time to perform AIG and MC was 194 ± 37 and 121 ± 26 minutes, respectively. The mean volume of blood loss during the operation was 140 ± 41 and 79 ± 25 mL for AIG and MC, respectively. The mean length of hospitalization due to AIG and MC was 9.7 ± 0.7 and 5.9 ± 0.8 days, respectively. The clinical outcomes were identical, and fusion rates were similar between the two groups. This study shows effective results of MC in anterior cervical intersomatic fusion. MC may be a promising substitute for autologous iliac crest bone graft.

Keywords: Bone Graft Substitute, Mineralized Collagen, Anterior Cervical Intersomatic, Fusion.

1. INTRODUCTION

Since Robinson and Cloward achieved anterior interbody fusion of the cervical spine with autogenous iliac crest bone grafts, anterior cervical discectomy and fusion (ACDF) has been established as one of the procedures to distract the disc distance, enlarge the neural foramen and obtain anterior interbody fusion.1–3 With a solid anterior interbody fusion, neural irritation can be reduced as a result of diminished motion within the neural foramen. Although the use of autograft in anterior interbody fusion of the cervical spine is the gold standard, complications associated with the harvest of autograft, such as limited quantities, donor site morbidity, blood loss, and increased operative time have prompted the search for suitable alternatives. Numerous bone substitutes using metals, ceramics and polymers have been developed. However, each has specific disadvantages, and none of them can perfectly take the places of autograft and allograft in current clinical practice. One important reason for the priorities of autograft and allograft is that their compositions and microstructures are the same as the bone to be repaired and therefore possess biological advantages.

According to the principle of biomimetic strategy, we have developed a new bone-resembling composite: mineralized collagen (MC) (Fig. 1), which has similar composition and microstructure to natural bone. Bone tissue is a particularly complex composite because it contains multiple levels of hierarchical organization. The lowest level of this hierarchy is the organization of collagen fibrils with respect to hydroxyapatite (HA) crystals. Briefly, we prepared this composite following two steps. Firstly, mineralized type I collagen fibrils were produced using self-assembling method.4 The collagen fibrils are formed by self-assembly of collagen triple helices and the HA crystals grow within these fibrils in such a way that the collagen fibers regulate the HA crystal nucleation and growth. Secondly, the mineralized type I collagen fibrils were added to dissolved polylactyl acid (PLA), and then the three-dimensional porous composite was prepared by lyophilization. It is a porous scaffold with pore size of 100–300 μm and about 90% porosity. The compressive...
2. METHODS

2.1. Patients

A total of 91 patients with multi-level symptomatic cervical disc disease refractory to conservative treatment were recruited prospectively for this study. The trial was approved by the ethical committee of the hospital. Allocation of surgical procedure (e.g., AIG or MC) for each patient was random after consultation with the patient. Both grafts were offered, and possible complications of the donor sites with autograft and the nature of MC were explained. Irrespective of graft types, smokers were urged to follow a smoking cessation program before and after surgery. The demographic data of patients showed that both groups were fairly homogeneous (Table I). Clinically, 52 patients (57.1%) had radiculopathy, 24 patients (26.4%) had myelopathy, and 15 patients (16.5%) had radiculomyelopathy. Neck pain was noted in all the patients. Pre-operative clinical and radiographic evidences indicated mechanical strength of this scaffold is close to 3 MPa. Results of our previous studies have shown that the scaffold not only mimics the composition of cancellous bone, but also has excellent biocompatibility, biodegradability, high osteoconductive activity, and promotes bone repair and regeneration. After the clinical trial was approved by the Food and Drug Administration (FDA) of P. R. China, 91 patients with multi-level symptomatic cervical disc disease were included in this preliminary study and divided into two groups of different graft usage: autologous iliac crest bone graft (AIG) group and mineralized collagen group (MC). Smith-Robinson technique was used in all cases in conjunction with cervical screw-plate instrumentation. Patients were followed-up routinely in the outpatient clinic, where clinical and radiologic evaluations were taken to determine whether MC was a promising alternative for autologous iliac crest bone graft in anterior cervical intersomatic fusion. The details of these observations are reported.

2.2. Surgical Techniques

All operations were performed using Smith-Robinson technique. A right-sided transverse skin incision was performed. Following discectomy, all endplate cartilage was removed with a curette. A high-speed burr was used to perforate the subchondral bone of the end plates. Posterior longitudinal ligament was excised systematically to ensure adequate neural decompression. Somatosensory-evoked potential monitoring was used in patients with marked cord compression. Autografts were harvested from the anterior iliac crest using the triple osteotome technique, where one osteotome was used to elevate the iliac crest with its muscle attachment, and then two osteotomes were used to cut the graft, which was, therefore, bicortical and a few millimeters larger in height than the decompressed disc space. Working 5 cm behind the anterosuperior iliac spine and without dissection of the iliac crest, this technique avoided cutaneous nerve injury, preserved the iliac crest, and was associated with minimal pain. The MC composite was properly fashioned with a scalpel, which was similarly a few millimeters larger in height than the decompressed disc space. To provide compressive forces on the graft-end plate interface, an approximately 2-mm graft height distraction of the motion segment was obtained before insertion of each graft. Each graft was countersunk 2 mm from the anterior vertebral border and impacted with the cortical surface positioned anteriorly.

Table I. Demographic data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MC</th>
<th>AIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>48.6 ± 12.4</td>
<td>49.5 ± 13.1</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>28/18</td>
<td>26/19</td>
</tr>
<tr>
<td>Operated levels (two-level/three-level)</td>
<td>39/7</td>
<td>36/9</td>
</tr>
<tr>
<td>Disc type (soft/hard)</td>
<td>21/25</td>
<td>23/22</td>
</tr>
<tr>
<td>Mean follow-up period (mo)</td>
<td>42.7 ± 0.8</td>
<td>42.6 ± 0.7</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>5.9 ± 0.8</td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td>Operative time (min)</td>
<td>121 ± 26</td>
<td>194 ± 37</td>
</tr>
<tr>
<td>Estimated blood loss (ml)</td>
<td>79 ± 25</td>
<td>140 ± 41</td>
</tr>
</tbody>
</table>

AIG: autologous iliac bone graft; MC: mineralized collagen group.

Fig. 1. Mineralized collagen (MC) implants.
(autograft). A bone drill was used to prepare the proper application of the plating device by smoothing the anterior cervical surface of the operative vertebrae for fixation. An anterior cervical plate was selected and guided by surgical midline markers for proper positioning. Each plate was contoured to cervical lordosis and to accommodate vertebral convexity in order to provide desired alignment and accomplish optimal bone-plate contact. A drill guide was used for screw drilling, allowing no more than 14 to 16 mm of penetration. Drilling was done directing screw insertion toward the midline and parallel to the vertebral end plate of the vertebral body. Unicortical screw purchase was obtained in all cases avoiding penetration into the cranial and caudal intervertebral space. Meticulous hemostasis was performed in all cases, and closure was achieved in layers. All patients were placed in a soft collar for 3 to 4 weeks post operation. Neck exercises were initiated at this time, and gradual return to normal activities was permitted.

2.3. Follow-Up Evaluations

Patients were followed-up routinely in outpatient clinics for clinical and radiologic assessment according to the follow-up protocol.

2.4. Clinical Evaluation

The clinical outcome was established based on available clinical follow-up data according to the Odom Grading Scale (Table II). The results were categorized as excellent, good, satisfactory, and poor. Excellent results meant that there were no reports referable to cervical disease and the patients were able to carry on with their daily occupation without impairment; good results meant that there was intermittent discomfort related to cervical disease that did not significantly interfere with the work of the patients; satisfactory results meant that there was subjective improvement but the physical activity of the patients were still limited; and poor results meant that there was no improvement or that the symptoms of the patients were worse than before surgery. Surgical treatment was considered successful when the clinical outcome of a patient was excellent, good or satisfactory. Otherwise it was considered a failure.

Table II. Clinical outcome of procedure performed and in relation to graft source.

<table>
<thead>
<tr>
<th>Group</th>
<th>Excellent</th>
<th>Good</th>
<th>Satisfactory</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIG (n = 45)</td>
<td>15</td>
<td>26</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>MC (n = 46)</td>
<td>13</td>
<td>31</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total (n = 91)</td>
<td>28 (31%)</td>
<td>57 (62%)</td>
<td>6 (7%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

AIG: autologous iliac bone graft; MC: mineralized collagen group.

2.5. Radiologic Evaluation

In this study, changes in the lordotic angle and the height of fused segment, as well as fusion pattern have been evaluated using plain radiographs-posteroanterior, lateral, and dynamic study, computed tomography (CT), or magnetic resonance imaging (MRI) (Fig. 2). Evaluation of the fusion was performed at 3, 6, 12 months and more than 24 months after surgery. The MC porous matrix was radiolucent on plain radiographs. With the formation of new bone, the radiologic density grew through the implant through the follow-up. Vertebral fusion was classified as absent, partial or complete. The absence of bone fusion was defined as no change in the radiologic aspect of the intersomatic space. Partial fusion was defined as the persistence of the intersomatic limits but with radiological signs of intervertebral changes. Complete fusion was achieved when bony trabeculae were clearly seen across the disc space. The change of height of the fused segments was expressed as the change of the distance from the midpoint of the superior end plate of the upper vertebra to the midpoint of the inferior end plate of the lower vertebra involved in the fusion. The vertical distance was expressed in millimeters. We calculated the lordotic angle formed by lines from the superior end plate of the upper vertebra and the inferior end plate of the lower vertebra with the cervical spine in neutral position.

2.6. Statistical Analysis

Qualitative and quantitative variables were evaluated using chi-square test and t-test, respectively, using the Statistical Package for Social Science (version 10.0) for Windows. A probability of less than 0.05 was regarded as significant.

3. RESULTS

The average length of hospital stay was 9.7 ± 0.7 days and 5.9 ± 0.8 days for those who had AIG and MC implants, respectively, which was statistically different (Table I). The mean operation time was 194 ± 37 minutes for the AIG group and 121 ± 26 minutes for the MC group (Table I). The mean estimated blood loss was 140 ± 41 mL and 79 ± 25 mL for the AIG and MC group, respectively (Table I). All patients have completed 2-year follow-up evaluations. The post-operative clinical outcome evaluation was conducted based on Odom’s criteria. According to the criteria, 28 patients (31%) had excellent results, 57 (62%) had good results, and 6 (7%) had fair results. The clinical outcome was not significantly different between the two groups (Table II). No patient exhibited a poor clinical outcome.

Radiographs revealed persistently enlarged pre-vertebral soft-tissue shadow and decreased disc height at C5–6 and C6–7 (Fig. 2(a)). MRI revealed features of cervical disc
Yu et al.  
Clinical Evaluation of Mineralized Collagen as a Bone Graft Substitute for Anterior Cervical Intersomatic Fusion

![Fig. 2](image-url)  
Preoperative and postoperative X-ray film and magnetic resonance imaging (MRI) showing the cervical spine of a patient. (a), (b) Preoperative X-ray and MRI image shows spinal cord was compressed by C5–6 disc and C6–7. (c) Postoperative immediate lateral images, a wedge of MC was placed into the C5–6 (low density) and intervertebral cage (placed MC) was placed into the C6–7. (d) Lateral X-ray at 14 weeks after the surgery C5–6 and C6–7 have achieved spinal fusion.

Fig. 2. Preoperative and postoperative X-ray film and magnetic resonance imaging (MRI) showing the cervical spine of a patient. (a), (b) Preoperative X-ray and MRI image shows spinal cord was compressed by C5–6 disc and C6–7. (c) Postoperative immediate lateral images, a wedge of MC was placed into the C5–6 (low density) and intervertebral cage (placed MC) was placed into the C6–7. (d) Lateral X-ray at 14 weeks after the surgery C5–6 and C6–7 have achieved spinal fusion.

disease at C5–6 and C6–7 (Fig. 2(b)). X-rays performed after 3 months revealed solid fusion at all levels (Fig. 2(d)). All 91 patients were assessed for radiographic fusion.

The results of bone fusion achieved at 3, 6, 12 and more than 24 months after surgery are shown in Table III. A total of 24 patients (26.4%) (17 in the AIG group and 7 in MC group) presented radiological signs of complete bone fusion 3 months after surgery. 41 patients (91.1%) in the AIG group and 31 patients (67.4%) in the MC group achieved radiological grade of fusion 6 months after surgery. One year after the surgery, 89 (97.8%) patients achieved successful bone fusion (100% autograft and 95.7% MC). These results suggested that patients in the MC group had a relatively slower fusion than patients in the AIG group. However, the rate of fusion observed in the MC group was similar to that observed in the AIG group 3, 6, 12 and more than 24 months after surgery ($p = 0.079$, $p = 0.06$, $p = 0.311$, $p = 0.425$). No loss of height of the fused segments was observed in both groups, and the lordotic angle of fused segments was maintained in all cases through the follow-up. No screw loosening and migration was observed in the radiographic evaluation.

Clinical Evaluation of Mineralized Collagen as a Bone Graft Substitute for Anterior Cervical Intersomatic Fusion

Yu et al.

Table III. Results of bone fusion at 3, 6, 12 and more than 12 postoperative months.

<table>
<thead>
<tr>
<th>Grade of fusion</th>
<th>Non fusion</th>
<th>Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Partial</td>
</tr>
<tr>
<td>3rd month</td>
<td>AIG</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6th month</td>
<td>AIG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12th month</td>
<td>AIG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last follow-up</td>
<td>AIG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>0</td>
</tr>
</tbody>
</table>

AIG: autologous iliac bone graft; MC: mineralized collagen group.

4. DISCUSSION

To treat degenerative disease, trauma and tumors of the spine, Robinson and Smith have introduced the anterior cervical decompression technique without microscope, in which fusion is achieved by inserting a bone graft harvested from the iliac crest of the patient for neural elements decompression and spinal stabilization. The indications of ACDF include radiculopathy, myelopathy, myelo-radiculopathy and traumatic instability at single or multiple levels. Anterior cervical disectomy without interbody fusion is practiced for treating degenerative disease by some authors; however, many authors now advocate for fusion in addition to disectomy. There are various techniques of ACDF including the Cloward technique, disectomy, and interposition graft. ACDF has been further modified using anterior locking plates for stabilization (ACDFP). The additional costs involved in ACDFP have led investigators to examine the difference in fusion and clinical outcome between patients with ACDF and ACDFP.9

Single-level ACDF is a highly successful procedure with high reported fusion rates ranging from 83% to 99% for autograft.10–12 However, as the number of grafts increases, in multilevel ACDF, the cervical spine is predisposed to decreased fusion rates as contact stress increases between the graft-body interfaces, which further contributes to unacceptable micromotion.13 In 1991, in patients undergoing a two-level ACDF, Zdeblick and Ducker14 have noted nonunion in 17% of those with autograft. Higher nonunion rates at 37% to 70% have been reported for three-level ACDFs.15–17 Then anterior cervical plating devices have increased fusion rates for multilevel instrumented ACDF.18–21 Although plating increases operative time, intensifies the risk for potential vascular, neural and soft tissue injury, is more costly in the short term, and requires extensive training, it is beneficial to bone fusion, avoids prolonged and uncomfortable post-operative immobilization, and is cost-effective in the long term by avoiding revision surgery required to address nonunions highly associated with patients with no internal fixation. The risk of complications associated with anterior cervical plating is always present but may be decreased with proper patient and plate selection, improved fixation techniques and increased experience.22 Fusion depends on four main factors:

1. the stability of the graft in the intersomatic space,
2. the state of the grafting bed,
3. regional and systemic factors of the host, and
4. the graft itself. The stability of the graft in the intersomatic space is necessary for a favorable creation of bony bridges around and through the graft.

Three ideal graft characteristics for successful fusion are osteogenesis, osteoinduction and osteoconduction, which create new bone, stimulate osteoblastic differentiation of progenitor cells, and provide a scaffold for bone deposition, respectively. A successful fusion will avoid pain at autograft sites and associated soft tissue morbidity, obviate the need for cervical orthosis, and does not impair with subsequent radiologic investigations. It will also provide immediate stability in compression, resist axial displacement, minimize neck pain, and maintain spinal alignment and foraminal height. A variety of graft substitute materials include autologous bone, allograft, synthetic material and metallic cages.23–27 Autograft has relatively few incidences of graft complication, such as graft collapse or migration. It is biocompatible, non-immunogenic, and poses no risk on disease transmission. For these reasons, autograft remains the standard of care for ACDF. Unfortunately, the harvest site for autogenous iliac crest bone grafts is associated with an amplified risk of morbidity, operation time, hospital stay and post-operative recovery.28–30 Furthermore, patient dissatisfaction with the cosmetics of the
iliac crest incision also contributes to the shortcomings of such a harvesting method. Although the use of allograft does avoid harvest site morbidity, is available in desired quantity, can be configured, decreases hospitalization time and reduces costs, disadvantages are also associated with allograft. These include histocompatibility differences that may affect proper healing, increased risk of infection, lack of availability, size variations, possible structural weakness, and lack of a strict program of quality control.\textsuperscript{31–33}

Complete fusion is aided by appropriate graft architecture. New bone graft materials are being designed to mimic one or more of the bone-forming components of autograft. The ideal graft substitute would be structurally sound in maintaining distraction across a disc space, porous enough to facilitate bony in-growth, non-immunogenic, biodegradable, and biocompatible. The mineralized collagen (MC) porous matrix is such a new bone-resembling composite developed by our laboratory. This composite not only possesses high porosity to approximately 90%, but also has necessary mechanical strength to support the space. The hierarchical microstructure of MC simulates cancellous bone and osteoconductive capabilities, which are characteristics mainly exist in collagen and nano-hydroxyapatite components.\textsuperscript{4, 34} In natural bone, collagen makes up about 30% of the dry weight of bone and 90–95% of its non-mineral content. Bone mineral is a nonstoichiometric carbonated apatite with low crystallinity and nanometer size. It makes up about 60–70% of the dry weight of bone, which show the same characters of mineral in the MC composite.\textsuperscript{35–37} Several authors have reported the usages of mineralized collagen, hydroxyapatite ceramic, and coralline hydroxyapatite bone graft materials for spinal fusion.\textsuperscript{38–40} Our studies show that MC scaffold mimics the composition of cancellous bone, has excellent biocompatibility, biodegradability and high osteoconductive activity, and promotes bone repair and regeneration.\textsuperscript{7–8} In this preliminary study, 91 patients have two- or three-level ACDF with rigid anterior plate fixation are included and divided into AIG group and MC group. They have also completed 2-year follow-up evaluations. The results demonstrated a 97.8% overall osseous union with a 100% and 95.7% fusion rate for autograft and MC one year after surgery, respectively.

5. CONCLUSIONS

This study shows effective results of MC in anterior cervical intersomatic fusion. MC may be a promising substitute for autologous iliac crest bone graft.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

References and Notes

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Yu et al.


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