Macroscopic and Histologic Analyses of De Novo Bone in the Posterior Spine at Time of Spinal Implant Removal

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Study Design: Case series report.

Objective: To evaluate the macroscopic and histologic appearance of bone after implantation of bone marrow aspirate on mineralized collagen fiber (Healos) at 12 to 24 months postoperative.

Summary of Background Data: Numerous bone graft substitutes are currently used for spinal fusion. The success of these technologies is usually determined by radiographic visualization at 1-year or 2-year follow-up. Although x-rays and computed tomography scans are among the best tools to identify bone, interpretation of these images can still lead to error.

Methods: Six subjects with prior spinal fusion were reoperated for spinal implant removal. All subjects had reached at least 11-month follow-up from their initial spinal surgery. The graft material used in all fusion sites was bone marrow aspirate with Healos (BMA-H), except in a case where autograft was used on one side and BMA-H on the other. At implant removal, macroscopic appearances of the fusion sites were manually tested, photographed, and biopsies were taken and processed for routine histology.

Results: Macroscopic observations were critical to determine presence of mineralized or fibrous tissue. In the site implanted with autograft, fibrous tissue was observed macroscopically. This finding was confirmed by histology. In all sites implanted with BMA-H, solid bone was observed macroscopically. In 1 particular case, the newly formed fusion masses had a rectangular shape, reminiscent of that of the implanted strips. Histologically, BMA-H biopsies revealed mature cortico-cancellous bone.

Conclusions: Published accounts of histologic evaluations of bone graft materials are uncommon, due to ethical and clinical limitations to harvesting tissue samples. Reoperations for instrumentation removal present rare occurrences when biopsies can be performed. In all cases, BMA-H implants resulted in solid, mature bone. These findings provide additional data on the effectiveness of BMA-H as a bone graft substitute for posterolateral fusion.

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Despite a wealth of scientific literature describing the optimal biologics for spinal fusion,[1] de novo bone formation in the posterior lumbar spine remains challenging. As discussed by others, good quality bone grafting material must be placed in a well-prepared host bed, in a carefully selected subject, followed by, first, a period of immobility and, later, a period of stress.

The graft material should ideally be osteoconductive, osteoinductive, and osteogenic and traditionally has been autologous cancellous or cortico-cancellous bone harvested from the iliac crest. However, there are numerous reports of complications after the iliac crest bone harvest.[2,3] As a result, there has been an intense search for viable alternatives to iliac crest autograft for over 30 years.

Osteoconductive materials such as collagen and various bioactive ceramics (eg, hydroxyapatite and tricalcium phosphate) have no intrinsic power to produce bone, but act as scaffolds onto which blood vessels can grow and osteoprogenitor cells can attach before proliferation and differentiation,[4] which is achieved via activation by naturally occurring or synthetic osteoinductive agents. Bone grafts contain viable osteoprogenitor cells capable of differentiating into osteoblasts thus becoming osteogenic.

One of the main and most easily accessible source of allogeneic osteoprogenitor cells is the bone marrow within the iliac crest and the vertebral bodies.[5] When added to a bone graft material, bone marrow has been shown to increase the bone-forming potential of that graft material.[6]

The clinical and preclinical efficacy of bone marrow-soaked grafts has been previously published, especially with hydroxyapatite-coated cross-linked collagen grafts (Healos)[7–9]; however, only 1 abstract has been published on the histologic appearance of the newly formed bone resulting from the use of bone marrow-soaked grafts.[10]

Radiographic imaging techniques, whether plain x-rays or computed tomographies, are used in most of the studies to determine fusion. In those rare instances when surgical exploration was used in conjunction to radiographs, agreement between radiographic fusion and

actual surgical findings only reached 69%.[11,12] Therefore, although radiographs still remain critical and in most cases, the only ethically acceptable method of assessing fusion, histologic evaluations provide a more accurate and informative basis to evaluate de novo bone formation and bridging.

MATERIALS AND METHODS

The present study is a series of 6 cases (Table 1), examining the macroscopic and histologic appearance of posterior and posterolateral fusion masses in the thoracolumbar and lumbar spine at the time of implant removal. All cases at the time of original spinal fusion were implanted with grafts consisting of 5 cm^3 ($2 \times 5 \times 0.5$ cm) strips of hydroxyapatite-coated collagen Type I graft (Healos) soaked in bone marrow aspirate, henceforth referred to as BMA-H.

The surgical technique in each case was standard for that procedure. The spine was exposed through a midline incision carried laterally to the tips of the transverse processes and pedicle screw instrumentation was carried out in all cases. The posterolateral fusion beds were prepared by meticulous decortication of the transverse processes, pars interarticularis, and lateral aspects of the facet joints. BMA was aspirated with a Jamshidi needle from the posterior iliac crest through the main incision. BMA was obtained in 3 mL aliquots to prevent venous blood dilution, with redirection of the needle for subsequent withdrawals. Six milliliters of BMA were mixed with each 5 cm^3 strip of Healos and were allowed to stand for at least 20 min before implantation to allow complete wicking of the BMA into the collagen-hydroxyapatite matrix.[8] The grafts were carefully delivered to the fusion site in a fashion designed to optimize graft-host bed surface contact. Four strips of BMA-H were used for each level of fusion, 2 per side. In case 1, which was the first time Healos was used in our hospital, abundant autograft was used on the left side consisting of finely morcellised facet and laminar bone taken from the wide decompression site.

The mean time to reoperation was 15.83 months (range, 11 to 24 mo). In the cases of degenerative disc disease (cases 1 and 3), the reason for implant removal was persistent pain at the index level, thought to be attributable to the presence of the pedicle screw instrumentation. A further patient with degenerative disc disease (case 2) had progressive adjacent level degeneration, above and below her fusion, hence requiring an additional procedure to extend her fusion. In the fracture cases (cases 4 to 6), implants were used as temporary internal struts and removal was planned approximately 1 year postoperatively. Radiographic imaging was also performed before implant removal and bone formation was assessed by manual palpation at the time of operation. Biopsies of the bone formed at the implant

TABLE 1. Summary of Case Reports

Patient Profile	Age/ Sex	Fusion Level	Fusion Material	Radiography Findings	Histology
Case 1 Professional water-skier L3/4 postdiscectomy instability	41/M	L3/4 PLF + Pedicle screw instrumentation	(R), BMA-H; (L) autograft	(R), Fused; (L), not fused, L3 screw broken	(R)–Cortico-cancellous bone with remodeling. Reactive changes in soft tissue; (L)–Mature fibrous tissue, no evidence of bone formation
Case 2 Fitness instructor L2/3 DDD	42/F	L2/3 PF + Pedicle screw instrumentation	Bilateral, BMA-H	Bilateral, fused	Bilateral–Mature cortical bone with adjacent fibrous tissue
Case 3 L4/5 degenerative spondylolisthesis L5/S1 DDD	55/F	L4/5 and L5/S1 Circumferential fusion	Bilateral, BMA-H	Bilateral, fused	Bilateral–Mature cortical bone with remodeling
Case 4 T12/L1 Traumatic distraction injury	22/M	T12/L1 PLF + T11–L3 Pedicle screw instrumentation	Bilateral, BMA-H	Bilateral, fused	Bilateral–Mature cortico-cancellous bone with little osteoclastic activity
Case 5 Burst fracture L1	14/M	T12/L1 PF + T12–L2 Pedicle screw instrumentation	Bilateral, BMA-H	Bilateral, fused	Bilateral–Mature trabecular bone with active osteoblastic activity. Occasional foci showing osteoclasts
Case 6 Burst fracture L1	38/M	T12/L1 PF + T11–L2 instrumentation	Bilateral, BMA-H	Bilateral, fused	Bilateral–Mature cortical bone with focal remodeling as evidenced by endochondral ossification

DDD indicates degenerative disc disease; PF, posterior fusion; PLF, posterolateral fusion.

site were then collected and compared histologically to normal bone to ensure there was no untoward inflammatory reaction associated with the graft material.

RESULTS

Radiographic imaging, in all cases, with the exception of case 1, was indicative of fusion at the concerned levels. In case 1, the 1-year radiograph (Fig. 1A) showed a solid fusion on the right, but no evidence of fusion on the left. This was clearer on an x-ray taken after implant removal (Fig. 1B). At operation, it was found that the left upper screw was broken with a fibrous nonunion in the left intertransverse space, but on the right side the fusion mass was solid (Fig. 2). Removal of metalwork, with further noninstrumented, posterolateral regrafting on the left side, using a combination of iliac crest autograft and BMA-H, resulted in a fusion subsequently.

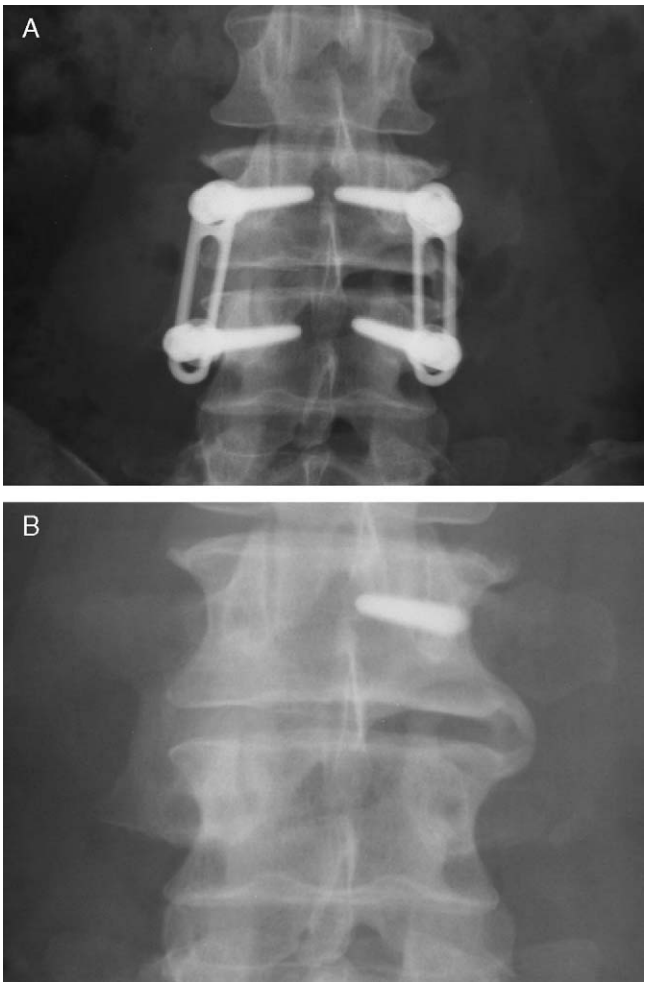

FIGURE 1. Anterioposterior radiographs of L3/4 fusion before and after pedicle screw removal, at 12 months postoperative (case no. 1). BMA-H was implanted on the right side, whereas autograft was implanted on the left side. BMA-H indicates bone marrow aspirate with Healos.

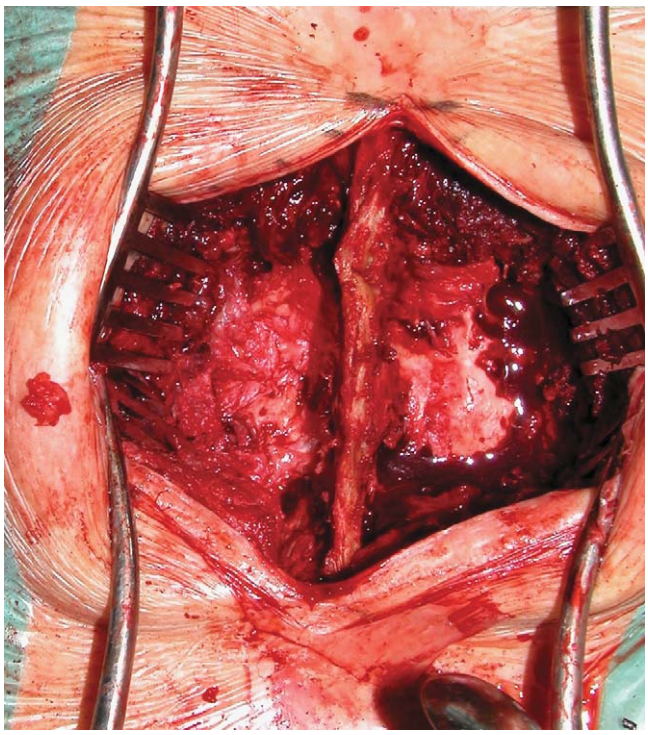

FIGURE 2. Photograph of the intraoperative appearance of L3/4 fusion, after implant removal (case no. 1). A fusion mass is visible on the right, but a fibrous nonunion can be seen on the left.

Manual palpation at the time of reoperation was supportive of the radiographic and histologic findings in all cases. Histologic analysis of all specimens taken from the BMA-H sites in the degenerative disc disease cases (cases 1 to 3) revealed mature cortico-cancellous bone formation with evidence of good remodeling. In the patients who sustained fractures (cases 4 to 6), histology showed the presence of mature cortico-cancellous bone at the BMA-H sites (Fig. 3). However, remodeling was incomplete, as evidenced by active osteoblastic activity but early limited osteoclastic activity, with focal areas of cartilage and fibrous tissue showing new bone formation. All histologic samples were free of inflammation, neoplasia, or cellular atypia.

Good functional recovery was noted in all 6 patients with their return to near premorbid levels of activity. One patient (case 6), had a residual selective loss of bladder and bowel control and erectile failure but no other neural injury, which was secondary to a conus medullaris lesion, at the time of his initial injury.

DISCUSSION

Iliac crest autograft remains the "Gold Standard" graft material for spinal fusion, but it is not free, nor is its harvest, complication-free, as recently shown by Dhawan et al.[13] In this case series, BMA-H was found to be a viable alternative to autograft in posterior and posterolateral

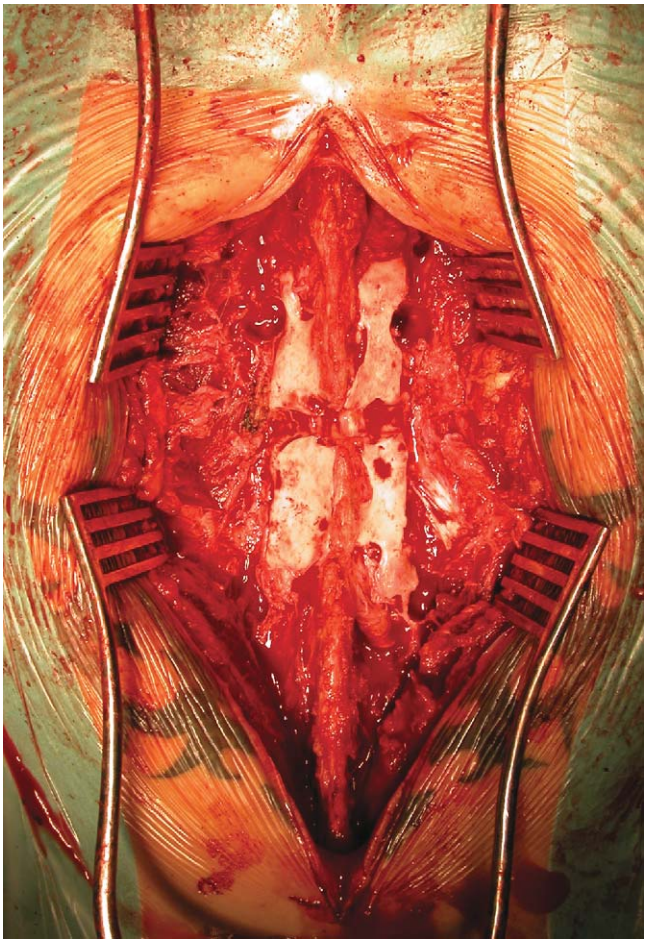

FIGURE 3. Photograph of the intraoperative appearance at T12/L1 (case no. 4). Both sides were implanted with BMA-H, 12 months prior. The fusion masses at T12/L1 adopted the shape of the implanted bone grafts. BMA-H indicates bone marrow aspirate with Healos.

thoracolumbar spinal fusions. Its use was not associated with any significant complications; specifically none of these 6 cases reported any type of morbidity from the bone marrow harvest site. This report complements previous published evidence supporting the use of Healos and BMA for spinal fusion in the posterior space.[7–9]

The debate over the efficacy of bone marrow for bone repair has prompted investigations into the cellular content and osteogenic potential of bone marrow cells, based on subject age and sex.[14] In our series, the average age was 35, so it can be hypothesized that the marrow aspirates obtained for this cohort were rich in osteoprogenitor cells, hence the high fusion rates observed herein.

In this study, biopsies were obtained between 11 and 24 months postoperatively. All histologic samples showed evidence of new, mature bone, with some active osteoblastic activity. One sample showed evidence of endochondral ossification (case no. 6). Residual graft material could not be seen, most likely due to complete remodeling of the graft into bone. This finding is consistent with that reported in a previous histologic analysis of Healos used as a bone graft. Faundez et al[10] described histologic findings for 4 cases at 15.3 months postoperative and found that all biopsies showed areas of remodeling and in some cases, foci of endochondral ossification, but no signs of residual graft material.

In our study, radiographic, macroscopic, and histologic evaluations provided similar fusion rates. On the basis of published literature, however, this 100% agreement may be due to our small sample size and may not be maintained with a larger patient population. Especially as, reported agreement rates between radiographic assessments and actual surgical findings only reach 69%.[11,12]

Several limitations in our study should be addressed. This report only includes a small sample size, with heterogeneous demographic and clinical profiles. We acknowledge that the age and fitness level of our patients, posed a low risk to fusion failure, and that the use of BMA-H in a more demanding environment may not be associated with a similar outcome. However, Neen et al[8] did show a rate of fusion in the posterolateral gutter using BMA-H, which indicates that the graft material will be effective in a wider selection of cases. In addition, to minimize the clinical impact of performing biopsies, collected samples were small and taken from specific locations along the posterior fusion masses that may or may not be representative of the entire new bone.

Despite its limitations, this report further confirms the results of prior studies that described the efficacy of Healos and autologous BMA used in bone graft sites, as an acceptable alternative to autograft in suitably selected patients.

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