Autologous Cell Concentrate from Bone Marrow Aspirate Combined with Demineralized Bone Matrix for Bone Grafting
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Introduction
Despite many alternatives, autologous bone is still considered the gold-standard grafting material [1]. Autografting has been reported for over a century with positive results in a variety of bone grafting applications [2-6]. Autograft bone has unique properties when compared to grafting alternatives due to the osteoblasts and osteogenic precursor cells present in autograft that can contribute to the formation of new bone [7]. Autograft also provides osteoconductive properties and osteoinductive factors that can be released during graft resorption [8-9]. Despite these positives, there are substantial risks associated with the procurement of autograft. Autograft procurement has been associated with significant donor-site morbidity [1,10,11]. Other complications include infection, prolonged drainage, hematomas, sensory loss, and subluxation [10-12]. It would be ideal to identify an autologous cellular source that retains osteogenic and osteoinductive properties while avoiding the complications associated with traditional autograft harvest.

Use of aspirated bone marrow from skeletal and long bones in bone grafting retains the osteogenic benefits of traditional autograft use, while avoiding the associated complications. Autologous bone grafts derive a large percentage of their osteogenic capacity from the bone marrow component, which is a well-documented source of progenitor cells capable of maturing down the osteoblastic lineage [13-18]. There are positive clinical reports for when bone marrow aspiration was used with xenograft [19], demineralized bone matrix [20-22], allograft [23], and ceramics [24-27]. Several studies have concluded that bone marrow-enriched graft provides results similar to those seen with autograft [21,23,25,28,29].

Recent works suggest that concentration of the cellular fraction of bone marrow aspirate may provide results superior to those obtainable using current bone-grafting techniques incorporating bone marrow aspirate. Connolly, et al [30] demonstrated a pronounced osteogenic response in an ectopic rabbit model using a concentrated cellular fraction of bone marrow aspirate. In a case series of 17 patients, Oyama, et al demonstrated alveolar bone regeneration with the application of a concentrated fraction of bone marrow aspirate with beta-tricalcium phosphate [31]. Hernigou et al, used centrifugation to obtain a concentrated cellular fraction from bone marrow aspirate. This fraction was injected percutaneously at nonunion sites and compared to a group injected with non-concentrated bone marrow. The patients where union was not obtained were seen to have received a statistically lower concentration and total number of progenitor cells [32].

The following case series provides examples of the use of concentrated bone marrow aspirate in various bone grafting procedures. A disposable system was used to concentrate the nucleated cell fraction of a bone marrow aspirate at the patient’s point-of-care. This concentrated cell fraction was then mixed with a freeze-dried DBM product and delivered to the graft site. All three cases resulted in a fully healed fracture with no adverse events related to the bone marrow aspiration technique.

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Methods and Surgical Technique

*Marrow Aspiration*- Bone marrow was obtained through needle aspiration of the iliac crest for each patient. Percutaneous aspiration was done through a stab incision utilizing a 10 gauge Jamshidi needle. Fifty ml of marrow was aspirated into a syringe containing 10ml of citrate anticoagulant. To minimize venous blood collection, the needle was repositioned within the iliac crest throughout the aspiration.

*Marrow Processing* - A nucleated cell concentrate (NCC) was obtained from the BMA using the MarrowStim Concentration System (Biomet Biologics Inc., Warsaw, IN). In brief, the BMA was centrifuged at 3200 RPM (#755VES, The Drucker Company, Philipsburg, PA) for 15 minutes in a dedicated disposable resulting in 6ml of NCC. This technique has been demonstrated to result in a NCC with 79% of the total nucleated cells from the bone marrow aspirate input (Figure 1) [33]. This cell population has been demonstrated to contain significant concentrations of hematopoietic, mesenchymal, and endothelial progenitor cells [34].

![Figure 1](image-url)  
*Figure 1*- The percent recovery of different cell populations in the NCC with the use of the MarrowStim device.

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*Grafting procedure*- The NCC obtained following processing of BMA was used to hydrate 5-10cc of a freeze-dried DBM product (Bonus DBM, Biomet Biologics, Inc.) [Figure 2]. The combined product was then packed into the bone defect following placement of internal fixation.

![Figure 2](image-url)

**Figure 2**- 10cc of Bonus® DBM being hydrated with 6cc of NCC
Results

Table I summarizes the series of 3 patients treated using NCC combined with DBM.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Comorbidities</th>
<th>Surgeon</th>
<th>Bone Involved/Procedure type</th>
<th>Time to Last Follow-up</th>
<th>Final Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>M</td>
<td>Smoker</td>
<td>JJI</td>
<td>Humerus/Initial repair of comminuted fracture</td>
<td>20 weeks</td>
<td>Fully healed fracture</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>M</td>
<td>None</td>
<td>BLV</td>
<td>Humerus/Initial repair of 3-part fracture with significant displacement</td>
<td>18 weeks</td>
<td>Fully healed fracture</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>M</td>
<td>Osteoarthritis</td>
<td>BLV</td>
<td>Tibia/Atrophic nonunion</td>
<td>35 weeks</td>
<td>Fully healed fracture</td>
</tr>
</tbody>
</table>

Table I-Patient Data

For International Use Only, this manuscript describes indications which may not be cleared by the FDA for use in the USA.
Patient 1

A 26-year-old male smoker presented with a fracture of the left proximal humerus with significant comminution [Figure 3A]. The humeral head had subluxed secondary to a nonfunctioning deltoid. The medial fragment, humerus and tuberosities were reduced and a plate was placed lateral to the fracture using lag screws, reduction pins and multiple locking screws to hold the plate in place. Ten cc of DBM hydrated with 6cc of NCC was then packed into the comminuted medial area. The follow-up visit at 6 weeks showed radiographic evidence of early healing. At the last follow-up, 20 weeks postoperative, the patient had full range of motion (ROM) of the elbow and rotation and flexion of the shoulder within 5° of the uninjured arm. Radiographs showed a fully healed fracture [Figure 3B].

Figure 3-A. X-ray at time of presentation showing fracture of the left proximal humerus with significant comminution. B. X-ray at final follow-up (20 weeks) showing fully healed fracture.

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Patient 2

A 50-year-old male presented with a 3-part fracture of the proximal area of the right humerus that had significantly displaced resulting in loss of function of the right arm [Figure 4A]. A proximal humeral fracture plate was placed along the posterior margin of the bicipital groove using screw fixation. Ten cc of DBM hydrated with 6cc of NCC was placed along the medial aspect of the fracture [Figure 4B]. At the seven week follow-up visit, the patient demonstrated full passive ROM, with active flexion of 85° and external rotation of 4° [Figure 4C]. At the last follow-up, 18 weeks postoperative, the patient had ROM of 110° in active flexion and 20° during external rotation. Radiographs showed a fully united fracture [Figure 4D].

Figure 4-A. X-ray at time of presentation showing a three-part proximal fracture of the humerus. B. Postoperative x-ray show placement of proximal humeral fracture plate. C. Image taken seven weeks postoperative. D. X-ray at final follow-up (18 weeks) showing fully healed fracture.

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Patient 3

A 56 year-old male presented with a non-union of the distal tibia and severe osteoarthritis related to a fracture that occurred one year prior [Figure 5A]. The talus had anteriorly subluxed preventing true anatomic fusion. The talus and the subtalar joint were decorticated and a guide rod was aligned with the calcaneus and the tibia. Proximal screws, a calcaneal screw and a single lateral to medial calcaneal screw were placed to fixate the joint in a proper alignment. 10cc of DBM was hydrated with 6cc of NCC and placed on the posterior aspect of the tibia at the fracture site of the subtalar and ankle joints. At the 21 week follow-up, the radiograph demonstrated good placement and alignment of the hardware and early evidence of consolidation at the fracture site [Figure 5B]. At the last follow-up, 35 weeks postoperative, radiographs showed a fully united fracture [Figure 5C].

Figure 5- A. X-ray at time of presentation showing a non union of a distal fracture of the tibia suffered one year prior. B. X-ray at 21 weeks postoperative showing early consolidation of tibial fracture. C. X-ray at final follow-up (35 weeks) showing union of tibial fracture.

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Discussion

The three cases presented went on to radiographic union. There were no complications involving the bone marrow aspiration and concentration technique. Since the centrifugation process was performed during the placement of the internal fixation, the only time added to the procedure was related to the aspiration of the bone marrow. In the surgeon’s experience, aspiration of 50cc of bone marrow typically took less than 5 minutes. Although for these cases the NCC was infused into a freeze-dried DBM product for delivery to the grafting sites, it could be delivered in a variety of grafting products.

The ability to concentrate nucleated cells from an autologous bone marrow aspiration at the point-of-care could prove to be a valuable bone grafting tool. The cells obtained in the concentrated fraction of bone marrow include various progenitor populations, including cell populations with the ability to mature down the osteoblastic lineage. A series of cases using this technique have resulted in favorable outcomes. There were no adverse events related to the marrow aspiration and concentration. This bone-grafting technique may provide results comparable to autografting procedures while reducing the risks, including donor-site morbidity that have been associated with autograft harvest. This novel bone-grafting solution merits further investigation.
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**References**