Novel Technology to Increase Concentrations of Stem and Progenitor Cells in Marrow Aspiration

David B Harrell, PhD, Brt, OF, FAARM, FRIPH, DABRM*
Joseph R Purita, MD, FAAOS, FACS+

*Chief Scientific Officer, Harrell BioScience Consulting, LLC; +Stem Cell Centers of America/Boca Raton Orthopaedic Group

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ABSTRACT

Use of centrifuged bone marrow aspirate for regenerative medicine is a growing practice. However, such centrifugation systems require aspirating large volumes (30-240 mL) in order to obtain sufficient stem/progenitor cellularity in a large enough post-centrifugation final volume for therapeutic administration. Presented here are the results of a series of 27 marrow aspirations using **Marrow Cellution™** (www.marrowcellution.com), a bone marrow access and retrieval device designed to increase the stem/progenitor cell concentrations from the aspirate. The samples were collected under field conditions from eight separate clinicians using three different independent laboratories. The quality of the marrow aspirate was determined by performing a CFU-f test to determine the number of osteo progenitor cells.[1] Stem cells capable of forming a CFU-f are routinely found in marrow but rarely in peripheral blood. Consequently, CFU-f represents the standard test to determine the number of immature stem and progenitor cells that are present in the aspirate.[1] Previous work done by a single clinician in a controlled setting demonstrated that **Marrow Cellution™** delivered superior regenerative potential (as measured by CFU-f counts) to existing BMAC (Bone Marrow Aspiration Concentration) systems.[2] This pilot study represents true field conditions as not all clinicians followed the exact same protocol with respect to heparin rinse, orientation (posterior or anterior) and volume of aspirate taken.

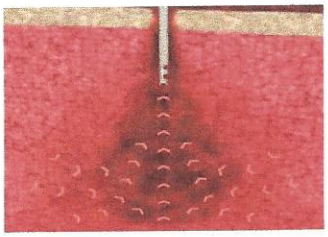
BACKGROUND

Industry often cites TNC (total nucleated cells) counts as a meaningful measure of the regenerative potential of a marrow-sourced biologic sample. TNC counts are less expensive and time-intensive to determine compared to counting osteoblast progenitor cells (as measured by CFU-f's - fibroblast-like colony-forming units). Peer reviewed literature however routinely cites CFU-f's rather than TNC's as the clinically relevant measure.[3-6] Academic studies have demonstrated a correlation between clinical outcomes and the the number of osteo-progenitor stem cells (as measured by CFU-f counts) and not TNC's.[3-6] TNC counts have limited clinical relevance because it includes nucleated red blood cells and white blood cells from peripheral blood that have reduced regenerative capability compared to marrow cells. This is especially true with biologic products that have been centrifuged because a nucleated cell from peripheral blood has the same density as a quiescent stem cell.[7-9] However, cycling progenitor stem cells have a greater density and are routinely discarded with the red cell component after centrifugation. Consequently, a centrifuge will concentrate peripheral blood nucleated cells preferentially over stem cells.

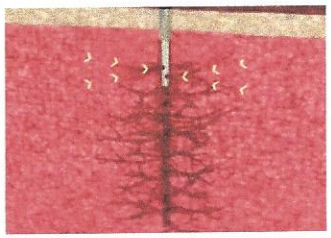
Traditional bone marrow aspiration needles were designed to aspirate 1-2 mL of marrow from a single location for diagnostic purposes.[1] When 1 mL of marrow is aspirated with a traditional needle, counts of 1451 CFU-f/mL are typical (40 x 10^6 TNC/mL).[1] When used to aspirate greater volumes that are typically required for regenerative therapies, traditional needle design results in excess peripheral blood infiltration due to basic fluid mechanics. Blood and marrow are non-Newtonian fluids and the traditional needle has a large open port at its distal end. As such it is known that peripheral blood infiltrates marrow aspirates greater than 1-2 mL when using a traditional needle due to the dramatically reduced viscosity of blood that fills the void in the medullary space that is in contact with the distal open ended lumen.

Using a traditional needle to aspirate volumes greater than 2 mL results in the initial small volume containing the most pure marrow.[10] Volume over 2 mL retrieved from a single site introduces peripheral blood into the aspiration. This peripheral blood dilutes further aspiration volume from the site and significantly reduces the stem/progenitor cell quantity of the aspiration.[1,11,12] Marrow aspiration volumes of greater than 2 mL using traditional needles typically contain only 200-300 CFU-f/mL (15-20 x 10^6 TNC/mL).[7,13] The lower viscosity of blood results in preferential aspiration of peripheral blood and a resultant precipitous decline in the stem/progenitor cells of the aspirate when larger volumes are drawn.[12,14,15] Moreover, traditional needles are technique-sensitive and not well matched to the requirement for larger aspiration volumes (60 mL) for the centrifuge to produce a final volume of 7-10 mL of autologous marrow-based therapies.[16]

Centrifuge-based systems are routinely used to overcome the limitations of lower-quality (reduced cellularity) marrow aspirations from traditional needles. These systems remove excess plasma and mature red cell count while recapturing a portion of nucleated cell content from both the marrow and the infiltrated peripheral blood components of the aspiration. These centrifuge volume reductions have become a common practice in many regenerative medicine procedures. Howev-



Traditional Needle



Marrow Cellution™

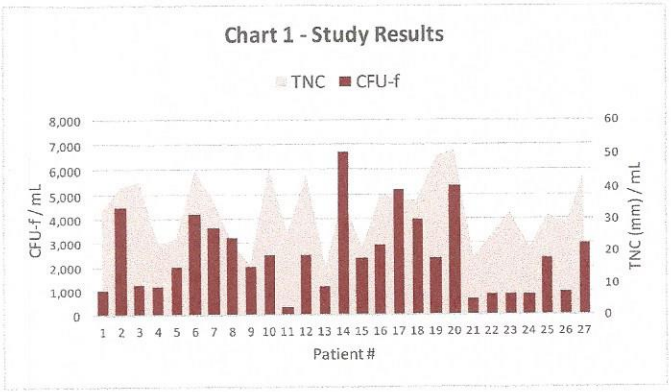
er, subsets of the nucleated cells obtained from the peripheral blood component of the aspirate may actually limit the success of procedures because nucleated cells derived from peripheral blood, rather than marrow, may stimulate an inflammatory response that can decrease the regenerative potential of the marrow-derived stem/progenitor cells.[17] More importantly, the inefficiencies of centrifuge-based systems, which have average recovery yields ranging from 32.5% to 65.2%, leads to a substantial discarding of cells in the final product.[7]

In this pilot study with **Marrow Cellution™** (Ranfac, Avon, MA), a novel bone marrow access and retrieval device co-developed by Endocellutions Corp (Marshfield, MA) and Ranfac Corp (Avon, MA), the limitations of traditional design aspiration needles and BMAC systems were substantially overcome. Flow into the aspiration system is collected laterally rather than from an open-ended cannula. This design allows for collection of marrow perpendicular to and around the channel created by the tip of the device, thus avoiding the aspiration of peripheral blood caused by the placement of the needle itself. Additionally, **Marrow Cellution™** incorporates technology to precisely reposition the retrieval system to a new location in the marrow after each 1 mL of aspiration. The effect of these two features is that multiple small volumes of high quality bone marrow aspiration are collected from a number of distributed sites within the marrow geography while also retaining clinicians' desire for a single entry point. The design enables a total volume of 8-20 mL of high quality biologic to be collected. In effect, a single puncture with **Marrow Cellution™** is functionally equivalent to repeated small aspirations (1 mL) from a number of puncture sites using traditional needles, but with substantial savings of time, effort, as well as reduced patient trauma and risk of infection.

The single-step **Marrow Cellution™** device produced the same (as counted by CFU-f's) stem/progenitor cell concentrations as a combination of traditional needles and industry-leading centrifugation systems. **Marrow Cellution™** allows the clinician to keep the product entirely on the sterile field rather than requiring the product to leave the sterile field for centrifugation. This further reduces time for the final product to be delivered to the patient (no centrifugation necessary), reduces procedural expenses, and retains all the cells and growth factors obtained in the aspiration.

Study Design

Informed consents were obtained from all patients for inclusion into the study according to ethical committee approval.



A series of 27 patients were seen by eight different clinicians and underwent marrow aspiration from the iliac crest with the **Marrow Cellution™** device using either a posterior (N=25) or anterior (N=2) orientation. A heparin rinse ranging from 500 to 2000 units/mL was used prior to aspiration. No additional heparin or anti-coagulant was used. Primary endpoints included fibroblast-like colony-forming units (CFU-f) and total nucleated cells (TNC).

Three of these patients had bilateral marrow aspiration using **Marrow Cellution™** from one iliac crest and using a traditional marrow aspiration needle the other iliac crest. The aspirations with the traditional needle were then centrifuged to produce a volume-reduced concentrate. Additionally, the aspiration volumes as well as the total volumes of the final product (aspirate for **Marrow Cellution™**; post-centrifugation for BMAC) were recorded. Descriptive statistics were used for the aspirates produced by **Marrow Cellution™**, the traditional needles, and the traditional needle/centrifuge combinations. Moreover, published literature were used to ascertain historical values for CFU-f counts from various centrifuge-based systems and compared with the aspirates produced by **Marrow Cellution™**. Finally, clinician reported estimates were gathered to determine relative preference for **Marrow Cellution™**, a traditional needle alone, or a traditional needle with centrifugation.

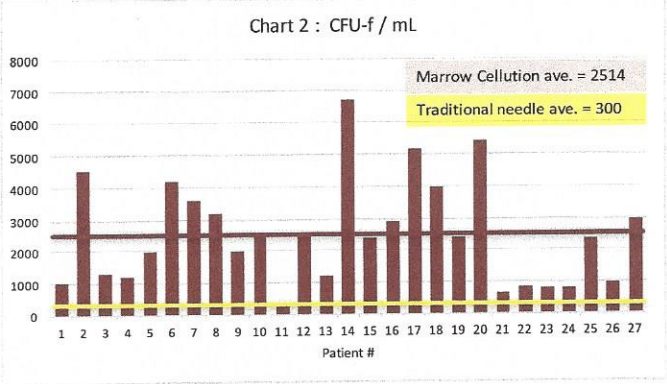
Results

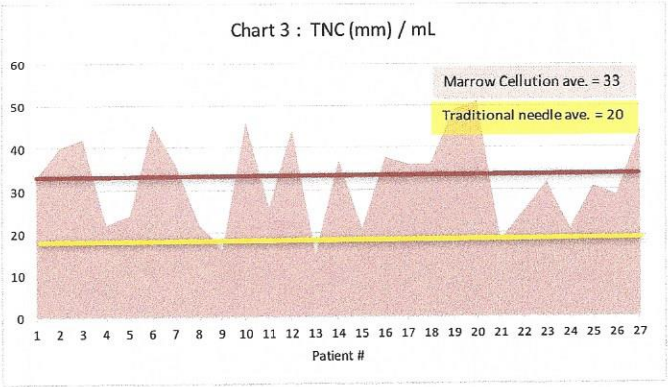
In 27 patients, 8-14 mL of marrow was collected from one iliac crest using the **Marrow Cellution™** device (aspirating from various marrow geographies from a single puncture site). Each sample was analyzed for CFU-f and TNC counts. Results for all 27 patients are depicted in Chart 1.

The average CFU-f count using **Marrow Cellution™** was 2514 (Chart 2) as compared to 200-300 CFU-f/mL using traditional needle technology.[7,13] The average TNC in the study was 33 x 10^6 TNC/mL (Chart 3) as compared to 15-20 x 10^6 TNC/mL using traditional needle technology.[7,13]

Marrow Cellution™ vs. traditional needle aspiration

In 3 patients, 8-20 mL of marrow was collected from one iliac crest using **Marrow Cellution™** (aspirating from various marrow geographies from a single puncture site); in the opposite iliac crest, 60-100 mL of marrow was collected using a





single puncture with a traditional needle. The larger volume was collected to reflect that this material is the substrate for subsequent volume reduction following centrifugation in such systems (e.g., BMAC). Two procedures used anterior entry and one used posterior. One clinician operated on two patients; and a second clinician operated on one patient. Samples of 0.5-1 mL were sent for laboratory analysis. Comparison of TNC (Chart 4) and CD34+ (Chart 5) cells were compared between **Marrow Cellution™** and the traditional needle to determine if there was a significant advantage between the two designs. With patient number 4, flow cytometry was also performed for CD34+ cells in the volume-reduced BMAC concentrate (0.140 x 10^6/mL) and was comparable to **Marrow Cellution™** (0.137 x 10^6/mL).

In three separate patients, **Marrow Cellution™** was used to collect a total of 8-10 mL of marrow aspirate. Two different clinicians performed the procedure; one surgeon used posterior access to the iliac crest, while one surgeon used anterior access. In these samples, both TNC (Chart 6) and CFU-f (Chart 7) were determined. These values were compared with published TNC and CFU-f counts from a traditional needle used to aspirate either 1 or 8 mL of marrow. The traditional needle had a significant decline in the number of stem cells aspirated per mL as the volume increased from 1 mL to 8 mL. By minimizing peripheral blood, **Marrow Cellution™** had similar number of stem cells per mL in 8 mL as the 1 mL sample from the traditional needle.

Marrow Cellution™ vs. centrifuged-based systems

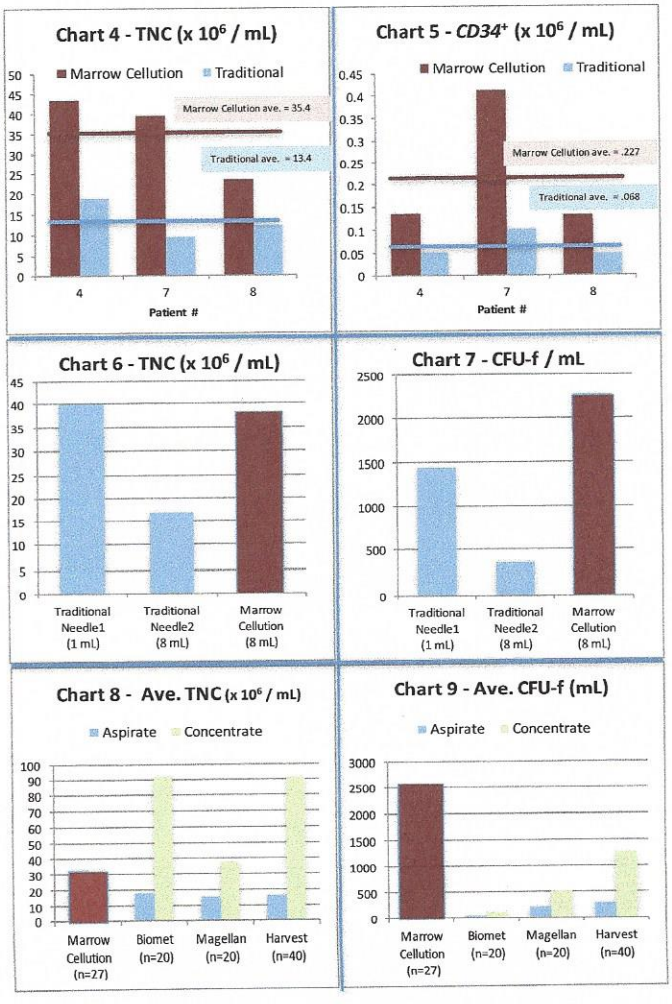
The average **Marrow Cellution™** CFU-f and TNC counts from this pilot study are compared to the average counts reported from leading centrifuged-based systems[7,16] in Charts 8 & 9.

Clinician comments on marrow aspiration technologies

Users of **Marrow Cellution™** reported that one significant advantage of the device is the ability to advance into and retreat from the marrow space in a controlled and precise manner. Along with the ability to aspirate more uniformly across the marrow geography, the **Marrow Cellution™** device produced a higher quality aspirate with the need to draw only the volume needed for the regenerative medicine treatment procedure. The clinicians also noted an improved safety profile, as the material produced does not need to leave the sterile field; in contrast, centrifuge-based technologies must leave the sterile field. Additionally, it was anticipated that substantial efficiency and cost savings would be obtained due to requiring less operating room time to prepare the marrow for use, and by eliminating the need for any specialized training beyond marrow aspiration.

Discussion

This study investigated a method to obtain equivalent stem/progenitor cells with less aspiration volume than centrifuge-based bone marrow aspirate concentrate. The **Marrow Cellution™** device provided a high quality bone marrow aspiration with reduced time and expense. The lower volume of bone marrow aspiration required can also be less traumatic on the patient and because the product remains entirely on the sterile field, risk of infection is also reduced. Our comparison



study used BMAC because of previous studies that demonstrated that BMAC produced the highest concentrations of CFU-f and CD34+ cells than other centrifuge-based systems.[7]

Conclusion

In this pilot study, the **Marrow Cellution™** device produced results suggesting that it can effectively replace aspiration of large volumes of marrow using traditional needles combined with the volume reduction of centrifuge-based systems. Traditional technologies typically discard 35-65% of cells and growth factors when reduced in centrifuge-based systems through the separation into the supernatant. These cells and growth factors are not discarded in the **Marrow Cellution™** device.

Marrow Cellution™ has a number of distinct procedural advantages: (1) the biologic produced by the device never leaves the sterile field; (2) the device requires minimal O.R. staff support and time; (3) the entire sample generated is used; (4) the device minimizes peripheral blood contamination; (5) the device requires minimal anti-coagulation; (6) the biologic does not require filtering, and (7) the design automatically repositions the aspiration cannula and aspirates from side ports across a greater geography of the marrow space so that it mimics multiple puncture sites with 1 mL aspirations. We were able to demonstrate that **Marrow Cellution™** was successful in obtaining CFU-f and TNC counts similar to what is expected from numerous insertion points along the iliac crest for multiple 1 mL-only draws; however, with **Marrow Cellution™**, only one insertion point was required.

In summary, the results documented herein from true field conditions were less than Scarpone achieved in the controlled study[2], nevertheless this pilot study clearly demonstrated superior results to previously published results from multiple centrifuged-based systems. This further suggests that the **Marrow Cellution™** device could provide even better results than BMAC alternatives as clinicians become more familiar and proficient with the device.

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