MARROW CELLUTION™ INCREASES CONCENTRATIONS OF STEM AND PROGENITOR CELLS IN MARROW ASPIRATION

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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 cell numbers.2 The quality of the marrow aspirate was determined by performing a CFU-f assay on 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. Stem cells capable of forming a CFU-f test are routinely found in bone 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.2 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 systems.3-6 The single-step Marrow Cellution™ device produced the same (as counted by CFU-f) 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 the cells and growth factors obtained in the aspiration.

BACKGROUND

Industry often cites TNC (total nucleated cell) 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). Colony-forming units (CFU-f) are a gold standard in regenerative medicine.19

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 million TNC/mL). 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.9 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.19-21 Marrow aspiration volumes of greater than 2 mL using traditional needles typically contain only 200-300 CFU-f/mL (15–20 million TNC/mL).1,21 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.22-24 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.18

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 (Figures 1 and 2). 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 reposist 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.

Figure 1. Marrow Cellution™ Flow Closed tip maximizes stem and progenitor cell recovery while minimizing infiltration of peripheral blood.

Figure 2. Traditional Needle Flow Open end tip allows infiltration of peripheral blood.
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).

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

Marrow Cellution™ vs. traditional needle aspiration

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. The average TNC in the study was 33 million TNC/mL (Chart 3) as compared to 15-20 million TNC/mL using traditional needle technology.
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 in Charts 4 & 5.

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.1

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; 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.

INDICATIONS

The Marrow Cellution™ Bone Marrow Aspiration Needle is intended for use for aspiration of bone marrow or autologous blood using a standard piston Syringe.

CONTRAINdications

Use only for bone marrow or autologous blood aspiration as determined by a licensed physician. The device is intended to be used by a physician familiar with the possible side effects, typical findings, limitations, indications and contraindications of bone marrow aspiration. The procedure should be performed on patients that are suitable for such procedure only.
REFERENCES


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