Novel Technology to Increase 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/progenitor cellularity in a large enough post-centrifugation final volume for therapeutic administration. It is well known that the highest quality (highest quantity of stem/progenitor cells) bone marrow aspirations require aspirating small volumes of bone marrow (1-2 mL). In this study, it was hypothesized that the need for centrifugation, and consequent volume reduction, was due to the limitations of the traditional bone marrow aspiration needle.

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 bone marrow aspirates greater than 1-2 mL. In this pilot study with Marrow Cellution™ (Ranfac, Avon, MA), a novel bone marrow access and retrieval device requiring substantially less bone marrow aspirate, the limitations of standard bone marrow aspiration needles (e.g., reduced stem/progenitors cell concentrations due to dilution with peripheral blood) were substantially overcome. Further, the single-step Marrow Cellution produced the same (as counted by $CD34^+$ cells) or greater (as counted by fibroblast-like colony-forming units, CFU-f) stem/progenitor cell concentrations as a combination of traditional needles and centrifugation with the SmartPReP 2 Bone Marrow Aspirate Concentrate® (BMAC) centrifuge-based

cellular processing system (Harvest Technologies®, Plymouth, MA). In addition, because there is reduced peripheral blood infiltration in bone marrow harvesting, Marrow Cellution allows the clinician to keep the product entirely on the sterile field rather than requiring the product the leave the sterile field for centrifugation and re-enter the sterile field for administration in the patient, 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.

BACKGROUND

The design of a traditional bone marrow aspiration needle has a removable stylet and a hollow cannula; such needle designs are decades old and were designed to aspirate 1 mL of marrow from a single location for diagnostic purposes.[1] Using a traditional needle to aspirate volumes greater than 2 mL (for which it was not designed) results in the initial small volume containing the most pure marrow.[2] Traditional bone marrow aspiration needles operate most optimally when aspiration volumes of approximately 1 to 2 mL are obtained.[1] Volume over this 2 mL, retrieved from a single site, introduces peripheral blood into the aspiration due to nearby ruptured capillaries. This peripheral blood dilutes further aspiration volume from the aspiration site and significantly reduces the stem/progenitor cell quantity of the aspiration.[1,3,4] Marrow aspiration volumes of greater than 2 mL using traditional needles typically contain total nucleated cell (TNC) counts of 15-20 x 10^6/mL and 200-300 CFU-f/mL;[5,6] however, when 1 mL of marrow is aspirated with a tradition needle, counts of 40 x10^6/mL TNC and 1451 CFU-f/mL are typical.[1] It is well known that peripheral blood has a dramatically reduced viscosity compared to bone marrow; 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 aspiration volumes are drawn.[4,7,8] 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.[9]

To overcome the limitations of lower-quality (reduced cellularity) marrow aspirations from traditional needles, aspirates are enhanced using a centrifuge-based system (e.g., BMAC). 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, in a volume compatible with tissue regeneration therapies. These centrifuge volume reductions have become a common practice in many regenerative medicine procedures. However, 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.[10] Additionally, 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.[5]

Marrow Cellution is a novel bone marrow access and retrieval device, co-developed by Endocellutions Corp (Marshfield, MA) and Ranfac Corp (Avon, MA), that incorporates features designed to minimize the limitations of traditional needles. 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; traditional needles aspirate through an open-ended cannula that aspirate peripheral blood caused by ruptured vessels during 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 volume 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 minimizes peripheral blood infiltration and enables a total volume of 8-20 mL 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.

STUDY DESIGN

Informed consents were obtained from all patients for inclusion into the study according to ethical committee approval. A series of nine consecutive patients from three clinical sites with different physician operators underwent marrow aspiration from the iliac crest with the Marrow Cellution device with either posterior (N=7) or anterior (N=2) entry into the iliac crest. Three of these patients had bilateral marrow aspiration with one iliac crest for Marrow Cellution and the other iliac crest for the traditional marrow aspiration needle; these aspirations with the traditional needle were then centrifuged to produce a volume-reduced concentrate. Primary endpoints included total nucleated cell (TNC), fibroblast-like colony-forming unit (CFU-f), and $CD34^+$ cell concentration. 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, traditional needle alone, or traditional needle with centrifugation.

RESULTS

<u>Comparison of Marrow Cellution to traditional needle aspiration</u>: 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 for all analyses. Comparison of TNC and $CD34^+$ cells were compared between Marrow Cellution and the traditional needle to determine if there was a significant advantage between the two designs (Table 1). 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)

Table 1. TNC and $CD34^+$ concentrations (x10^6/mL) using either Marrow Cellution or Traditional Needle.

Patient ID	Cell Type	Marrow Cellution	Traditional	Ratio (Marrow Cellution:Traditional)
4	TNC	43.5	18.7	2.32
	$CD34^+$	0.137	0.053	2.58
7	TNC	39.4	9.5	4.14
	$CD34^+$	0.412	0.102	4.03
8	TNC	23.3	12.1	1.92
	$CD34^+$	0.133	0.048	2.77
Average	**TNC**	**35.4**	**13.4**	**2.6**
	CD34+	**0.227**	**0.068**	**3.4**

In three separate patients, Marrow Cellution was used to collect a total of 8-10mL 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 and CFU-f 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.

Table 2. TNC (x10^6/mL) and CFU-f (/mL) concentrations using either Marrow Cellution or Traditional Needle.

	Volume	CFU-f	TNC
Traditional Needle[1]	1 mL	1451	40
Traditional Needle[11]	8 mL	356	17
Marrow Cellution (N=3)	8 mL	2275	38

Comparison of Marrow Cellution to Centrifuge-Based Technologies. In 5 patients, 8 mL of aspirate was collected from one iliac crest using Marrow Cellution;

60 mL of aspirate was collected from the opposite iliac crest using a traditional needle. All procedures used anterior entry and were performed by the same clinician. Following aspiration with the traditional needle, the BMAC system was used as per the manufacturer-supplied protocol to reduce the volume to 7 mL or 10 mL.

Table 3. TNC (x10^6/mL) concentrations of aspirations from Marrow Cellution, traditional needle, and traditional needle plus centrifugation.

Patient ID	Marrow Cellution	Traditional Needle	BMAC (post-centrifugation)	Volume (post-centrifugation)
4	43.5	18.7	69.3	10
5	30.8	8.6	29.9	10
6	86.1	21.4	126.8	7
7	35.6	9.8	50	7
9	29.6	10.6	28.4	10
Average	**45.1**	**13.8**	**60.9**	**8.8**

Comparison of Marrow Cellution TNC and CFU-f to historical data. In three patients, Marrow Cellution was used to collect 8-10 mL of marrow aspirate. Both TNC and CFU-f were determined for these samples (Table 4). The average Marrow Cellution values along with the average values of different centrifuge-based systems[5,9] were compared (Table 5).

Table 4. TNC (x10^6/mL) and CFU-f/mL in three patients treated with Marrow Cellution

Patient ID	TNC	CFU-f
1	33	1040
2	40	4513
3	42	1273
Average	**38**	**2275**

Table 5. Average TNC (x10^6/mL) and CFU-f (per mL) in three patients treated with Marrow Cellution and published values for various centrifuge-based systems.

Device	Avg TNC	Avg CFU-f
Marrow Cellution	38	2275
Biomet	Aspirate: 18 Concentrate: 92	Aspirate: 54 Concentrate: 134
Arteriocyte Magellan	Aspirate: 16 Concentrate: 38	Aspirate: 223 Concentrate: 514
Harvest BMAC	Aspirate: 17 Concentrate: 91	Aspirate: 303 Concentrate: 1270

Clinician reported comments on marrow aspiration technologies. Users of Marrow Cellution reported that one significant advantage of Marrow Cellution is the ability to advance into and retreat from the marrow space in both a controlled and a 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 aspirate only the volume needed for the regenerative medicine treatment procedures. 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 then centrifuge-based bone marrow aspirate concentrate. The Marrow Cellution device provided a high quality bone marrow aspiration with reduced time and expenses. 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.[5]

This pilot study does have limitations. First, while suitable for a pilot study, the sample size is small. Future studies utilizing Marrow Cellution should incorporate a larger sample size. However, even with out small sample size, Marrow Cellution consistently produced equivalent $CD34^+$ cell counts and higher CFU-f cell counts than BMAC. Second, while the numbers of stem/progenitor cells have been associated with either regeneration and healing or lack thereof,[12,13] future studies should include patient follow-up. Third, while the time to centrifuge is eliminated (16-20 minutes), an accurate measure of time difference between Marrow Cellution and a traditional needle with centrifugation should be calculated.

CONCLUSION

There are several benefits of the Marrow Cellution novel design. First, the design automatically repositions the aspiration cannula and only aspirates from side ports across a greater geography of the marrow space so that it mimics multiple puncture sites with 1 mL aspirations. The number of TNCs and CFU-f was greater than traditional aspirations of similar volumes and was comparable or greater than centrifuge-based final products. 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. Secondly, Marrow Cellution allows the clinician to retain the product on the sterile field. Centrifuge-based systems require the bone

marrow aspiration to leave the sterile field for centrifugation and the final product to re-enter the sterile field after centrifugation and product withdrawal. The ability to keep the product on the sterile field reduces the risk of infection to the patient undergoing the procedure. Thirdly, cells and growth factors reduced in centrifuge-based systems through the separation into the supernatant. This accounts for the yields of 35-65% in such systems. These cells and growth factors are not discarded in the Marrow Cellution device. Finally, the Marrow Cellution device is anticipated to result in significant cost savings, not only due to the reduced time of the procedure, but also because no specially-designed disposable and centrifuge are necessary for the final product used for treatment. Marrow Cellution allows the clinician to aspirate bone marrow and immediately use the product for treatment to facilitate the surgical needs.

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