Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells

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ABSTRACT

It is well known that the highest quality bone marrow aspirations (greatest quantity of stem/progenitor cells) require aspirating small volumes of bone marrow (1-2 mL) from different locations.[1-4] It is also known that peripheral blood infiltrates bone marrow aspirates when greater than 1-2 mL is drawn from any single location.[1-3] In this pilot study of Marrow Cellution™ (www.marrowcellution.com), a novel bone marrow access and retrieval device, greater stem/progenitor cell concentrations (as counted by fibroblast-like colony-forming units, CFU-f) were demonstrated compared to previously published works that used a combination of a traditional needle with a centrifuge-based cellular processing system. A CFU-f test was conducted to determine the quality of the marrow aspirate because cells capable of forming a CFU-f are routinely found in marrow but rarely in peripheral blood.[1,4-6] Consequently, CFU-f represents a standard test to determine the number of immature stem and progenitor cells that are present in the aspirate. A comparison of Marrow Cellution CFU-f data collected in this study to historical CFU-f data from traditional needle plus centrifugation systems are found in Table 1.

Table 1: Comparison of CFU-f mean values

	Volume (mL)	CFU-f per mL	CFU-f Total in Graft
Marrow Cellution	11	3,290	37,230
Celling[7]	7	2,713	18,991
Harvest[5]	7	1,270	8,890
Magellan[5]	7	514	3,598
Biomet[5]	7	134	938

BACKGROUND

Stem and progenitor cells are enriched in the spongy marrow that is located within the pockets created by the honeycomb of trabecular bone within the medullary space. Only a finite number of stem cells reside within any given pocket

of spongy marrow. Volume over 1 mL retrieved from a single site introduces significant peripheral blood into the aspiration.[1] This peripheral blood dilutes further aspiration volume from any given site and significantly reduces the stem/progenitor cell quantity of the aspiration per mL.[1,3,4] It is well known that peripheral blood has a dramatically reduced viscosity compared to bone marrow.[8] In response to vacuum pressure, lower viscous fluid flows preferentially compared to higher viscous fluids. The channel created by the needle upon its re-traction from the marrow space will fill immediately with peripheral blood. Modifying the aspiration technique by re-positioning a traditional needle via retraction from the marrow space and aspirating through the open lumen results in preferential aspiration of peripheral blood and a resultant precipitous decline in the stem/progenitor cells of the aspirate, per mL.[4,8,9] This decline is because the channel created by the needle fills with blood upon its retraction and the lower viscous blood enters through the large lumen at the distal end of the needle, limiting the flow that comes through the side ports of the needle.[10,11]

The design of a traditional marrow aspiration needle that has a removable stylet and hollow cannula is decades old and was designed to aspirate 1mL of marrow from a single location for diagnostic purposes. Marrow aspiration volumes of greater than 2 mL at any one site using traditional needles typically contain total nucleated cell (TNC) counts of 15-20 x 10^6/mL and 200-300 CFU-f/mL;[5,12,13] 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]

To overcome the limitations of lower-quality (reduced cellularity) high volume marrow aspirations from traditional needles, clinicians attempt to enhance the marrow biologic by using a centrifuge-based system (e.g., BMAC). These systems remove 85% of the starting aspirate volume by discarding lower density plasma and higher density cells comprised primarily of red cells while retaining a majority of the platelets, lymphocytes and monocytes, granulocytes and young red cells from both the marrow and the infiltrated peripheral blood components of the aspiration. These systems do not distinguish between nucleated cells from the peripheral blood component of the aspirate compared to the marrow component of the aspirate, (both sets of cells have the same density). In the case of a poor aspirate comprised primarily of peripheral blood, the only difference between the biologic that a PRP kit produces compared to what a BMAC kit produces is that the BMAC kit has a higher red cell content and more granulocytes. The higher red cell and granulocyte content is because the BMAC protocol captures a higher density range of cells. Higher granulocytes in certain situations can result in greater inflammation.[14]

Marrow Cellution is a novel bone marrow access and retrieval device, co-developed by Endocellutions Corp (475 School Street, suite 12, Marshfield MA) and Ranfac Corp, (30 Doherty Ave. Avon MA) that incorporates features designed to minimize the limitations of traditional needles. Flow into the aspiration system is collected mainly laterally because the tip of the aspiration cannula is closed. This design allows for collection of marrow perpendicular to and around the channel created by the tip of the device; traditional needles, even ones with side ports, aspirate primarily through an open-ended cannula which leads to excess peripheral blood in the aspirate.[10] 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 effects of these two features are 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 10 mL to be collected. In effect, a single puncture with Marrow Cellution appears to be functionally equivalent to repeated small aspirations (1 mL) from a number of puncture sites using traditional needles, but with substantial savings of time, effort, and reduced patient trauma and risk of infection.

STUDY DESIGN

A series of five patients were seen by the same clinician and laboratory and underwent marrow aspiration from the iliac crest with the Marrow Cellution device using a posterior orientation. A 2000 unit per mL heparin rinse was used prior to aspiration. No additional heparin or anti-coagulant was used as the biologic was used within a short period of time from collection and was not administered systemically. Primary endpoints included total nucleated cell (TNC) and fibroblast-like colony-forming unit (CFU-f). 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.

RESULTS

In 5 patients, 10-14 mL of marrow was collected from one iliac crest using Marrow Cellution (aspirating from various marrow geographies from a single puncture site). Each sample was analyzed for TNC and CFU-f; these data are shown in Table 2.

Table 2. TNC and CFU-f values obtained with Marrow Cellution

Patient	Aspirate Volume Per mL	TNC per mL (millions)	CFU-f per mL	Total CFU-f in Graft
1	10	45	4,222	42,220
2	10	31	3,400	34,000
3	10	22	3,000	30,000
4	14	45	3,050	42,700
5	10	44	2,780	27,800
Average	**11**	**37**	**3,290**	**35,344**

DISCUSSION

Centrifuge systems discard 85% of the aspirate by removing lower density plasma and higher density cells composed primarily of red cells while retaining 15%

of the starting volume that contains a majority of the platelets, lymphocytes, monocytes, granulocytes and young red cells. However, within the discarded higher density red cells are a great number of very potent, cycling, high-density, proliferating progenitor cells. These cells increase in density as they build up nucleic mass prior to division and are always found in the red cell component after centrifugation and consequently, are discarded by all centrifuge protocols.[15,16] In addition, many protocols require filtering of the marrow prior to centrifugation. Filtering removes cell aggregates and clots that contain many stem cells.[7,16,17]

The rationale for centrifugation protocols based on aspirating large volumes of marrow is challenged by the above data set. Centrifugation protocols 1) require larger aspiration volumes that are associated with excess peripheral blood 2) have inherent inefficiencies that leaves significant numbers (approximately 40%) of stem cells behind in the discarded red cell portion of the processed marrow 3) require at least 10% dilution by volume for the addition of anti-coagulant to allow the sample to separate 4) and require another 10% dilution in the form of a neutralizing agent such as thrombin and calcium chloride in order for the marrow to clot in the graft. Finally, centrifugation protocols require the marrow to be filtered prior to centrifugation. Cells bound within a clot cannot be counted but they can be delivered to the patient when mixed with graft material or injected. This is not the case when clots are filtered out prior to centrifugation. This sentiment is best summarized by Muschler et al who concluded "A larger-volume of aspirate (more than 2mL) from a given site is contraindicated with the additional volume contributing little to the overall number of bone-marrow cells and results principally in unnecessary blood loss" (p 1707).[1] At the clinician's discretion, adding additional heparin to the aspiration syringe to keep the sample stable for an extended period of time outside the body is possible.

Despite demonstrating higher CFU-f counts from Marrow Cellution needles, there are a number of caveats associated with this pilot study. First, while suitable for a pilot study, the sample size is small. Future studies utilizing Marrow Cellution should incorporate a larger sample size. Second, while higher numbers of stem/progenitor cells have been associated with regeneration and healing,[7,15,18,19] future studies should include patient follow-up. Third, comparison to historical values of CFU-f data has limitations given the significant patient-to-patient variability.

Marrow Cellution System has advantages over centrifugation devices; the biologic produced by the System never leaves the sterile field, the System requires less O.R. staff support and time, the entire sample generated by the System is used, the System minimizes peripheral blood contamination, the System requires minimal anti-coagulation, and the biologic does not require filtering. We were able to demonstrate that Marrow Cellution was successful in obtaining TNC and CFU-f 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. While this pilot study was not designed to be an equivalence study, the comparison of the CFU-f data to previously published results from multiple centrifuged-based systems is intriguing and suggests that Marrow Cellution

could provide at least as many CFU-f, if not more, than traditional needles and centrifugation systems (Table 1).

CONCLUSION

There are several benefits of the Marrow Cellution novel design. First, 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. The system does not require filtering or 10% dilution with anti-coagulant. 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 process and product entirely on the sterile field. Centrifuge-based systems require the bone marrow aspiration to leave the sterile field for centrifugation and the final product then re-enters the sterile field after centrifugation and product withdrawal. The ability to keep the product on the sterile field reduces risk of infection to the patient undergoing the procedure. Thirdly, cells and growth factors are reduced in centrifuge-based systems through filtration and discarded material. This accounts for the yields of 35-65% in such systems. These cells and growth factors are not discarded in the Marrow Cellution device.

Our testing was performed independently without third party commercial sponsorship or influence.

REFERENCES

1. Muschler GF, Boehm C, Easley K. Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. J Bone Joint Surg Am 1997;79:1699-709.
2. Batinic D, Marusic M, Pavletic Z, et al. Relationship between differing volumes of bone marrow aspirates and their cellular composition. Bone marrow transplantation 1990;6:103-7.
3. Bacigalupo A, Tong J, Podesta M, et al. Bone marrow harvest for marrow transplantation: effect of multiple small (2 ml) or large (20 ml) aspirates. Bone marrow transplantation 1992;9:467-70.
4. Hernigou P, Homma Y, Flouzat Lachaniette CH, et al. Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. International orthopaedics 2013;37:2279-87.
5. Hegde V, Shonuga O, Ellis S, et al. A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. Journal of orthopaedic trauma 2014;28:591-8.

6. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005;87:1430-7.
7. Pettine KA, Murphy MB, Suzuki RK, Sand TT. Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. Stem Cells 2015;33:146-56.
8. Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. Annals of biomedical engineering 2008;36:1978-91.
9. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nature reviews Immunology 2006;6:93-106.
10. Tanikawa S, Sakamaki H, Mori S, et al. [Relationship between the presence of side-holes in bone marrow aspiration needle and the number of harvested bone marrow mononuclear cells]. [Rinsho ketsueki] The Japanese journal of clinical hematology 1997;38:1249-53.
11. Lannert H, Able T, Becker S, et al. Optimizing BM harvesting from normal adult donors. Bone marrow transplantation 2008;42:443-7.
12. Castro-Malaspina H, Ebell W, Wang S. Human bone marrow fibroblast colony-forming units (CFU-F). Progress in clinical and biological research 1984;154:209-36.
13. Marx RE, Harrell DB. Translational research: The CD34+ cell is crucial for large-volume bone regeneration from the milieu of bone marrow progenitor cells in craniomandibular reconstruction. Oral Craniofac Tissue Eng 2012;2:263-71.
14. Dragoo JL, Braun HJ, Durham JL, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. The American journal of sports medicine 2012;40:1274-81.
15. Juopperi TA, Schuler W, Yuan X, Collector MI, Dang CV, Sharkis SJ. Isolation of bone marrow-derived stem cells using density-gradient separation. Experimental hematology 2007;35:335-41.
16. Bhartiya D, Shaikh A, Nagvenkar P, et al. Very small embryonic-like stem cells with maximum regenerative potential get discarded during cord blood banking and bone marrow processing for autologous stem cell therapy. Stem cells and development 2012;21:1-6.
17. Ahmadbeigi N, Soleimani M, Babaeijandaghi F, et al. The aggregate nature of human mesenchymal stromal cells in native bone marrow. Cytotherapy 2012;14:917-24.
18. Hernigou P, Flouzat Lachaniette CH, Delambre J, et al. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. International orthopaedics 2014;38:1811-8.
19. Hernigou P, Beaujean F. Treatment of osteonecrosis with autologous bone marrow grafting. Clin Orthop Relat Res 2002:14-23.