Marrow Cellution:
The Impact of Volume on Related Cell Counts Using The Marrow Cellution Bone Marrow Aspiration System

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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 and that significant peripheral blood infiltration occurs with larger volume bone marrow aspirates. [1-4] In this pilot study of Marrow Cellution ™ (MC) (www.ranfac.com/marrowcellution), we investigated whether using the MC device and reducing the volume of aspirate per location from 1 mL to ½ mL across a similar trajectory would improve the stem/progenitor cell concentrations (as counted by fibroblast-like colony-forming units, CFU-f and the CD 34 marker). These tests represent a standard to determine the number of immature stem and progenitor cells that are present in the aspirate. 1,[4-6] Table 1 shows a comparison of CFU-f and CD 34+ data collected in this study using the MC device compared to historical data previously published by us using the MC device and the 1 mL technique. In addition, it contains CFU-f data for autograft that was previously published by others.

Table 1:

Device	TNC	CFU	CD34+	Volume	Ref
MC	43.4 x 10^6/ml	5653/ml	441,000 cells/ml	4 mL	
MC	36.5 x 10^6/ml	2263/ml	237,000 cells/ml	8-10 mL	20
Autograft	55.7 x 10^6/ml	4564/ml	Not measured	0.7 mL	21

BACKGROUND

Greater volumes retrieved from a single site introduces significant peripheral blood into the aspiration.[1] This peripheral blood 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 such as blood flows preferentially compared to higher viscous fluids such as marrow.[4,8,9,10,11] Higher granulocytes from peripheral blood in certain situations can result in greater inflammation. [14]The design of a traditional marrow aspiration needle has a removable stylet and hollow cannula with an open lumen that may have side holes and is used primarily 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 x106/mL TNC and 1451 CFU-f/mL are typical. [1] Flow into the MC aspiration system is collected mainly laterally because the tip of the aspiration cannula is closed.

Our previous work demonstrated the MC design that allows for collection of marrow perpendicular to and around the channel created by the tip of the device combined with technology to precisely reposition the retrieval system to a new location in the marrow after each 1 mL of aspiration significantly improved the cellularity of the aspirate. Stem and progenitor cells are enriched in the spongy marrow that is located within the pockets created by the honeycomb-like trabecular bone within the medullary space. Only a finite number of stem cells reside within any given pocket of spongy marrow while certain cell populations that are enriched for CFU-fs appear to reside on the inner surface of bones. (21) These areas are targeted by the side ports that promote lateral flow of marrow into the needle. In this study, we wanted to determine if the effects of these two features of the MC device could be improved by reducing the multiple small volume aspirations that are collected from the distributed sites within the marrow geography from 1 mL to a ½ mL.

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 MC system using a posterior orientation. A 2000 unit per mL heparin rinse of the cannula 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), fibroblast-like colony-forming units (CFU-f), and CD34_+ cell counts using the ISHAGE protocol. Published literature were used to ascertain historical values for CFU-f and CD 34+ counts.

RESULTS

In 5 patients, 3.5-6.0 mls of marrow was collected from one iliac crest using the MC system and multiple 0.5 ml aspirations (aspirating from various marrow geographies from a single puncture site). Each sample was analyzed for TNC, CFU-f, and CD34+_cell counts; these data are shown in Table 2.

Patient	TNCC	FU	CD34+	Aspirate Volume
1	54.0 x 10^6/ml	7644/ml	726,000 cells/ml	3.5 ml
2	7.4 x 10^6/ml	7514/ml	405,000 cells/ml	3.5 ml
3	3.6 x 10^6/ml	3840/ml	442,000 cells/ml	3.0 ml
4	31.2 x 10^6/ml	4157/ml	270,000 cells/ml	4.0 ml
5	38.2 x 10^6/ml	5109/ml	364,000 cells/ml	6.0 ml
	44.9 x 10^6/ml	5653/ml	441,000 cells/ml	

DISCUSSION

Reducing the aspiration volume per location within the iliac bone from approximately 1mL to ½ mL using the MC device significantly improved the cell counts. This finding is consistent with others who have demonstrated a smaller volume aspirate has a higher concentration of stem cells per mL. Additionally, our results had CFU-f counts that are similar to what is typically found in autograft bone.

The MC device aspirate is not filtered or manipulated and contains a full range of cells typically found in marrow. The entire density range of marrow cells has been shown to have stem cells present and filtering marrow can cause a loss of these valuable cells. [15],[16],[7],[16],[17] Despite demonstrating higher CFU-f counts from the MC system when the aspiration volumes per site is reduced, 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 MC 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.

We were able to demonstrate that MC was successful in obtaining TNC and CFU-fs similar to what is expected from autograft; however, with MC, minimal morbidity is incurred as only one insertion point using a 13gauge needle is required. While this pilot study was not designed to be an equivalence study, the comparison of the CFU-f data to previously published results on autograft is intriguing and suggests that the MC system could provide comparable CFU-f to autograft without the morbidity (Table 1).

CONCLUSION

There are several benefits of using this protocol with the MC System. 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 ½ mL aspirations. The system does not require filtering or 10% dilution with anti-coagulant. The number of CFU-fs was comparable to autograft. In this pilot study, the MC system produced results suggesting that it can effectively be combined with an appropriate carrier as a substitute for autograft. Secondly, MC allows the clinician to retain the process and product entirely on the sterile field. Thirdly, all cells and growth factors are retained in the MC aspirate and because of its minimal morbidity, a second aspiration site can be accessed if a volume of more than 3.5 mL is desired. 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.

20. Michael A. Scarpone, D.O., Daniel Kuebler, Ph.D. Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells

21. George F. Muschler, MD The Cleveland Clinic Foundation, Cleveland, OH COMPARISON OF BONE MARROW ASPIRATION AND BONE CORE BIOPSY AS METHODS FOR HARVEST AND ASSAY OF HUMAN CONNECTIVE TISSUE PROGENITOR Paper #41 FIFTY-EIGHTH ANNUAL MEETING APRIL 3-7, 2006 Buenos Aires, Argentina

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