Technique Matters:

Small Draw, Diverse Geography, Precise Repositioning, Lateral Draws Only, Pace, and Syringe Plunger Yields Clinically Relevant CFU-f Counts

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ABSTRACT

Positive clinical outcomes for orthopedic treatments utilizing autologous bone marrow-derived stem cells have been linked to the cellular content of the marrow aspirate graft as measured by fibroblast-like colony-forming units (CFU-f) as certain minimum thresholds have been associated with beneficial outcomes. The purpose of this study was to examine if a modified technique could produce a graft in which the CFU-f content exceeded those thresholds. The modified technique utilized the Marrow Cellution™ device (MC), which aspirates small aliquots of marrow across a large geography from the posterior iliac spine, and aspirating solely from the side ports of the harvesting device.[20] In this pilot study, we investigated the cellular recovery using the MC device by altering the published protocol by reducing the volume of aspirate to only 5mL across the first half of the geographic trajectory before switching the syringe to aspirate another 3mL to 5mL per location across the remaining geographic trajectory. Stem/progenitor cell concentrations as counted by CFU-f and TNC (total nucleated cells) were performed on a 1mL sample taken from the first syringe containing 5mL of native aspirate. These tests represent a standard to determine the number of immature stem and progenitor cells that are present in the aspirate.[1,4,6]

Study Design

A series of six patients who presented to the clinic for regenerative medicine treatment utilizing autologous bone marrow aspirate (BMA) underwent marrow aspiration from the posterior iliac crest with one patient having bilateral aspiration. Following standard protocol, signed informed consent was obtained from each patient. A strict adherence to procedural aspirating protocol for the MC device was observed, with two exceptions noted below. All samples taken were obtained by the same clinician. All samples were analyzed independently at the same laboratory.

For each aspiration, a 2000U/mL heparin rinse of the cannula was used prior to aspiration. An additional 0.5mL of 2000U heparin was added to each aspiration syringe. 10mL syringes were used for all aspirations, but each syringe was only filled to 5mL. Therefore, a total of 10mL of harvest was obtained from each iliac crest, but in two separate 5mL aliquots. After the first 5mL was obtained from each ilium and thoroughly mixed with the 0.5mL of heparin, 1mL was removed from that first syringe to obtain a representative sample. The 1mL sample was shipped for lab analysis in 10% by volume ACD-A and all samples were analyzed within 24 hours of harvest.

Primary endpoints included total nucleated cell (TNC) counts using a Nexcelom Vision 2000 and fibroblast-like colony-forming unit (CFU-f) counts. CFU counts were performed by adding 500,000 nucleated cells into a T-25 tissue culture flask containing 5mls of growth media. After three days, the vial was washed and fresh media was added. At day 14, the cultures were stained and colonies with more than 100 cells were counted. Published literature were used to ascertain historical values for TNC and CFU-f counts using the MC System.

Results

Table 1 shows the TNC and CFU-f results for the seven samples in our study. Of the seven samples, two were discarded due to suboptimal harvesting technique. Specifically, the aspiration for Patient 1 was performed using a heparin rinse of 500U, whereas the recommended heparin strength is 2000U. This resulted in an untoward dilutional effect. For the first hip of Patient 3 (aspirations

were performed bilaterally), the harvesting device was determined to be improperly placed and had to be repositioned, but only after 1mL of marrow had already been aspirated. Therefore, strict harvesting technique was not observed. Not surprisingly, the two samples that did not follow a strict protocol yielded the lowest CFU-f counts.

Table 1:

Date		TNC (10^6/mL)	CFU-f /mL	
6 Feb	pat 1	53.8	2,722	*
19 Mar	pat 2	40.1	4,828	
19 Mar	pat 3 (1st hip)	33.6	2,779	**
19 Mar	pat 3 (2nd hip)	69.5	4,056	
20 Mar	pat 4	56.5	3,555	
20 Mar	pat 5	72.6	4,720	
20 Mar	pat 6	36.8	3,332	
Average			***3,713***	

* 500 unit Heparin rinse vs. recommended 2,000 unit rinse
** Needle repositioned

Discussion

It is well known that the highest quality bone marrow aspirations (greatest quantity of stem/progenitor cells) are generated by a maximum pressure gradient change using a 10mL syringe and aspirating small volumes of bone marrow (1-2mL) from different locations. In addition, studies have shown that significant peripheral blood infiltration occurs with larger volume bone marrow aspirates.[1,2,3,4] It has also been previously established 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]

"Legacy" aspiration devices, such as the Jamshidi needle, have a large open-ended cannula as well as smaller side ports at the distal end of the cannula. Lower viscous fluid, such as the peripheral blood from bone marrow, will preferentially be aspirated through the open end of the cannula, rather than more highly viscous fluids, such as stem cell-rich marrow, being aspirated through the small side ports. This preferential aspiration of peripheral blood thereby necessitates centrifugation in an attempt to separate and dispose of the peripheral blood and retain and concentrate the regenerative cells.

However, submitting bone marrow aspirates to centrifugation in an attempt to isolate and concentrate regenerative cells while minimizing peripheral blood cells has the potential to alter the characteristics of the final centrifuged products in undesirable ways. For example, centrifugation can result in higher granulocyte counts which can then potentially lead to a greater inflammatory response. In addition, centrifugation depends on creating a separation gradient based on molecular weight. However, the molecular weight of actively dividing, though not completely separated, stem cells will be close to, or even greater than that of the cells that are being removed via the centrifugation process, such as erythrocytes and polymorphonuclear leukocytes. This may result in a decreased total number of regenerative cells in the final centrifuged product.

The MC device obviates the pitfalls of legacy needles and subsequent centrifugation. It accomplishes this by sealing the end of the aspirating cannula while leaving the side ports open. This dramatically reduces the potential for aspirating peripheral blood while enhancing the aspiration of stem cell-rich marrow from the side ports. As a result, the need for centrifugation is eliminated, thereby dramatically reducing the total amount of time required to perform the therapeutic procedure, significantly reducing the need for staff to process the aspirate, and ultimately yielding a favorable autologous orthobiologic product (see table below).

Aspirating a volume of marrow greater than 2mL from any one site using traditional needles typically yields total nucleated cell (TNC) counts of 15-20 x10^6/mL and 200-300 CFU-f/mL; however, when only 1 mL of marrow is aspirated with a tradition needle, counts of 40 x10^6/mL TNC and 1451 CFU-f/mL are typical.[1,4,22]

As noted above, syringe vacuum pressure during aspiration has also been shown to influence stem cell yield. Grønkjær et al showed that a rapid forceful aspiration yielded a statistically significant higher quality aspirate than slow aspiration.[24]

Our test indicated that increasing the syringe vacuum pressure across the marrow geography accessed by switching the 10mL syringe half way through the aspiration process improved the cell counts. This finding is consistent with others who have demonstrated a smaller volume aspirate using a 10 mL syringe has a higher concentration

of stem cells per mL.[4] This makes sense insofar as, as the 10mL syringe fills with fluid, the ability to generate high syringe vaccum pressure necessarily decreases (there is less distance for the plunger to be retracted, resulting in less vacuum force). This is why in our study we filled a 10mL syringe only to 5mL, aspirating only 1mL per geographic region, while using maximum vacuum force applied to the plunger, and then switched to another empty 10mL syringe to harvest the next 5mL in similar fashion.

Because the Marrow Cellution aspirate is not filtered or manipulated it contains the full range of cells typically found in marrow

Our results demonstrate CFU-f counts that are similar to what is typically found in autograft bone.[21] Because the MC device aspirate is not filtered or manipulated it contains the 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 and centrifuging marrow can cause a loss of these valuable cells as noted above. [5,7,15,17]

Table 2 shows the comparison of the average CFU-f data collected in this study (using only the five samples that followed the proper aspiration protocol) to clinically relevant CFU-f thresholds previously established for different therapeutic indications. It is clear that the results of MC device far exceeded the suggested CFU-f levels.

Table 2:

Indication	CFU-f /mL Average	Reference
Non Union	2,835	6
Osteonecrosis *	1,123	19
Rotator Cuff Repair	4,200	18
Spine Fusion	865	22
Intradiscal	2,702	7
This Paper	4,828	

* Excludes patients with co-morbidities

Limitations

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, future studies should include patient follow-up. [6,7,13,18,19,22] Third, comparison to historical values of CFU-f data has limitations given the significant patient-to-patient variability. While this pilot study was not designed to be an equivalence study, the comparison of the CFU-f data to previously published results is intriguing and suggests that the MC system could provide comparable CFU-f without the need to centrifuge and filter the harvest.

Conclusion

Following a strict protocol for aspirating, sample preparation, and shipping for laboratory testing of marrow aspirate is critical. The two samples that deviated from the protocol had the lowest counts. The number of CFU-fs was comparable to, or surpassed, clinically relevant thresholds of CFU-f per mL established in the literature. MC allows the clinician to retain the process and product entirely on the sterile field. 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 greater volume is desired.

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