COMPARATIVE ANALYSIS OF MARROW CELLUTIONS AND EMCYTE® BMC SYSTEM

June 2017

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INTRODUCTION

Research has demonstrated that the number of colony forming unit - fibroblast (CFU-f) in the graft is positively correlated with clinical outcomes. [2,3]. Cells capable of forming a CFU-f are found in marrow but not in blood and therefore are an indication of the number of early stage stem and progenitor cells present in the biologic. Several alternative systems are available for harvesting autologous bone marrow and optionally centrifuging it to further concentrate cells to treat local bone defects [1,2,3].

OBJECTIVE

This study was designed to compare the Marrow Cellution™ system (MC) to the EMCYTE® BMC system.

MATERIALS AND METHODS

Three sets of bone marrow aspirate samples were collected from the hemi-pelvis / PSIS from each iliac spine, randomly assigned, using either the EMCYTE BMC system or the Marrow Cellution™ System. After careful review of the manufacturer's most recent instructions for use by the clinical staff, (written instructions and on-line video) BMA was aspirated using the MC system and aspirated and processed using the EMCYTE system. All bone marrow aspirates (BMA) were processed during the procedure and samples arrived at the lab within 8hrs of collection. For each sample, a TNC count, CD 34+ count and CFU-f count was conducted at Franciscan University, Steubenville, OH.

RESULTS

Processing Time

The Marrow Cellution™ System requires approximately 1 ½ minutes to aspirate 8 to 10 cc of marrow from a single puncture. The biologic never leaves the sterile field, the entire sample is used, no manipulation such as filtering is required and no extra anti-coagulation is needed.

In this case series, the EMCYTE BMC System for bone marrow aspirate concentrate (BMC) required approximately 3 minutes to aspirate. To obtain the required 60 mL of volume, after the initial insertion, the needle was removed from the body as the aspirate was taken using the mechanical assist of the Vaclok syringe (Merit Medical Systems) provided in the kit. In addition, the EMCYTE BMC System requires approximately 12 minutes of combined technician setup and centrifugation time that is conducted outside the sterile field.

Analysis of BMA and BMAC

The same donor, alternate hemi-pelvis / PSIS, was utilized to evaluate each system. On average, the MC system had more stem cells per mL as defined by CD 34+ and CFU-f compared to the aspirate or centrifuged product from the EMCYTE system. Cells capable of forming a CFU-f in culture, arise from

early stage CD34+ cells. (4,5) On average, MC system aspirate had a higher TNC count compared to the EMCYTE system aspirate but had approximately half as many TNC as the EMCYTE centrifuged product. Consequently, the ratio of CFU-f to TNC or CD34+ to TNC was significantly higher in the MC product compared to the centrifuged product. The ratio of stem cells to TNC may be an important indicator of the clinical effectiveness of the biologic.

CFU-f per mL

	MC	EMCYTE	
	BMA	BMA	BMC
Patient 1	3,610	**	350
Patient 2	1,560	100	250
Patient 3	1,620	150	200
Average	2,263	125	267

CD 34+ per mL

	MC	EMCYTE	
	BMA	BMA	BMC
Patient 1	326,000	**	151,200
Patient 2	149,957	45,771	109,609
Patient 3	236,100	64,604	175,620
Average	237,319	55,188	146,476

TNC per mL (millions)

	MC	EMCYTE	
	BMA	BMA	BMC
Patient 1	57	**	121
Patient 2	25.6	10.5	18
Patient 3	27	12.5	42.2
Average	36.53	11.5	60.40

** No sample was taken on the BMA for patient 1

The CD 34 + counts were conducted by flow cytometry using the ISHAGE protocol. To measure CFU-f levels in the samples, 5 microliters of undiluted bone marrow was plated into 6-well dishes containing 3mls of DMEM/F12 media (GIBCO) supplemented with 15% MSC qualified FBS (GIBCO) and an antibiotic/antimycotic mix (Gibco). Cells were cultured under standard conditions, 370C, 5% CO2. After 48 hours, the wells were washed four times with HBSS to remove non-adherent cells. The cells were then cultured for 12 additional days with the media being changed every three days. After 14 days total, the colonies were stained with 0.5% crystal violet solution in methanol. Colonies with 100 or more cells were counted as CFU-fs. All samples were processed in duplicate and the CFU-f counts presented are the average of the two counts.

CONCLUSIONS

- The Marrow Cellution aspiration system had significantly more CD 34+ and CFU-f per mL as compared to the EMCYTE BMC system.
- The Marrow Cellution System had significantly less contaminating peripheral blood compared to the EMCYTE BMC system as indicated by the higher ratio of cfu-f to nucleated cells.
- The Marrow Cellution System required significantly less preparation time compared to the EMCYTE BMC system.
- The Marrow Cellution System required significantly less aspirate (8mL compared to 60mL) compared to the EMCYTE BMC system).
- The Marrow Cellution System did not require additional manipulative steps outside the sterile field compared to the EMCYTE BMC system.

	Marrow Cellution™	EMCYTE BMC®
Aspiration Volume	≈7-10mL	≈60mL
Final Volume	≈7-10mL (no change)	≈7 mL
Aspiration Sites	1	1
Aspiration time	1-2 Minutes	2-3 Minutes
Manipulated off sterile field	NO	YES
Processing Time	0 Minutes	12 Minutes
Avg. CFU-f	2,263	267

REFERENCES

1. Connolly J. et al. JBJS 1989;71: 684-91.
2. Hernigou P. et al. JBJS 2006; 88 Suppl 1: 322-27.
3. Hernigou P. et al. JBJS 2005; 87: 1430-7.
4. Busser H. et al. Stem Cells & Development 2015 vol. 24
5. Lin C. et al. Cytotherapy 2012; 1-7