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CLINICAL CASE STUDY

FlexMetric bone marrow aspirator yields laboratory and clinically improved results from mesenchymal stem and progenitor cells without centrifugation

Robert E. Marx[1] | Paul Amailuk[1,2,3] | Neal Patel[1,3] | Andre Ledoux[1,3,4] | Dani Stanbouly[5]

[1]University of Miami Miller School of Medicine, Retired Hospital Corporation of America, Mercy Hospital, Consultant, Miami, Florida, USA

[2]Gold Coast University Hospital Australia, Southport, Queensland, Australia

[3]Jackson Health Systems, Miami, Florida, USA

[4]US Army Walter Reed Military Medical Center, Private Practice, Baptist Health Systems, Miami, Florida, USA

[5]Columbia University, College of Dental Medicine, New York, New York, USA

Correspondence
Robert E. Marx, University of Miami Miller School of Medicine, Retired Hospital Corporation of America, Mercy Hospital, Consultant, Miami, FL, USA.
Email: Robertmarx42@yahoo.com

Abstract

Several devices used to harvest stem/progenitor cells from bone marrow are available to clinicians. This study compared three devices measuring stem cell yields and correlating those yields to bone regeneration. A flexible forward aspirating system Marrow Marxman (MM), a straight needle aspirating on withdrawal system Marrow Cellutions (MC), and a straight needle aspirating on withdrawal and centrifuging the aspirate (BMAC) were compared in a side-to-side patient comparison, as well as tissue engineered bone grafts. The FlexMetric system (MM) produced greater CFU-f values compared to the straight needle (MC) $\Delta = 1083$/ml, $p < 0.001$ and 1225/ml, $p < 0.001$ than the BMAC system. This increased stem/progenitor cell yield also translated into a greater radiographic bone density at 6 months $\Delta = 88.3$ Hu, $p \leq 0.001$ versus MC and $\Delta = 116.7$, $p < 0.001$ versus BMAC at 6 months and $\Delta = 72.2$, $p < 0.001$ and $\Delta = 93.3$, $p < 0.001$ at 9 months respectively. The increased stem/progenitor cell yield of the MM system clinically translated into greater bone regeneration as measured by bone volume $p < 0.014$ and $p < 0.001$ respectively, trabecular thickness $p < 0.007$ and $p < 0.002$ respectively, and trabecular separation $p = 0.011$ and $p < 0.001$. A flexible bone marrow aspirator produces higher yields of stem/progenitor cells. Higher yields of stem/progenitor cells translate into greater bone regeneration in tissue engineering. Flexmetric technology produces better bone regeneration due to a forward aspiration concept reducing dilution from peripheral blood and its ability to target lining cells along the inner cortex. Centrifugation systems are not required in tissue engineering procedures involving stem/progenitor cells due to nonviability or functional loss from g-forces.

KEYWORDS
bone regeneration, FlexMetric® Technology, lining cells, mesenchymal stem cells, osteoprogenitor cells, tissue engineering

All authors have read and approve this manuscript.

1 | INTRODUCTION

The enhancement of clinical outcomes from bone marrow aspirates has been associated with greater yields of certain stem/progenitor cells (Colnot, 2009; Marx & Harrell, 2014). While suggested to produce clinically relevant outcomes in orthopedic (Barakat, 2019) (Wakitani, 2007) and oral and maxillofacial bone regeneration surgery (Melville et al., 2019) as well as pain management (Josi et al., 2021) and numerous regenerative medicine applications (Longo, 2011; Schmidtt et al., 2012), it has not been directly correlated. Several techniques, devices, and concepts have been advanced to increase the yield of stem/progenitor cells in each aspirate.

The challenge to increase stem cell yields from bone marrow requires small aspiration volumes, guidance to the greatest intra marrow reservoir of stem/progenitor cells (niche), avoidance of dilution from intra marrow bleeding, and prevention of contamination or breakage of the marrow aspirator.

There are three current devices utilizing different concepts that are frequently utilized to aspirate stem/progenitor cells. The first is the original Harvest Technologies Terumo Bone Marrow Aspirate Concentrate System (BMAC). The second is the Marrow Cellutions™ straight needle aspirating on withdrawal non-centrifuged system. The third recently introduced device is the FlexMetric flexible Marrow Marxman™ non centrifuged system that advances a flexible needle that seeks out the inner cortical surface which is the location of the greatest number of stem/progenitor cells (Everts, 2002; Matic et al., 2016).

This study compared the total nuclear cell counts and colony forming units of the CD34+ and CD105+ stem/progenitor cells from side-to-side comparisons in the 10 patients each. It then compared the radiographic and histomorphometry bone density regenerated after placement into mandibular continuity defects of 6 cm or greater.

2 | MATERIALS AND METHODS

General: All bone marrow aspirations were accomplished by surgeons with a minimum case experience of 20 cases in each of the three techniques and devices. Bone regeneration via in situ tissue engineering at the point of care was achieved by combining 10 ml of the bone marrow aspirate or bone marrow concentrate with cancellous allogeneic bone, particle size 150–300 microns, and rhBMP-2/ACS (Infuse Bone Graft® Medtronic) 1 mg/1 cm of defect.

3 | PATIENT SELECTION

Patients were selected to have a single continuity defect of the mandible greater than 6 cm from bone loss due to nonmalignant pathology or trauma.

4 | EXCLUSION CRITERIA

1. Age less than 25 years or greater than 90 years
2. Previous head and neck malignancy
3. Previous radiotherapy or chemotherapy
4. Previous or current use of bisphosphonates or RANKL inhibitors
5. History of medications known to directly alter bone marrow cells or bone regeneration.
6. Known allergy to recombinant human bone morphogenetic protein, heparin, ACD-A, or bovine collagen
7. Religious or cultural objection to allogeneic bone, bovine products, or blood replacement

5 | BONE MARROW ASPIRATION CONCENTRATION (BMAC)

All bone marrow aspirations were accomplished using a heparin (0.5 ml–2000 units/ml) coated aspiration trocar from the anterior ilium 4 cm posterior to the anterior spine as per the manufacturer's instructions for use. For this device, four separate punctures were used to harvest 15 ml each of a Bone Marrow Aspirate (BMA) using the rotational concept of Hernigou et al. (2021). Each 15 ml aliquot was placed into a transfer bag containing 4 ml of Anticoagulant Citrate Dextrose-A (ACD-A).

The 60 ml total was then withdrawn from the transfer bag through a micro pore filter and taken off the sterile field where it was placed into the Terumo Harvest Technologies proprietary centrifuge within a cannister containing a floating shelf designed to collect nucleated cells, megakaryocytes and platelets. The automated centrifuge ran a 14-min double spin program designed to separate cellular elements from noncellular elements. The 10 ml of Bone Marrow Aspirate Concentrate (BMAC) was then aspirated from the bone marrow plasma off the sterile field again to yield 10 ml then returned to the sterile field for application in bone regeneration surgery.

6 | MARROW CELLUTIONS NON-CENTRIFUGATION SYSTEM (MC)

For this device, a single puncture was used in the same location of the anterior ilium. The trocar needle combination was hand driven 2.5 cm into the marrow space. The inner sharp trocar was then withdrawn and a blunt multi side port needle was inserted. One ml of bone marrow was initially drawn followed by activating a screw device to upward reposition the needle approximately 2 mm while also rotating the needle based upon a marker at its top to adjust the side port positions. This maneuver was repeated several more times as per manufacturer's instructions withdrawing 1 ml of bone marrow from each location so as to yield 10 ml of anticoagulated bone marrow. Anticoagulation was achieved using a 0.5 ml of a heparin solution 2000 units/ml (1000 units total).

7 | MARROW MARXMAN™ NON-CENTRIFUGATION SYSTEM (MM)

For this device a single puncture was also used in the same location of the anterior ilium. The trocar needle combination was hand driven to a stable position just through the iliac crest. The rigid introduction needle was removed and leaving the FlexMetric® trocar inserted. An initial 1 ml of bone marrow was aspirated. The flexible trocar was then advanced toward the medial cortex and 1 ml of bone marrow aspirated for every 2 mm of advancement (one complete turn of the device handle). As the flexible trocar scythed off the medial cortex another 1 ml of anticoagulated bone marrow was aspirated at every 2 mm of advancement to a total of 10 ml. Anticoagulation was achieved using a 0.5 ml of a heparin solution, 2000 units/ml (1000 units total).

8 | CLINICAL BONE REGENERATION

Five of the 10 patients in each arm were randomized to receive 10 ml of the bone marrow stem cell/progenitor cell yield from each device. Each of the ten-bone marrow stem cell/progenitor cell yields was mixed into cancellous allogeneic bone 150–300-micron particle size, 1 cc per cm of mandibular continuity defect, and 1 mg of recombinant human Bone Morphogenetic Protein/Acellular Collagen Sponge [(rhBMP-2/ACS) Infuse Bone Graft® Medtronic] per cm of defect. All mandibular continuity defects were 6 cm or greater in a nonirradiated tissue bed. All grafts received a titanium mesh to house the particulate graft and a rigid 2.8 mm rigid titanium reconstruction plate applied. All patients were limited to a soft diet for 6 weeks. None were placed in maxillomandibular fixation. Each graft was followed radiographically with a cone beam CT scan at 3 months, 6 months, and 9 months. Dental implants were placed in each patient at 6 months of graft maturity with a core bone trephine taken at the site of implant placement.

9 | LABORATORY METHODS

Total nuclear cell counts were counted using a 1 ml sample from each aspirate or aspirate concentrate diluted 1:20 in a balanced salt solution. Cells were dyed with Acridine Orange/Propidum (Attune Flow Cytometry Thermofisher, Waltham, Massachusetts). Red fluorescent cells were interpreted as nonviable nucleated cells and were not reported as part of the TNC count. Green/blue fluorescent cells were interpreted as viable nucleated cells and were reported as part of the TNC count.

CFU-f counts used 3–10 μl of each sample plated into five wells containing 3 ml of RPMI media supplemented with fetal bovine serum (R&D Systems®). Culture wells were incubated at 37°C and 5% $C0^2$. Nonadherent cells were removed with Hank's Balanced Salt Solution (HBSS) four times at 48 h. Each well was cultured for 18 days total with media change every third day. Colonies were then stained with 0.50% crystal violet solution in methanol. Colonies containing 100 cells or greater were counted as CFU-f.

10 | BONE DENSITY MEASUREMENTS

Standard cone beam CT scans were obtained at 3 months, 6 months, and 9 months using the I-CAT FLX series cone beam CT scanner (Henry Schein, Ft. Lauderdale, FL). Hounsfield density units (HU) were measure at three locations; the center of the graft and 2 cm from each bone end. The average of the three measurements were taken as the HU count for that graft.

11 | GRAFT HISTOMORPHOMETRY

At the time of dental implant placement, 2 mm diameter bone cores were taken as a routine part of the implant placement and processed for standard structural histomorphometry. Two cross sections were read 2 mm distant from the central part of the core (Olympus Microscope [BX 60 Hitschfels Instruments, St. Louis, MO]). Each section was read using a semi-automatic analyzer system (Bioquant, Nashville, TN linked to a camera). Lucida Captronics EDI-750 CE (Meyer Instruments Inc, Houston TX).

Results were reported using the standard nomenclature of the American Society of Bone and Mineral Research (ASBMR) histomorphometry nomenclature committee (Dempster, 2012) that is, bone regenerated BR = Bone Volume BV/Total Volume TV (BR = BV/TV), Trabecular bone thickness (TbTh) was directly measured. Trabecular numbers (Tbn) = BV/TV/TbTh and trabecular bone separation Tbsp = 1/Tbn − TbTh.

12 | DATA ANALYSIS

Descriptive statistics (mean, frequency, range, standard deviations) were calculated for all study variables. 2-tailed Independent sample *t* tests and ANOVA were used to determine significant differences among the treatment systems (MM, MC, T-BMAC) for the continuous outcome variables (TNC, CFU-f, HU, bone regeneration [BV/TV], Trabecular Number [BV/TnTh], Trabecular Separation [Tbsp]). A *p* value of less than 0.05 was considered statistically significant. All statistical calculations were completed with SPSS version 28 for Mac (IBM Corp., Armonk, N.Y., USA).

13 | RESULTS

13.1 | Patient distribution

The patient distribution was 17 males 13 females. Mean age 44 years, range 26–62 years. The defect size as well as the gender

and age distribution of those receiving the bone marrow aspirate or bone marrow aspirate concentrate is shown: Table 1.

13.2 | Laboratory stem cells/progenitor cells

13.2.1 | MM versus MC systems

The MM system's TNC average of 10 patient's aspirates was $36.38 \times 10^6 \pm 10.8 \times 10^6$/ml and its CFU-f's; yield was 3202 ± 1505/ml. The MC system's average TNC's in the same 10 patients was $29.4 \times 10^6 + 16.4 \times 10^6$ ml. and its CFU-f's yield was 2119 ± 1755/ml, **a difference of 7.4×10^6 TNC's/ml and 1083 CFU-f's/ml**, both systems did not require centrifugation. The difference was statistically significant at $p < 0.001$.

13.2.2 | MM versus T-BMAC systems

The MM system's TNC average of 10 patient's aspirates was $33.4 \times 10^6 \pm 12 \times 10^6$/ml and its CFU-f's were 2848 ± 936/ml. The T-BMAC system's average TNC's in the same 10 patients was $22.1 \times 10^6 + 18 \times 10^6$/ml and the CFU-f's yield was 1593 ± 114.7/ml, **a difference of 11.3×10^6 TNC's/ml and 1225 CFU-f's**. The T-BMAC system required centrifugation of an initial 60 ml aspirate. The MM system did not require centrifugation of its initial 10 ml aspirate. The difference was statistically significant at $p < 0.001$.

13.2.3 | MC versus T-BMAC systems

The MC system's TNC average of 10 patient's aspirates was $26.2 \times 10^6 \pm 14.4 \times 10^6$/ml and its CFU-f yield was 2214 ± 1128/ml. The BMAC system's average TNC's in the same 10 patients was $20.8 \times 10^6 \pm 15.2 \times 10^6$/ml and the CFU-f yield was 1391 ± 1011/ml, **a difference of 5.4×10^6 TNC's/ml and 823 CFU-f/ml**. The T-BMAC system required centrifugation of an initial 60 ml aspirate. The MC system did not require centrifugation of its initial 10 ml aspirate. The difference was statistically significant at $p < 0.005$ (Table 2).

13.3 | Cone bean CT scans for bone graft densities

Traditional DXA scans for bone density of long bones are not suitable for mandibular grafts with indwelling titanium plates and screws. However, standard Cone Beam CT scans eliminate metal scatter and artifact and have been found to be useful in comparisons studies. In this study, 10 patients from each system were treated using the bone marrow final product in the in situ tissue engineering concept applied to bone regeneration in the mandible. The results are reported for cone beam CT scan taken at the 3 months, 6 months, and 9 months in Tables 3–5.

At 3 months the average Hounsfield Units (HU) were comparable between the three systems—MM versus MC Δ = 0.9, MM versus T-BMAC Δ = 1.3, MC versus T-BMAC Δ = 0.4. The difference was not statistically significant among the three systems ($p = 0.812$).

TABLE 1 Gender–age–size–distribution

MM			MC			T-BMAC		
Gender	Age	Size	Gender	Age	Size	Gender	Age	Size
M	35	6.5 cm	F	59	7.4 cm	M	26	8.5 cm
F	48	8.0 cm	M	48	8.1 cm	F	37	6.0 cm
M	62	7.1 cm	M	33	10.0 cm	F	60	7.7 cm
M	58	6.0 cm	F	27	8.1 cm	M	49	10.4 cm
F	27	8.7 cm	M	51	6.4 cm	F	47	8.0 cm
F	45	9.5 cm	M	26	6.9 cm	M	38	9.5 cm
M	39	6.5 cm	F	31	7.4 cm	F	46	10.1 cm
M	40	9.7 cm	M	45	6.8 cm	M	28	7.5 cm
F	37	7.3 cm	M	38	10.2 cm	F	32	7.8 cm
M	29	11.1 cm	F	30	7.1 cm	M	39	7.3 cm
	46.6	8.84 cm		43.3	8.31 cm		45.2	8.96 cm
Average			Average			Average		
$M = 7$			$M = 6$			$M = 5$		
$F = 3$			$F = 4$			$F = 5$		

Abbreviations: MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate.

TABLE 2 TNC and CFU-f Values

MM/ml	MC/ml	Δ	*p* Value
TNC 36.38 × 10^6 ± 10.8 × 10^6	29.4 × 10^6 ± 16.4 × 10^6	7.0 × 10^6	
CFU-f 3202 ± 1505	2119 ± 1755	1083	<0.001
MM/ml	**T-BMAC/ml**	**Δ**	***p* Value**
TNC 33.4 × 10^6 ± 12 × 10^6	22.1 × 10^6 ± 18 × 10^6	11.3 × 10^6	
CFU-f 2848 ± 1505	1593 ± 1147	1225	<0.001
MC/ml	**T-BMAC/ml**	**Δ**	***p* Value**
TNC 26.2 × 10^6 ± 14.4 × 10^6	20.8 × 10^6 ± 15.2 × 10^6	5.4 × 10^6	
CFU-f 2214 ± 1128	1391 ± 1011	823	<0.005

Abbreviations: CFU-f, Colony Forming Units-Fibroblasts; MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate; TNC, Total Nucleated Cells.

TABLE 3 Cone beam CT scans (3 months)

	MM	MC	T-BMAC	*p* Value
HU	33	31	30	-
	40	39	42	-
	31	38	28	-
	36	28	27	-
	42	32	36	-
	29	33	37	-
	33	30	31	-
	27	34	34	-
	34	40	36	-
	41	32	32	-
Average	34.6 HU	33.7 HU	33.3 HU	0.812

Abbreviations: Hu, Hounsfield Units; MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate.

TABLE 4 Cone beam CT scans (6 months)

	MM	MC	T-BMAC	*p* Value
HU	288	252	194	-
	390	311	212	-
	315	277	236	-
	339	249	218	-
	292	259	251	-
	277	182	280	-
	385	278	191	-
	316	132	202	-
	392	190	228	-
	415	216	230	-
Average	340.9 HU	234.6 HU	224.2 HU	<0.001

Abbreviations: Hu, Hounsfield Units; MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate.

At 6 months the average Hounsfield Units were significantly greater than at 3 months. The difference was statistically significant among the three systems ($p < 0.001$)—MM versus MC Δ = 88.3, MM versus T-BMAC Δ = 116.7 and MC versus T-BMAC Δ = 28.4.

At 9 months, the average Hounsfield Units were again greater for each system and a similar, statistically significant variance was noted among the three systems ($p < 0.001$)—MM versus MC Δ = 72.2, MM versus T-BMAC Δ = 93.3 and MC versus T-BMAC Δ = 21.1.

14 | BONE HISTOMORPHOMETRY DATA

14.1 | Bone regeneration (BV/TV)—Table 6

Bone regeneration data was obtained from the core specimens taken at the time of implant placement. Each comparison used the same volume of bone marrow mixed with the cancellous allogeneic bone and rhBMP-2/ACS size and dosing as previously discussed. Therefore, each comparison included five matched patients for each comparison MM versus MC, MM versus T-BMAC, and MC versus T-BMAC. The difference was statistically significant between MM versus MC (58.4% vs. 43.3%, $p = 0.014$) and MM versus T-BMAC (60.1% vs. 39.0%, $p < 0.001$). The difference was not statistically significant between MC versus T-BMAC (44.2% vs. 38.4%, $p = 0.154$).

14.2 | Trabecular thickness (TbTn measured)—Table 7

These same five specimens yielded average trabecular bone thickness numbers. The difference was statistically significant for MM versus MC (86.2 vs. 73.2 μm, $p = 0.007$) and MM versus T-BMAC (85.0 vs. 65.6 μm, $p = 0.002$). The difference was not statistically significant between MC versus T-BMAC (73.8 vs. 67.8 μm, $p = 0.269$).

TABLE 5 Cone beam CT scans (9 months)

	MM	MC	T-BMAC	*p* Value
HU	477	368	281	-
	429	391	329	-
	390	272	312	-
	382	303	294	-
	400	364	212	-
	351	324	284	-
	412	320	252	-
	448	326	327	-
	388	401	311	-
	419	305	277	-
Average	409.6 HU	337.4 HU	287.9 HU	<0.001

Abbreviations: Hu, Hounsfield Units; MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate.

TABLE 6 Bone histomorphometry at 6 months

MM versus MC bone regeneration				
BV/TV bone regeneration	**MM%**	**MC%**	**Δ**	***p* Value**
	58.2	40.4	-	-
	67.3	39.3	-	-
	62.1	37.4	-	-
	48.7	57.7	-	-
	55.5	41.5	-	-
Average	58.4	43.3	15.1%	0.014
MM versus T-BMAC				
	MM%	**T-BMAC %**	**Δ**	***p* value**
	64.2	38.9	-	-
	69.4	40.4	-	-
	51.6	38.8	-	-
	56.0	39.6	-	-
	59.3	37.3	-	-
Average	60.1	39.0	21.1%	<0.001
Nine months	**MC**	**T-BMAC**	**Δ**	***p*-value**
	41.6	36.7	-	-
	38.0	41.9	-	-
	36.9	36.3	-	-
	55.4	40.0	-	-
	49.1	37.1	-	-
Average	44.2	38.4	5.8%	0.154

Abbreviations: BV, Bone Volume; MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate; TV, Total Volume.

TABLE 7 Trabecular thickness: TbTh μm

	MM (μm)	T-BMAC (μm)	Δ	*p* Value
	80	76	-	-
	87	68	-	-
	91	80	-	-
	79	72	-	-
	94	70	-	-
Average	86.2	73.2	13.0	0.007
	MM (μm)	**T-BMAC (μm)**	**Δ**	***p* Value**
	96	64	-	-
	77	69	-	-
	82	74	-	-
	80	61	-	-
	90	60	-	-
Average	85.0	65.6	19.4	0.002
	MC (μm)	**T-BMAC (μm)**	**Δ**	***p* Value**
	79	60	-	-
	84	58	-	-
	66	76	-	-
	68	75	-	-
	72	70	-	-
Average	73.8	67.8	6.0	0.269

Abbreviations: MC, Marrow Cellutions; MM, Marrow Marxman; T-BMAC, Terumo - Bone Marrow Aspirate Concentrate; Tb, Th Trabecular Thickness; μm, microns.

14.3 | Trabecular separation (Tbsp) Tbsp = 1/TbTn = TbTn–TbTh—Tables 8–10

Trabecular separation refers to the diameter of the cavity containing marrow between bone trabecule and is an index of that part of a graft which is not bone. The difference was statistically significant for MM versus MC 97 (97.6 vs. 160.9 μm, $p = 0.011$) and MM versus T-BMAC (83.4 vs. 190.8 μm, $p < 0.001$). The difference was not statistically significant for MC versus T-BMAC (152.2 vs. 193.8 μm, $p = 0.051$).

15 | DISCUSSION

15.1 | Laboratory component

The distribution of stem cells and progenitor cells in bone marrow is not uniform. The greatest concentration of stem cells and progenitor cells is located along the inner cortex of bone as adherent cells consistent with the definitional characteristics of stem cells (Caplan, 1991, 2017). Other locations are those cells adherent to the internal trabeculae of bone and about blood vessels as perivascular

TABLE 8 Trabecular separation TbSp (TbSp = 1/Tbn-TbTh)

	MM/μm	MC/μm	Δ	*p* Value
	172.4–80 = 92.4 μm	227.7–76 = 151.7 μm	59.3	-
	199.3–8.37 = 112.3 μm	256.4–68 = 188.4 μm	76.1	-
	161.3 = 91 = 70.3 μm	270.3–80 = 190.3 μm	120.0	-
	204.1–79 = 125.1 μm	172.4–72 = 100.4 μm	25.1	-
	181.8–94 = 87.8 μm	243.9–70 = 173.9 μm	86.1	-
Average	97.6 μm	160.9 μm	73.2 μm	0.011

Abbreviations: MC, Marrow Cellutions; MM, Marrow Marxman; Tbn, Trabecular Numbers; TbSp, Trabecular Separation; TbTh, Trabecular Thickness.

TABLE 9 Trabecular separation TbSp (TbSp = 1/Tbn − TbTh)

	MM/μm	T-BMAC/μm	Δ	*p* Value
	156–96 = 60 μm	256.4–64 = 192 μm	132	-
	144–77 = 68 μm	250.0–69 = 181 μm	113	-
	192–82 = 110 μm	256.4–74 = 182 μm	72	-
	179–80 = 99 μm	250.0–61 = 189 μm	90	-
	170–90 = 80 μm	270.0–60 = 210 μm	130	-
Average	83.4 μm	190.8 μm	107.4 μm	<0.001

Abbreviations: MM, Marrow Marxman; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate; Tbn, Trabecular Numbers; TbSp, Trabecular Separation; TbTh, Trabecular Thickness.

TABLE 10 Trabecular separation TbSp (TbSp = 1/Tbn − TbTh)

	MC/μm	T-BMAC/μm	Δ	*p* Value
	212–79 = 133 μm	270–60 = 210 μm	77	-
	263–84 = 179 μm	238–58 = 180 μm	1	-
	270–66 = 204 μm	278–76 = 202 μm	−2	-
	182–68 = 114 μm	250–75 = 175 μm	61	-
	204–73 = 131 μm	270–67.8 = 202.2 μm	71	-
Average	152.2 μm	193.8 μm	41.6 μm	0.051

Abbreviations: MC, Marrow Cellutions; T-BMAC, Terumo-Bone Marrow Aspirate Concentrate; Tbn, Trabecular Number; TbSp, Trabecular Separation; TbTh, Trabecular Thickness.

cells (Crisan et al., 2008). Therefore, stem cell/progenitor cell harvesting devices which can remove adherent cells from the inner cortex will likely yield the greater numbers of these cells. Selecting donor sites with a rich content of trabecular bone such as the ilium, proximal tibial plateau, and proximal and distal ends of the femur will also yield the greatest numbers of these cells. This has been shown in several studies but not necessarily shown to translate into an enhanced clinical outcome (Scarpone et al., 2019; Schafer et al., 2019).

In this study, the flexible forward aspirating concept of the FlexMetric® device that targets the inner cortical lining cells (MM) showed a greater yield of stem/progenitor cells, CFU-f"s 1083/ml more than the straight needle aspirating upon withdrawal device (MC) $p > 0.001$. It also showed a great number of stem/progenitor cells CFU-f's 1225/ml more than a straight needle aspirating upon withdrawal device harvesting 60 ml of bone marrow aspirate centrifuged down to 10 ml $p > 0.001$ (Table 2).

The greater yield of the FlexMetric® device is due to its ability to seek out and course down the inner cortex taking advantage of this stem cell/progenitor cell rich location. It is also due to its concept of aspirating forward which directs the aspiration to a new previously uninjured area rich in stem/progenitor cells. The concept of aspirating via a straight needle limits the harvesting of stem/progenitor cells by its location within the center of the marrow space thereby harvesting areas with less resident stem/progenitor cells. Moreover, the concept of aspirating upon withdrawal encourages dilution from blood. While side port aspiration attempts to limit dilution from marrow bleeding these side ports continually become located in the injured and bleeding portion of the bone marrow by its initial insertion. After the initial 1 ml withdrawal of bone marrow such devices cannot escape aspirating from a pool of blood.

In this study, the FlexMetric® device showed an even greater yield of stem/progenitor cells when compared to a straight needle

aspirating on withdrawal system centrifuged to create a concentrate. This is again due in part to the two main limitations of a straight needle aspiration upon withdrawal just discussed. It is also somewhat due to the deleterious effects on cell membranes and cellular organelles caused by the g-forces of centrifugation. Studies have shown such detrimental effects of centrifugation on several cell types, that is, spermatozoa (Alvarez et al., 1993), bacteria (Peterson et al., 2012), human red blood cells (Wiegman et al., 2017), embryonic cells (Wong, 2008), and human adipose cells (Conde-Green et al., 2010) but none on human bone marrow. Cell viability loss may be from 1% to 20% but the centrifuged cell's functionality has not been well studied. The data from this study where a straight needle aspirating upon withdrawal was compared to a straight needle aspirating upon withdrawal but also centrifuged the non-centrifuged system yielded a higher stem/progenitor cell yield, 1203/ml. This suggests a centrifuged apoptosis of a certain number of the stem cell-progenitor cell yield (Figure 1).

16 | TRANSLATIONAL COMPONENT

16.1 | Bone density assessment

The null hypothesis for this study is that increased stem/progenitor cells do not enhance clinical outcomes in bone regeneration. The radiographic density data and the several histomorphometry measurements deny that hypothesis. The bone density data of the grafts at 3 months show no significant difference in all the grafts placed $p = 0.812$. This is consistent with known time phase of bone graft maturity (Griffin, 2015). Autogenous cellular grafts and in situ tissue engineered grafts which are cellular from bone marrow, the only mineral density at 3 months would be the residual un-resorbed allogeneic bone particles. At this stage, new bone formation is in the osteoid stage with minimal mineralization and will not register a significant radiographic bone density (Table 3).

The radiographic bone density of all three grafting systems at 6 months showed a distinct increase in density consistent with the maturing phase of cellular bone grafts (Griffin et al., 2015; Marx, 1992). However, the increased stem/progenitor cells of the FlexMetric® (MM) device produced an average of 340.9 HU as compared to the straight needle without centrifugation (MC) device 252.6 HU, $\Delta = 88.3$, HU, $p < 0.001$ (Figures 2 and 3) and the straight needle with centrifugation (T-BMAC device) 224.2 HU, $\Delta = 116.7$ HU, $p < 0.001$. Stem cells are not only proliferating cells, they have

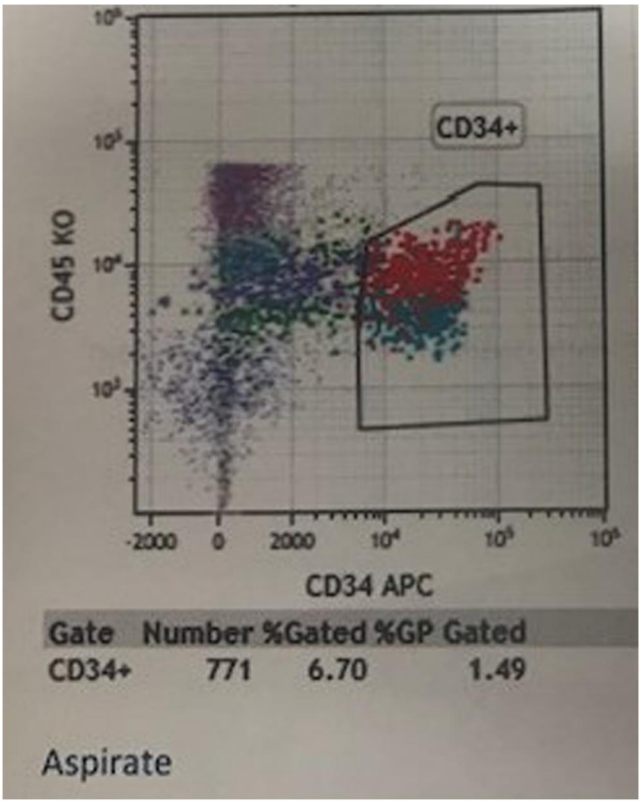

FIGURE 1 Flow cytometry of centrifuged bone aspirates (BMAC) indicate a large number of nonviable cells (RED)

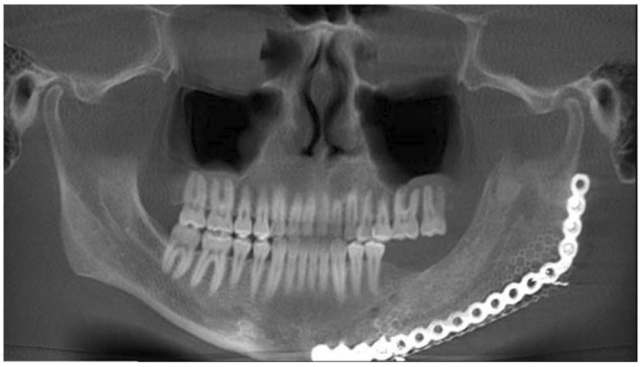

FIGURE 2 Radiograph of bone regeneration from Flexmetric device bone marrow aspiration with robust mineral density Hu = 340.9

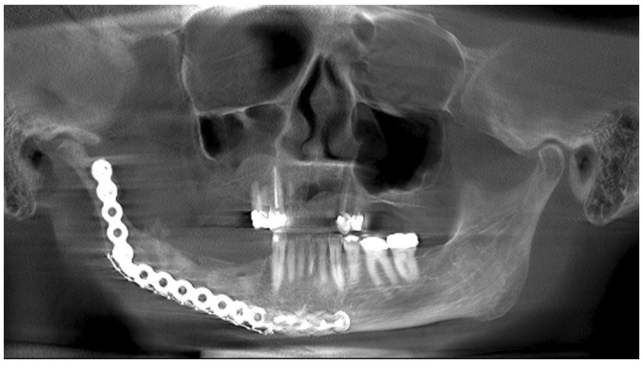

FIGURE 3 Radiograph of bone regeneration from a straight needle marrow aspiration device with modest mineral density Hu = 252.6

been shown to be signaling cells (Caplan, 2017). This increased number of stem/progenitor cells advanced and enhanced the maturity phase of bone formation for early mineralization by virtue of their proliferation and signaling abilities (Table 4).

The bone densities of all three grafting systems at 9 months showed a continued increase in bone density consistent with the maturity and remodeling of a cellular bone graft. Once again, the FlexMetric® (MM device) produced an average of 409.6 HU as compared to the straight needle without centrifugation (MC device) 337.4 HU, $\Delta = 72.2$ HU, $p < 0.001$, and the straight needle with centrifugation (T-BMAC device) 316.3 HU, $\Delta = 93.3$ HU $p < 0.001$. These results indicate a maintenance of mineral density through the remodeling phase of a tissue engineered graft that is linked to the initial amount stem/progenitor cells in the graft (Table 5).

16.2 | Histomorphometry assessment

The three primary functions of the human skeleton are structural support, an attachment for muscle and a reservoir of calcium. All three of these are based upon the mineral component of bone. Therefore, to translate stem cell/progenitor cell yields to clinical functionality histomorphometry of the model used in this study (mandibular grafts) is required. Functional bone regeneration in mandibular continuity defects as well as in long bones requires sufficient regenerative bone volume, mineralization and size of the trabecular component. Additionally, it requires a bone without large marrow spaces such as is noted in osteoporosis which can weaken the structural integrity and lead to fracture (Downey & Siegel, 2006; Woods et al., 2000).

The bone regeneration in an in situ tissue engineered graft is expected to be greater than established norms in long bones in an adult. This is because it is newly formed bone as much as the mandible itself has a somewhat greater bone density than long bones due to the presence of teeth and chewing functions. Nevertheless, this study compared bone volume, trabecular thickness, trabecular numbers and trabecular separation to size matched grafts in age matched patients.

16.3 | Bone regenerative volume

The percentage of bone volume measured from the FlexMetric® device (MM) averaged between 58.4% and 60% and gained 15.1% greater bone volume than the straight needle device without centrifugation (MC) (Figures 4 and 5) and 21.1% greater than straight needle device with centrifugation $p = 0.014$. This gain links stem/progenitor cell numbers to a greater bone regeneration. It is noteworthy and somewhat confirmatory that the two straight needle devices each produced a lesser bone formation but their difference was only 5.8% with the centrifugation device $p = 0.0154$. The lesser of the two is most likely due to loss of viability or a functionally disabled cell composite (Table 6).

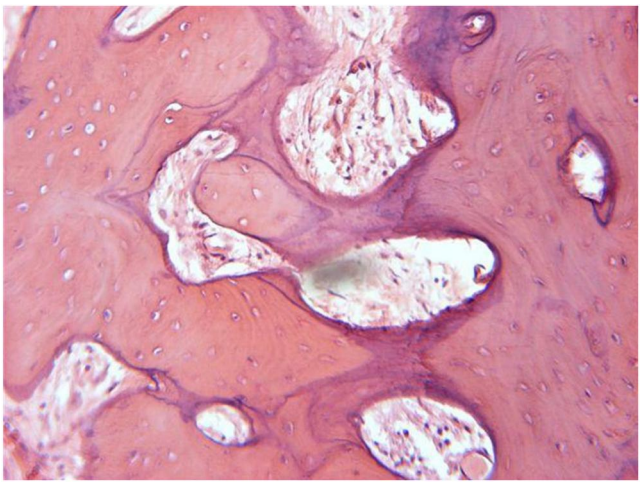

FIGURE 4 Histology of core specimen at 6-months from Flexmetric device used in tissue engineering. Significant bone volume, trabecular thickness and decreased marrow spacing is noted. All bone was mineralized and mature without residual allogeneic particles. H&E stain original magnification 4X

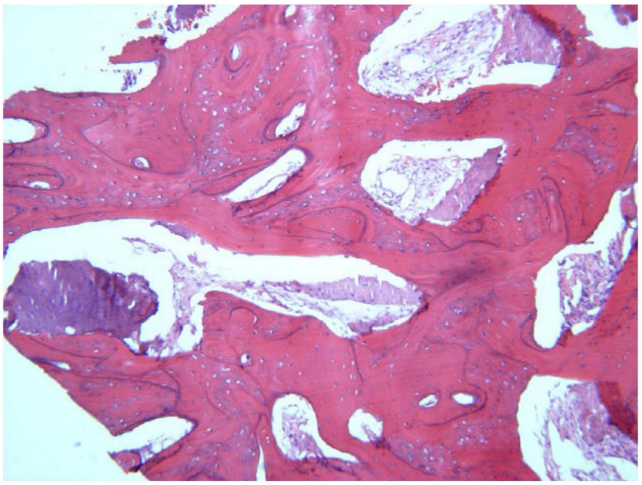

FIGURE 5 Histology of core specimen at 6 months from straight needle device used in tissue engineering. Bone volume is modest with less trabecular thickness and larger marrow spaces. Some immature and less mineralized bone is also noted. H&E stain original magnification 4X

16.4 | Trabecular thickness

The measured trabecular thickness corelated to the stem/progenitor cell counts with the higher yields from the FlexMetric® device without centrifugation (MM) regenerating a trabecular thickness 13 μm more than the straight needle device also without centrifugation (MC) $p = 0.007$ (see Figures 4 and 5) and 19.4 μm more than straight needle device with centrifugation (T-BMAC) $p = 0.002$. The difference between the two straight needle devices was only 6 μm $p = 0.0269$ and not statistically significant with the non-centrifugation system producing a slightly better trabecular bone thickness.

16.5 | Trabecular separation

The separation of trabecule is another index of the closeness of trabecule and the density of trabecule in a given volume of bone between the two cortices. This value measures the distance between trabecule that is occupied with either hematopoietic marrow or fibro fatty marrow not contributing to the structural integrity of the bone. The higher stem/progenitor cell yields of the FlexMetric® device without centrifugation (MM) correlated with a 73.2 μm reduced separation between trabecule as compared to the straight needle without centrifugation (MC) $p = 0.011$. It also corelated a 107 μm reduced separation as compared to the straight needle with centrifugation $p = 0.001$ (T-BAMC).

The correlation of trabecular separation with stem cell progenitor cell yields is also noted in the reduced separation of 41.6 μm of the non-centrifugation versus the centrifugation straight needle device $p = 0.51$ (MC versus T-BMAC).

17 | CONCLUSIONS

- This study documented that stem cells/progenitor stem cell yields from a bone marrow harvest are directly correlated to bone formation, bone density, and bone maintenance.
- A flexible trocar that is directed to harvest lining cells and aspirates as it advances rather than a straight needle that aspirates upon withdrawal within in the center of the marrow space captures a greater number of stem/progenitor cells.
- Centrifugation seems to somewhat reduce the viability of stem cells/progenitor cells or possibly their ability to function as bone forming or signaling cells.
- This study confirmed previous studies identifying that the lining cells along the inner cortex are the most osteogenic.

AUTHOR CONTRIBUTIONS

Robert E. Marx, Paul Amailuk, and Neal Patel designed and performed the research study. Neal Patel, Andre Ledoux, and Dani Stanbouly analyzed the data. Robert E. Marx and Neal Patel wrote the paper.

CONFLICT OF INTEREST

Robert E. Marx is a consultant to Lenkbar LLC and Hospital Corporation of America. Other authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available at Lenkbar LLC following an embargo from the date of publication to allow for commercialization of research findings.

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