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Title: A prospective comparison of three approved systems for autologous bone marrow concentration demonstrated non-equivalency in progenitor cell number and concentration

Vishal Hegde MD*, Owolabi Shonuga MD**, Scott Ellis MD***, Austin Fragomen MD****, John Kennedy MD***, Valery Kudryashov**, Joseph M. Lane MD**

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Corresponding Author:
Vishal Hegde, MD
Orthopaedic Surgery Resident
University of California Los Angeles
10833 Le Conte Avenue 76-143 CHS
Los Angeles, CA 90095
Telephone: 908-410-2101
Fax: 212-772-1061
Email: vhegde530@gmail.com

*Department of Orthopaedic Surgery, University of California Los Angeles, Los Angeles, CA.

**Metabolic Bone Disease Service, Hospital for Special Surgery, New York, NY.

***Foot and Ankle Service, Hospital for Special Surgery, New York, NY.

****Limb Lengthening and Complex Reconstruction Service, Hospital for Special Surgery, New York, NY.

Abstract:
Objectives: To evaluate the efficacy of three commercially available systems: the Harvest SmartPReP 2 BMAC™, Biomet BioCUE™, and Arteriocyte Magellan® systems. We compared the number and concentration of progenitor cells achieved both before and after centrifugation and the percentage of progenitor cells salvaged after centrifugation.

Methods: 40 patients, mean age 47 ± 18 years (range: 18-92 years, 19 male/21 female) were prospectively consented for bilateral iliac crest aspiration. The first twenty aspirations compared the Harvest and Biomet systems, and based on those results, the second twenty compared the Harvest and Arteriocyte systems. One system was randomly assigned to each iliac crest. Each system's unique marrow acquisition process and centrifugation mechanism was followed. Samples for analysis were taken both immediately prior to the marrow being put into the centrifugation system (after acquisition), and after centrifugation. The number of progenitor cells in each sample was estimated by counting the connective tissue progenitors (CTPs).
Results: The Harvest system achieved a significantly greater number and concentration of CTPs both before and after centrifugation when compared to the Biomet system. There was no difference in the percent yield of CTPs after centrifugation. There was no significant difference in the number and concentration of CTPs between the Harvest and Arteriocyte systems before centrifugation, but the Harvest system had a significantly greater number and concentration of CTPs after centrifugation. The Harvest system also had a significantly higher percent yield of CTPs after centrifugation compared to the Arteriocyte system.
Conclusion: The Harvest system resulted in a greater CTP number and concentration after centrifugation when compared to the Biomet and Arteriocyte systems and may thus provide increased osteogenic and chondrogenic capacity.

Key words: bone marrow concentration, non-union, percutaneous autologous bone marrow harvesting, fibroblast colony-forming units, bone healing

Introduction:

Autologous bone grafting continues to be the gold standard in the treatment of fracture nonunion. However, due to several limitations such as donor site morbidity, limited graft supply, limited space at the bone graft site and patient dependent co-morbidities, alternative treatment methods are increasingly being sought to facilitate bone healing. One such biological agent is the combination of autologous connective tissue progenitor cells and the various osteogenic proteins found in bone marrow.[1-4] Several investigators have successfully utilized bone marrow aspirates in the treatment of nonunions and other musculoskeletal tissue repair, including cartilage repair, for which direct intra-articular injection of connective tissue progenitor cells has been widely attempted.[2, 5-18]

It has been demonstrated in both animal and human studies that the osteogenic capacity of bone marrow is impacted by cell concentration.[19] There is a positive correlation between the number and concentration of osteoprogenitor cells delivered in autologous bone marrow grafting procedures and both the volume of mineralized callus and rate of healing of nonunions.[13] In addition, increasing osteoprogenitor cell concentrations decrease the time needed to obtain union. Although such studies examining cartilage repair have not been done in humans, animal studies have indicated that increasing concentrations of mesenchymal progenitor cells mobilize more effectively into injured tissue, including cartilage.[20]

As a result of its initial clinical success, autologous bone-marrow grafting procedures have become an increasingly popular method of treating bony and cartilaginous defects.[2, 15, 21] Consequently, a number of companies have produced centrifugation systems for the purpose of bone marrow aspirate concentration. These systems have different methods of marrow processing and centrifugation and may differ in their efficacy. Since efficacy of concentration can impact the clinical outcome of these procedures, it is important to compare these systems to confirm their equivalence.

In this study, three different bone marrow concentration systems were sequentially and prospectively compared for effectiveness in salvaging progenitor cells for autologous bone-marrow grafting procedures: the Harvest SmartPReP 2 BMAC™ (Harvest Technologies Corporation, Plymouth, MA), the Biomet BioCUE™ (Biomet Biologics, Warsaw, IN), and the Arteriocyte Magellan® (Arteriocyte Medical Systems, Cleveland OH) systems. In our comparison we asked: (1) is there a difference in the number, concentration and/or prevalence of CTPs as a consequence of bone marrow harvesting prior to concentration, (2) is there a difference in the number, concentration and/or prevalence of CTPs after centrifugation, and (3) is there a difference in the percent yield of cells or CTPs (percentage of cells or CTPs in the initial sample that are retained in the processed sample) after centrifugation between the three systems?

Materials and Methods:

Patient Eligibility Criteria and Demographics

Inclusion criteria for this study were adult patients who were undergoing percutaneous autologous bone marrow grafting as a part of their scheduled surgery. Patients were being treated by four independent surgeons, and received concentrated marrow injections for a variety of indications (Table 1). Exclusion criteria were those factors that could potentially impair bone marrow quality, including patients with hematologic malignancy, patients undergoing chemotherapy, patients taking medications that have bone marrow suppression as a side effect, and previous iliac crest bone marrow aspiration.

Between December 2010 and May 2012, 40 consecutive patients with a mean age of 47 ± 18 years (range 18 to 92 years, 19 male and 21 female), were consented for participation in this study.

Study Structure

This study was separated into two arms. The first arm compared the Biomet and Harvest systems. Based on the results from that arm, the Harvest system was chosen for the second arm of the study to be compared to the Arteriocyte system. This study was approved by the hospital's institutional review board and supervised by the biologics committee as a quality assessment project. The risks and benefits of participation in the study were explained to the study participants and consent was obtained prior to surgery.

Bone Marrow Aspiration

In the operating room, after the patients received either spinal or general anesthesia depending on the subsequent procedure being performed, patients underwent bilateral anterior iliac crest aspiration. Each system was randomly assigned to be drawn either from the right or left anterior iliac crest, with the other system used on the contralateral iliac crest. The operating surgeon was blinded to the result but not the system being used. The surgeon obtained bone marrow aspirate by Jamshidi aspiration first from the right, and then the left iliac crest using the technique described by Hernigou et al.[6] The Jamshidi utilized for aspiration was the one included in the packaging of the system being used. A parallel approach was used to aspirate the bone marrow, where the needle was directed parallel to the iliac wing between the inner and outer tables. Two starting holes were made, one to collect the first 30cc and the second for the second 30cc of marrow. At each individual site, 5cc were aspirated and the Jamshidi was then rotated 45 degrees. After the next 5cc was aspirated, the needle was advanced 1-2cm and this process was repeated. For each reposition, the obturator was reinserted into the needle to clear the bore of the needle of possible debris. Each surgeon used an identical technique to improve uniformity. The aspirations and subsequent concentrations were performed in accordance with the manufacturers' respective usage protocols. Representatives from Harvest, Biomet and Arteriocyte were invited to observe each aspiration and concentration to ensure compliance.

Prior to marrow aspiration, the Harvest aspiration system, including the Jamshidi needle and two 30ml suction locking syringes, were coated with heparin. Sixty milliliters of bone marrow was then aspirated and put into a blood transfer bag containing 8ml of anticoagulant citrate dextrose solution A and passed through a 200-micron filter into a 60 ml syringe. At this point a 1ml sample was taken and put into 2ml of Hank's

Buffered Salt Solution (Cellgro®, Mediatech, Inc., Manassas, VA) in a 15ml conical tube (BD Biosciences, San Jose, CA) and transported on ice to the lab for analysis. This sample was taken immediately before the bone marrow aspirate was put into the centrifugation system and was thus considered representative of the unconcentrated bone marrow aspirate produced by the Harvest system (Figure 1 and 2).

The Biomet system used 5ml of the anticoagulant citrate dextrose solution A in each of two 30ml standard non-suction locking aspiration syringes to prevent clotting (a total of 10cc of the anticoagulant citrate dextrose solution A). Each syringe was then used to aspirate 25ml of bone marrow using an identical Jamshidi needle compared to those used by the Harvest system, for a total of 50ml of bone marrow aspirate. At this point, a 1ml sample was taken from the first syringe used to aspirate for the Biomet system and put into solution as described above. This sample was also taken immediately before the bone marrow aspirate was put into the centrifugation system and was thus considered representative of the unconcentrated bone marrow aspirate produced by the Biomet system (Figure 1).

Prior to marrow aspiration, the Arteriocyte aspiration system, including an identical Jamshidi needle compared to those used by the Harvest and Biomet systems, and two standard, non-suction locking aspiration syringes, were coated with heparin. Each syringe was then filled with 4ml of anticoagulant citrate dextrose solution A. The syringes were then used to aspirate 26ml of bone marrow using the Jamshidi needle, for a total of 52ml of bone marrow aspirate. This bone marrow was then passed through a 200-micron filter into a 60 ml syringe. At this point, a 1ml sample was taken and put into solution as described above. This sample was taken immediately before the bone marrow aspirate was put into the centrifugation system and was thus considered representative of the unconcentrated bone marrow aspirate produced by the Arteriocyte system (Figure 2).

Each system's remaining bone marrow aspirate was then placed in the respective centrifuge for concentration and run in accordance with the manufacturer's directions. After centrifugation, the Harvest system has the ability to produce either 7ml or 10ml of bone marrow concentrate. For this study, 7ml was chosen to most closely approximate the output of the Biomet system (6ml). A 1ml sample was taken from the 7ml of bone marrow concentrate produced and put into solution as described above. This sample was considered representative of the bone marrow concentrate produced by the Harvest system. As noted above, centrifugation results in 6ml of concentrated bone marrow aspirate for the Biomet system, of which a 1ml sample was taken and put into solution as described above. This sample was considered representative of the bone marrow concentrate produced by the Biomet system. The Arteriocyte system has the ability to produce between 3ml to 10ml of bone marrow concentrate after centrifugation. For this study, 7ml was chosen to approximate the output of the Harvest system. A 1ml sample was taken from the 7ml of bone marrow concentrate produced and put into solution as described above. This sample was considered representative of the bone marrow concentrate produced by the Arteriocyte system.

Nucleated Cell Count

Complete blood counts were performed on the four samples collected in the operating room using an automated cell counter (ADVIA® 120 Hematology System, Siemens Healthcare Diagnostics, Deerfield, IL) to obtain the number of nucleated cells (million/ml) in each sample. The percent yield of concentration was calculated by dividing the total number of nucleated cells in each system's concentrated sample by the total number of nucleated cells in each system's unconcentrated aspirate.

Colony-Forming Unit Analysis

Connective tissue progenitors (CTPs) were cultured and used as an indicator of progenitor cell activity as previously described.[8, 13, 22-25] CTPs are a population of cells in native tissue that are capable of being activated to proliferate and generate colonies of progeny that can differentiate to express one or more connective tissue phenotypes. This cell population is heterogeneous, as demonstrated by large differences in the biological performance of individual colonies. The CTPs measured using this assay do not include other

types of progenitors that may also be present in bone marrow aspirates, including hematopietic and endothelial cells. [26, 27]

To further isolate progenitor cells prior to cell culture, 3ml of Hank's Buffered Salt Solution was added to each sample and the samples underwent Ficoll density gradient centrifugation (NAPCO 2028R Multi-Function Centrifuge, BridgePath Scientific, Frederick, MD) on top of 6ml of Ficoll-Paque™ PLUS (GE Healthcare, Piscataway, NJ) at 5°C for 30 minutes at 2,000 rpm. A thin layer of mononuclear cells remained suspended above the Ficoll layer and were taken from the centrifuged samples and washed with 6ml of modified Eagle's minimum essential media (Gibco® Opti-MEM® I Reduced Serum Media, Life Technologies, Grand Island, NY) supplemented with 20% fetal bovine serum (Life Technologies), 100U/ml penicillin, and 100µg/ml streptomycin.

The mononuclear cells were then resuspended and cultured in duplicate at a density of $1x10^5$ cells/cm^2 on 100mm diameter cell culture dishes (BD Biosciences) containing 10ml of Opti-MEM® I, supplemented with 20% fetal bovine serum, 100U/ml penicillin, and 100µg/ml streptomycin. The culture plates were placed in a humidified incubator with 20% FiO_2 and 5% CO_2 and maintained at 37°C as previously described.[13] The growth medium was renewed every three days, and the cultures were evaluated on the eleventh day. CTPs were identified by staining with crystal violet solution (Sigma-Aldrich, St. Louis, MO) and were counted once by visual inspection of distinct circumscribed colonies with the plate on a white piece of paper (Figure 3). A colony was counted if it was 1 mm or larger in diameter. The counts from the duplicate plates were then averaged. The researcher counting the colonies was a fourth year medical student with greater than one year of experience working in a tissue culture laboratory and was blinded as to which system the plate corresponded.

Results for each system, after harvest and after processing, were expressed as: the mean number of nucleated cells and CTPs; the concentration of cells and CTPs ([cells] and [CTPs] in units of cells/ml or CTPs/ml); and the prevalence of CTPs (PCTP - in units of CTPs per million nucleated cells). The yield of total cells and CTPs for each system was calculated for each system, as well as the percent yield of cells and CTPs (the fraction of total cells and CTPs in the starting sample that was retained in the processed sample).

Statistical Methods

Descriptive statistics were calculated and the significance level was set at $p < 0.05$. This kind of comparative study between different systems has not been performed before, so the actual sample size needed to power the study was not known. Results for each arm were reviewed after each subset of 10 patients was tested. When the results indicated that one system was more effective, that study arm was terminated. Comparisons between the Biomet and Harvest arm, and the Harvest and Arteriocyte arm of the study for all of the outcome variables were made using the non-parametric Mann-Whitney U test to identify the significance of differences between groups.

Source of Funding

There was no external funding source for this study. The study was funded internally by our hospital's independent biologics review committee because of the study's potential importance from a quality assessment perspective.

Results:

Biomet vs. Harvest

Aspirate Data:

There was no significant difference between the mean concentration of nucleated cells obtained for the Harvest system (18.62 ± 12.16 million/ml, range 0.82 to 43.23 million/ml) and the Biomet system (17.77 ± 12.47 million/ml, range 2.76 to 51.03 million/ml) ($p = 0.705$) (Table 2). The mean prevalence of CTPs (CTPs per million nucleated cells) was significantly greater for the Harvest system (14 ± 11, range 3 to 43) than the

Biomet system (6 ± 7, range 1 to 26) (p = 0.002). The mean yield of CTPs was also significantly greater for the Harvest system (12,282 ± 9,157, range 350 to 40,437) than the Biomet system (2,684 ± 2,532, range 146 to 9,743) (p < 0.001). Finally, the mean concentration of CTPs (CTPs/ml) in the aspirate for the Harvest system (205 ± 152, range 6 to 674) was significantly greater than the Biomet system (54 ± 51, range 3 to 195) (p < 0.001) (Figure 4).

Concentrate Data:

There was no significant difference between the mean concentration of nucleated cells obtained for the Harvest system (101.48 ± 64.13 million/ml, range 25.33 to 240.42 million/ml) and the Biomet system (90.81 ± 61.05 million/ml, 5.76 to 262.35 million/ml) (p = 0.766). The mean prevalence of CTPs (CTPs per million nucleated cells) was significantly greater for the Harvest system (18 ± 10, range 5 to 43) than the Biomet system (4 ± 4, range 1 to 18) (p < 0.001). The mean yield of CTPs was also significantly greater for the Harvest system (7,100 ± 6,705, range 799 to 28,262) than the Biomet system (806 ± 946 range, 74 to 3,614) (p < 0.001). Finally, the mean concentration of CTPs (CTPs/ml) in the aspirate for the Harvest system (1,014 ± 958, range 114 to 4,037) was significantly greater than the Biomet system (134 ± 158, range 12 to 602) (p < 0.001).

Percent Yield Data:

There was no significant difference in the percent yield of concentration of nucleated cells between the Biomet (62.34% ± 28.29%, range 10.49% to 100%) and Harvest (66.52% ± 19.47%, range 27.10% to 100%) systems (p = 0.725). There was also no significant difference in the percent yield of CTPs between the Harvest (57.19% ± 25.55%, range 16.21% and 100%) and Biomet (44.47% ± 36.97%, range 0.81% and 100%) systems (p = 0.480).

Harvest vs. Arteriocyte

Aspirate Data:

There was no significant difference in any aspirate measurements between the Harvest and Arteriocyte systems. The mean concentration of nucleated cells obtained for the Harvest system (16.62 ± 8.42 million/ml, range 4.32 to 31.8 million/ml) and the Arteriocyte system (15.49 ± 7.83 million/ml, range 4.14 to 38.34 million/ml) were similar (p = 0.685) (Table 3). This was also true for the prevalence of CTPs (CTPs per million nucleated cells) obtained for the Harvest system (17 ± 10, range 5 to 40) and the Arteriocyte system (14 ± 7, range 3 to 27) (p = 0.992), as well as the mean yield of CTPs for the Harvest system (18,157 ± 16,329, range 1,672 to 74,551) and the Arteriocyte system (11,349 ± 8,057, range 1,550 to 25,424) (p = 0.156). Finally, the mean concentration of CTPs (CTPs/ml) in the aspirate for the Harvest system (303 ± 271, range 28 to 1,243) was also comparable to the Arteriocyte system (223 ± 156, range 33 to 488) (p = 0.582) (Figure 5).

Concentrate Data:

There was a significant difference between the mean concentration of nucleated cells obtained for the Harvest system (90.8 ± 48.90 million/ml, range 21.66 to 197.67 million/ml) and the Arteriocyte system (38.17 ± 22.58 million/ml, 13.23 to 101.82 million/ml) (p < 0.001). The prevalence of CTPs (CTPs per million nucleated cells) was not significantly different for the Harvest system (21.7 ± 9.7, range 3.4 to 38.7) than the Arteriocyte system (22.6 ± 12.1, range 3.4 to 40.7) (p = 0.857). The mean yield of CTPs was significantly greater for the Harvest system (8,888 ± 7,064, range 1,300 to 25,607) than the Arteriocyte system (3,600 ± 2,483, range 714 to 10,291) (p = 0.004). Finally, the mean concentration of CTPs (CTPs/ml) in the aspirate for the Harvest system (1,270 ± 1,009, range 186 to 3,658) was significantly greater than the Arteriocyte system (514 ± 355, range 102 to 1,470.2) (p = 0.004).

Percent Yield Data:

There was a significant difference in the percent yield of concentration of nucleated cells between the Harvest (65.07% ± 19.29%, range 28.53% to 93.74%) and Arteriocyte (32.55% ± 13.72%, range 12.89% to 64.93%) systems (p = < 0.001). There was also a significant difference in the percent yield of CTPs between the Harvest (56.10% ± 28.03%, range 22.39% and 100%) and Arteriocyte (39.41% ± 22.23%, range 11.19% and 100%) systems (p = 0.028).

Discussion:

Our study showed that the Harvest system is able to achieve a significantly greater number and concentration of progenitor cells than the Biomet system, both before and after centrifugation of bone marrow aspirate. As the Biomet and Harvest systems acquire and process marrow differently, the Harvest system is likely to have superior acquisition and presentation of progenitor cells to the concentration device. The study also showed that although there is no significant difference in the aspirate before centrifugation between the Harvest and Arteriocyte systems, the Harvest system is able to achieve a significantly greater number of nucleated cells and a greater number and concentration of progenitor cells after centrifugation. The difference between the Harvest and Arteriocyte systems is likely the centrifugation device itself, which leads to a superior yield of concentrated progenitor cells by the Harvest system.

There are currently multiple companies marketing systems for the aspiration and concentration of bone marrow, each using differing methods. As our study showed, some methods may be more efficacious than others. Previous studies have shown that there is a positive correlation both between bone-marrow osteogenic capacity and progenitor cell concentration as measured using CTPs, impacting callus formation, time to union and healing rate, as well as chondrogenic capacity, cell number, and cell mobilization into injured tissue.[13, 19, 20] Therefore, the system selected may impact the clinical outcome of procedures utilizing the osteogenic or chondrogenic capacity of bone marrow.

Some limitations should be noted. First, because the method each system uses to prepare and concentrate the bone marrow is different, we could not blind the aspirating physicians to which system they were using to aspirate marrow. However, the systems were randomly assigned to the iliac crests, and the right crest was always aspirated first. Second, the volume of marrow aspirated for each system was different. Harvest aspirated a total of 60cc of marrow, while Arteriocyte aspirated 52cc and Biomet aspirated 50cc. Although this would impact the final CTP yield, this study followed each manufacturer's protocol. Thus, the CTP yields are considered representative of what would be available for reinjection during a standard procedure using any one of these systems.

Third, although CTPs have been correlated with osteogenic and chondrogenic capacity in-vivo, the in-vivo survival of progenitor cells after centrifugation is probably what is most critical for clinical results. Unfortunately, we are unable to provide any data to support that one system offers better survivability of these cells in-vivo. Yet by measuring the prevalence of CTPs (number of CTP/million nucleated cells) obtained after centrifugation for each system, we are able to comment on in-vitro survivability. Previous studies have discussed prevalence as the most likely factor that may influence the survivability of CTPs following transplantation. Increasing prevalence means that more CTPs can be implanted into a site with fewer other nucleated cells as competitors for oxygen and other nutrients.[28-32] By that measure, the Harvest and Arteriocyte systems may offer comparable survivability of these cells, while the Harvest system may offer superior survivability compared to the Biomet system. In addition, while leukocytes have been shown to increase catabolic signaling molecules in-vitro, our study did not factor in the variability in leukocyte concentration of the various systems.[33] CTPs were plated using a standard number of leukocytes per plate, so the role of leukocytes was not studied and did not impact our results.

Finally, it is important to note that no conclusions can be made directly comparing the Biomet and Arteriocyte systems, as there was no arm of this study pairing the two for sample collection and analysis. We attempted to limit the number of patients in this study due to the morbidity (post-operative donor site pain) and cost associated with a bilateral iliac crest aspiration compared to a single crest aspiration. As each patient

only has 2 iliac crests, only a head to head study with two arms was possible. After the first two arms were completed, we felt that the clear superiority demonstrated by the Harvest system compared to the Biomet and Arteriocyte systems made a comparison between the Biomet and Arteriocyte systems unnecessary. Despite these limitations, we report a prospective comparative study of three autologous bone-marrow concentration systems used in the same hospital by four independent surgeons.

The significant difference in the aspirate measurements between the Harvest and Biomet systems, and the equivalence of the aspirate measurements between the Harvest and Arteriocyte systems, indicates that techniques used by both Harvest and Arteriocyte that are not used by Biomet can be important in the preparation of marrow prior to centrifugation. We were able to identify two key differences in the Harvest and Arteriocyte systems compared to the Biomet system before the marrow is centrifuged.

The first is the type and amount of anticoagulant used. While the Biomet system uses 10ml of anticoagulant citrate dextrose solution A to prevent clotting in their aspiration syringes, the Harvest and Arteriocyte systems coat the syringes with heparin, and then add 8ml of anticoagulant citrate dextrose solution A during marrow processing. It is possible that either the extra anticoagulant citrate dextrose solution A used by the Biomet system is harmful to the progenitor cells, or that the heparin coating used by the Harvest and Arteriocyte systems may be beneficial. This may occur either because heparin inhibits CTP binding to the surface of syringes and the transfer bag, resulting in less CTP loss during processing, or because it stimulates CTP formation. Heparin has been demonstrated to be crucial in the interaction of fibroblast growth factors and fibroblast growth factor receptors.[34, 35] Low concentrations of heparin can facilitate the binding of fibroblast growth factors and receptors, ultimately stimulating cell proliferation.[36, 37]

The second difference is the use of a filter prior to injecting the marrow into the centrifugation device. The Harvest and Arteriocyte systems require the marrow to pass through a large 200-micron filter into a 60ml syringe before centrifugation, while the Biomet system does not. This filter may remove cellular aggregates, clot and fat globules from the marrow prior to centrifugation.[13] These particles could have interfered with efficient separation and recovery of progenitor cells in both the Biomet system centrifuge and the Ficoll density gradient centrifugation, resulting in lower CTP counts.

A potential difference is the type of aspiration syringe each system utilizes. While Biomet and Arteriocyte use two regular 30ml aspiration syringes, Harvest uses two 30ml suction locking syringes. The suction lock theoretically provides a more consistently powerful suction in the iliac crest compared to manually pulling the syringe back and forth, thereby aspirating a greater amount of true marrow and less peripheral blood. Yet as the Arteriocyte system had comparable aspirate CTP counts to the Harvest system, these syringes were not thought to contribute to the difference in samples in either arm of the study.

It is important to note that there was a great variability in the CTPs measured between the patients in this study, including the younger patients. This variability can be a limiting factor to the success of bone marrow grafting procedures, as the number of progenitors that are being delivered to the healing site can be unpredictable. The average amount of colony-forming units in this study was 1.4 to 1.8 CTP/cm^2 for the Harvest system in the Biomet arm and 1.7 to 2.2 CTP/cm^2 for the Harvest system in the Arteriocyte arm. For the Biomet system, it was 0.4 to 0.6 CTP/cm^2 and for the Arteriocyte system it was 1.4 to 2.3 CTP/cm^2. These values were obtained using a seeding density of $1x10^5$ cells/cm^2. They are comparable to Jäger et al. with 2.8 to 4.1 CTP/cm^2 and Castro-Malaspina et al. with 0.6 to 1.9 CTP/cm^2.[8, 38] Muschler et al. had 5.5 CTP/cm^2 (seeding density of $1.2x10^5$ cells/cm^2), but counted CTPs as groups of 8 cells. Colonies that small would not have been noticed by visual inspection in this study.[39]

The Harvest SmartPReP 2 BMAC™, Biomet BioCUE™ and Arteriocyte Magellan® systems were all approved through the FDA's 510(k) regulation process. Although the FDA determined the three systems to be substantially equivalent to previously approved devices, this study demonstrates that when it comes to the aspiration and concentration of bone marrow to obtain progenitor cells, the Harvest system is more effective than both the Biomet and Arteriocyte systems.

In the future, other systems that are FDA-approved for autologous bone marrow aspiration and concentration should be studied to ensure that they are at least equivalent to the Harvest system in their ability to concentrate CTPs. If another system is found to yield better results, it should then become the benchmark by which new systems for the aspiration and concentration of bone marrow are measured. In addition, further studies should be done to directly compare the in-vivo efficacy and clinical outcomes of procedures in which these systems are commonly used, such as non-union, joint fusion, and neo-cartilage formation.

In conclusion, we compared three systems for autologous bone marrow concentration to analyze the variability that exists between systems in producing a concentrated population of progenitor cells. We found that, compared to the Biomet and Arteriocyte systems, the Harvest system produced a greater number and concentration of progenitor cells as measured by CTPs in the concentrated marrow. Thus, it is possible that the Harvest system can provide greater healing capacity, and thus better union, fusion and cartilage formation rates when compared to the Biomet and Arteriocyte systems.

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Figure Legend

Figure 1. Flowchart demonstrating the study marrow procurement protocol in the operating room for the Harvest vs. Biomet arm.

Figure 2. Flowchart demonstrating the study marrow procurement protocol in the operating room for the Harvest vs. Arteriocyte arm.

Figure 3. Crystal violate stained colony forming units provide an estimate for the number of connective tissue progenitors (CTPs) in the sample. Representative samples are shown. A) Harvest concentrate sample was counted to have 226 colonies, which vary widely in size, cell density, and cell number. B) Biomet concentrate sample was counted to have 60 colonies. C) A 40x magnification image of a single medium sized colony, containing approximately 800 cells.

Figure 4. Charts demonstrating the distribution of fibroblast colony-forming units per ml of aspirate and concentrate in the Harvest vs. Biomet arm.

Figure 5. Charts demonstrating the distribution of fibroblast colony-forming units per ml of aspirate and concentrate in the Harvest vs. Arteriocyte arm.

BMAC Indications and Procedures

Table 1. Indications and procedures for which patients received concentrated bone marrow aspirate

Indication	Procedure	Reason for BMAC	Number of Patients
Ankle arthritis	Ankle distraction	Accelerate neo-cartilage formation	11
First MTP arthritis	First MTP joint fusion	Enhance fusion	14
Leg length discrepancy	Tibial and fibular osteoplasty	Enhance healing	4
Osteochondral defect	BMAC injection	Enhance repair	1
Osteochondral defect	Retrograde drilling	Enhance repair	3
Osteochondral defect	OATS	Enhance repair	4
Bone cysts	BMAC injection	Enhance healing	2
Tibial non-union	BMAC injection	Enhance healing	1

MTP, metatarsophalangeal; TMT, tarsometatarsal; BMAC, bone marrow concentrate; OATS, osteochondral autograft transfer system

Harvest v Biomet: Cellular Composition of Aspirate and Concentrate

Table 2. Comparison of Cellular Composition of Harvest and Biomet System Bone Marrow Aspirate and Bone Marrow Concentrate

		Harvest	Biomet	p
Aspirate	Nucleated cells (million/ml)	18.62 ± 12.16	17.77 ± 12.47	0.705
	CTP Prevalence (CTP/million nucleated cells)	14 ± 11	6 ± 7	0.002
	Total CTPs	12,282 ± 9,157	2,684 ± 2,532	< 0.001
	CTP/ml	205 ± 152	54 ± 51	< 0.001
Concentrate	Nucleated cells (million/ml)	101.48 ± 64.13	90.81 ± 61.05	0.766
	CTP Prevalence (CTP/million nucleated cells)	18 ± 10	4 ± 4	< 0.001
	Total CTPs	7,100 ± 6,705	806 ± 946	< 0.001
	CTP/ml	1,014 ± 958	134 ± 158	< 0.001
Yield (%)	Nucleated cells	66.52 ± 19.47	62.34 ± 28.29	0.725
	CTP	57.19 ± 25.55	44.47 ± 36.97	0.480

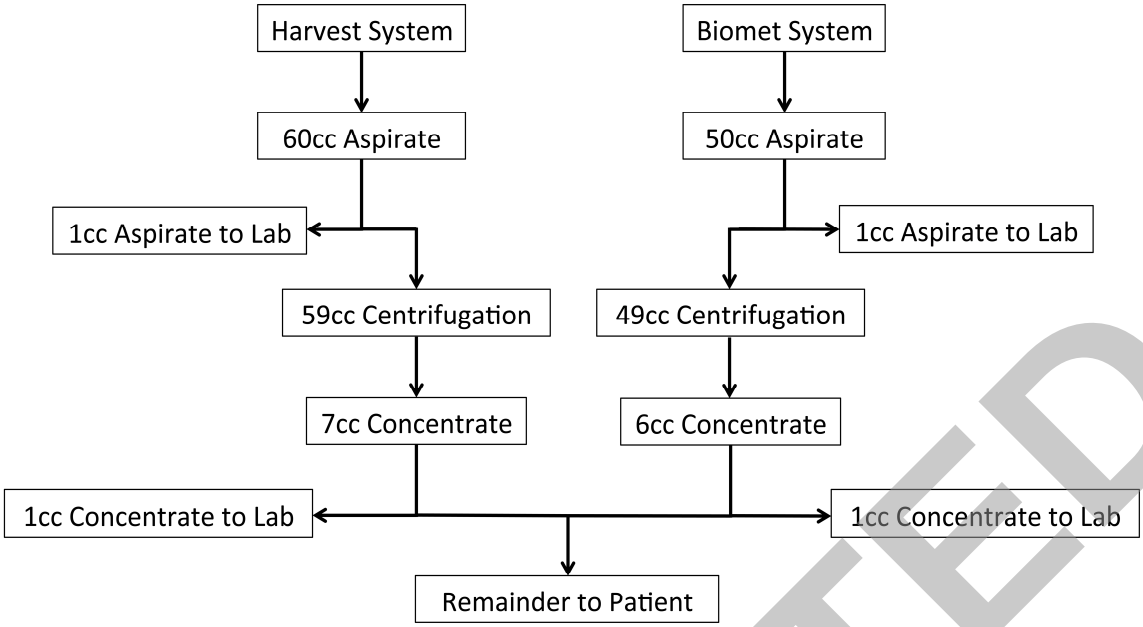
CTP, connective tissue progenitor.

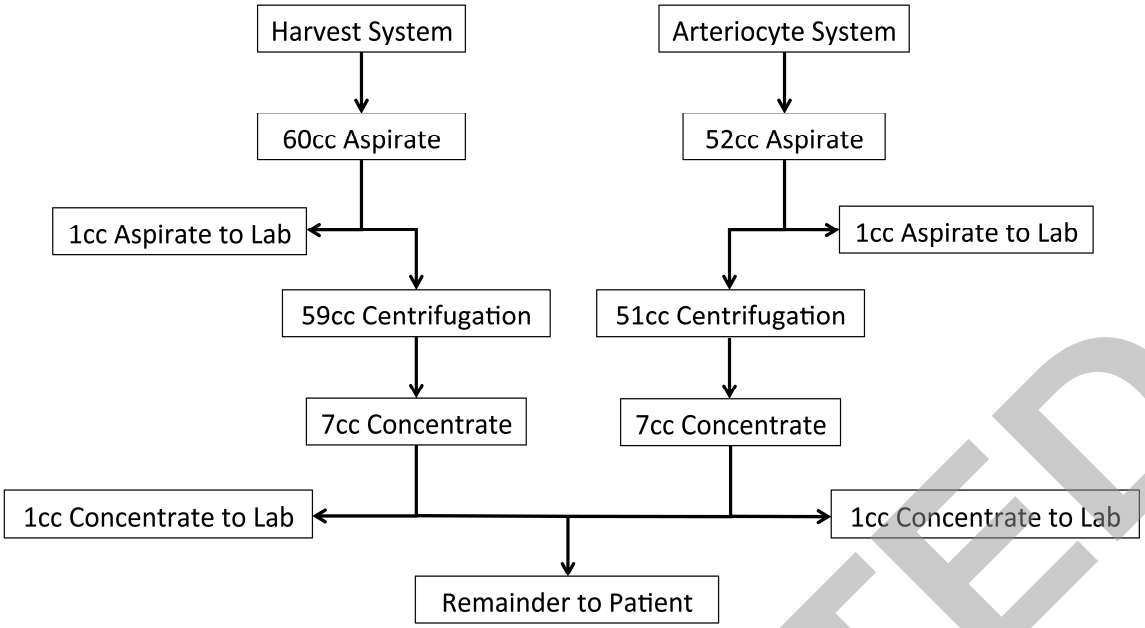
Harvest v Arteriocyte: Cellular Composition of Aspirate and Concentrate

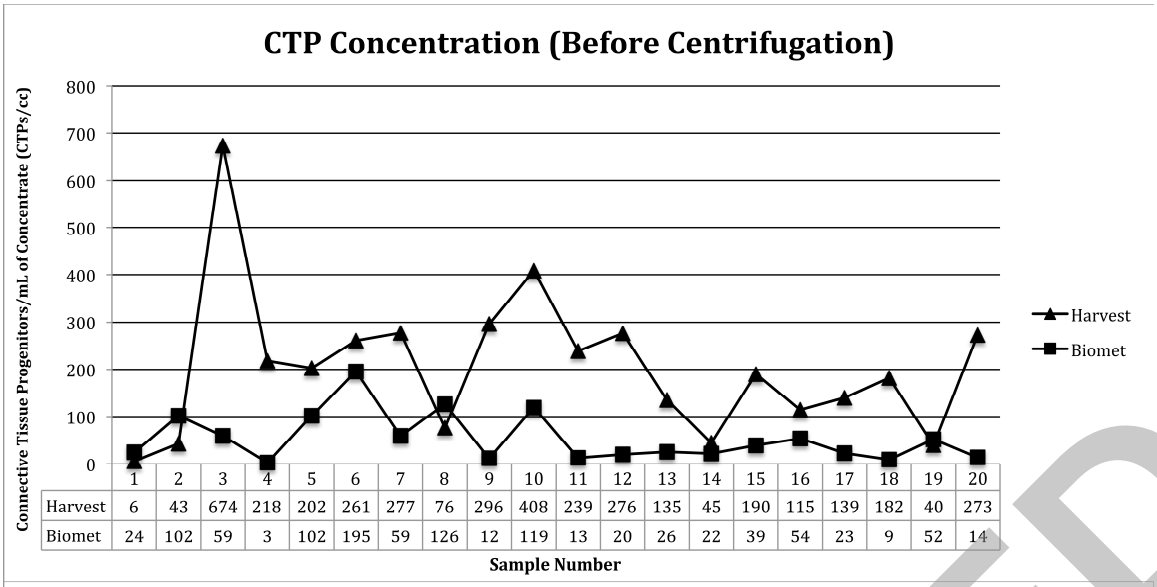
Table 3. Comparison of Cellular Composition of Harvest and Arteriocyte System Bone Marrow Aspirate and Bone Marrow Concentrate

		Harvest	Arteriocyte	p
Aspirate	Nucleated cells (million/ml)	16.62 ± 8.42	15.49 ± 7.83	0.685
	CTP Prevalence (CTP/million nucleated cells)	17 ± 10	14 ± 7	0.992
	Total CTPs	18,157 ± 16,329	11,349 ± 8,057	0.156
	CTP/ml	303 ± 271	223 ± 156	0.582
Concentrate	Nucleated cells (million/ml)	90.8 ± 48.90	38.17 ± 22.58	< 0.001
	CTP Prevalence (CTP/million nucleated cells)	21.7 ± 9.7	22.6 ± 12.1	0.857
	Total CTPs	8,888 ± 7,064	3,600 ± 2,483	0.004
	CTP/ml	1,270 ± 1,009	514 ± 355	0.004
Yield (%)	Nucleated cells	65.07 ± 19.29	32.55 ± 13.72	< 0.001
	CTP	56.10 ± 28.03	39.41 ± 22.23	0.028

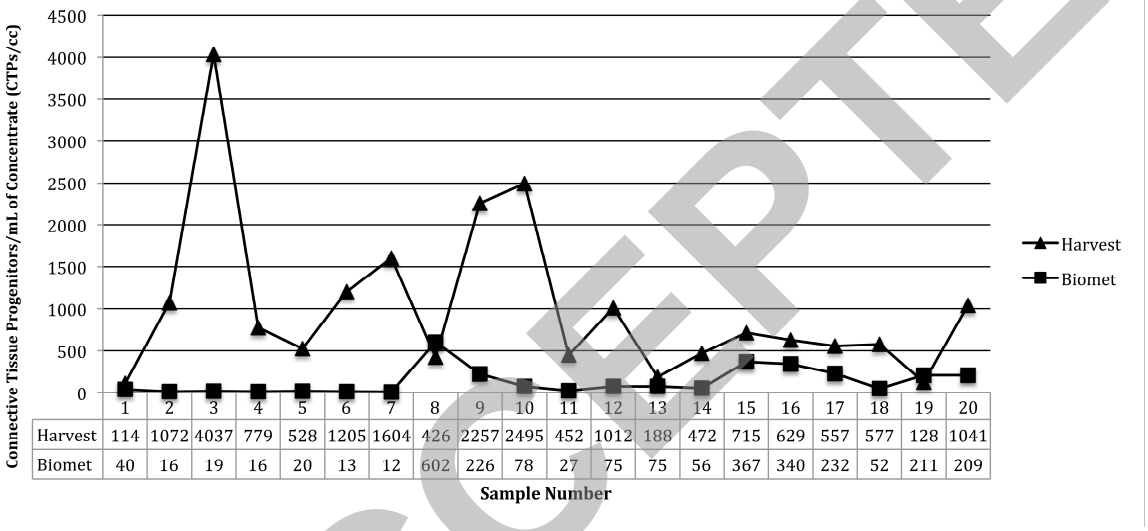
CTP connective tissue progenitor.



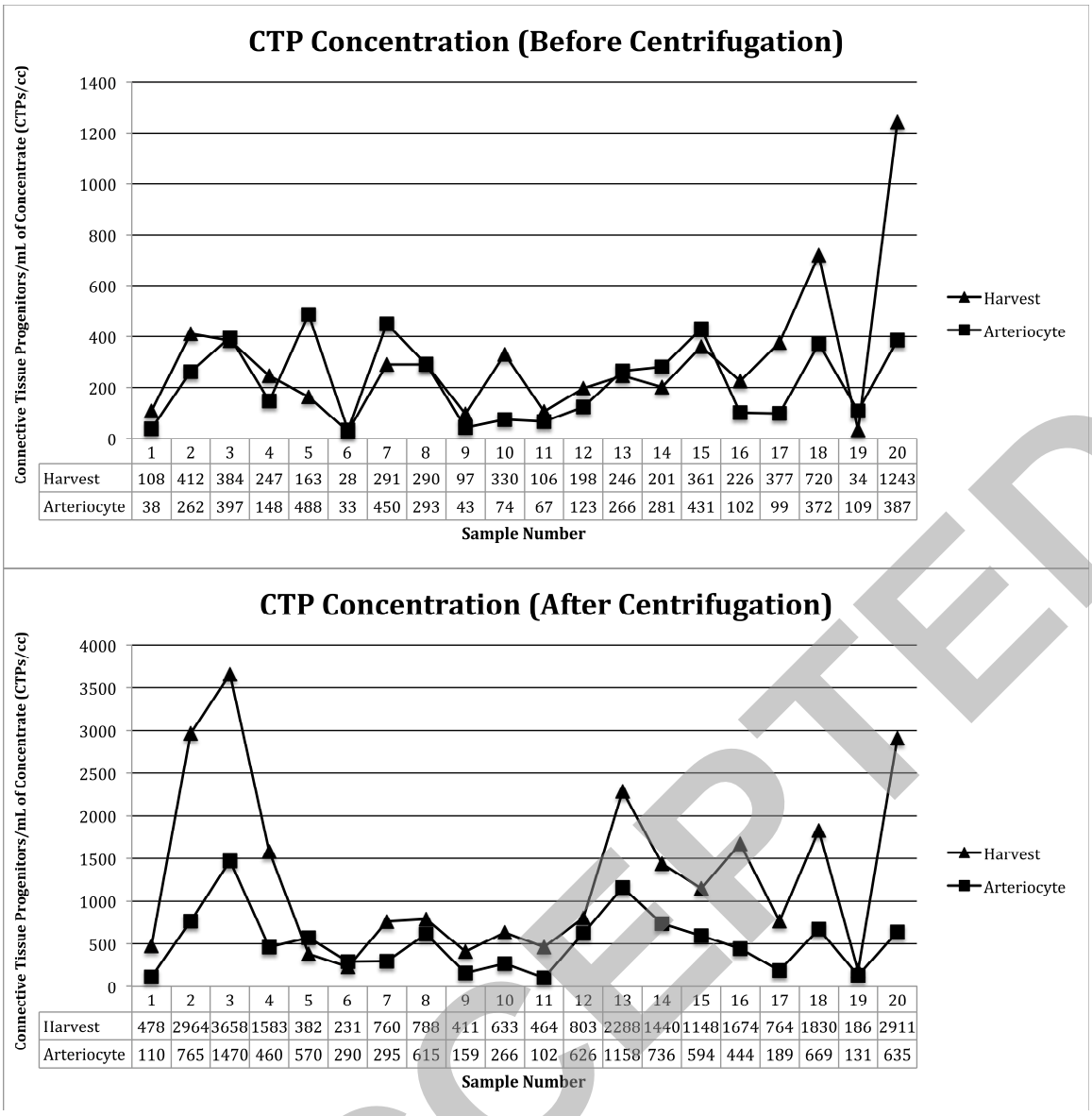




	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Harvest	6	43	674	218	202	261	277	76	296	408	239	276	135	45	190	115	139	182	40	273
Biomet	24	102	59	3	102	195	59	126	12	119	13	20	26	22	39	54	23	9	52	14



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Harvest	114	1072	4037	779	528	1205	1604	426	2257	2495	452	1012	188	472	715	629	557	577	128	1041
Biomet	40	16	19	16	20	13	12	602	226	78	27	75	75	56	367	340	232	52	211	209



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Harvest	108	412	384	247	163	28	291	290	97	330	106	198	246	201	361	226	377	720	34	1243
Arteriocyte	38	262	397	148	488	33	450	293	43	74	67	123	266	281	431	102	99	372	109	387

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Harvest	478	2964	3658	1583	382	231	760	788	411	633	464	803	2288	1440	1148	1674	764	1830	186	2911
Arteriocyte	110	765	1470	460	570	290	295	615	159	266	102	626	1158	736	594	444	189	669	131	635