

AUTOGRAFTX

BMA & AUTOGRAFT HARVESTING SYSTEM

AGENDA

1. Key benefits
2. Peripheral blood infiltration during marrow aspiration – the cause of low stem cell counts
3. How AutograftX™ overcomes the problem of peripheral blood infiltration of the aspirate
4. Comparison AutograftX™ to:
 - a) PSIS and traditional needle
 - b) Pedicle and traditional needle
 - c) Centrifugation systems
5. What is the importance of cfu-f counts compared to nucleated cell counts?
6. How many osteo-progenitor cells are needed for successful clinical outcomes
7. Conclusion

KEY BENEFITS

Reduce the Cost of Utilizing Biologics

The AutograftX™ System delivers a regenerative solution at a reduced cost compared to the industry leading solutions. To investigate the cost benefits of smarter engineering, please contact a AutograftX™ representative.

Minimize O/R Time

Centrifugation systems typically require 20 minutes or more of spin time during the surgical procedure, not to mention the additional personnel (i.e. perfusion) and support time needed for preparation and cleanup of the equipment.

Minimize Sterility Challenges

Centrifugation systems require passing the BMA off the sterile field for processing and back on for implantation. The AutograftX™ System eliminates the additional steps where infection concerns must be managed.

KEY BENEFITS (CONTINUED)

Minimize Sample Waste

Centrifugation systems typically discard 80% of the aspirate due to the high levels of peripheral blood. Worse, significant numbers of the desired cells (approx. 40%) are discarded because as these cells increase in density prior to division, they are processed into the undesired red cell centrifuge component and thus discarded, substantially limiting the regenerative potential of the resulting sample.

Minimize Use of Anti-Coagulants

Centrifugation systems require at least 10% dilution by volume for the addition of anti-coagulant to allow the sample to separate, and also require another 10% dilution in the form of a neutralizing agent such as thrombin and calcium chloride in order for the marrow to clot in the graft. The AutograftX™ System eliminates these requirements.

Eliminate the Need to Filter

Protocols require the marrow to be filtered prior to centrifugation. Cells bound within a cell aggregate can be delivered to the patient when mixed with graft material or injected. This is not the case when aggregates are filtered out prior to centrifugation. Filtering takes additional time, but more importantly, filtering reduces regenerative potential.

PERIPHERAL BLOOD INFILTRATION DURING MARROW ASPIRATION

Traditional needles result in a precipitous decline in the stem/progenitor cells of the aspirate in larger than 1 mL aspiration

Advancing the needle breaks a channel in the trabecular bone.

The channel created by the needles placement will immediately fill with peripheral blood as the needle is retracted.

Peripheral blood has a dramatically reduced viscosity compared to bone marrow in vivo.

Fluid will follow the path of least resistance which means that in response to the negative pressure of the syringe, the lower viscosity peripheral blood, will preferentially flow over higher viscous marrow into the lumen.

Virtually of the fluid enters the end lumen with the side holes contributing nothing to overall cell yield.

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.

Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. *Annals of biomedical engineering* 2008;36:1978-91.

Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nature reviews Immunology* 2006;6:93-106.

Tanikawa 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* 1997 Dec;38(12):1249-53.



PERIPHERAL BLOOD INFILTRATION DURING MARROW ASPIRATION(continued)

The data shows that as the aspiration volume increases from any one given site, the concentration of bone marrow derived cells decreases quickly and the fraction of the sample composed of peripheral blood increases

MUSCHLER G, et al "Aspiration to Obtain Osteoblast Progenitor Cells from Human Bone Marrow: The Influence of Aspiration Volume" The Journal of Bone and Joint Surgery; VOL. 79-A, NO. 11. Cleveland Clinic

Nucleated cell concentration was 3-fold, and CFU-GM concentration 10-fold higher per mL in the first- puncture 1.0 ml bone marrow sample than in the transplant

Batinic, D et al "Relationship between differing volumes of bone marrow aspirates and their cellular composition." Bone Marrow Transplant. 1990 Aug;6(2):103-7.

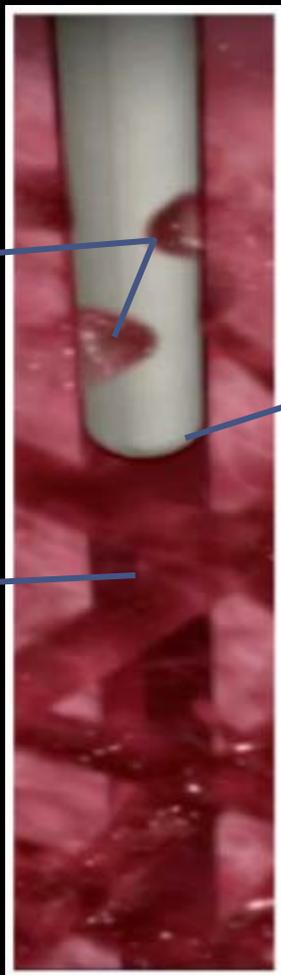
This study shows that marrow harvesting by means of multiple small volume aspirations minimizes the dilution with peripheral blood and results in greater numbers of cells and hemopoietic progenitors.

Bone marrow harvest for marrow transplantation: effect of multiple small (2 ml) or large (20 ml) aspirates. Bacigalupo A et al; Bone Marrow Transplantation. [1992, 9(6):467-470]

These results confirmed that strict adherence to a specific collection procedure, involving small volume marrow aspirations and multiple puncture sites, results in a product with a high number of early hematopoietic progenitor cells and minimal contamination by peripheral blood.

Spitzer et al "The impact of harvest center on quality of marrows collected from unrelated donors." J Hematother. 1994 Spring;3(1):65-70. Massachusetts General Hospital, Boston 02114.

AUTOGRAFTX™ OVERCOMES THE PROBLEM OF PERIPHERAL BLOOD INFILTRATION OF THE ASPIRATE



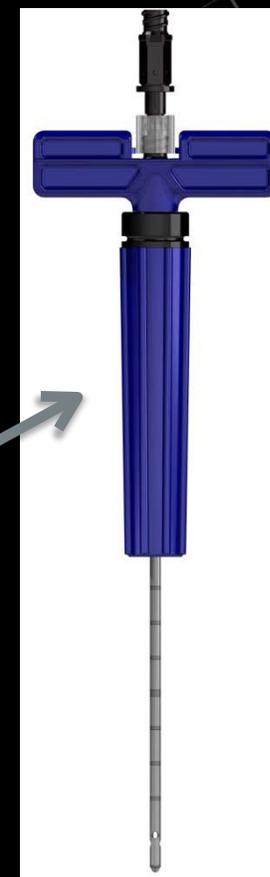
Four channel, closed tipped, aspirating cannula prevents exposure of the needle tip to the channel filled with peripheral blood created by the needle as it is being retracted from the bone space.

Channels

Closed Tip

Peripheral Blood Canal

Screw guide to precisely relocate the aspiration cannula during the procedure



COMPARISON TO A TRADITIONAL NEEDLE

ILIAC SPINE :

Technique #	Technique Description	CFU-f per m/L	
		Volume	
		10	50
1	Traditional Needle Single Puncture (2)	376	95
2	Traditional Needle, insert deep and aspirate as you withdraw (1, 4)	356	52
3	Traditional Needle Aspirate as you advance needle by re-inserting blunt stylet, advancing, remove stylet, attaching syringe, aspirate, repeat process (3)	N/A	54
4	Traditional Needle - Same as 3 but use a vac lock syringe to more precisely control the vacuum pressure (3)	N/A	205
5	AutograftX™ (5)	3,290	N/A

(1)McLain RF, Fleming JE, Boehm CA, Muschler GF. Aspiration of osteoprogenitor cells for augmenting spinal fusion: comparison of progenitor cell concentrations from the vertebral body and iliac crest. J Bone Joint Surg Am 2005;87:2655-61.

(2)Hernigou p; et al. Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. Int Orthop. 2013 Nov;37(11):2279-87.

(3)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.

(4) Jones et al; Rapid intraoperative concentration of mesenchymal stem cells from bone marrow aspirate Spine Care, Houston TX; Biomet Inc.

(5)Annual Orthopedic Update 2016, Allegheny Health Network; "AutograftX Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells". Lecture- Machael A Scarpone MD, Daniel Kuebler

COMPARISON TRADITIONAL NEEDLE

Traditional Needle Iliac Crest & Vertebral Body compared to AutograftX™ Iliac Crest:

All Amounts Shown per mL

	Traditional Needle		AutograftX
	Iliac Crest	Vertebral Body	Iliac Crest
Average	356	465	3,290

AutograftX™ gives significantly more cfu-f than a traditional needle in the pedicle or Iliac spine

Technique: Traditional Needle

- Use a sharp Marrow Needle to make the pilot hole in the pedicle
- Remove the sharp stylet from the marrow needle and aspirate 2 mL from each pedicle hole at both the proximal and distal end of the channel created. Total volume aspirated was 16mL comprised of 8mL from each of the two vertebra from each patient
- Using Same Needle & in the same patient, take 8mL from 4 separate punctures (2mL per puncture) from both the left and right iliac crest for a total of 16 mL per patient

A COMPARISON TO CENTRIFUGE SYSTEMS:

	No Centrifuge	Centrifugation of 60 mL		
	<u>AutograftX™ (1)</u>	<u>Harvest BMAC (2)</u>	<u>Biomet (2)</u>	<u>Magellen (2)</u>
Volume mL	11	7	7	7
Osteo-progenitor per mL *	3,290	1,270	134	514
Total Osteo-progenitor in Graft	35,344	8,890	938	3,598
* TNC (MM) **	37	90.8	92	38

*Osteo-progenitor measured by industry standard CFU-f test

** Centrifugation protocol does not distinguish between nucleated cell from peripheral blood and marrow

The high TNC count and low stem cell count in the centrifuged biologic is the result of a high level of peripheral blood contamination

(1)Annual Orthopedic Update 2016, Allegheny Health Network; "AutograftX Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells". Lecture- Machael A Scarpone MD, Daniel Kuebler Ph.D.

(2)Vishal Hegde MD et al; "Title: A prospective comparison of three approved systems for autologous bone marrow concentration demonstrated non-equivalency in progenitor cell number and concentration." Journal of Orthopaedic Trauma Publish Ahead of Print

WHAT IS THE IMPORTANCE OF CFU-F COUNTS COMPARED TO NUCLEATED CELL COUNTS?

Authoritative studies in orthopedics (Hernigou et al) demonstrated statistical significance in non-union and osteonecrosis to the number of cfu-f cells in the graft.

In those same studies, nucleated cells in the graft did not rise to significance. Interestingly, cfu-f is found frequently in marrow and very rarely in peripheral blood.

From Hernigou “Therefore, it seems reasonable to suggest that a graft needs to contain greater than 1,000 progenitors / cm³”

Hernigou et al Treatment of Osteonecrosis with autologous bone marrow grafting Clinical Orthopaedics and Related Research number 405, pp 14-23

Hernigou P, et al Percutaneous Autologous Bone-Marrow Grafting for Nonunions – Influence of the Number and Concentration of Progenitor Cells The Journal of Bone and Joint” Volume 87-A No 7 July 2005

A COMPARISON - SUCCESS / FAILURE LINKED TO CFU-F

Author	Indicaiton	Success	Failure
Pettine	Intradiscal - Lumbar Spine (pain management)	2,000	NR *
Hernigou	Rotator Cuff	4301	1500
Gan	Lumbar Spine Fusion	865	NR
Hernigou	Osteonecrosis	562	143 **
Hernigou	Non Union	1,500	600

*All patients responded; statistically significant difference in level of pain response between patients receiving less than or greater than 2,000 cfu-f

** Excludes sickle cell, alchohol, steroid, organ transplant, co-morbidity

*** AutograftX comfortably exceeded the failure threshold

Pettine K et al Percutaneous Injection of Autologous Bone Marrow Concentrate Cells Significantly Reduces Lumbar Discogenic Pain Through 12 Months STEM CELLS Jan 2015. Vol 33. issue 1 pg 146-156

Hernigou P 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; 1811-1818

Gan Y et al The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion Biomaterials, 2008, 3973-3982

Hernigou et al Treatment of Osteonecrosis with autologous bone marrow grafting Clinical Orthopaedics and Related Research number 405, pp 14-23

Hernigou P, et al Percutaneous Autologous Bone-Marrow Grafting for Nonunions – Influence of the Number and Concentration of Progenitor Cells The Journal of Bone and Joint” Volume 87-A No 7 July 2005

CONCLUSION

Properly aspirating marrow using the AutograftX™ System will minimize peripheral blood dilution and will result on average in same osteo-progenitor stem cell count (cfu-f count) per mL as aspirating larger volumes of aspirate and centrifuging to reduce the peripheral blood contamination.

A larger-volume of aspirate (more than 2mL) from a given site is contraindicated with the additional volume contributing little to the overall number of bone-marrow cells and results principally in unnecessary blood loss.

MUSCHLER G, et al “Aspiration to Obtain Osteoblast Progenitor Cells from Human Bone Marrow: The Influence of Aspiration Volume” The Journal of Bone and Joint Surgery; VOL. 79-A, NO. 11 Cleveland Clinic

Clinical outcomes are linked to the stem cell content of the graft and not the nucleated cell content.

Hernigou P, et al Percutaneous Autologous Bone-Marrow Grafting for Nonunions – Influence of the Number and Concentration of Progenitor Cells The Journal of Bone and Joint” Volume 87-A No 7 July 2005

Migiaccio AR et al “Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity.” Blood: 2000 Oct 15;96(8):2717-22.