Marrow Cellution

Index

The importance of cfu-f counts

Comparison:
A) Marrow Cellutions B) Trinity / OsteoCel
C) Centrifugation Protocols

Allograft Processed cells are damaged and do not function

Bone Marrow Aspirate– A proven driver of bone regeneration

Peripheral blood infiltration during marrow aspiration
Peripheral blood = Low Stem Cell Yield

How Marrow Cellutions overcomes the problem of peripheral blood infiltration of the aspirate

Marrow Cellutions - Detail of cfu-f collected

Conclusion

The Importance of cfu-f 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 1000 progenitors / cm ^3"*

Cfu-f has been established as the gold standard in determining the quality of a biologic in orthopedic regenerative medicine

Hernigou et al Treatment of Osteonecrosis with autologous bone marrow grafting Clinical Orthopaedics and Realted 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:

A) Marrow-Cellutions B) Trinity C) Centrifugation

Product	Marrow Cellutions (1)	Trinity / Osteocell (2)	Centrifugation of 60 mL *		
			SmartPrep (3)	Biomet GPS (3)	Magellan (3)
Concentrate Volume mL	10	Per mL	7	7	7
CFU-f	3,405	33	1270	134	514
TNC (MM) *	33.1	0.4	90.8	92	38

* Centrifugation protocol does not distinguish between TNC (total nucleated cell) from peripheral blood verses marrow

The high TNC count and low stem cell count in the centrifuged biologic is due high levels of peripheral blood contamination

(1) Harvesting bone marrow without centrifugation. Scarpone et al Presented at the Allegheney orthopedic update meeting, 2016
(2) Brown LS. Darmoc MM, Clineff TD Investigation of Mesenchymal Stem Cell Phenotype and Function in an Allograft Cellular Bone Matrix. Stryker Corp. Malvern. PA
(3) 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

Marrow-Cellution

Case #	Nucleated Cells/ml	CFU-f/ml	Sex	Age	Comorbidities
1	4.46 x 10^7	4200	male	52	HTN, GERD
2	3.63 x 10^7	3600	male	45	Crohns, GERD
3	2.16 x 10^7	3200	male	72	HTN, CAD
4	1.55 x 10^7	2000	male	59	none
5	4.61 x 10^7	2500	male	37	low IGF-1
6	2.64 x 10^7	300	male	85	RA, Diabetes, Gout, used Humira
7	4.42 x 10^7	2500	male	57	none
8	1.56 x 10^7	1200	female	65	Hypothyroid
9	3.74 x 10^7	6700	male	22	none
10	2.09 x 10^7	2400	male	69	none
11	3.82 x 10^7	2900	female	76	HTN
12	1.78 x 10^7	700	male	58	Diabetes, Obesity
13	3.63 x 10^7	5200	female	34	Psoriatic arthritis
14	3.57 x 10^7	4000	female	34	Psoriatic arthritis
15	4.85 x 10^7	2400	male	46	GERD, smoking
16	5.1 x 10^7	5400	male	44	HTN, GERD, Gout, Obesity
17	1.53 x 10^7	800	male	56	none
18	4.46 x 10^7	4200	male	65	Ulcers
19	1.01 x 10^7	7300	female	61	none
20	1.5 x 10^7	6600			

"Harvesting bone marrow without centrifugation" Scarpone et al Presented at the Allegheney orthopedic update meeting, 2016

Average cfu 3,405

Processed Allograft Cells Do Not Function Due to Damage Caused By the Processing

	Fresh Autograft (1)	Marrow Cellution (3)	OsteoCel (2)	Trinity (2)
cfu-f	4,564	3,405	28	38
TNC (millions)	56	33.1	0.25	0.4
All amount are reported on a per mL basis				

- Cells capable of forming a cfu-f are easily separated from fresh autogenous bone (autograft). (Standard collagenase protocol)
- The proprietary processing preparation of the OsteoCel & Trinity products results in the cells unnaturally adhering to the bone surface such that they could not be tested using established protocols. (Standard collagenase protocol).
- The ability of cells to migrate in response to cytokines is critical to their ability to function in a trauma environment. The un-natural adherence of the cells that results from the proprietary processing of the allograft raises concern over the ability of those cells to contribute post transplantation.
- Cells need to be supported by a functioning blood supply to survive over an extended period of time. (typically, within 100um of a blood vessel
- Total nucleated cells (TNC) cells from the fresh autograft sample reflects that no immune matching is required for autologous use.

(1) Comparison of bone marrow aspiration and bone core biopsy as methods for Harvest and Assay of Human Connective Tissue Progenitors Rozic et al Cleveland Clinic NIH grant RO1 AR049686

(2) Brown LS. Darmoc MM, Clineff TD Investigation of Mesenchymal Stem Cell Phenotype and Function in an Allograft Cellular Bone Matrix. Stryker Corp. Malvern. PA

(3) Harvesting bone marrow without centrifugation. Scarpone et al Presented at the Allegheney orthopedic update meeting, 2016

Processed Allograft Cells Do Not Function Due to Damage Caused By the Processing

Clinical Evidence – Trinity / OsteoCel

In a standard athymic murine model for bone formation, their was no difference shown at time 14 days and time 28 days between standard human allograft tissue (study group 1) compared to Trinity (study group 2) and OsteoCel (study group 3)

Brown LS. Darmoc MM, Clineff TD Investigation of Mesenchymal Stem Cell Phenotype and Function in an Allograft Cellular Bone Matrix. Stryker Corp. Malvern. PA

One year arthrodesis rates in ACDF using standard a-cellular human allografts compared to OsteoCel did not show any statistical difference. Although not statistically significant, patients treated with MSC allografts demonstrated lower fusion rates compared to a matched non-MSC cohort.

Steven McAnany, MD, et al Mesenchymal Stem Cell Allograft As A Fusion Adjunct In One And Two Level Anterior Cervical Discectomy And Fusion: A Matched Cohort Analysis The Spine Journal Accepted Date: 18 February 2015

Cells from a different person have an immune profile. For example, if you have 500,000 cells and 100,000 cells are MSC, the other 400,000 will cause a rapid immune response.

Regulatory Concerns

These Allografts Are Required to Submit for Regulatory Clearance

Value added bone graft materials harvested from recently deceased donors "rely on the metabolic activity of living cells" for their primary function and are not for "autologous use"; and are regulated as a "drug, device, and/or biological product under the FD&C Act, and/or section 351 of the PHS Act (42 U.S.C. 262), and applicable regulations, including21 CFR Part 1271, and premarket review" is required. Based on the recently finalized guidance, none of these products have submitted for the applicable clearance from FDA and use of such materials is the equivalent to using a drug that is not approved for any indication. The recently issued final guidance where FDA has documented that such products are not cleared for use in humans puts heightened risk on any institution that continues to use them in their clinical practice. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585403.pdf

Bone Marrow Aspirate and Dowels –

A proven driver of bone regeneration

There are over 150 references supporting the use of bone marrow for grafting.
Marx, J Bone Marrow Bone Marrow: A Validated Driver for Bone Regeneration, White Paper

BMA is a safe alternative to iliac crest harvest without associated complications or morbidity.
Muschler, GF Nakamoto, C Griffith, L.G. Engineering Principles of Clinical Cell-Based Tissue Engineering, Journal of Bone and Joint Surgery, 2004; 86(7): 1541.

The addition of autologous marrow is a value driven and highly effective way for achieving graft incorporation.
Deakin, D.E., Bannister, G.C.; Graft Incorporation After Acetabular and Femoral Impaction Grafting With Washed Irradiated Allograft and Autologous Marrow. The Journal of Arthrorplasty, 2007 January; 22(1): 89-94

A combination of marrow aspirate, trephine assisted dowel graft and TCP graft material had the same fusion rate as autograft in the gutter of the lumbar spine without the associated morbidity.
Yamada T et al. Hybrid grafting using bone marrow aspirate combined with porous β-tricalcium phosphate and trephine bone for lumbar posterolateral spinal fusion: a prospective, comparative study versus local bone grafting.
Spine (Phila Pa 1976). 2012 Feb 1;37(3)