

# Point of Care Concentration of Bone Marrow

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## ABSTRACT INTRODUCTION:

Until recently, adult stem cells were presumed to be committed to differentiation of specific tissues. Adult hematopoietic stem cells (HSCs) originally believed to be limited to hematopoietic differentiation are capable of dedifferentiation and transdifferentiation to generate cells of all lineages. Mesenchymal stem cells (MSCs) have also been shown to transdifferentiate into various tissues. This capability is referred to as stem cell plasticity. In this study we evaluated a methodology to concentrate bone marrow at point of care utilizing existing technology within 15 minutes of collection.

## METHODS:

Sixty (60) mL of bone marrow was withdrawn with a single aspiration from the posterior iliac crest into a syringe containing preservative free heparin (20-50 U heparin per mL of bone marrow). The procedure had IRB approval; all donors were evaluated for transmissible diseases, and were financially compensated. All *in vitro* analyses were performed in our laboratory within 24 hrs of collection. The concentration process utilizes the standard Harvest Technologies SmartPreP/DePuy Symphony centrifuge device and disposable. The density of the floating shelf of the processing disposable has been modified to enhance the collection of the cellular contents of the bone marrow. The system can process two bone marrow samples, or concomitantly 60 mL of whole blood for the preparation of platelet gel or utilize a designed counter balance. The bone marrow aspirates were analyzed numerically and morphologically pre and post concentration. Hematopoietic stem cells (CD34<sup>+</sup>) were used to evaluate bone marrow stem cell concentration because of their higher frequency as compared to MSCs. The process of bone marrow derived stem cell (BMSC)/MSC isolation and expansion was evaluated utilizing specially formulated media supplements to differentiate into osteogenic and chondrogenic lineages.

## RESULTS:

The bone marrow concentrates were prepared in various volumes depending upon their intended use. A summary of the results in a volume that would be used in orthopedic surgery is shown in the following table.

Mean SD	MNC x 10 <sup>3</sup> /uL	WBCx 10 <sup>3</sup> /uL	CD34 <sup>+</sup> x 10 <sup>3</sup> /mL	% Y CD34 <sup>+</sup>	#MNC in BMC Post	#CD34 <sup>+</sup> in BMC Post
Pre mean ± SD	4.72	20.03	241.3			
	1.57	7.28	164.07			
Pre mean ± SD	28.43	115.69	1661	70.0	160.3 x 10 <sup>6</sup>	9.36 x 10 <sup>6</sup>
	7.19	30.59	851.64	0.14	40.87	4.85

Table 1. Summary data for 9 samples prepared in 6 mL volume for use in orthopedic bone grafting procedures (% Y = percent yield).

HSCs have also been demonstrated to transdifferentiate into skeletal and cardiac myocytes. Marrow concentrates for these clinical uses are prepared in 10 to 15 mL volumes. The results obtained following concentration of bone marrow aspirates from 17 donors are shown in the following table:

Product	Mean % Yield	Mean # Cells
MNCs	70.2 ± 29.9	190 million ± 79
CD34 <sup>+</sup> cells	74.6 ± 13.7	9.02 million ± 4.3
Platelets x 10 <sup>3</sup> /uL	73.9 ± 17.1	794 ± 429

MNCs = mononuclear cells.

Table 2. The results obtained from concentrating 17 bone marrow aspirates to volumes of 6 mL to 18 mL.

Concentrated bone marrow was cultured in MSC media. The cultures demonstrated viable hMSCs that were identical to a commercially available cell line. The cultures were transferred into osteogenic media to determine whether there was differentiation from the pleural potent MSC toward osteogenic cells. After 10 days bone marrow derived cells and a commercial cell line were stained with Von Kossa silver stain and for alkaline phosphatase. Both lines demonstrated osteoblastic differentiation.

## DISCUSSION:

Bone and blood formation have traditionally been thought to be distinct and unrelated processes. Bone and bone marrow are closely aligned compartmentally which suggests that these tissues may have a common bone marrow progenitor that gives rise to both osteoblasts and hematopoietic cells. Recent work confirms that the bone marrow contains a primitive cell able to generate both hematopoietic lineages.

Hematopoietic precursors reside close to endosteal surfaces which suggest that osteoblasts play a central role in hematopoiesis. It has been shown that osteoblasts produce factors essential for the survival, removal, and maturation of hematopoietic stem cells (HSCs).

## CONCLUSION:

- Research has demonstrated that growth factors derived from platelet gel increase the proliferation of stem cells both *in vitro* and *in vivo*.
- Current research suggests a reciprocal relationship between osteoblasts and HSCs.
- Plasticity of adult bone marrow derived stem cells is fact.
- Bone marrow derived stem cells can be:
  - concentrated at point of case with 70% efficiency
  - concentrated within 15 minutes
- The system can effectively and reproducibly collect mononuclear cells in concentrations up to 7 times native levels while maintaining the viability and function of the cellular components.

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