# EFFICIENT HARVEST OF A MONONUCLEAR CELL-RICH FRACTION FROM ASPIRATED BONE MARROW

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**Introduction**

The use of autologous bone marrow aspirate for bone grafting has been advocated as a means to provide an osteogenic cell source while avoiding the donor site morbidity associated with traditional autograft [1]. Concerns that the concentration of osteoprogenitors in aspirated samples is insufficient to have a clinical impact have led to various efforts to further concentrate the cell component. In general, long processing times or culturing steps that make point of care implementation infeasible have largely restricted the use of these concentration methods to experimental efforts [2]. The limited clinical evidence suggests that cellular concentration positively affects the clinical outcome of bone grafting procedures [3]. The following study analyzes the use of a commercially available platelet concentrate system to produce a mononuclear cell-rich fraction (CRF) from human bone marrow aspirate samples. The mononuclear CRF was compared to the base aspirate samples using a hematology analyzer and flow cytometry. Colony forming unit assays were performed on the CRF and compared to results obtained using a standard laboratory concentration technique [4].

**Method**

*Aspirate Concentration*- 120ml aspirate samples (heparinized) were obtained from healthy volunteers (Cambrex, Rockville MD). 10ml was preserved to serve as base sample. 55ml was centrifuged for 15 minutes using the GPSII™ Platelet Concentrate System to obtain approximately 6ml of CRF. The remaining 55ml was processed via Hetastarch sedimentation followed by Ficoll separation to obtain approximately 6ml of an isolated cell fraction.

*Analysis*-Cell counts were obtained in triplicate for all base and cell-rich samples using a Cell-Dyn 3700 (Abbot, Dallas, Texas). Baseline and concentrate samples were antibody labeled, fixed, and analyzed with flow cytometry per instructional labeling for the FACS Caliber device (BD Bioscience, San Jose, CA). Fibroblast colony forming unit assays (StemCell Technologies, Vancouver, BC) were performed on three CRF samples and compared to three samples collected using Hetastarch/Ficoll separation. In brief, cells were cultured for 14 days and visualized with Wright stain, followed by enumeration of the colonies.

**Results**

Table I shows the average (±SE) total nucleated (TNC) and mononuclear (MNC) cell concentration, concentration factor (CRF/baseline), and recovery of mononuclear cells in the cell-rich fraction obtained from human bone marrow samples.

| | Base | CRF |
|---|---|---|
| [TNC] ($x10^3/\mu l$) | 16.6 ± 2.7 | 106.1 ± 17.0 |
| TNC Concentration Factor | NA | 6.45 ± 1.02 |
| TNC Recovery (%) | NA | 69.2 ± 10.9 |
| [MNC] ($x10^3/\mu l$) | 3.6 ± 0.6 | 22.1 ± 3.7 |
| MNC Concentration Factor | NA | 6.11 ± 0.97 |
| MNC Recovery (%) | NA | 65.6 ± 10.5 |

Table I-Cell count data for human aspirate samples (n=6)

The average concentration of mononuclear cells in the CRF was 22.1 x $10^3$ cells/μl. This represented a 6.11 fold increase in concentration and a 65.6% recovery compared to the baseline levels.

Table II shows the average (±SE) concentration of various antibody positive cells in the cell-rich and baseline samples, as well as the percentage of the baseline cells that were recovered in the cell-rich fraction. These calculations were made using the results obtained from the flow cytometry analysis and the cell count data presented above. The antibodies selected represent a variety of progenitor cell populations including hematopoietic (CD34, CD45, CD133, VEGFR2), mesenchymal (CD105), and endothelial (CD34, CD105, CD133, VEGFR2) progenitor cells. Antibodies representing T-cell (CD3, CD45, CD56) and macrophage (CD14, CD45) populations were also selected. The concentration factor of the CRF compared to baseline ranged from 5.93-8.84 (mean of 7.41). The percent recovery in the CRF ranged from 59.3-91.8% (mean of 76.9%) for the selected antibodies.

| | [Base] ($x10^3/\mu l$) | [CRF] ($x10^3/\mu l$) | Concentration Factor (CRF/Base) | CRF Recovery (%) |
|---|---|---|---|---|
| CD3 | 1.40 ± 0.22 | 11.99 ± 2.11 | 8.84 ± 1.65 | 88.4 ± 16.5 |
| CD14 | 0.86 ± 0.19 | 4.57 ± 1.00 | 5.85 ± 0.99 | 58.5 ± 9.9 |
| CD34 | 0.12 ± 0.04 | 0.86 ± 0.18 | 7.87 ± 1.33 | 91.8 ± 15.5 |
| CD45 | 10.74 ± 2.92 | 68.31 ±18.31 | 6.49 ± 1.02 | 64.9 ± 10.2 |
| CD56 | 1.17 ± 0.38 | 7.22 ± 1.59 | 7.67 ± 2.07 | 89.5 ± 19.1 |
| CD105 | 0.72 ± 0.20 | 2.93 ± 0.51 | 5.93 ± 1.37 | 59.3 ± 13.7 |
| CD117 | 0.50 ± 0.27 | 2.03 ± 0.59 | 7.84 ± 1.87 | 78.4 ± 18.7 |
| CD133 | 0.03 ± 0.01 | 0.18 ± 0.03 | 7.45 ± 1.28 | 74.5 ± 12.8 |
| VEGFR2 | 0.95 ± 0.17 | 7.98 ± 1.58 | 8.72 ± 1.62 | 87.2 ± 16.2 |

Table II-Antibody positive cell concentration and percent recovery for human aspirate samples (n=6)

Table III shows the average (±SE) number of fibroblast colony forming unit cells that were sequestered from three human aspirate samples using the GPSII™ Platelet Concentrate System and standard Ficoll separation. Identical input volumes (55ml) were processed using both techniques. The average output volume of the cell-rich fraction was 5.83ml for the GPS method and 6ml for the Ficoll method.

| | Processing Time (min) | CFU-F per ml | Total CFU-F |
|---|---|---|---|
| GPS | 31.7 ± 3.3 | 1506 ± 371 | 8779 ± 2162 |
| Ficoll | 193.3 ± 22.4 | 588 ± 189 | 3530 ± 566 |

Table III-Fibroblast colony forming unit data for GPS and Ficoll separation methods from equal volumes of human aspirate samples (n=3)

The average number of CFU-F cells recovered using the GPS method was 8779 with an average of 3530 being recovered using the Ficoll technique. This represents a 2.49 fold increase in the number of CFU-F colonies recovered using the GPS method compared to the Ficoll method. Figure 1 shows a typical cell colony at the termination of the CFU-F assay.

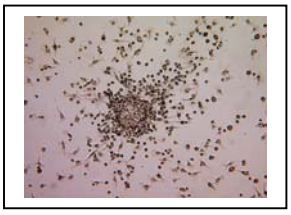

Figure 1-Example of a cell colony following a CFU-F assay

**Conclusions**

A method to concentrate progenitor cells from human bone marrow aspirate samples was analyzed using cell counts, flow cytometry, and colony forming unit assays. This preliminary data demonstrated the ability of this method to concentrate mononuclear cells. Flow cytometry revealed the ability of the method to recover and concentrate a variety of progenitor, T-cell, and macrophage populations. Compared to a standard laboratory technique, this method recovered a greater number of fibroblast colony forming cells from the same baseline aspirate samples and did so in approximately one-sixth the time.

The method of concentration primarily consisted of a 15-minute centrifugation process making implementation in a point of care setting feasible. Further analytical work will provide a greater understanding of the cellular population being harvested with this method.

**References**

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