A Rapid Technique for the Isolation and Concentration of Stem Cells From Human Bone Marrow

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Statement of Purpose: Adult stem cells such as mesenchymal stem cells (MSCs) are being examined for their potential application in a variety of clinical arenas, including orthopedic[1] and cardiovascular[2] tissue regeneration. Bone marrow remains a convenient source for obtaining MSCs. Ideally, one would like a simple, reproducible means to isolate and concentrate the cells, allowing the physician to reapply the enriched fraction according to the therapeutic requirements. The Gravitational Platelet Separation (GPS™-II) System is a simple device currently being used at the patient's point of care to isolate and collect platelet concentrate from a fraction of whole blood. The goal of this study is to determine if the GPS-II system could also be used to isolate and concentrate viable adult stem cells from whole marrow, and if the cells were still capable of being induced toward various lineages.

Methods: *Processing of Marrow:* Approximately 25 mL of human bone marrow (Cambrex, Walkersville, MD) was aspirated from the iliac crest of ten (n=10) adult donors, mixed with 100 U heparin/mL of marrow, and shipped overnight. An aliquot of marrow was removed and used for baseline nucleated cell counts using a Neubauer hemacytometer. The GPS™-II Mini Platelet Concentrate Separation Kit (Cell Factor Technologies, Inc., Warsaw, IN) was used to separate the remaining marrow fraction. This involved a 15 minute centrifugation cycle at approximately 1400g. After centrifugation, the buffy coat fraction was isolated and collected, and nucleated cell counts were again performed. Viability was determined using Trypan Blue exclusion.

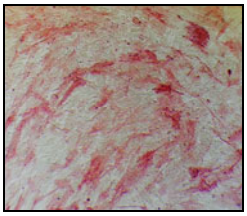
Cell Culture and Differentiation: The resultant cells obtained from the buffy coat isolation were plated and cultured in DMEM with 10% FBS/1% antibiotic at 37°C in 5% CO_2. Cells were culture expanded for a minimum of two weeks, harvested, and used for differentiation assays as described below.

Osteogenic Assay: Cells were replated in 6-well plates and cultured in osteogenic media supplemented with β-glycerophosphate, ascorbic acid, and dexamethasone as described previously[3]. After 16 days, cultures were stained for the presence of alkaline phosphatase.

Adipogenic Assay: Cells were replated in 6-well plates and cultured in adipogenic media supplemented with IBMX, dexamethasone, insulin, and indomethacin. After 21 days, cultures were stained with Oil Red O for lipid accumulation[4].

Chondrogenic Assay: Cells were replated in micromass cultures and maintained in chondrogenic media supplemented with dexamethasone, ascorbic acid, proline, ITS^+, sodium pyruvate, and TGF-β1 for 14 days. Resultant pellet cultures were papain digested and analyzed for sulfated glycosaminoglycans (s-GAG) using dimethylmethylene blue.

Results / Discussion: An average of 3.1 ± 0.1 mL of buffy coat was collected. Average cell counts on the marrow sample before and after centrifugation were $2.62x10^7$ ± $8.2x10^6$/mL and $1.23x10^8$ ± $5.0x10^7$/mL, respectively, indicating a 4.6-fold (±0.8) increase in nucleated cells. This correlates to a 59 ± 8% cell recovery from the whole marrow sample. Cell viability in all cases was >99%. After two weeks in growth media, cultures had not yet reached confluency, although several large colonies could be seen in each culture. After culture in osteogenic media, a dense network of nodules was observed, and abundant positive staining for alkaline phosphatase could be seen (Fig 1a). Cells cultured under adipogenic conditions gave evidence of progressing toward the adipogenic lineage. Clusters of intracellular lipid-containing droplets could be seen scattered throughout the culture. These droplets stained positive when treated with the lipid dye Oil Red O (Fig 1b). Control cultures for both cases failed to exhibit positive staining (Fig 1c). Pellet cultures exposed to chondrogenic media conditions had a glossy white appearance after two weeks, and contained 7.8 ± 4.3 µg s-GAG as compared to 0.3 ± 0.4 µg for control cultures. Although there was a large variation in s-GAG content and pellet size from donor to donor, in all cases the chondrogenic pellet culture was larger and contained more proteoglycan then the matched control.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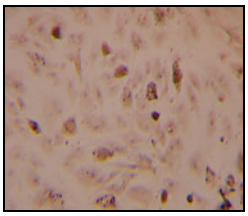
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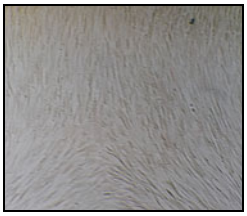
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Conclusions: The GPS-II Platelet Concentrate Kit facilitates the isolation and concentration of stem cells from whole marrow. The system is easy to use, reproducible, and provides for a fraction enriched in viable multi-potent cells. Further characterization of the cell population obtained is warranted.

References:

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