Maintaining Mixed Populations of Adult Stem Cells Enhances Osteogenic Potential

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Statement of Purpose:

Stem cell therapy is a growing focus for the emerging field of regenerative medicine. A significant challenge to future clinical advances is obtaining sufficient quantities of stem cells possessing the necessary regenerative potential. Protocols to culture expand these cells typically use standard tissue culture vessels, manual medium exchange and harvest procedures with serial passages. Aastrom Biosciences utilizes a GMP compliant single pass perfusion (SPP) bioreactor technology for automated expansion and harvest of stem and progenitor cells from human bone marrow (BM).[1] SPP is significant for the production of the stromal cell populations with gene activation for early stage growth factors, that support stem cell maintenance and replication, as well as the MSC populations. The Tissue Repair Cells (TRCs) produced are a mixed population of cells and have been characterized by cell surface markers, gene and protein expression, and functional in vitro assays for bone, cartilage, fat, and endothelial cells. TRCs are currently in clinical trials for a variety of tissue regeneration indications.

Most stem cell therapy strategies focus on purified populations of cells. However, some studies demonstrate enhanced tissue formation when purified stem and progenitor cells are co-transplanted with accessory cells.[3] We hypothesized that a mixed cell population would yield a more physiologically relevant regenerative potential, in terms of markers of bone generation, than a purified stromal cell population. To address this hypothesis, we tested the in vitro osteogenic differentiation potential of mixed BM-derived cell primary cultures versus the stromal fraction. We found that removing the non-adherent cells during culture of the stromal fraction decreases the osteogenic potential of BM-derived cells.

Methods:

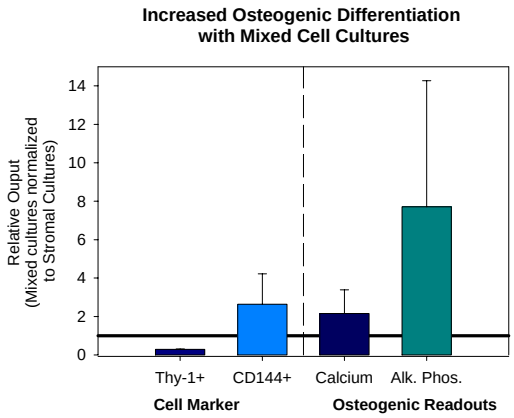
Fresh BM mononuclear cells (MNC) were obtained from healthy donors (Poietics Inc. Gaithersburg, MD) and cultured in Aastrom's bioreactor. Bioreactors contained a cell bed made of plasma treated, glycol-modified polyethylene terephlalate (PETG). BM MNC were seeded into bioreactors at 300,000 cells per cm^2 and cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% horse serum, 10% fetal bovine serum , hydrocortisone, vancomycin, and gentamicin.[2] For evaluation of stromal cultures, the non-adherent cells were drained from the respective bioreactors on day 2 of culture. Mixed cell cultures were not manipulated during culture. Cells were harvested on day 12 using Trypsin-EDTA, counted, and analyzed with flow cytometry.

Cells were analyzed for osteogenic potential upon harvest after being exposed to culture media enriched with dexamethasone, ascorbic acid, and β-glycerophosphate to promote osteoblast differentiation.[4] Calcium was measured as μg per 10^5 cells plated (Pointe Scientific kit). Alkaline phosphatase was measured as nmol p-Nitrophenol/minute per 10^6 cells plated (Sigma-Aldrich).

Results / Discussion:

When testing mixed TRC versus stromal bone marrow-derived cell cultures, mixed cultures produced 3.9±0.4-fold more total nucleated cells than stromal cell cultures. However, the frequency of Thy-1+ cells was 3-fold greater in the stromal cell cultures (82% vs. 24%), while the number of accessory cells, such as those bearing the CD144+ endothelial marker was higher in the mixed cultures. Our previous work has shown that Thy1+ cells contain the CFU-F population and those cells with potential to differentiate into osteoblasts. In addition, correlation between Thy-1+ cells and ectopic bone formation in vivo has been observed with TRCs.[5]

When the ability to induce bone formation markers was compared between mixed and stromal cell only cultures, the osteogenic capacity was much greater in mixed cell cultures, despite a decreased frequency of Thy-1+ cells. Mixed cell cultures showed 2-fold higher calcium content and 7.7-fold higher alkaline phosphatase activity, when compared to stromal only cell cultures. In summary, removing non-adherent cells during primary culture decreased the osteogenic potential of the bone forming stromal cell population.



Conclusions:

TRCs expanded as mixed cell cultures result in a cell population with increased osteogenic potential compared to stromal cell only cultures from the same source of bone marrow-derived cells. This suggests the hypothesis that increased osteogenic potential of mixed cell cultures may be due to signaling between the various stem cell populations, their progenitors and more mature cells acting as accessory "enhancing" cells.

References:

1) Paalson et al., Biotechnology, Vol. 11, 1993.
2) Mandalam et al., Ex Vivo Cell Therapy, pg.273, AP, 1999.
3) Krebsbach et al., FASEB Journal, Vol. 19, April 2005
4) Jaswal et al., J Cellular Biochemistry Vol. 64, 1997.
5) J. Dennis et al., ASBMR poster, 2004.