Hyaluronan as a Novel Marker for Selection of Connective Tissue Progenitors: Comparison of Two Methods for Optimal Enrichment

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INTRODUCTION:
An effective tissue-engineered bone graft requires consideration of several factors: an osteoconductive matrix, osteoinductive stimuli, osteogenic cells, and the appropriate biophysical environment, including suitable mechanical and electrical stimuli and mass transport properties that maximize the survival of transplanted cells. Osteogenic cells are available from a variety of sources; however, bone marrow aspirates provide the inherent advantages of being available from everyone, as well as easily accessible with little morbidity and no immunogenic risk to the patient. Due to the diverse population of cell types in bone marrow, coupled with the relative rarity of osteogenic progenitor cells, termed connective tissue progenitors (CTPs), a continual challenge in this field is the preferential selection of CTPs out of this heterogeneous mix of cells in a fresh bone marrow aspirate. A connective tissue progenitor is defined as a tissue-adherent stem or progenitor cell that proliferates and expresses one or more connective tissue phenotypes. The frequency of these cells in bone marrow is quite low, on average 1 in 20,000 nucleated cells, and this rarity highlights the need for clinically relevant selection strategies to optimize the impact of these cells in a graft site. A system for positive selection of CTPs would allow for not only an increased concentration of CTPs in a graft, but also the elimination of the more numerous, non-osteogenic cells that do not contribute to new bone formation. In fact, these abundant, non-osteogenic cells may hamper new bone growth by competing with CTPs for the limited oxygen and nutrients available at the graft site. This overwhelming disparity in metabolic demand limits the depth at which CTPs can remain viable in the graft, and these competing, non-osteogenic cells contribute to persistent inflammation as pro-inflammatory cytokines and cell debris are released after cell death.

This study evaluated surface-bound hyaluronan (HA), a glycosaminoglycan present in many pericellular matrices, as a novel target for positive selection of CTPs from a fresh bone marrow aspirate.

METHODS:
Bone marrow was aspirated from 4 patients from the iliac crest according to approved IRB protocol (#5119). Buffy-coated mononuclear cells from freshly aspirated marrow were labeled with an HA binding protein coupled to magnetic beads and magnetically separated using the EasySep® system from Stem Cell Technologies. Two protocols were evaluated: the traditional purification scheme and a simplified, OR-friendly single pass protocol. The purification scheme produced 3 fractions from the magnet: HA+++ (HA+++, HA+, HA-, HA-(1P)), and then standardized to the geometric mean of the unselected marrow control, correcting for the large variability seen between patients. The geometric means and 95% confidence intervals were then obtained to determine significance.

RESULTS:
Using the purification protocol, a mean of 1.5% of cells were retained in the HA+++ fraction after magnetic separation. The geometric mean demonstrates that the HA+++ population showed a 2.9-fold enrichment in colony prevalence compared to the unselected bone marrow aspirate (BMA), and a 9.0-fold enrichment when compared to the HA- cells, which were both statistically significant. In addition, the HA+++ CTPs were found to be significantly more proliferative than those in the BMA, as measured by the number of cells per colony.

The single pass protocol separated 14.7% of cells in the HA(1P) fraction. These cells showed similar prevalence to the unselected marrow control, while the HA(1P) were significantly depleted in CTPs. The HA(1P) cells were significantly more proliferative than the unselected marrow control, as well as more proliferative than the HA- (1P) fraction. Figure 1 illustrates the differential partitioning of CTPs isolated in the separation from a representative patient for both the purification protocol and the single pass protocol.

DISCUSSION:
One or more subsets of human marrow-derived CTPs appear to present or retain a hyaluronan rich matrix on their surface at the time of harvest or form such a matrix soon after harvest. This may provide a useful method of subfractionation of CTP populations, for both biological characterization and clinical transplantation. The HA+++ population is both enriched in CTPs and highly proliferative, which provides a valuable population for transplantation. The HA(1P) population, while also significantly more proliferative, doesn’t offer statistically significant enrichment as compared to the marrow control.

The highly proliferative HA+++ cells may, for example, offer superior performance in an in vivo graft environment, due to the elimination of the majority of non-essential, non-osteogenic cells that may hamper new bone growth by competing with CTPs for the limited oxygen and nutrients available at the graft site.