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The effect on cell isolation from bone marrow using subsequent mechanical agitations

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Even though bone marrow is a rich source of cells, it is a very heterogeneous tissue. Isolating cells from cadaveric bone marrow yields a complex combination of cell types, some of which may be undesirable for a specific application and others that would be beneficial. We utilized mechanical agitation of bone pieces in four subsequent stages with no additional separation enzymes to extract cells from the marrow of cadaveric donors, followed by density gradient separation. In order to understand the general composition of cells extracted from each stage, we assessed viability, performed flow cytometry and Complete Blood Count (CBC) on the density gradient separated cells from each stage. This study has revealed that although viability decreases with each subsequent stage, the percentage remains above 90% for all stages. In all cases, the solution isolated from the first stage before density gradient separation was too coagulated to analyze by CBC. Two of three cases had significant coagulation in stage 2. Although the erythrocyte concentration in stage 1 was highest directly after agitation, there was no significant difference between the stages following density gradient separation. The most notable flow cytometry result was the significant reduction in the percentage of HLADR positive cells with each successive stage. We were surprised to find that most SSEA4 positive and CD105 positive cells are in isolate from the second stage. These findings suggest that although each stage reduces antigen-presenting cells, stage 2 is responsible for the majority of Mesenchymal Stem Cells by this method.

Biography

Miguel Quevedo completed his Master of Science in Biomedical Engineering at the age of 23 from the University of Miami. Currently he is a process engineer with Vivex Biomedical Inc. Stuart Oglesby completed his Bachelor of Science in Biology at the age of 22 from the University of Georgia and is currently product director for orthobiologics at Vivex Biomedical Inc.

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