Lab. Work 14.

Methods for Isolation of Bone Marrow Stem Cells: Comparative Analysis

Stem cell harvest and isolation

Advances in medicine and medical technology have resulted in a tremendous improvement in health and welfare. However, we are still faced with various diseases that are difficult to treat using contemporary medicine. For organ failure (heart, renal & liver failure) and neurodegenerative diseases (Parkinson’s and Alzheimer’s disease), there is, at present, no effective treatment other than the transplantation of organs from human donors or cells from a fetus.

The many problems associated with transplantation, such as immunological rejection, infectious diseases, and a lack of donors have prompted the search for a novel treatment method. During the past decade, regenerative medicine has emerged as a key technology in the next generation of medical care, and cell therapy and organ repair using stem cells have become very attractive options for regenerative medicine.

Various multipotent stem and progenitor cells exist in adult tissues and organs to replace lost or injured cells and are almost comparable to embryonic stem (ES) cells with respect to their ability to differentiate into various tissues in vitro and in vivo. Thus, there has been tremendous progress in understanding the mechanism of tissue regeneration. Application of these cells in regenerative medicine has required modified methods for isolation.

The techniques (5).

These methods are commonly used for debulking heterogeneous samples. The second group comprises affinity methods such as capture on affinity solidmatrix (beads, plates, fibers) , fluorescence-activated cell sorting (FACS) and magnetic cell sorting, which are based on biochemical cell surface characteristics and biophysical criteria (in FACS). Here, we are going to review development of methods applied to the isolation of cells from bone marrow (BM). Enrichment of mononuclear cells.

Several techniques for the enrichment of mononuclear cells (MNC) from original BM have been developed.

These techniques include sedimentation with agents such as Hydroxyethyl starch (HES) density gradients using Buffy coat and blood cell separators. The latest blood cell separators, with fully automated methods, allow BM processing in a closed system, which simplifies separation procedures and improves the quality of the collected cells in terms of cell recovery, collection selectivity, and microbiologic safety. Manual MNC isolation Early attempts to process BM were carried out using manual techniques.

A simple technique to separate red blood cells (RBC) from BM by sedimentation after addition of HES was developed specifically for ABO-incompatible allogeneic bone marrow transplant (BMT) .

Although effective, manual methods are, by nature of the excessive handling and exposure to the environment, more likely to result in bacterial contamination of the BM. Inclusion of unwanted components, including neutrophils and platelets, is also problematic when using manual buffy coat preparation, as there is no difference in the differential count of BM nucleated cells before and after processing.

Density gradient separation using Ficoll-Hypaque as described by Wells et al. yielded a nearly RBC-free BM cell suspension. Ficoll-Hypaque gradient centrifugation allows rapid and efficient isolation of mononuclear cells from human peripheral blood and also bone marrow. Using these kinds of techniques MNC yields generally exceed 50% of original cell numbers, but such techniques are associated with potential cell injury from the reagents used, as demonstrated by a significant reduction in tritiated thymidine incorporation and loss of lymphocyte viability following culture with FicollHypaque.

The method is also inefficient for process of cell separation holds an important role in cell therapy and regenerative medicine using stem cells. Stem cells are usually present in only small quantities in adult tissues and organs, and an effective separation procedure for stem cells is always required.

The separation of cells is different from the separation of other materials. Since cells are living and variable, depending on the surrounding environment, the separation processes are limited by the medium and operation.

The speed of the operation and viability during separation are important operating factors. During the last decade, several technologies have been proposed to purify hematopoietic cells for clinical use.

We focus on different methods used for the separation of stem cells from bone marrow for transplantation and compare these methods, pointing out their advantages and limitations.

**Procedures for cell separation.**

The ability and efficiency of various techniques to purify adult stem cells from a heterogeneous cell population is an important factor in the successful characterization and application of stem cells. Existing cell separation methods can be classified into two main groups. The first is based on physical criteria like size, shape, and density differences and includes filtration and centrifugation

techniques.

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The method is also inefficient for large volumes and requires considerable technical expertise. As such, this technique is the starting point for most studies of human lymphoid cells. In general, Ficoll-Hypaque centrifugation does not change either the phenotype or the function of the isolated mononuclear cell population. However, it may be best to verify this in studies of cells from patients with various diseases.

Schwella et al. noted that BM products obtained by manual means are comparable to those processed by a blood cell separator and that separating cells by Ficoll-Hypaque centrifugation also often decreases the cytometry time for acquisition and removal of nonviable cells.

The majority of groups prefer applying unfractionated autologous BM MNC, manually isolated by Ficoll density gradient-based separation. This conventional method is a time-consuming process involving at least two washing steps that make the system ‘open’. With operator-dependent results, manual Ficoll density gradient, based MNC separation, is neither standardized nor reproducible and thus not optimally suitable for clinical implementation, although groups have tried to standardize it to good manufacturing practices (GMP) grade.