

A Comparative Study using Negative Selection and Standard Methods for Adult Mesenchymal Stem Cells Isolation

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INTRODUCTION: Realizing the therapeutic potential of MSCs to repair varieties of tissue, current research has been directed in establishing MSC isolation and manipulation techniques. As a result, several of these methods have been identified each claiming to be better than the other. Ficoll-Paque method involves the use of high density with low viscosity and low osmotic pressure liquid under gradient centrifugation to obtain the mononucleated fraction of bone marrow which contains MSCs (Bobis et al., 2006). However, this method involves several phases of manipulation and centrifugation process that may increase the risk of contamination. Study have also shown that separation of mononucleated cells using the ficoll-paque method resulted in a poor recovery rate of MSCs (Hung et al., 2002). Without using ficoll-paque gradient separation, our project intention was to look for an isolation method which is cheaper, easier, better efficiency and able to provide higher cell yield.

METHODS: In trying to establish the best of currently available methods, we conducted a study to compare standard isolation method (using Ficoll-Paque) and negative selection method (without using Ficoll-Paque). For standard isolation method, MSCs derived from bone marrow of New Zealand white rabbits were isolated from ficoll-plasma interface layers and later cultured in low glucose medium containing 10% Fetal Bovine Serum. For negative selection method, cell pellets attained from centrifugated bone marrow aspirate was first suspended in medium prior to cell culture. Observations were made at day-1, day-3, day-7 and day-14. The number of nucleated cells was estimated after counting into hemocytometer and cellular viability was determined by the Trypan Blue exclusion method. The isolated cells from the two different methods were then characterized by immunostaining analysis of specific surface antigens. Tested markers included MSC markers (CD 29 and CD 44).

The cell morphology was examined by hematoxylin and eosin (H&E) staining.

RESULTS: Our preliminary results show that in both methods; cell attachment were noticeable on flask surfaces as early as day-3 and achieved 70%-80% confluence within 2 weeks. Both methods have shown same efficiency in MSCs isolation with no significant differences in cell number, MSC morphology and expression. Immunostaining revealed that rBM-MSCs were positive in CD 29 and CD44. Hematoxylin and Eosin (H&E) staining showed mainly fibroblastoid-shaped elongated cells with darkly stained nuclei.

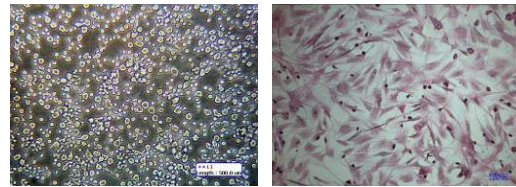


Fig1. Morphological appearance of MSCs after day 3 of culture and H&E staining of MSCs

DISCUSSION & CONCLUSIONS: Negative selection method was comparable to that of standard isolation method. In conclusion, a simpler and efficient MSCs isolation method without using Ficoll-Paque have been identified and established. Future work in characterizing the isolated MSC will be carried out by using a flowcytometer. The outcome of this study will contribute to the improvement of MSC isolation technique for future stem cell therapies.

REFERENCES: ¹Bobis S. et al., *Folia Histochemica Et Cytobiologica*, 2006, 44(4):215-230. ² Hung SC et al., *Stem Cells*, 2002, 20:249-258.

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