TITLE:
APPLICATION OF ADULT MESENCHYMAL STEM CELL TECHNOLOGY FROM HUMAN BONE MARROW IN BONE DEVELOPMENT

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CURRENT STATUS OF PROJECT: On going.

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INTRODUCTION

Everyday thousand of people suffer from bone diseases that lead to destruction of bone tissue. The disease need to be replaced. Various types of bone allograft, xenograft and synthetic biomaterial are now used for bone replacement therapy but the future of bone reconstruction lie in the use of stem cell technology for bone development. Mesenchymal stem cells (MSCs) have been paid increased attention because of their powerful proliferation and pluripotent differentiating ability. In this study we try to isolate MSCs from human bone marrow and to differentiate MSCs into osteoblast cells in osteogenic medium. Culture-expended of MSCs were characterized by single cell derived colonies (CFU-F) and the present of CD13/CD105 were characterized by using LSAB kit. Optimal osteogenic differentiation were determined from the formation of a mineralized extracellular matrix visualized by Von Kossa staining and Alkaline Phosphatase ALP assay . Moreover osteogenic differention were judge by RT-PCR profiling of gene expression.

OBJECTIVES

1. To isolate mesenchymal stem cell from human bone marrow.
2. To differentiate mesenchymal stem cell into osteoblast cells in osteogenic medium

METHODOLOGY

1. MSC Isolation and Culture

Human bone marrow mononuclear cells was isolated by using ficoll-paque to separate the mononuclear cells from red blood cells and granulocytes. Mononuclear was cultured in a 25 cm^2 flask containing 5.0ml of MSC culture medium (DMEM, 10% fetal bovine serum, 1% penicillin/ streptomycin) and incubated in 5% CO_2 in air at 37^oC and the cells was monitored daily.

2. Characterization of Mesenchymal Stem Cell (MSC).

i) Direct Plating.

Mononuclear cell (MNC) was cultured in medium (DMEM, 10% fetal bovine serum, 1% penicillin / Streptomycin). After 24 h, non-adherent cells was removed, then washed and changed medium. Adherent cell clusters will give rise to colony-forming fibroblastic cell (CFU-F).

3. To detect the present of CD 13/ CD 105 as a surface marker on mesenchymal stem cell.

i) LSAB kit (Labelled Streptavidin-Biotin Method)

4. To stain matrix mineralization associated with osteoblast.

i) Von kossa Staining.

MSCs were cultured for two weeks in osteogenic medium. Then the cells were fixed in 4% parafolmaldehyde after rinsing with Phosphate-buffered Saline (PBS). Then the cells were stained by Von Kossa staining method.

5. To detect gene expression.

i) RT-PCR
RNA were extracted from MSCs by using kit. The quality of the RNA was checked from the spectrophotometer reading A_{260}, Purity A_{260}/A_{280}=1.7-2.0 and RNA gel electrophoresis. Then two-step RT-PCR were run and were checked by doing gel electrophoresis.

EXPECTED OUTCOME:

i) A population of mesenchymal stem cell derived from human bone marrow can be identified.
ii) These MSCs can differentiated into osteoblast.