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Evaluation of the morphology of two types of Mesenchymal stem cells

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Abstract: Bone marrow-derived MSCs (BM-MSCs) is rare population represent about ~ 0.001–0.01% of bone marrow mononuclear cells. Because of their low abundance, in vitro culturing and expansion is essential requirement to obtain adequate numbers for research or clinical application. During long term culture, cells lose their proliferation, differentiation capacity and enter cellular senescence. In this study, we have tried to maximize number of cells separated from the same sample by allowing adhesion of bone marrow cells for 24h labeled with first adhesion cells (FACs) and re-culture of suspended cells again in a new flask labeled with re-culture of suspended cells (RSCs). We have characterized the morphology of FACs and RSCs. The results showed that the two types of cells have the same morphology. We concluded that we were able to isolate larger number of MSCs from the same sample

keywords: MSCs, reculture, morphology

1. Introduction

Mesenchymal stem cells (MSCs) are adult undifferentiated multipotent stem cells having the ability to self-renewal and differentiation into mesodermal lineage as osteocytes, adipocytes, and chondrocytes [16]. MSCs can be isolated from tissues and secretions of the adult body, such as adipose tissue, peripheral blood, dental pulp, yellow ligament, menstrual blood, endometrium, milk from mothers, as well as fetal tissues: amniotic fluid, membranes, chorionic villi, placenta, umbilical cord, Wharton jelly, and umbilical cord blood [2, 7].

MSCs are an attractive candidate for use in regenerative medicine because of their biological functions which include ability to migrate into sites of injury, differentiate into several cell types, secrete various trophic factors, low immunogenicity, and modulate the immune system [11, 14]. MSCs are not a pure population of stem cells having a heterogeneous mix of multipotent stem cells, committed progenitors, and differentiated cells with varying differentiation and self-renewal potential [15]. Unfortunately, MSCs do not have unique marker to be used in identification, so, international society for cellular therapy (ISCT) determined minimal criteria to define MSCs from other type of stem, progenitor and differentiated cells. MSC must be able to adhere to plastic surface, Positive for CD105, CD73, and CD90 but negative for hemato-poietic markers such as CD45, CD34, CD14, or CD11b, and capable of developing to osteoblasts, adipocytes, and chondroblasts under conventional in vitro differentiation conditions [6].

Bone marrow mesenchymal stem cells (BM-MSCs) represent the historically first and most common source of MSCs. BM-MSCs offer physical support to hematopoietic stem cells and differentiate into osteoprogenitors to guarantee a functional remodeling of the BM niche [4]. BM-MSCs represent a rare population ranging from 0.001 to 0.01% of the total nucleated cells [10]. Because of the low percentage of MSCs can be isolated from donor tissue, harvested primary MSCs should be expanded first in 2D culture surface, to reach sufficient numbers for usage in many research and clinical application [12]. However, the use of MSCs in cell therapy has been hampered by

a number of constraints, including decrease in proliferation rate, restricted life span, and gradual loss of stem-ness during *in vitro* expansion [9].There are many strategies which were developed to increase number of cells and improve efficiency of MSCs such as preconditioning of MSCs through exposure to sub-lethal cellular stressors(such as oxidative stress, heat, and nutrient depletion) , optimization of cell culture condition (such as hypoxia, and seeding density) and genetic modification [5, 12].

BM-MSCs are traditionally isolated from bones of femur and tibia by culturing in complete media containing (Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (FBS) and penicillin/streptomycin) for 24-72h, then discard suspended cells, and feeding the adhered cells [8]. In this study we hypothesized that the suspended cells still have a plastic adhered cells that similar to MSCs.

In this study, we tried to evaluate the morphology of two types of cells (cells after 24h culture labeled with first adhesion cells (FACs) and cells from re-culture of suspended cells (RSCs)).

2. Materials and methods

2.1. Rat bone marrow mesenchymal stem cells isolation and culture:

Bone marrow cells were collected by flushing of femur and tibia of female Sprague Dawley (SD) rats (150–200 g and 6–8 weeks, obtained from medical experimental research center (MERC).Bone marrow cells were cultured in 75cm^2 flask with 15 mL of complete culture medium (CCM), containing DMEM/ F12 (cat. no. 11554546, Gibco™) with 10% fetal bovine serum (cat. no. F7524, Sigma-Aldrich), 1% penicillin streptomycin (cat. no. P4333, Sigma-Aldrich). Cells were incubated at 37°C and 5% CO_2. After 24h, suspended cells were transferred to a new culture flask and allowed to re-adhere (labeled as RSCs). In the same time, the adhered cells were cultured in a new culture media (labeled as FACs).After another 24h of plating, non-adherent cells were removed, and medium was replaced at every 48 hours. Adherent cells from two types of cells were further propagated and when reaching 80–90% confluence, cells were detached by using 0.25% trypsin-EDTA (cat. no. 25-200-056; Gibco, United Kingdom) at 37°C for 3–5 min. Harvested cells were cultured in 75 cm^2 flasks for further expansion. During expansion time, media were changed every 3 days. Expanded cells were either used for downstream experiments or cryopreserved using freezing media (10%DMSO (cat. no. 67-68-5, sigma Aldrich), 70% DMEM/F12, and 20% FBS). Cells were examined under inverted microscope from P0 to P8.

3. Results and Discussion

3.1. Morphology of plastic adhered cells:

BM-MSCs were cultured in complete media and allowed to adhere to the plastic surface for 24h (FACs) flask. The suspended cells were transferred to another flask to allow re-culturing again for another 24h. The cells showed plastic adherence ability in the two groups and the morphology of adhered cells were examined by inverted microscope.

In P0, Number of adhered cells in FACs was more than that adhered in RSCs. But RSCs shows homogenous cells than FACs that showed heterogeneous mixture of cell. Both types of cells either FACs or RSCs cells were grown nearly in the same morphology with fibroblast-like spindle shaped cells, formed scattered colony and proliferated to form monolayer cells **(and**Error! Reference source not found.**)**.

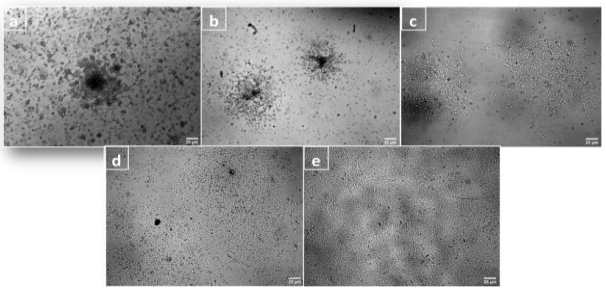
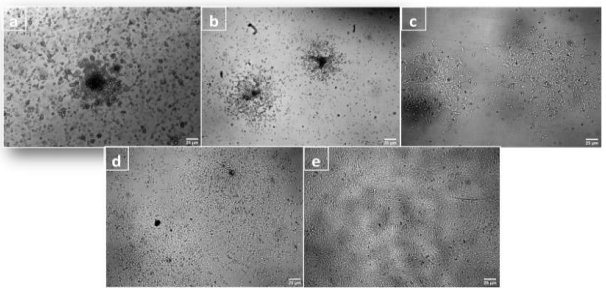


Fig1: Morphology of BM-MSCs after 24h adhesion (FACs) in P0 at different degree of confluence



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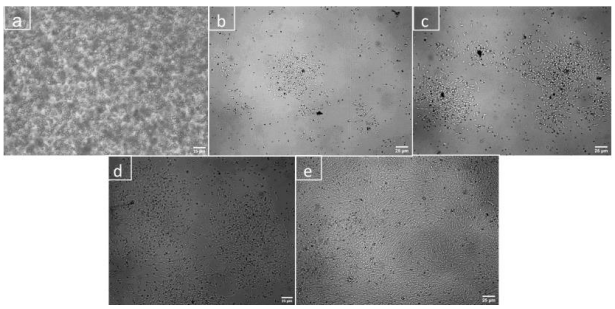

Fig 2: Morphology of BM-MSCs from re-culture of suspended cells (RSCs) in P0 at different degree of confluence

FACs and RSCs from P1 to P8 were able to proliferate linearly with normal morphology as shown in (Error! Reference source not found. andFig)

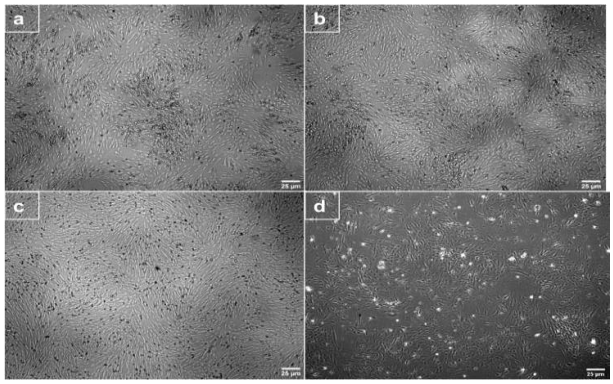

Fig 3: Morphology of FACs of (a) P1, (b) P2, (c) P3, and (d) P8

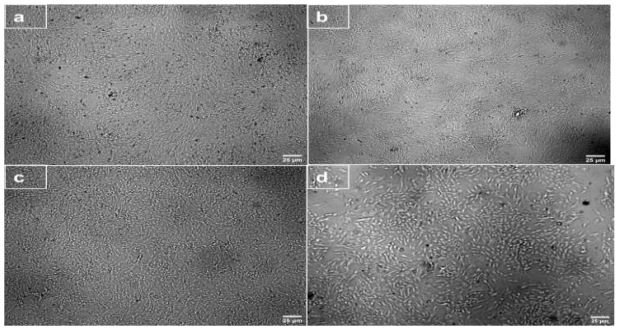

Fig 4: Morphology of BM-MSCs from RSCs in (a) P1, (b) P2, (c) P3

The clinical use of culture-expanded marrow derived MSCs in the fields of tissue engineering, cell therapy, and gene therapy has become popular and widely accepted. The most important step in MSCs-based cell therapy is to obtain sufficient quantity of cells in a clinically permitted period. Usually, this could be achieved by culturing bone marrow or bone derived cells in a relatively long period. There are various methods to isolate and expand MSCs, including density gradient isolation, immunomagnetic isolation, flow cytometry sorting and plastic-adherent culture [13]. Other methods have been employed to increase the proliferation of marrow-derived MSCs, including conditioned medium, autologous serum or plasma, and combinations of growth factors. We confirmed in this study that MSCs exist in the non-adherent cell population of primary bone marrow cell culture; when the non-adherent cells were collected and re-plated again as in the group of RSCs. This is a simple and cost-effective method to increase cell numbers and shorten the cell culture time, hence reducing the risks of contamination. MSCs derived from RSCs showed the same morphology when compared with MSCs derived from the first adherent cell (FACs) in agreement with Baksh, Davies [3] who demonstrated that BM-MSCs comprise a heterogeneous mix of adherent and non-adherent cells, both of them can give rise to multiple mesenchymal tissues including bone, cartilage or fat. However, these findings are in disagreement with Akiyama, *et al* who showed that the suspended MSCs failed to adhere to the plastic surface but adhered by using extra-cellular cell matrix (ECM)-coated dishes [1].

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