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Random Differentiation of Bone Marrow Derived Mesenchymal Stem Cells

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Abstract – AIM: To investigate and determine the capacity of mesenchymal stem cells (MSCs) toward differentiation and to which extent they can differentiate.
METHODS: MSCs were isolated from the bone marrow of Sprague dewaly rats, and tested for their self-renewal ability and then characterized using flowcytometer. For differentiation, cells were seeded in low density for 3 monthos. The morphology observed daily and documented.
RESULTS: cells propagated in culture for more than 30 passages, MSCs showed a high capacity for differentiated into adipocyte, osteocyte and neural cells after different time.
CONCLUSION: Bone marrow MSCs have great differentiation capabilities. MSCs can differentiate into functional cell lines, and may be used as a treatment for many diseases.

Keywords – Stem Cell, Random Differentiation, Neural Cells, Adipocyte and Osteocyte.

I. INTRODUCTION

Bone marrow mesenchymal stem cells carry much more important implications for future clinical strategies, because they are easily accessible for an autograft and routinely isolated from adults without the religious and ethical concerns like those of fetal embryonic cells.([1],[2]) Some researches showed that bone marrow-derived MSCs can differentiate into multiple types both in vitro and in vivo.([3],[4]) But other publications presented conflicting results.([5], [6])

Among bone marrow cells, there are two subsets, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Bone marrow (BM) derived mesenchymal stem(MSCs) were first defined by Friedenstein, who found an adherent fibroblast-like cells that can differentiate into bones so he referred to as osteogenic precursor cells.1 Subsequent researches showed that these populations have the capability to differentiate into multiple other mesodermal cell lineages, including chondrocytes, tenocytes, and myoblasts . Based on this multilineage differentiation capacity, Caplan called these populations mesenchymal stem cells.[7]

Based on their adhesiveness property to plastic surfaces [8], [9]),Mesenchymal stem cells can be isolated from the bone marrow, and from other tissues, including adipose tissue,[10] placenta, amniotic fluid, and fetal tissues such as fetal lung and blood. [11] In addition, umbilical cord blood (UCB) has been identified as a source of MSCs. Probably as a result of their low frequency in UCB, conflicting reports

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initially have been published on the presence of MSCs in UCB[12].

MSCs are thought to be pluripotent cells, these cells Under appropriate conditions can be differentiated into many types of cells or tissue types including cartilage, bone, adipose and fibrous tissues, and myelosupportive stroma([8], [9]).and can be ideal source of transplantation therapy. Moreover, seeding these cells with some hormones, amino acids and growth factors such as epithelial growth factor and brain-derived neurotrophic factor or chemical products such as dimethyl sulfoxide and butylated hydroxyanisole induced the bone marrow stroma cells to exhibit different phenotypes ([13], [14]). These cells exhibit a remarkable plasticity, with the ability to differentiate into cells with mesodermal, neuroectodermal, and endodermal characteristics in vitro [15].

Furthermore, upon transplantation, bone marrow (BM) cells developed into hepatocytes by in vivo transplantation ([16]-[19]). they can also differentiate into epithelium of the liver, lung, and gut. Bonnet et al demonstrated that single cell–derived populations of murine BM-derived MSCs characterized by stage-specific embryonic antigen-1 expression, were capable of differentiation in vivo,5 thus showing their true stem-cell properties. Another study showed that the marrow-isolated adult multilineage-inducible cells capable of differentiating in vitro into cell lineages from all three germ layers have been described [20].

These findings indicate that BM cells include pluripotent stem cells, but the mechanism of BM cell differentiation into a specific cell lineage is still not clear. Herein, we trying to induce MSCs to differentiate into multiple lineage without using growth factors, hormones or chemical substances to gather more information about the capability of these populations to differentiate.

II. Materials And Methods

2.1. Animals

Six males of Sprague-Dawley rats at 48 weeks of age were used as a source of Bone marrow. All animals were obtained from medical Experimental research center, Mansoura university. experiments were performed in accordance with the animal guidelines of Mansoura University.

2.2.1. Isolation and culture of rat bone marrow

All rats were killed using overdose of thiopental then immediately sacrificed, femurs and tibias were extracted, all tendons and muscle were removed then the bones washed using 70% ethanol and merged in phosphate buffer solution(PBS) until marrow extraction. The marrow of each bone was flushed using 2-3 ml of DMEM-Low glucose, all bone marrow was collected and centrifuged for 10 min. at 1500xg. The pellet then seeded into T-75 tissue culture flask and incubated at 37^0 C and 5% CO_2.

2.2.2 Subculture of mesenchymal stem cells

After 48 hrs, all suspended cells were discarded and only adhered cells left in the flask. Medium was changed regularly every 3 days until the confleuncy was 75 %, at this point cells were harvested and subcultured at 1:2 split ratio.

2.3. colony forming unit (CFU)

All cells seeded in this assay were from passage 3 where all populations had the same morphology and appeared homogenous. Cells were seeded in 6-well plate at a density of 200 cells/ well, mixed and distributed evenly in each well. Medium was changed every 5 days for 2 weeks. All colonies were stained using crystal violet stain and counted under an inverted microscope.

2.4. Cell characterization

According to the manufacturer's recommendations with the monoclonal antibodies The cells were harvested smoothly using EDTA soln., washed twice with PBS and were resuspended in flowcytometry washing buffer (10 mL/L bovine serum, 2 g/L sodium azide in PBS), and stained for: PE conjugated anti-rat CD 29, FITC conjugated anti-rat-CD90 and PCy5 conjugated mouse anti-rat-CD44. After washed three times with washing buffer, the stained cells were resuspended in 300 µL fixation buffer (20 g/L formaldehyde in PBS) and run on a flow cytometer (FACS Calibur, BectonDi-ckinson, USA). The results were analyzed by CellQuest software (BD, USA).

2.5. Inducing random differentiation

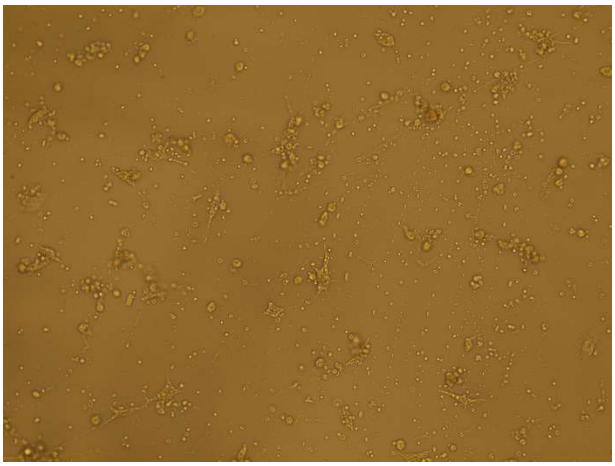
MSCs were cultured at low seeding density of about 100 cells/flask(T-25), the flask swirled good to confirm that each cell was seeded separately, the medium was not changed until PH turned yellow. Cells were not harvested for 3 months.

III. Results

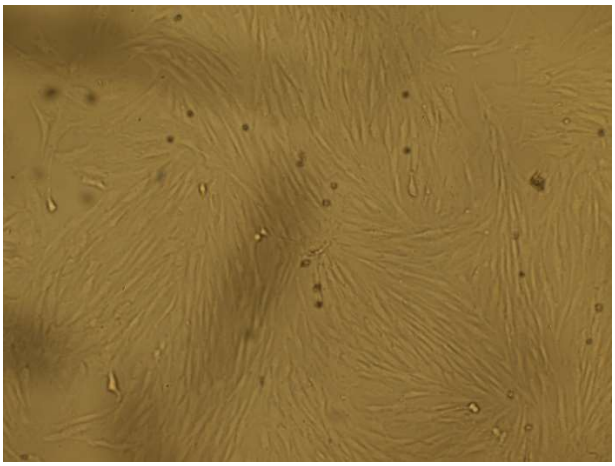
3.1. Cell morphology

3.1.1. Cell shape of MSC before differentiation

After passage 3 all populations were spindle shape-like cells, no round or polygonal cells. (figure 1A&B)



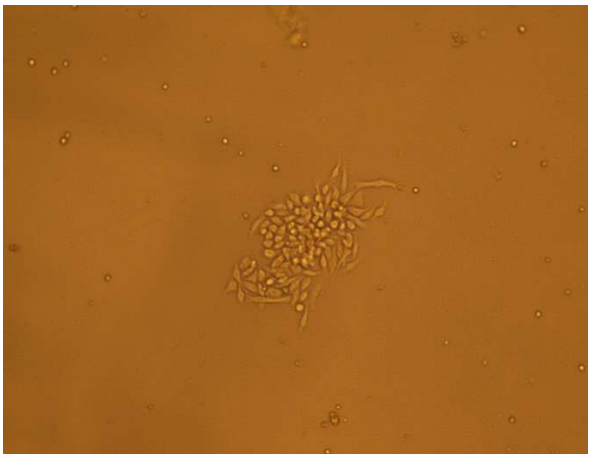
(figure 1A): bone marrow cells after 48 hrs display spindle shape morphology.



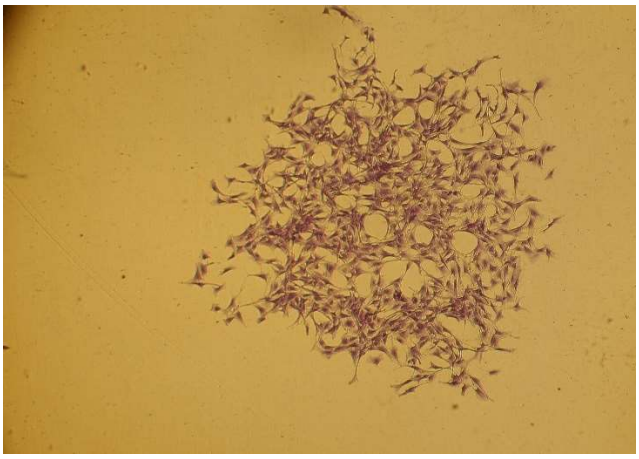
(figure B): MSCs after passage 3 showed homogenous clear morphology of spindle shape.

3.2. CFU assay

Colonogenic capacity of the cells were determined using CFU-F assay which provides a convenient tool to assess the proliferation and self-renewal capacity. Only colonies displayed more than 20 cells were scored under an inverted microscope (Figure 2A & B). Colonies with a morphology differing from the MSC morphology were excluded from the results. The results showed that each well gives more than 84% colonogenic efficacy after 2 weeks.



(Figure 2A)MSCs colony contain about 50 cells without stain.



(Figure 2B): MSCs colony stained with crystal violet.

3.3. Characterizing stem cell population with Flow cytometer

Flowcytometric analysis of all populations at passage 3 showed that the cells were stained positive for Mesencymal stem cell markers; $CD29^{+}$ And $CD90^{+}$ simultaneously and were negative for CD 45^{-} which considered a heamatopoitic marker as shown in (figure3).

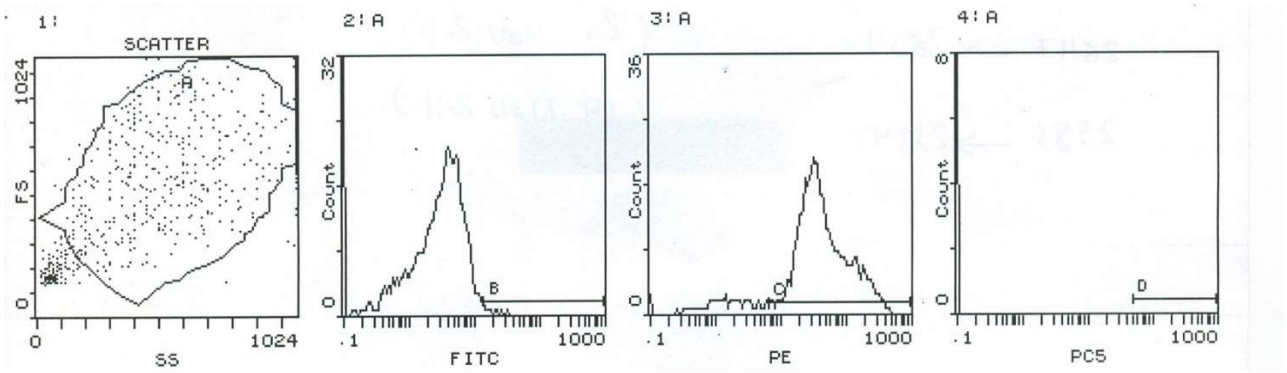


Figure 3: cells stained positive for CD29(FITC), CD90(PE) and Negative for CD 45(PCY5).

3.5. Reporting random differentiation

We have noticed that although all cells were cultured under the same culture conditions and the same type of medium, they differentiated in independent manner into many specific types of cells displaying the distinct morphology of each type clearly. They have differentiated into clusters of Osteocyte after 2 weeks (figure 4). In addition, they have differentiated into adipocyte in five weeks (figure 5) and into neural cells after 8 weeks (figure 6).

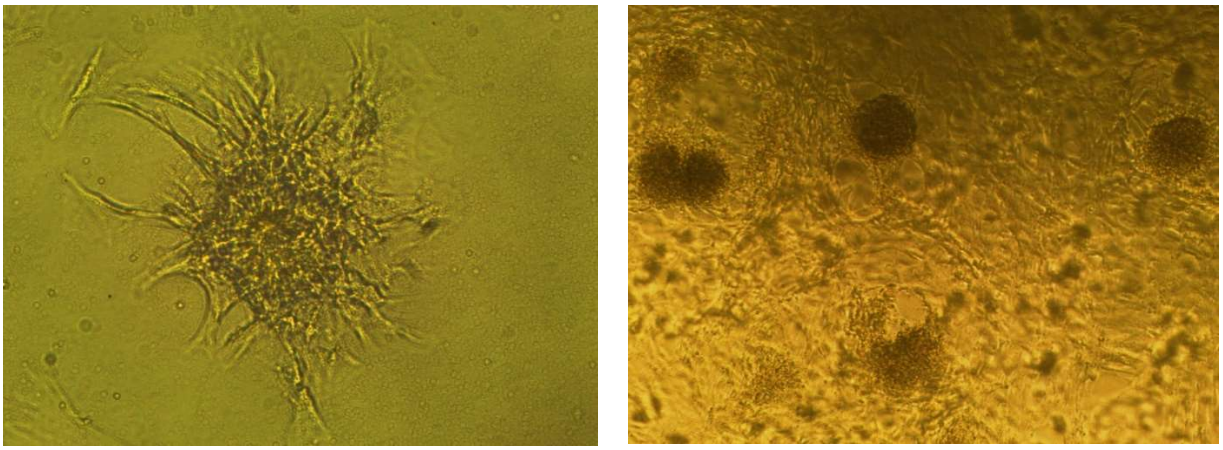


Figure 4: cells formed a distinct clusters of Osteocyte.

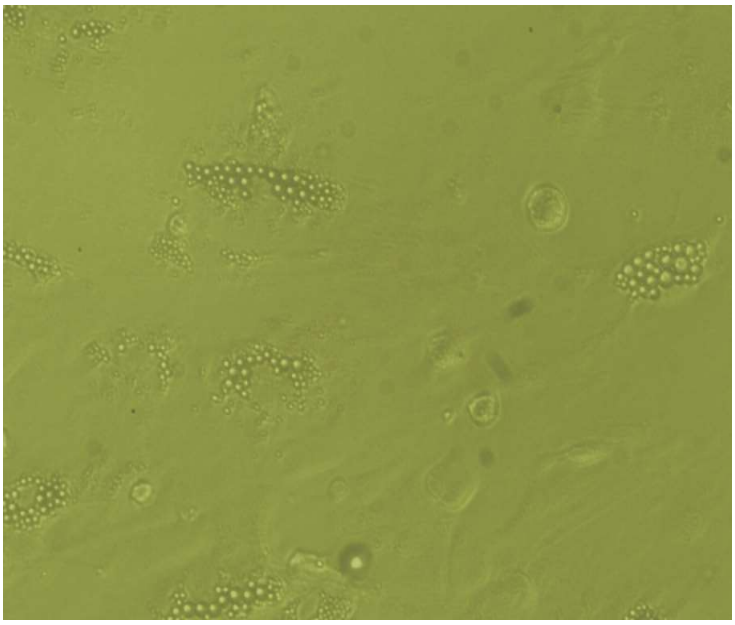


Figure 5: fat droplets appear show clearly in the cytoplasm without staining.

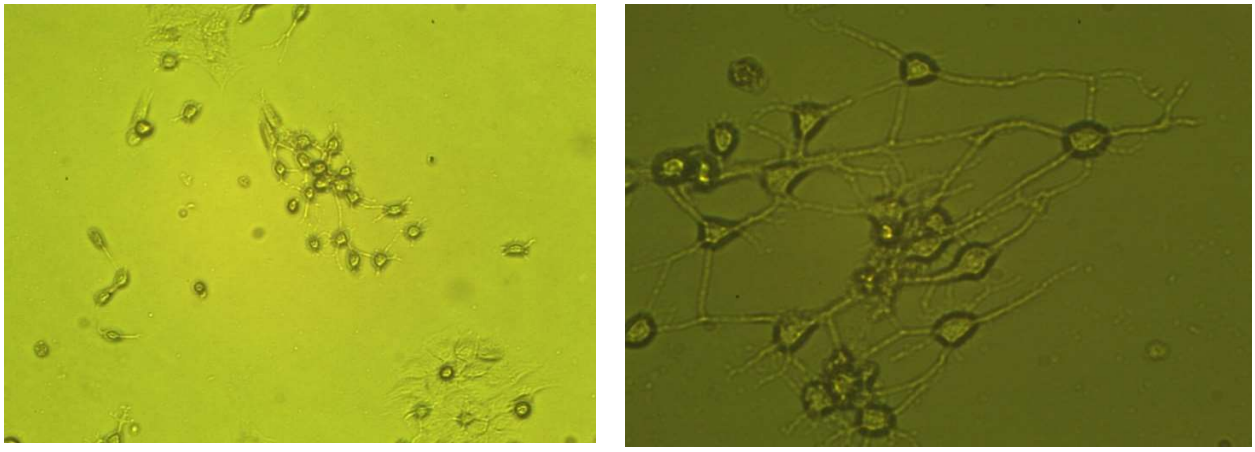


Figure 6: cells gained the morphology of neurons and connected via clear neural network.

IV. Discussion

Bone marrow has been known for years as a safe and abundant source for large quantities of adult stem cells. BM stem cells are normally present in adult marrow, pluripotent cells, and can easily differentiate into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, cardiomyocytes and marrow. They have also been shown to differentiate into cells of ectodermal origin, such as neurons, although with a much lower efficiency compared with mesoderm derivatives.

Hess et al [21] tested the capacity of transplanted bone marrow-derived stem cells to initiate endogenous pancreatic tissue regeneration in diabetic mice. Tang et al [22] also reported the same results. Yet other reports demonstrated that such events only occur at very low frequency, and the apparent plasticity has been shown in one case to be explained by cellular fusion. ([23], [24]) However, most of these studies mentioned above were based on animal models and little is known about the differentiation mechanism of bone marrow-derived stem cells in vitro.

Apart from MSCs, bone marrow also contains a hematopoietic stem cell population that can engraft in epithelial tissues and differentiate into epithelial cells of the liver, lung, gastroin- testinal tract, and skin [25].

In this study, we demonstrated that BM cells have the ability to differentiate into multiple cell lines and not restricted to one lineage in an independent manner. we identified, characterized, and propagated bone marrow cells for a long time and this provided the direct evidence that the differentiation capacity of bone marrow-derived MSCs in Vitro is unlimited.

Assady et al. show that pluripotent undifferentiated human ES cells spontaneously differentiate in vitro into cells with characteristics of insulin-producing cells. Mouse ES cells have also been shown to normalize blood glucose when transplanted into streptozotocin-induced diabetic mice. Similar results have been obtained by Castaing et al. by grafting human pancreatic tissue in beta cell–deficient severe combined immunodeficiency mice, but use of embryonic tissues raises ethical issues ([26], [27], [28]).

The present study demonstrates that, alternatively, MSCs can be shifted toward different phenotypes with distinct morphology. We characterized MSC phenotype and pluripotency more extensively.

Previous reports demonstrated that MSCs are relatively easy to expand ([29], [30]). In this study, we examined the extent to which the cells could be differentiated in culture by lowering seeding density and increasing culture period. Despite some tiny strain-dependent variations, exponential growth is maintained for more than 30 passages and phenotype is conserved.

MSCs could be induced to differentiate into endoderm and ectodermal derivatives. Different protocols have been tried to induce bone marrow-derived MSCs to differentiate into IPCs in vitro. High glucose concentration was considered as a potent inducer for pancreatic islet differentiation. [22] Data from other literatures report that MSCs could be transduced with chemical substances and using also high efficiency retroviral vectors ([31], [32], [33]). Other investigators suggest in vitro, cocultivation of hepatocytes and nonparenchymal cells has been used to preserve and modulate the hepatocyte phenotype [34].

So, it can be hypothesized that pluripotent stem cells reside in the adult bone marrow in a standby state but their differentiation potential is restricted by their natural environment.

Our cell population was negative for CD45, and we favor the hypothesis that cells undergoing differentiation were mesenchymal and not hematopoietic stem cells.

The induced differentiated cells were morphologically similar to pancreatic islet-like cells.

In our experiment, the phenotype of bone marrow-derived stem cells is negative for CD45, which indicated they were unlikely to be hematopoietic stem cells. On the contrary, CD29 and CD 90 are positive indicating that the bone marrow-derived stem cells might be bone marrow-derived MSCs. This is consistent with other reports. ([35], [36])

We hypothesized that cell-cell interactions are imperative for coordinated organ function, and cell specification. So, it is interesting to determine whether or not cell-cell interactions improve BM cell differentiation into a hepatocyte lineage.

In the present study, cell culture may have played a role in stimulation for differentiation of MSCs into Neural cells, adipocyte and osteocyte. In addition, this process may depend on the cell density and how cells are distributed in the flask.

V. Conclusion

In conclusion**,** cells in the BM may be a useful resource for transplantation therapy for many diseases and tissue damage regardless of the tissue type, they have a great potential to differentiate. We recommend that further studies are necessary to explain the mechanism of this process and how cel

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