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**RESEARCH ARTICLE**

# The effect of Bromodeoxyuridine on Mesenchymal Stem Cells viability and differentiation

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## *Abstract*

Mesenchymal stem cells are sufficient for cell therapy in a large range of diseases. In the process of cell therapy evaluation, tracing the differentiated cells inside the body is considered essential and can be achieved by labeling the stem cells by a marker. One of the markers that can be used for this purpose is the DNA synthesis marker 5-bromo-2-deoxyurudine (BrdU), a thymidine analog that incorporates to DNA of dividing cells during the S-phase of the cell cycle, and is used for birth dating and monitoring cell proliferation.

The present study aims to investigate the influence of stem cell labeling bromodeoxyuridine on the biological properties of the in vitro cultured rat bone marrow mesenchymal stem cells. This was demonstrated by monitoring morphological features, cluster of differentiation (CD), Reverse transcription-polymerase chain reaction (RT-PCR), immunocytochemistry and differentiation into adipogenic lineage.

The findings revealed that incorporated BM-MScs with BrdU showed less viability, changes on cell surface markers and genes expression and less adipogenic potential, So that BrdU is a toxic substance that has cytological effect so we suggest more studies on BrdU and find novel alternative.



## INTRODUCTION

Stem cells are the cells that have the capacity to self-renew and able to generate differentiated cells [1,2] , it also found in all multicellular organisms in small portion in body mass, but can divide through mitosis and differentiate into diverse specialized cell types or produce more stem cells [3] .

Adult stem cells are any stem cells taken from mature tissue. Because of the stage of development of these cells, they have limited potential compared to the stem cells derived from embryos and fetuses [4]. Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.) [5,6] ,They play important roles on local tissue repair and regeneration.

Adult Bone Marrow mainly comprises two populations of precursor cells, *hematopoietic stem cells* (HSCs) and marrow stromal cells (MSCs) [7]. Mesenchymal stem cells (MSC) obtained from bone marrow [8,9,10] have been reported to differentiate into several cell types such as those in muscle [11], fat [9], bone [12,13] , cartilage [8], liver [14], lung [15], hepatic [16], renal [17], cardiac [18], and neural cells [19,20]. Hence, the descriptive terms "pluripotent" or "multipotent" are reciprocally used to describe the capacity of MSCs to differentiate into a wide arrange of mammalian tissues [21].

Adipogenesis is a process whereby mesenchymal stem cells (MSCs) or preadipocytes differentiate to acquire phenotypic characteristics of mature adipocytes [22]. *In vitro* adipogenesis can be induced by treating MSCs with a hormonal cocktail containing dexamethasone, isobutyl methyl xanthine (IBMX) and indomethacin

[23,24,25]. The differentiation might be confirmed using oil-red staining technique and controlling the expression of specific proteins, such as peroxisome proliferation-activated receptor γ2 (PPARγ2), lipoprotein lipase (LPL), and the fatty acid binding protein aP2 [9].

Adipocytes are specialized cells that store triacylglycerols (fats). MSC differentiation into adipocytes requires the activity of a transcription factor called peroxisome proliferator activator receptor-gamma (PPAR-γ). PPAR-γ regulates the function of many adipocyte specific genes [26], and interacts with members of the CCAAT/enhancer binding protein (C/EBP) family to regulate adipogenesis [27].

Labeling or tagging of cells is necessary to track the migration and differentiation of cells by imaging after transplantation and to distinguish the implanted cells from the host tissue cells [28,29]. For decades, [$^3$H]thymidine ([$^3$H]dT) autoradiography dominated the studies of cell proliferation, birth dating, migration, and fate in the developing brain [30,31,32,33,34,35,36,37]. However, because of cost, logistic problems in handling radiolabeled substances, and the lengthy process of developing autoradiographs (3–12 weeks), contemporary studies are commonly performed with the thymidine analog bromodeoxyuridine (5-bromo-2'-deoxyuridine) (BrdU), a halopyrimidine with a different molecular structure than [$^3$H]dT. [38].

Bromodeoxyuridine (5-Bromo-2-Deoxyuridine, BrdU) is a pyrimidine analogue of thymidine that is selectively incorporated into cell DNA at the S phase of the cell cycle. The use of BrdU as a thymidine analogue has made the identification of DNA synthesis in cell suspensions, cell smears, and tissue sections possible. BrdU in culture is incorporated into the DNA during DNA synthesis. Cellular incorporation of BrdU can be detected by anti-BrdU specific antibodies following membrane permeabilization by flow cytometry or immunohistochemistry. BrdU labeling, is extensively documented in the biomedical literature. Monoclonal antibodies against BrdU have also proven valuable for studying cell cycle kinetics and DNA repair synthesis and also for assessing cell proliferation in the presence of growth factors or cytotoxic drugs and demonstrating sister chromatid exchange [39].

## Materials and methods

### *Harvesting and culturing of mesenchymal stem cells.*

Adult outbred Sprague Dawley (SD) rats that weighing 180 to 210 g were served as bone marrow donors. Bone marrow was obtained from the femurs and tibia of adult SD rats [40]. Rat bone marrow cells were cultured in basic media (Dulbecco's modified Eagle's medium [DMEM], Sigma Chemical Co., St. Louis, MO, USA) containing 10% fetal bovine serum (FBS; Sigma). The effluent was collected in sterile tubes. Gentle pipetting resulted in obtaining of a single cell suspension. Bone marrow cells were counted and plated with a concentration of $10\times10^6$/ml in T-75 flasks. The cells were then cultured in DMEM containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37oC in a humidified atmosphere that contained 5% CO2. Medium was changed after 4 days and every 3 days thereafter. Non adherent hematopoietic cells were removed when medium was changed. After a mean of 7 days, cells reached subconfluence and was detached with trypsin/EDTA, reseeded at $4\times10^3$ cells/cm$^2$, and used for experiments after the third passage. For each passage the cells were plated similarly and grown to confluency of 70% [41]. Mesenchymal stem cells features were demonstrated by typical spindle-shaped morphology [42].

### *BrdU labeling*

To label cells *in vitro*, carefully add 10 μl of BrdU solution (1 mM BrdU in 1× Dulbecco's PBS) for each ml of tissue culture medium. For this step, it is important to avoid disturbing the cells in any way (eg, by centrifugation steps or temperature changes) that may disrupt their normal cell cycling patterns. The cell culture density should not exceed $2 \times 10^6$ cells/ml. The treated cells are then incubated for the desired length of time. The incubation time with BrdU was 3 days before any step.

Cells from the same population that are not BrdU-labeled are the negative cell staining control.

### *Fibroblast-like colony-forming unit assay*

Fibroblast like colony growth was evaluated on primary cells grown on tissue culture six-well dishes. Total bone marrow-derived cells were plated at the density of $25 \times 10^6$ cells/well. After 7 days, the capability of mesenchymal stem cells to form fibroblast-like colonies was assessed. MSCs morphology was showed acquired by contrast-phase microscope.

***Cell Viability.***

Viability of cultured BM-MSCs incorporated or non-incropated with BrdU was evaluated through the trypan blue exclusion test. Equal volume of both solution 0.04% Trypan blue and stem cells were mixed and incubated for 10 minutes at 37 $^{o}$C then the number of viable cells unstained was counted using a haemocytometer and light microscope.

***Flow cytometry for cell surface antigen expressions.***

The bone marrow derived stem cells (control cells and BrdU-labeled cells) at passage 3 was released by trypsinization and were analyzed by flow cytometry. The cells were centrifuged at 1200 rpm for 5 minutes and then dissolved in phosphate buffered saline (PBS) at the concentration of (1 x $10^6$ / ml). The cells were stained with different fluorescently labeled monoclonal antibodies (mAb). In brief, 100 ul of cell suspension was mixed with 10 ul of the fluorescently labeled mAb and incubated in the dark at room temperature for 30 min. then washing with PBS and analyzed immediately on flow cytometry. The monoclonal antibodies were used in different combinations of fluorochromes; namely fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine.5 (PeCY5). CD90, CD29, CD14 are monoclonal antibodies against MSC antigens and CD45, CD34 are against hematopoietic antigens. The immunophenotyping was performed on EPICS-XL flowcytometry (Coulter, Miami, and Fl) in Cancer Institute, Mansoura University, Mansoura, Egypt. The cells were analyzed with the most appropriate gate using the combination of forward and side scatters.

***Adipogenic differentiation***

Cells labeled or non-labeled with BrdU were plated at $2\times10^4$ cells/cm2 in 6-well plates and cultured for one week. The medium was then changed to an adipogenic medium [10% FBS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 10 μg/mL insulin and 100 μM indomethacin in high glucose (HG)-DMEM] for an additional three weeks. In order to determine adipogenic differentiation, the cells were fixed in 4% paraformaldehyde for 10 minutes and stained with fresh Oil red-O solution (Sigma) to show lipid droplets in induced cells [43].

***Oil red O staining***

After 21 days, the adipogenic cultures were fixed in 4% paraformaldehyde for at least 1hr and fixative was carefully aspirated and cultures were rinsed three times with PBS.Then washed twice with water. Three ml of fresh 0.3% oil Red-O solution was added and incubated for 2hr at room temperature. After incubation ,the oil red O solution was removed and washed thrice with water. Then counterstained with haematoxylin for 5 to 15 minutes.

***Reverse transcription-polymerase chain reaction (RT-PCR)***

Gene expression was determined by RT-PCR on samples of *adipogenic differentiation* at 4, 7, 14 and 21 days. Total RNA was extracted from the cultured cells by using RNeasy Minikit (QIAGEN Inc., Valencia, CA) and treated with deoxyribonuclease I to remove contaminating genomic DNA following the manufacturer's instructions, The specific primers used for RT-PCR are listed in Table (1). The RT-PCR procedure was performed using the One Step RT-PCR kit, beginning at 50°C for 30 min and 95°C for 15 min for reverse transcription, then followed by 35 cycles, with each cycle consisting of denaturion at 94°C for 1 min, annealing at 57°C for 1 min, elongation at 72°C for 1 min, and the final extension at 72°C for 10 min. The amplified DNA fragments were visualized through 2% agarose gel electrophoreses and photographed under UV light [44] Reverse transcription was performed with 1 μg RNA in a total volume of 20 μl per reaction.( BIOMETRA).

**Table 1.** Primers sequence of rat adipocyte genes for RT-PCR

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| Adiponectin | GGGATTACTGCAACCGAAGG | CCATCCAACCTGCACAAGTTT |
| Leptin | TTCACACACGCAGTCGGTATC | GTGAAGCCCGGGAATGAAG |
| Lipoprotein Lipase | GTACAGTCTTGGAGCCCATGC | GCCAGTAATTCTATTGACCTTCTT GTT |
| PPAR-γ | CATACATAAAGTCCTTCCCGCTG | TTGTCTGTTGTCTTTCCTGTCAAGA |

***Immunocytochemistry using adherent cells***

Adipogenic differentiated stem cell which plated in 6-well plate in PBS for 15 minutes at room temperature was rinsed then block endogenous peroxidase activity with 3% H2O2 for 10 minutes at 37ºC after that rinse in PBS for 5 minutes at room temperature.

DNA Denaturation: Place 6-well plate in 2 N HCl for 30 minutes at 37oC. Rinse thoroughly in PBS (prerinsing in 0.1M Na2B4O7 (Borax) is optional).

Enzymatic pretreatment: Apply 100 µl of prewarmed pepsin or trypsin solutions onto sections. Incubate 30 minutes with pepsin or 20 minutes with trypsin at 37oC. Rinse in PBS, Blocking: Apply 100 µl blocking solution for 20 minutes at 37oC. Tap off excess solution. Do not wash.

Monoclonal Anti-BrdU (100 µl) diluted in diluent. Incubate 2 hours at 37oC. Rinse in PBS three times for 5 minutes each then proceed with the biotinylated second antibody and ExtrAvidinTM peroxidase using the immunohistology procedure with the mouse ExtrAvidinTM Staining Kit.

Develop the AEC color using the directions in the kit. Do not counterstain.

Apply coverslip with liquid glycerol gelatin.

***Statistical analysis***

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). All data were presented as mean±SE. One-way ANOVA and Duncan's post-hoc test were used to compare the groups. $P<0.05$ was considered to be statistically significant.

## Results

***Isolation and culturing of MSCs:***

We harvested the mesenchymal stem cells (MSCs) from the donor rat bone marrow; purify them in culture media with different passages. MSCs were generated by stander procedures and grown for at least two passages in culture. Contaminating hematopoietic cells were depleted during passage 1 and MSCs were morphologically defined by a fibroblast-like appearance.

***Fibroblast-Like Colony-Forming Unit Assay***

MSCs after isolation from bone marrow characteristic by their adherence to plastic consisted of a heterogeneous cell population with a predominant spindle-shaped morphology and were able to form fibroblast like colonies.

***Viability Test***

Since cells are very selective in the compounds that pass through the membrane, and they lose this selectivity when they are dead. Trypan blue is a vital stain used to selectively color dead tissues or cells blue. Our results showed that all BM-MSCs incorporated or not incorporated with BrdU obtained were viable and did not accept the stain, where viability percentage of BrdU labeled was between 75 and 85% compared to the unlabeled positive control that was >85%.

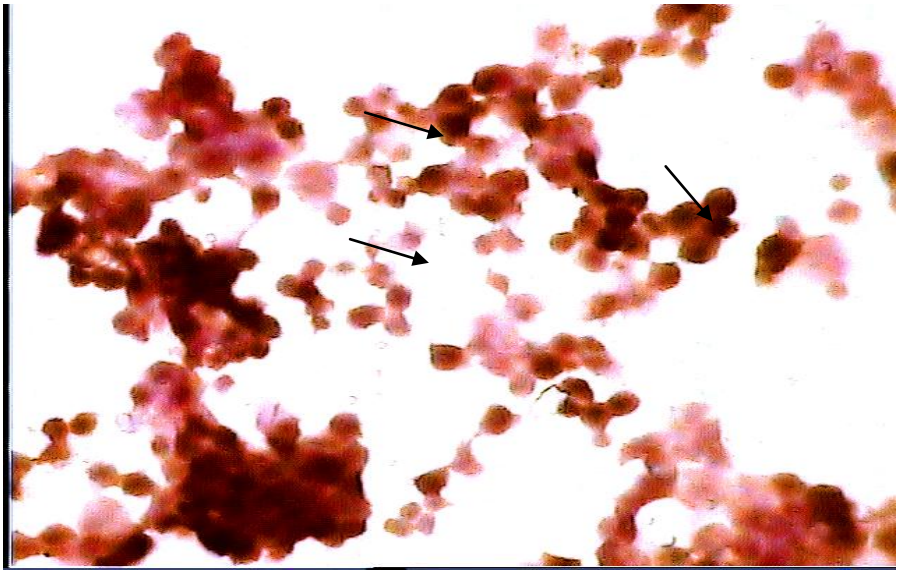

**Figure 1.** BM-MSCs were incubated with BrdU for72 h. The arrows show BrdU+ mononuclear cells that have round or oval shaped and brown nuclei and irregular cytoplasm.

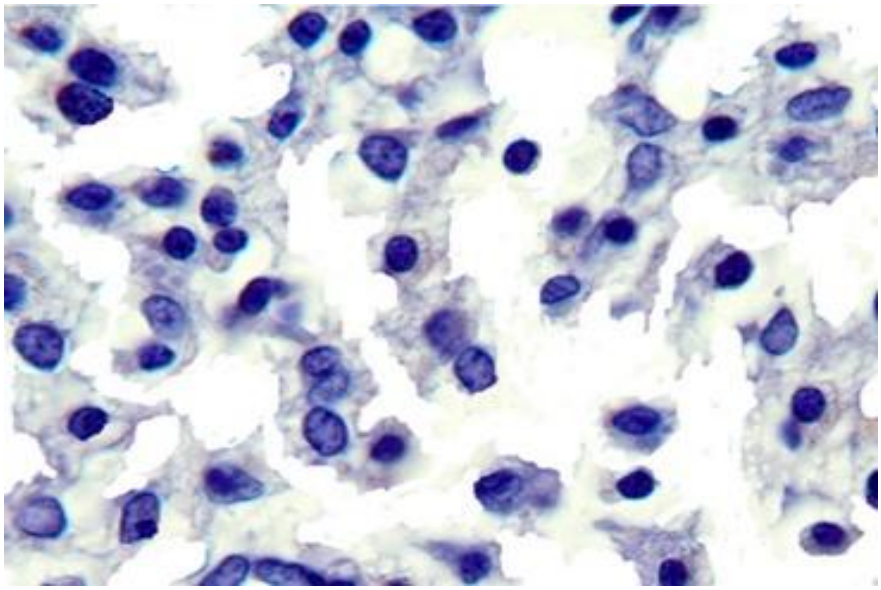

**Figure 2.** BM-MSCs stained with only Harris Hematoxylin without BrdU labeling. The image shows completed violet mononuclear cells.

***Phenotypic analysis.***

***Phenotypic analysis for rat BM-MSCs without BrdU.***

After three passages, flow cytometric analysis of undifferentiated cells showed purified mesenchymal cell as they were negative for CD45, and CD34, meanwhile they expressed high levels of CD29,CD14, and CD90 (Fig.3&4)

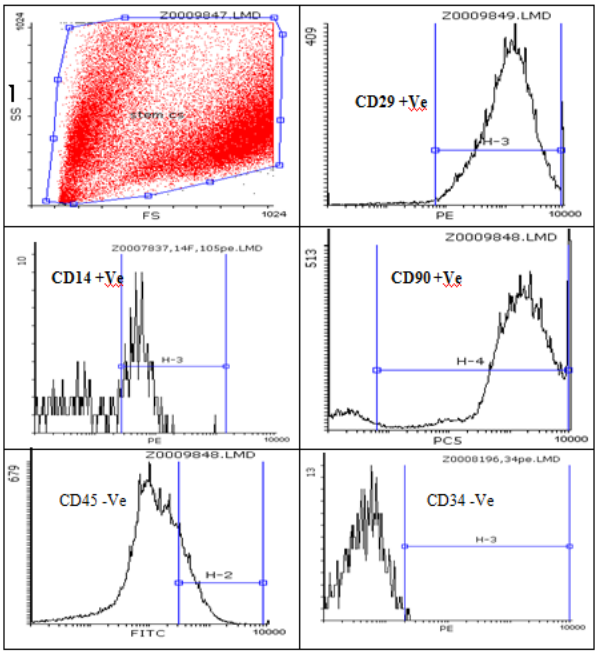

**Figure 3.** Phenotypic characteristic of rat bone marrow mesenchymal stem cells without BrdU, flow cytometry analysis revealed that their expression of surface antigens CD29, CD14 and CD90 (Passage 3) was strongly positive; while CD45 and CD34 was negative.

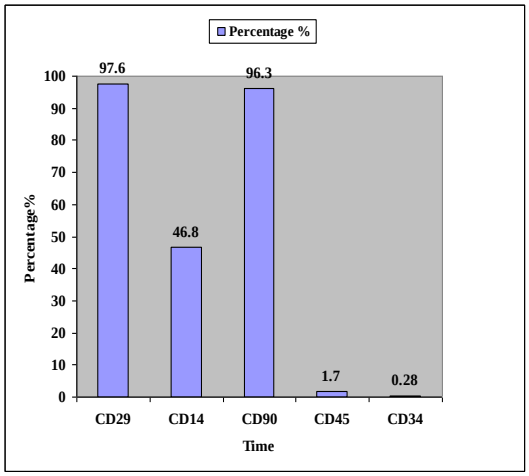


**Figure 4.** Show the percentage of flow cytometric surface markers for the undifferentiated MSCs without BrdU.

***3.3.2. Phenotypic analysis for BM-MSCs incorporated with BrdU.***

After three passages, flow cytometric analysis of undifferentiated cells incorporated with BrdU showed changes in mesenchymal cell as they were negative for CD45, CD29,CD14 and CD34, meanwhile they expressed high levels of CD90 (Fig.5&6)

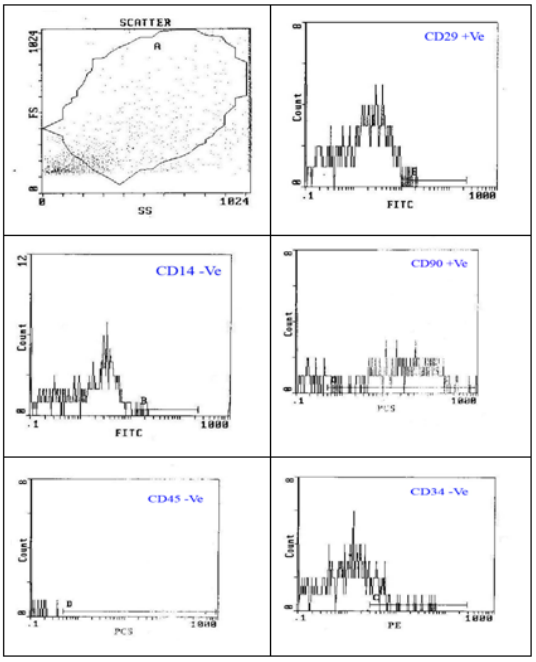


**Figure 5.** Phenotypic characteristic of rat bone marrow mesenchymal stem cells incorporated with BrdU, flow cytometry analysis revealed that their expression of surface antigens CD90 (Passage 3) was strongly positive; while CD29, CD14 ,CD45 and CD34 was negative.

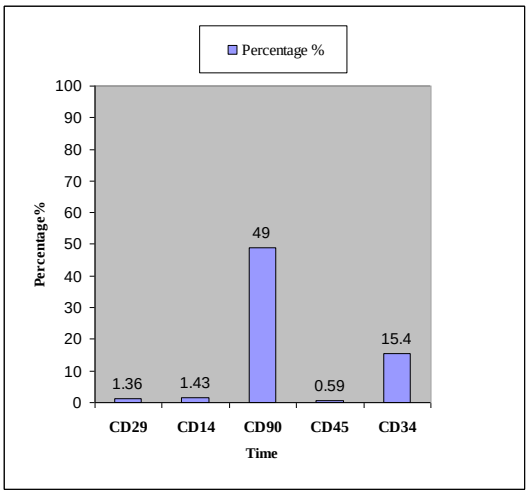


**Figure 6.** Show the percentage of flowcytometric surface markers for the undifferentiated MSCs incorporated with BrdU.

**Table 2.** Comparison of percentage of Cell surface markers (CD29, CD14, CD90, CD45, CD34) between BM-MSCs without BrdU and BM-MSCs incorporated with BrdU

| | % of surface marker in BM-MSCs without BrdU | % of surface marker in BM-MSCs incorporated with BrdU |
|---|---|---|
| CD29 | 97.6 | 1.36 |
| CD14 | 46.8 | 1.43 |
| CD90 | 96.3 | 49 |
| CD45 | 1.7 | 0.59 |
| CD34 | 0.28 | 15.4 |

***Adipocytic differentiation:***

Using phase contrast microscopy, BM-MSCs monolayer cultures treated with adipogenic induction media were found to contain more vacuoles compared to the untreated control cells. Adipogenic differentiation was slightly appeared at day 4 after incubation with adipogenic induction medium. Intensity of stained cells was gradually increased with days 7, 14 and 21 of culture periods. Oil Red O staining for fat revealed that these vacuoles contain neutral lipids (Fig. 7{A-D}). In contrast to this, control cells cultured for the same time period in normal culture medium, containing 10% FBS, stained negative for fat vacuoles (Fig. 7E). Furthermore, in the induced cultures, Oil Red O staining could be found in nearly 80–90% of the cells. This demonstrates that adipogenic differentiation of BM-MSCs was highly efficient.

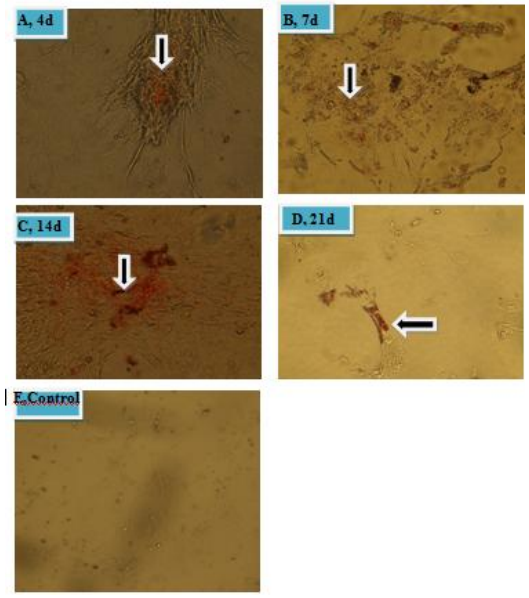


**Figure7.** Photomicrographs of mesenchymal stem cells (MSC) without BrdU showing differentiative potential at different time's intervals. Adipocyte differentiation is visualized by highly refractive intracellular lipid vacuoles and droplets appear as cherry red spheres within the cells and by Oil Red O staining {original magnification 200}.

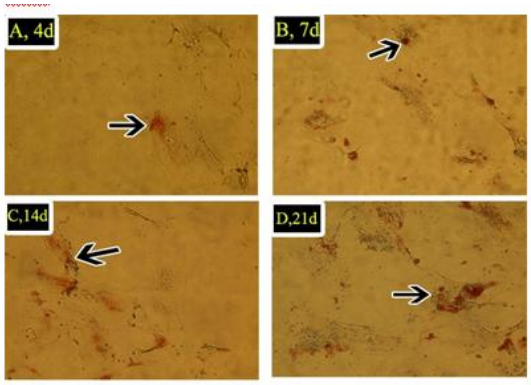


**Figure.8,** Photomicrographs of mesenchymal stem cells (MSC) incorporated with BrdU showing differentiative potential at different time's intervals. Adipocyte differentiation is visualized by highly refractive intracellular lipid vacuoles and droplets appear as cherry red spheres within the cells and by Oil Red O staining {original magnification 200}

***Gene expression***

***Gene expression in BM-MSCs without BrdU.***

Differentiation was further demonstrated by RT-PCR analysis of adipocytic markers gene expression was observed at different time intervals, after 4, 7 14 and 21 days (Fig. 9). Gel electrophoresis for mRNA expression of PPAR-γ showed that: lane 4d, 7d, 14d and 21d made bands appeared at 100 bp and proved the formation of PPAR-γ adipocytic transcription factor. Gel electrophoresis for mRNA expression of LPL, Leptin and Adiponectin showed that: lanes 4d, 7d, 14d and 21d made bands appeared of adipocytic marker, at 95 bp, 230 bp and 100 bp respectively proved the formation of LPL, Leptin and Adiponectin adipocytic marker.

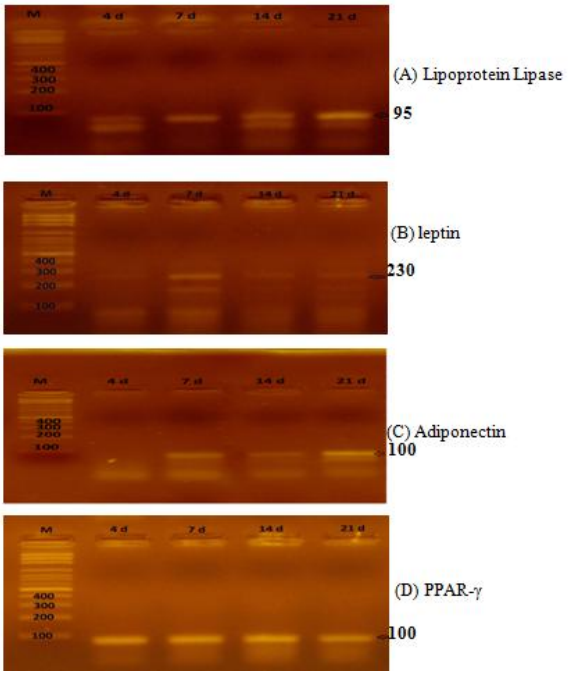


**Figure 9.** Gel electrophoresis for Lipoprotein Lipase, leptin, Adiponectin and PPAR-γ showed that: lane M (DNA marker) made base pairs and lane 4d, 7d, 14d and 21d showed bands present and proved the genes expression of the differentiated BrdU non-labeled BM-MSCs into adipocyte.

***Gene expression in BM-MSCs incorporated with BrdU.***

Differentiation was further demonstrated by RT-PCR analysis of adipocytic markers gene expression was observed at different time intervals, after 4, 7 14 and 21 days (Fig. 10). Gel electrophoresis for mRNA expression of PPAR-γ showed that: lane 4d, 7d, 14d and 21d made bands appeared at 120 bp and proved the formation of PPAR-γ adipocytic transcription factor. Gel electrophoresis for mRNA expression of LPL, Leptin and Adiponectin showed

that: lanes 4d, 7d, 14d and 21d made bands appeared of adipocytic marker, at 95 bp, 230 bp and 90 bp respectively proved the formation of LPL, Leptin and Adiponectin adipocytic marker.

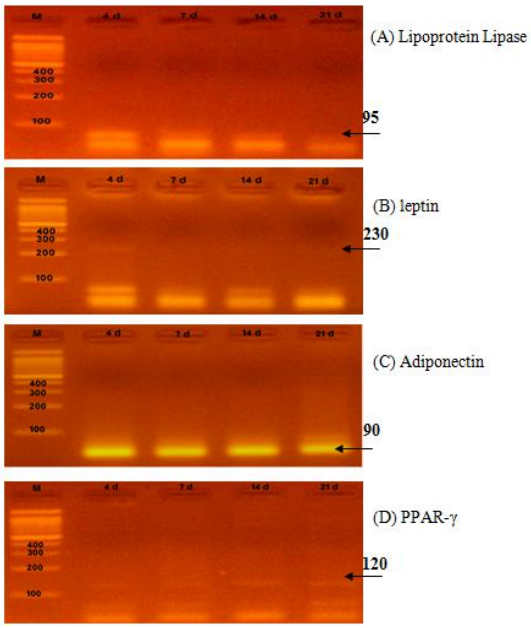


**Figure 10.** Gel electrophoresis for Lipoprotein Lipase, leptin, Adiponectin and PPAR-γ showed that: lane M (DNA marker) made base pairs and lane 4d, 7d, 14d and 21d showed bands present and proved the genes expression of the differentiated BrdU incorporated BM-MSCs into adipocyte.

**Table 3.** Comparison between presence of bands of gene expression in BrdU non-labeled cells and BrdU incorporated cells.

| | **Bands of gene expression of BrdU non-labeled cells** | **Bands of gene expression of BrdU incorporated cells** |
|---|---|---|
| PPAR-γ | 100 bp | 120 bp |
| LPL | 95 bp | 95 bp |
| Leptin | 230 bp | 230 bp |
| Adiponectin | 100 bp | 100 bp |

***Immunocytochemistry of differentiated BM-MSCs with anti BrdU***

Immunoreactivity of stained adipocytes differentiated stem cells incorporation with BrdU has been shown in Figure 11. (A&B). After the immunocytochemistery, the BrdU reactive cells have round or oval shaped and brown nuclei and thin irregular cytoplasm and in the control group brown cells were not observed .

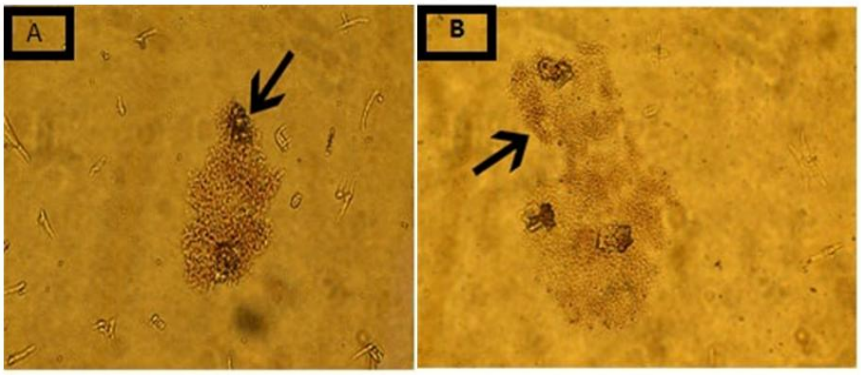


**Figure 11.** BMCs were incubated with BrdU for 72 h (A); (B). The arrows show BrdU+ adipocyte cells that have round or oval shaped and brown nuclei and irregular cytoplasm.

# Discussion

Stem and progenitor cells from various sources have been shown to increase the functional effect for treating cardiac, liver, lung and neurological diseases. Regardless of stem cell origin, future clinical trials will require that the location and number of such cells can be tracked in vivo. Several methods have been described for tracking of injected cells utilizing nuclear stains, e.g., BrdU, fluorescent dyes (e.g., Dil, DAPI, or PKH26), GFP, fluorescently labeled DNA probes (e.g., fish), MRI and isotope labeling techniques to detect specific cells of interest [45,46].

In earlier studies, the BrdU labeling method was used mainly for analyzing cell cycle in cultured cells [47] and for visualizing proliferating cells in the central nervous system [48]. Later in more recent studies, BrdU labeling has been used to track stem or non-stem cells that are labeled in vitro and subsequently transplanted in vivo [49, 50]. As an analog of thymidine, BrdU is a marker for DNA synthesis and not necessarily a marker for cell proliferation. BrdU labels only cells that are synthesizing DNA; thus, a single injection of BrdU will label cells in the S-phase and the S-phase is a small proportion of the whole cell cycle [51].

The objectives of this work was examined the BrdU labeling effect on BM-MSCs viability and differentiation. We achieved our purpose by comparing between incorporated and non-incorporated BM-MSCs with BrdU stain through characterization of viability and ability of differentiation into adipocytes.

Our data revealed that isolated and culture BM-MSCs were plastic-adherent cells and morphologically defined by fibroblast–like appearance. After a few days, these adherent cells, of hetero-geneous appearance, start to proliferate. The initial clones of adherent cells expanded into round-shaped colonies composed of fibroblastoid cells, thus the term of CFU-F. These results are consistent with those reported in many studies [52,40].

In this study, BM-MCs were labeled with BrdU. After immunocytochemistery our results demonstrated that 10 μM BrdU was an appropriate marker for labeling rat BMCs *in vitro*. With this labeling method, viabilities of BM-MCs until 72h incubating time were between 75 and 85% compared to the unlabeled positive control that was >85%. Our data supported the work done by Lequeux's (2011) [53] findings about BrdU labeling of human ASCs that revealed, with BrdU labeling cell MCs viability.

Cell surface marker of present data demonstrated that non- labeled stem cells with BrdU expressed mesenchymal stem cell surface antigens CD29, CD14 and CD90 as markers for MSCs and lacking of the hematopoietic stem cell markers CD45 and CD34. These data was confirmed by results of De Macedo Braga et al. [54] and Ayatollahi et al. [54] which demonstrated that rat bone marrow-MSCs express of cell-surface markers (CD markers) such as CD29, CD14 and CD90. In the contrary, Ayatollahi et al. [55] found that rat BM-MSCs were negative for CD45.

In the other hand results of surface marker obtained from incorporated stem cells with BrdU expressed changes in mesenchymal stem cell surface marker as they were negative for CD45, CD29,CD14 and CD34, meanwhile they expressed high levels of CD90 .

As a stem cell type, MSCs exert self-renew ability and multipotent differentiation potential. Particularly, MSCs can be induced into osteoblasts, adipocytes, chondrocytes, neurosphere and neural lineage in vitro [56,57,58]. In the present study both incorporated and non-incorporated with BrdU rat bone marrow MSCs claimed that they possess stem cell potential, capable of differentiating into adipocyte cells. Adipogenic differentiation was slightly appeared at day 4 after incubation with adipogenic induction medium. Intensity of stained cells was gradually increased with days 7, 14 and 21 of culture periods. Oil Red O staining for fat revealed that these vacuoles contain neutral lipids. These represent the major source of multilineage of cells [59,60,61].

The current study reports that, BrdU non-labeled gene expression profiles throughout the differentiation process were examined in adipogenic differentiated genes at 4, 7, 14, and 21 days. Gel electrophoresis for mRNA expression of PPAR-γ showed that: lane 4d, 7d, 14d and 21d made bands appeared at 100 bp and proved the formation of PPAR-γ adipocytic transcription factor. Gel electrophoresis for mRNA expression of LPL, Leptin and Adiponectin showed that: lanes 4d, 7d, 14d and 21d made bands appeared of adipocytic marker, at 95 bp, 230 bp and 100 bp respectively proved the formation of LPL, Leptin and Adiponectin adipocytic marker. While data obtained from BrdU incorporated gene expression showed changes in bands of PPAR-γ and Adiponectin 120 bp and 100 bp respectively but bands of LPL and Leptin were the same results of non-incorporated with BrdU observed at 95 bp and 230 bp respectively.

In conclusion, Evidence had been provided that BrdU is a toxic substance [62,63,64] and also induce mutations due to its incorporation into DNA, and in view of the correlation between mutagenic and carcinogenic

potential [65], exposure to BrdU might be expected to result in the appearance of tumors in longterm [66] and large amount tests [67]. For this reason investigators decided to find the best amount and time of cell exposure with BrdU. Feng et al, in 2005, to identify the optimal BrdU concentration and incubating time for bone marrow derived mesenchymal stem cells (MSCs) labeling [68].

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