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# In Vitro Differentiation of Bone Marrow Stromal Cells into Oligodendrocyte-like Cells Using Triiodothyronine as Inducer

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## ABSTRACT

An in vitro technique was devised to induced autologous adult stem cells into oligodendrocyte-like cells. In this study, a protocol was developed for the induction of bone marrow stromal cells (BMSCs) into oligodendrocyte-like cells. BMSCs were incubated in one of these three pre-inducers: dimethyl sulfoxide (DMSO), $\beta$-mercaptoethanol ($\beta$ME) or biotylated hydroxyanisol (BHA), each followed by retinoic acid (RA) treatment. The percentage of viable cells in BHA-RA preinduced cells was significantly lower than the others. The results showed that the preinduced cells were immunoreactive for nestin and NF-68; among the mentioned protocols, the immunoreactivity yielded by following the DMSO-RA protocol was significantly higher than the others. Moreover, no significant immunoreactivity was observed for preinduced cells to O4, O1, MBP (myelin basic protein), S100, and GFAP (glial fibrillary acidic protein). The cells were immunoreactive to oligo-2. Two phases of induction were done: the first was a combination of basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and heregulin (HRG), followed by either triiodothyronine (T3) or Forskolin (FSK) as the second phase. The conclusion is that the trans-differentiation of BMSCs by DMSO followed by RA (preinduction stage) then bFGF-PDGF-HRG followed by T3 (10 ng/ml) (induction stage) can be a potential source for oligodendrocyte-like cells preparation.

**KEYWORDS:** bone marrow stromal cells, oligodendrocyte-like cells, triiodothyronine

## INTRODUCTION

Oligodendrocytes form a myelin sheath around axon to support the rapid nerve conduction in the central nervous system (CNS) [1]. The loss of myelin from intact axons is a prominent feature of a wide range of CNS disorders affecting many patients for example spinal cord injury [2], brain injury [3], stroke [4, 5], Alzheimer's disease [6], schizophrenia [7], multiple sclerosis, [8] and aging [9–11]. Generally, remyelination can be achieved by transplantation of oligodendrocytes derived from stem cells [12].

The differentiation of stem cells into oligodendrocyte lineage has been done so far using different sources including embryonic stem cells (ESCs), fetal-derived stem cells, and adult stem cells. Fraichard et al. reported the induction of ESCs differentiation into O4- and GFAP-immunoreactive cells as well as neurons [13]. A more pure population of oligodendrocytes derived from ESCs was produced by forming intermediate oligospheres [14], and Nistor et al. were able to obtain oligodendrocytes with high purity using positive selection and mechanical enrichment [15]. However, Sadowski et al. documented the teratogenic

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potential of mouse ESCs induced to differentiate into oligodendrocyte phenotype [16]. Also, the differentiation of ESCs was compared with induced pluripotent stem cells and the results suggested that induced pluripotent stem cells contained intracellular inhibitory factors that prevented the cells from terminal differentiation into oligodendrocytes [17]. On the other hand, fetal cells such as neural crest stem cells generated oligodendrocytes that were reported to ameliorate the myelin deficiency in shiverer mice [18]. Also, human umbilical cord blood cells were used to derive oligodendrocytes using special medium containing FGF4 and SCF [19]. Tracy et al. were able to derived oligodendrocytes from cryopreserved umbilical cord blood [20], but these sources of transplantation are allografts. In another study, adult stem cells were used to derive oligodendrocytes from the mesenchymal stem cells (MSCs) derived from adipose tissue, and were reported to differentiate into oligodendrocytes [21]; however, regulation of multiple sclerosis [22, 23], or improve the engraftment of allogeneic oligodendrocyte progenitors [24]. Neurosphere derived from BMSCs were induced to differentiate into oligodendrocytes [25]; some investigators confirmed these findings [26, 27], while some others reported that the in vivo administered MSCs derived from the bone marrow stromal cells (BMSCs) could instruct the neural stem cells to differentiate into oligodendrocytes [28]. One of the most important advantages of the BMSCs is the autologous property of these cells that essential for avoiding the immunological rejection [29].In this study, we attempted to devise a protocol to evaluate the transdifferentiation potential of BMSCs into oligodendrocyte-like cells.

## MATERIALS AND METHODS

### Preparations of BMSCs

The Animal Studies Ethical Committee at Tarbiat Modares University, Tehran, Iran approved the experimental work done in this study. BMSCs were collected from the tibias and the femurs of adult Sprague–Dawley rats at 6–8 weeks of age (Razi Institute, Tehran, Iran). The proximal and distal ends of the bones were removed under aseptic conditions and the bone marrow was aspirated with 5 ml of $\alpha$-MEM (Gibco, UK) containing 500 units of heparin using a 21G needle. The cell pellet was obtained and suspended in $\alpha$-MEM (containing 15% fetal bovine serum (FBS), 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, 25 ng/ml amphotericin B, and 2 mM L-glutamine: all from Gibco, UK). The harvested cells were seeded on a 75 $cm^2$ flask (Nunc, Denmark) at 37°C, 5% $CO_2$ incubator for 24 hr. The flasks were washed with PBS in order to remove the hematopoietic cells. The cells were incubated for 2–3 days, where the cells reached the confluency and the culture was repeated for four passages (P4), one week for each passage. The cells were then removed with 0.25% trypsin and 1 mM EDTA for 5–10 min at 37°C in order to

TABLE 1. The experimental design of the study, presenting the controls, the preinduction, and induction protocols

| Groups | Phase | Treatment groups | Dose | # of day(s) | Evaluated marker(s) |
|---|---|---|---|---|---|
| Control | BMSCs at P4 | – | – | 70–80% confluency | Fibronectin, CD44, CD90, CD45 Nestin, NF68, 04, O1, MBP, GFAP, S100, Oct-4, and NeuroD |
| Preinduction | PP-1 | $\beta$ME/DMSO/BHA | 1 mM/2%/200 $\mu$M | 1 day | – |
| | PP-2 | RA | 1 $\mu$M | 3 days | Fibronectin, CD44, CD90, CD45 Nestin, NF68, 04, O1, MBP, GFAP, S100, Oct-4, and NeuroD |
| Induction [the best treatment group of PP-2 (DMSO-RA)] | IP-1 | bFGF, PDGF, and HRG | 5 ng/ml, 10 ng/ml, and 200 ng/ml, respectively | 2 days | Fibronectin, Nestin, NF68, and 04 |
| | IP-2 | FSK or T3 | 5 $\mu$M or (0, 5, 10, 50, 100, and 200 ng/ml) | 2 days | Fibronectin, CD44, CD90, CD45 Nestin, NF68, 04, O1, MBP, GFAP, S100, Oct-4, and NeuroD |

Note: The preinduction and induction protocols of the bone marrow stromal cells (BMSCs) were used in the study. The preinduction consisted of two phases of treating at PP-1 (***first phase of the preinduction stage) and PP-2 (second phase of the preinduction stage). At PP-1 BMSCs at P4 (fourth passage) were incubated with either $\beta$-mercaptoethanol ($\beta$ME), dimethyl sulfoxide (DMSO) or butylated hydroxyanisol (BHA); and at PP-2 followed by all-retinoic acid (RA).

The induction consisted of two phases of treating at IP-1 (first phase of the induction stage) and IP-2 (second phase of the induction stage). At IP-1, NELCs incubated with platelet derived growth factor (PDGF-AA), basic fibroblast growth factor (bFGF) and heregulin (HRG), and at IP-2 incubation with Forskolin (FSK) or one of dosages of triiodothyronine (T3: 0, 5, 10, 50, 100, and 200 ng/ml).

TABLE 2. The percentages of the immunoreactive cells to different types of markers used in the study

| Groups antibodies | BMSCs | βME-RA | DMSO-RA | BHA-RA |
|---|---|---|---|---|
| **Fibronectin** | 92.75 ± 3.86 | 6.6 ± 1.84*† | 3.1 ± 1.48* | 3.8 ± 1.54* |
| **CD44** | 94.3 ± 4.66 | 7.8 ± 2.34* | 6.8 ± 2.3* | 7.1 ± 1.86** |
| **CD90** | 97.4 ± 4.18 | 9.12 ± 2.7* | 11.4 ± 3.47* | 10.5 ± 2.74 |
| **CD45** | 4.5 ± 2.18 | 0 ± 0* | 0 ± 0* | 0 ± 0* |
| **Nestin** | 0.95 ± 0.75 | 62.05 ± 6.45*† | 75.03 ± 4.63* | 40.1 ± 7.46*† |
| **NF68** | 1.05 ± 0.75 | 57.7 ± 6.90* | 72 ± 5.62* | 39 ± 6.44*† |
| **GFAP** | 0 ± 0 | 4.2 ± 2.76* | 4.25 ± 2.22* | 1.25 ± 1.16*† |
| **O4** | 0 ± 0 | 1.95 ± 1.98* | 4.8 ± 1.73* | 1.2 ± 1.28*† |
| **O1** | 0 ± 0 | 2.45 ± 2.16*† | 6.5 ± 2.94* | 2.4 ± 1.81*† |
| **MBP** | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| **S100** | 0 ± 0 | 1.95 ± 1.82* | 4.7 ± 1.78* | 1.5 ± 1.23*† |

Note: The markers of the bone marrow stromal cells (fibronectin), mesenchymal stem cells (CD44 and CD90), hematopoietic cell (CD45), neuroblast markers (nestin and neurofilament 68: NT and NF68, respectively), astrocyte glial fibrillary acidic protein: GFAP), oligodendrocyte lineage (O4, O1, and MBP), and Schwann cell (S-100) markers. They were used to evaluate the differentiation of the preinduction in undifferentiated bone marrow stromal cells (BMSCs), the cells treated with β-mercaptoethanol (βME) followed by retinoic acid (βME-RA), dimethyl sulfoxide (DMSO) followed by retinoic acid (DMSO-RA) or butylated hydroxyanisol followed by retinoic acid (BHA-RA).

The means and the standard deviations of the mean percentage of immunoreative cells to the above markers are also mentioned. The percentage was expressed as the number of immunoreactive cells to the total cell number of the counted cells.

*Indicates statistically a significant difference between the BMSCs and all of preinduction groups. The significance level was designated as $p < .05$.

†Shows a statistically significant difference between DMSO-RA and two other preinduction groups. The significance level was designated as $p < .05$.



obtain a single-cell suspension. Nearly 5,000 cells/cm$^2$ were replated on gelatin-coated 24-well plates containing gelatin-coated glass coverslip in order to evaluate the cellular markers. At P4, the cells were checked for the properties of BMSCs using fibronectin (+), CD44 (+), CD90 (+), and CD45 (−) immunostaining, as well as for Oct-4 (to check the stemness) using RT-PCR. The lipogenic and osteogenic differentiation of the isolated cells were evaluated according to Neuhuber et al. [30], and the lipogenic cells were stained with oil red, while the osteogenic cells were stained with Alizarin red [31].

The differentiation protocol consisted of preinduction and induction stages were listed in Table 1; each was divided into two phases. The protocol was performed in each of the subgroups. The subgroups were treated at the first phase of the preinduction stage (PP-1) with either βME (1 mM) (Invitrogen, UK), dimethyl sulfoxide (DMSO) (2%) (Merck, Germany) or BHA (200 μM) (Merck, Germany) in α-MEM medium without FBS for one day, and the cells were subsequently washed with phosphate buffer saline (PBS). In the second stage (PP-2), the pre-induction medium was changed with α-MEM and 15% FBS containing all trans retinoic acid

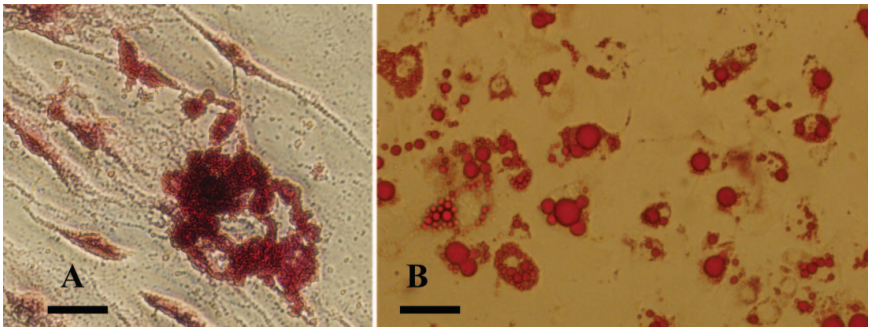


FIGURE 1. The osteogenic and lipogenic differentiation of cultured bone marrow stromal cells. A: Represents the osteogenic differentiation following the treatment of the bone marrow stromal cells with differentiation medium. There are small nodules stained with Alizarin red stain (scale bar = 20 μm). B: represents the lipogenic differentiation following the treatment of the bone marrow stromal cells with differentiation medium. There are many cells with multilocular lipid materials stained with oil red stain (arrow) (scale bar = 40 μm).

(RA: 1 $\mu$M; Sigma, USA) for three days. The best subgroup was selected on the basis of the percentage of immunoreactive cells to the listed differentiation markers. PP-2 was followed by the induction stage. At the first phase of the induction (IP-1), the platelet-derived growth factor (PDGF-AA) (5 ng/ml), basic fibroblast growth factor (bFGF) (10 ng/ml), and heregulin (HRG) (200 ng/ml) (bFGF-PDGF-HRG; all purchased from Sigma, USA) were added to the medium, followed by the second phase of the induction (IP-2), which was done by the addition of either Forskolin (FSK: 5 $\mu$M; Merck, Germany) or triiodothyronine (T3: 0, 5, 10, 50, 100, and 200 ng/ml; Sigma, USA). The incubation time at each phase was two days.

The cells of the PP-2 and IP-2 as well as the untreated BMSCs were harvested and their mRNAs were extracted to evaluate NeuroD and Oct-4 expression by RT-PCR. Furthermore, immunocytochemical analysis was performed on the adherent cells.

## Immunocytochemistry

The isolated and induced BMSCs were plated on a gelatin-coated (Merck, Germany) glass coverslip, washed in PBS, and fixed with 4% paraformaldehyde (Invitrogen, UK) in PBS for 15 min. The fixed cells were washed twice with PBS before staining. Permeabilization and blocking nonspecific antigen reaction were carried out in blocking buffer consisting of 0.1% Triton X-100 (Sigma, USA) and 10% goat serum in PBS for 1 hr.

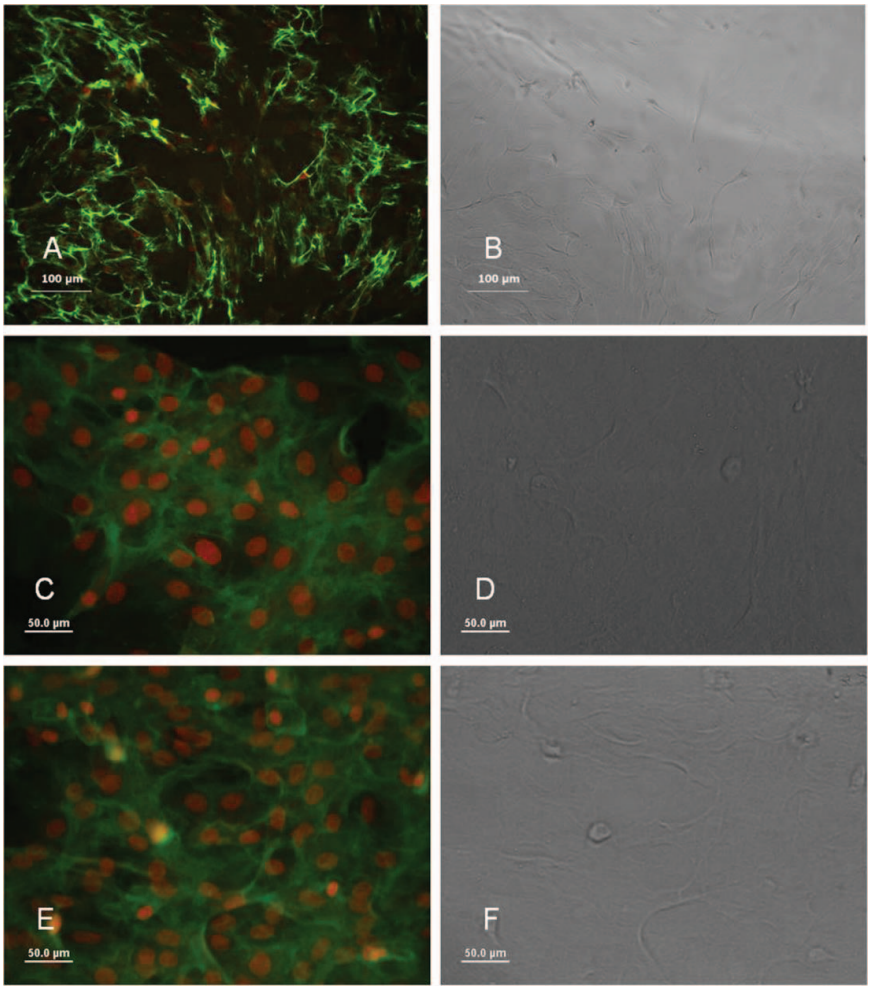


FIGURE 2. Characterization of the undifferentiated bone marrow stromal cells using immunocytochemistry. The differentiation markers used in the study included fibronectin (a marker for BMSCs), CD44, and CD90 (markers for mesenchymal stem cells). They were incubated with anti-fibronectin, anti-CD44, and anti-CD90 (primary antibodies), followed by the secondary antibody conjugated with FITC, (A), (C), and (E), respectively, and counterstained with ethidium bromide. (B), (D), and (F) show phase contrast images of (A), (C), and (E), respectively [scale bar: 100 $\mu$m (A and B), 50 $\mu$m (C)].

The primary antibodies: mouse anti-fibronectin monoclonal antibody (1:100; Chemicon, USA), mouse anti-CD44 monoclonal antibody (1:300; Santa Cruz Biotechnology, USA), mouse anti-CD90 monoclonal antibody (1:200; Santa Cruz Biotechnology, USA), mouse anti-CD45 monoclonal antibody (1:300; Santa Cruz Biotechnology, USA), rabbit anti-nestin polyclonal antibody (1:100; Chemicon, USA), mouse anti-NF-68 monoclonal antibody (1:50; Chemicon, USA), mouse anti-O4 monoclonal antibody (1:200; Chemicon, USA), mouse anti-O1 monoclonal antibody (1:300; Chemicon, USA), mouse anti-oligo-2 monoclonal antibody (1:300; Chemicon, USA), mouse anti-MBP monoclonal antibody (1:400; Chemicon, USA), mouse anti-S100 monoclonal antibody (1:150; Chemicon, USA), and mouse anti-GFAP monoclonal antibody (1:800, Sigma, USA) were incubated overnight at 4°C washed three times in PBS, incubated with the relevant secondary antibody (anti-mouse FITC-conjugated or anti-rabbit FITC-conjugated; 1:100, both from Chemicon, USA) for 2 hr at room temperature, washed in PBS twice, and counterstained with ethidium bromide (EB) (1:10,000; Sigma, USA) for 1 min to demonstrate the nuclei. Then, they were washed in PBS and examined using a fluorescence microscope at 200 × magnifications (Axiophot, Zeiss, Germany). For negative controls, the primary antibodies were omitted and the same staining procedure was conducted as above.

### RT-PCR

After the preinduction and the induction periods, the total RNA was extracted from the induced BMSCs and the noninduced cells using RNX plus (Cinnagen, Iran) and was stored at −80°C. cDNA synthesis was carried out from 5 $\mu$g total RNA using Fermentas kit (Fermentas, Lithuania) according to the manufacturer's instructions. Primer annealing temperatures and primer sequences were:

$\beta_2$ microglobulin ($\beta_2$M, internal control) [32, 33]: 58°C
forward (F): 5′-CCGTGATCTTTCTGGTGCTT-3′,
reverse (R): 5′-TTTTGGGCTCCTTCAGAGTG-3′,
Oct-4: 57 °C F: 5′- AAGCTGCTGAAACAGAAGAGG -3′,
R: 5′-ACACGGTTCTCAATGCTAGTC -3′ and
NeuroD: 56 °C F: 5′- AAGCACCAGATGGCACTGTC -3′,
R: 5′- CAGGACTTGCATTCGATACAC -3′.

The 25 $\mu$l PCR product contained the following components: 0.2 pM of each primer, 0.3 mM dNTP, 1.5 mM $MgCl_2$, 1U *taq* DNA polymerase, and 1× PCR buffer (Fermentas, Lithuania). The PCR reactions were conducted in a programmable thermocycler (Biorad, USA) with the following temperature profile: 94°C for 5 min, 35 cycles at 94°C for 45 s, 55°C for 45 s and 72°C for 45 s, and a final extension at 72°C for 10 min. Afterwards 10 $\mu$g of the PCR product was separated, run on a 1.5% agarose gel, stained with EB and visualized by UV transilluminator.

### Statistical Analysis

The statistical analysis was carried out using ANOVA with Tukey's multiple comparison and Student *t*-test. For each parameter, the significance level was determined using SPSS 10 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Bone Marrow Stromal Cells

The mean percentages of immunoreactive cells to fibronectin, CD44 and CD90 at the P4 were 92.75 ± 3.86%, 94.3 ± 4.66, and 97.4 ± 4.18 (mean ± SE),

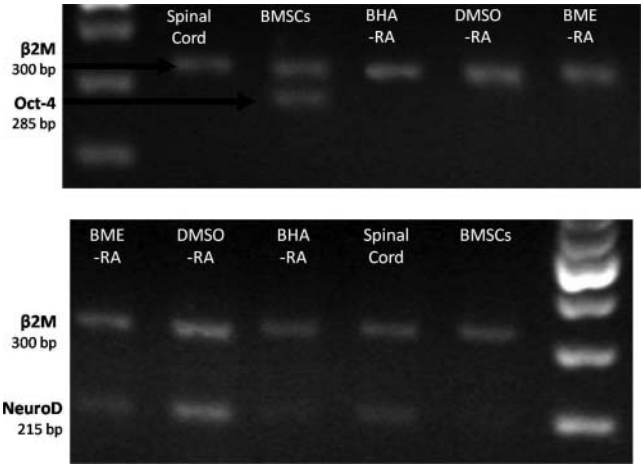


FIGURE 3. A study using RT-PCR to characterize the undifferentiated bone marrow stromal cells (BMSCs). The upper panel is an electrophorogram used to characterize the stemness of BMSCs, and the lower panel shows a comparative study for preinduction stage. In the preinduction stage, three types of preinducers were used in the study including $\beta$-mercaptoethanol ($\beta$ME), dimethyl sulfoxide (DMSO), and butylated hydroxyanisol (BHA), followed by retinoic acid (RA) treatment. Also, mRNA was extracted from the BMSCs to run the reverse transcriptase polymerase chain reaction (RT-PCR). The upper panel presents the RT-PCR electrophorogram of $\beta$ME-RA, DMSO-RA, or BHA-RA, using Oct-4 gene primers. The gene segment was 285 bp and the house-keeping gene was $\beta_2$ microglobulin as an internal control ($\beta$2M: 300 bp); "spinal cord" was the adult spinal cord used as a control, and the leftmost lane represents the DNA ladder. Oct-4 gene was expressed in undifferentiated BMSCs only. Accordingly, the lower panel represents the electrophorogram of RT-PCR products of the NeuroD gene (segment size: 215 bp).

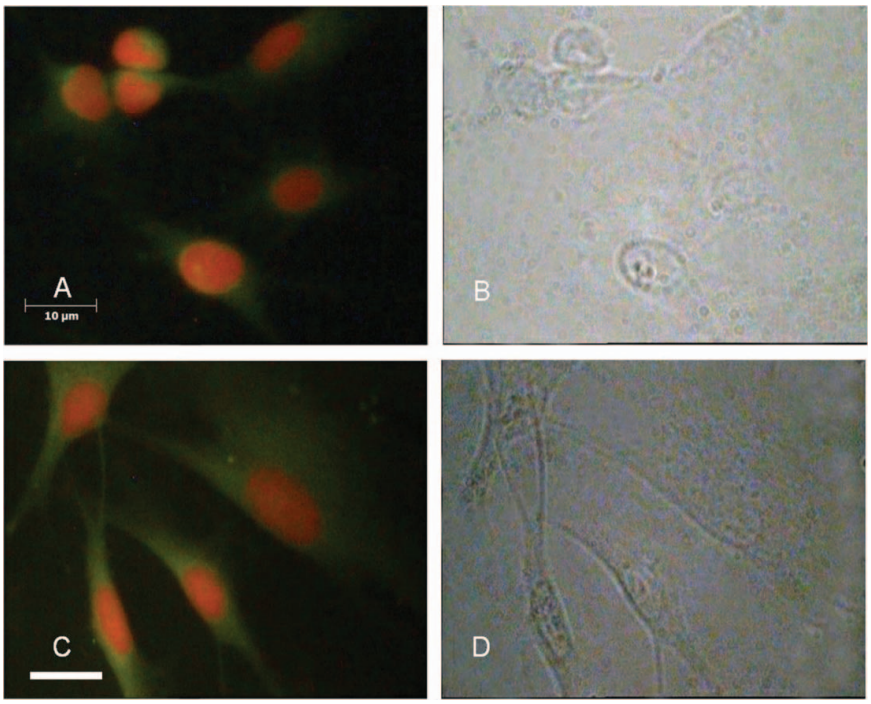


FIGURE 4. Characterization of the undifferentiated bone marrow stromal cells (BMSCs) using immuocytochemistry. The neuroblast markers used in the study included nestin and neurofilament 68. The cells were incubated with anti-nestin and anti-neurofilament 68 antibodies (primary antibodies), followed by the secondary antibody conjugated with FITC, (A) and (C), respectively, and counterstained with ethidium bromide (EB). (B) and (D) show phase contrast images of (A) and (C), respectively (scale bar: 10 $\mu$m).



respectively. None or very few cells were immunoreactive for CD45 (hematopoietic marker), nestin, NF68, O4, O1, MBP, GFAP, and S100 (Table 2). The lipogenic and osteogenic differentiation of the isolated BMSCs are presented in Figure 1. The immunoreactivity of the undifferentiated BMSCs to fibronectin, CD44 and CD90 antibodies in P4 is provided in Figure 2. In addition, mRNA of Oct-4, a stemness marker, was expressed in these cells; but NeuroD, a transcription factor transiently expressed in neuronal precursor cells [34], was not detected (Figure 3).

## Preinduction

Our findings showed that treatment of BMSCs with DMSO followed by all-trans-retinoic acid (DMSO-RA) at the PP-2 resulted in the NeuroD expression, while Oct-4 was not detected. However, the expression of NeuroD gradually decreased in $\beta$ME-RA and BHA-RA groups, respectively (Figure 3).

Transdifferentiation of BMSCs into preinduced cells was evaluated by nestin and NF-68 expression using immunocytochemistry (Figure 4). The results showed that nestin expression was significantly higher in DMSO-RA than in $\beta$ME-RA and BHA-RA (Table 2). Nevertheless, oligodendrocytes, astrocytes, and Schwann cell markers were insignificantly expressed at the preinduction stage (Table 2). Fourth passage of untreated BMSCs viability

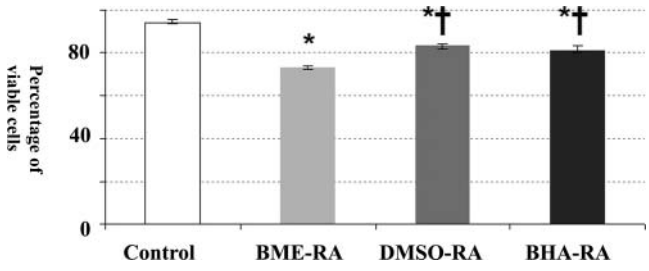


FIGURE 5. The percentage of the viable cells in untreated bone marrow stromal cells (BMSCs) of preinduction stage. Three types of preinducers were used in the study including $\beta$-mercaptoethanol ($\beta$ME), dimethyl sulfoxide (DMSO), and butylated hydroxyanisol (BHA), followed by retinoic acid (RA) treatment. A histogram of the percentage presents the viable cells in undifferentiated BMSCs (control), $\beta$ME-RA, DMSO-RA, and BHA-RA. The percentage of viable cells in the control was significantly higher than that in the other groups. The lowest percentage was in BHA-RA group. *Significant difference with control or untreated BMSCs group. †Significant difference with BME-RA.

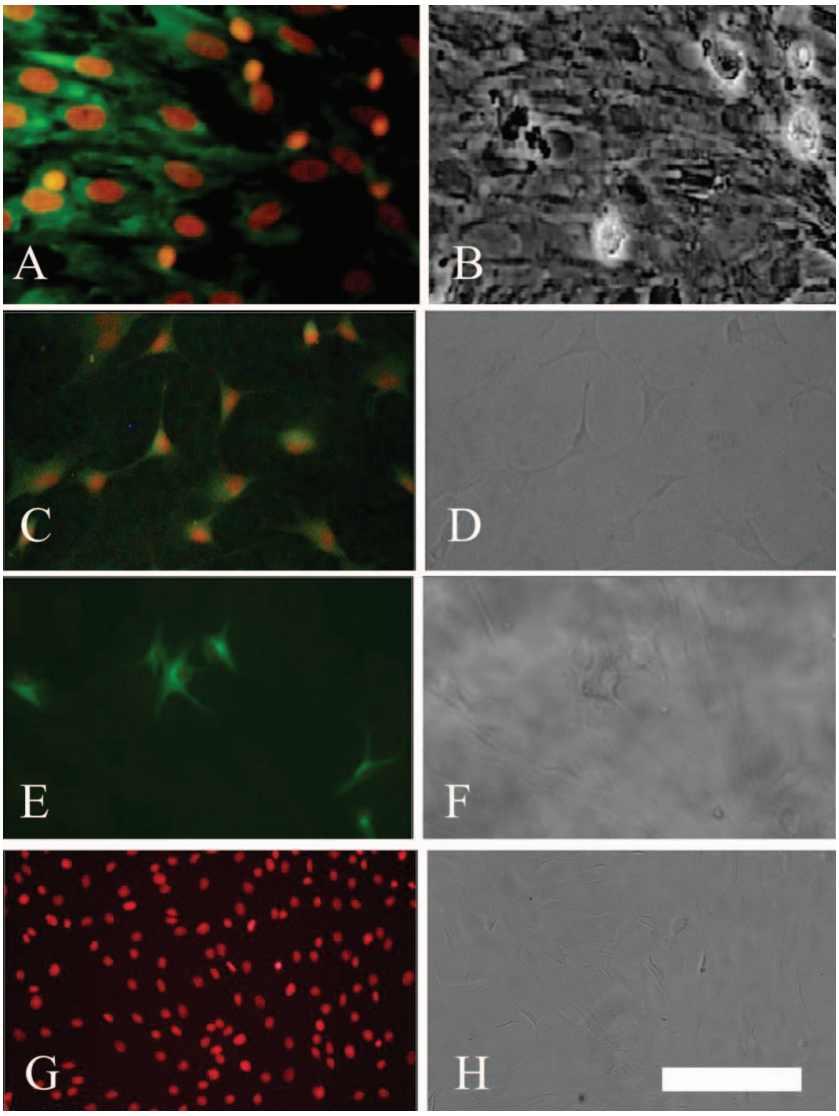


FIGURE 6. Characterization of the differentiated bone marrow stromal cells (BMSCs) using immuocytochemistry. The oligodendrocyte lineage markers used in the study included oligo-2, O4, O1, and MBP. The cells were incubated with anti-oligo-2, anti-O4, anti-O1, and anti-MBP antibodies (primary antibodies), followed by the secondary antibody conjugated with FITC, (A), (C), (E), and (G), respectively. The cells were counterstained in (A), (C), and (G) with ethidium bromide (EB). (B), (D), (F), and (H) show phase contrast images of (A), (C), (E), and (G), respectively (scale bars: 23.75, 47.5, 47.5, and 237.5 $\mu$m; A, C, E, and G, respectively).

was above 95%. The lowest viability among preinduction groups was noticed in BHA-RA group (Figure 5).

## Induction

At the end of the second phase of induction (IP-2), about 30% of the cells had spindle or irregular shapes with the processes greater than or equal to the long diameter of the cell body and approximately 60% of the cells became spindle-shaped or triangular with obvious processes stretching out. The cells expressed oligo-2, O4, and O1 (markers of oligodendrocyte lineage), but no immunoreactivity to MBP was observed (Figure 6).

Trypan blue exclusion staining showed a similar viability for 5 and 10 ng/ml of T3 doses; however, FSK and other doses of T3 (50, 100, and 200 ng/ml) had significantly toxic effect on the cells (Figure 7).

These observations confirmed that induction with growth factors followed by T3 could effectively induce NELCs into oligodendrocyte-like cells (OLCs) in vitro.

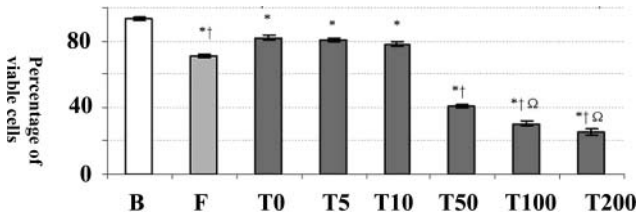


FIGURE 7. The percentage of the viable cells at induction stage of preinduced cells. The preinducer was dimethyl sulfoxide (DMSO) followed by retinoic acid (RA) treatment, and the inducers were the platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and heregulin (HRG) followed by either Forskolin (FSK) or a dose response of triiodothyronine (T0, T5, T10, T50, T100, and T200: 0, 5, 10, 50, 100, and 200 ng/ml, respectively). A histogram presents a dose response of triiodothyronine (T0, T5, T10, T50, T100, and T200: 0, 5, 10, 50, 100, and 200 ng/ml, respectively) and compared with F and undifferentiated bone marrow stromal cells (B). *Significant difference with BMSCs group. †Significant difference with 0, 5, and 10 ng/ml of T3. ΩSignificant difference with 50 ng/ml of T3.

## DISCUSSION

The selection of a proper cell candidate for replacement therapy of demyelinating diseases requires satisfying the following criteria: the transplant should form myelin-forming cells, be able to proliferate, be genetically stable, be able to migrate, satisfy safety issues (not to be tumorogenic), be immunocompatible, and ethically acceptable [35]. Dezawa et al. reported that BMSCs satisfied the above criteria [36]. In addition, BMSCs are easily accessible, readily adhere to plastic culture dishes [37], and by appropriate induction, they can differentiate into other lineages including glial cells [38]; therefore, BMSCs can be a feasible source for cell replacement therapies [39].

The results of this investigation showed that the incubation of BMSCs with DMSO followed by RA resulted in transdifferentiation of the BMSCs into NELCs where the percentage of NELCs was higher than those with the other preinducers ($\beta$ME-RA and BHA-RA).

DMSO was used to preinduce the BMSCs into cardiomyocytes and the results showed that DMSO was not a suitable preinducer [40]. Chu et al. reported that DMSO could preinduce the BMSCs into neurons, astrocytes and oligodendrocytes [41]. Other investigators disputed the preinduction with DMSO as an artifact [30, 42]; nonetheless, the microarray study showed that the neural markers were expressed in the BMSCs treated with DMSO [43]. In this study, DMSO was used to preinduce BMSCs together with RA into oligodendrocytes. The use of RA has been reported to contribute to preinduction of the BMSCs into oligodendrocyte precursor cells [39].

The inducer used in this protocol (bFGF-PDGF-HRG followed by 10 ng/ml T3) showed significantly higher percentage of OLCs among the other T3 dosages as well as FSK. The generation of OLCs from NELCs is consistent with findings of other investigators who reported the generation of oligodendrocytes from the neural stem cells [44].

Because the progenitor cells can express common marker for both oligodendrocytes and neurons, the immunoreactivity of preinduced BMSCs to nestin and other neuronal precursors indicates that they have a common origin [45–47].

Myelination depends on the differentiation of oligodendrocytes. In turn, this event is influenced by several factors, for example, thyroid hormone (T3) affects the timing of differentiation and regulates the expression of several enzymes involved in the synthesis of complex lipids and in the expression of some myelin structural proteins [48].

In this investigation, the final transdifferentiation of rat BMSCs was accomplished by T3 (10 ng/ml) where the highest percentage of O4 and O1 expression was yielded among other groups; however, no immunoreactivity to MBP could be detected because the cells had not yet reached maturity. The use of T3 as an inducer is consistent with results of other investigators [49–51]. The transplantation of transdifferentiated OLCs in the contused spinal cord resulted in improvement of the behavioral test as compared with those treated with undifferentiated BMSCs (unpublished data). Lu et al. reported that human marrow stromal cells (hMSCs) treated with $\beta$ME for 1 day followed by RA for 3 days resulted in myelinating glial-like cells and very low level of O4 immunoreactivity [39]. At the end of IP-2, mRNA of NeuroD gene was not detectable in the experimental groups (Figure 8). The highest expression level of S100 was seen only in the FSK group at IP-2 (Figure 9). The O4 and O1 immunocytochemical results showed that treatment with DMSO-RA followed by bFGF-PDGF-HRG and T3 (10 ng/ml) had the optimal effects on differentiation of neuroepithelial-like cells (NELCs) compared to other experimental and control groups. However, the differentiated cells showed no immunoreactivity for MBP (Figure 9).

On the one hand, Garcia et al. showed that BMSCs have a potential to produce glial derived neurotrophic factor (GDNF) and nerve growth factor (NGF) [52], which can assist in spontaneous transdifferentiation of BMSCs into neurons. Our results about undifferentiated BMSCs revealed that there was low immunoreactivity for nestin (1%) and NF-68 (1%), and no O4, O1, MBP, and GFAP expression, which are consistent with the findings of other investigators [31]. On the other hand, the Oct-4 gene, a stem cell marker [53], was detected by RT-PCR in the undifferentiated BMSCs but

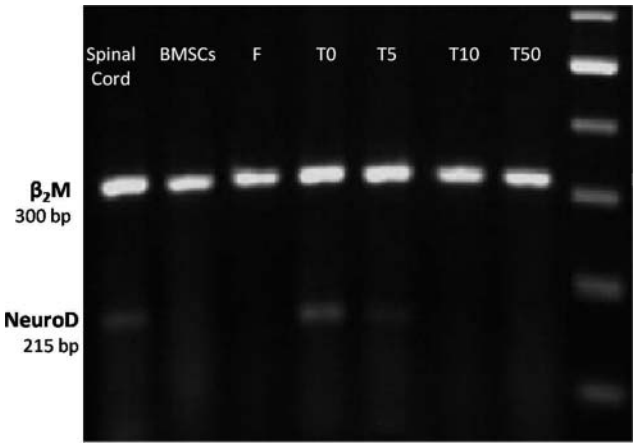


FIGURE 8. A study using RT-PCR to evaluate the expression of the NeuroD gene, a neural differentiation marker, in the induced cells. At induction stage, the preinduced cells were induced with platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and heregulin (HRG) followed by forskolin (FSF) or different doses of Triiodothyronine. Also, mRNA was extracted from the bone marrow stromal cells (BMSCs) to run the reverse transcriptase polymerase chain reaction (RT-PCR). An electrophorogram represents the expression of the NeuroD gene using a dose response of triiodothyronine (T0, T5, T10, and T50: 0, 5, 10, and 50 ng/ml, respectively) and is compared to untreated BMSCs (BMSCs). F and the fetal spinal cord "spinal cord" were used as controls.

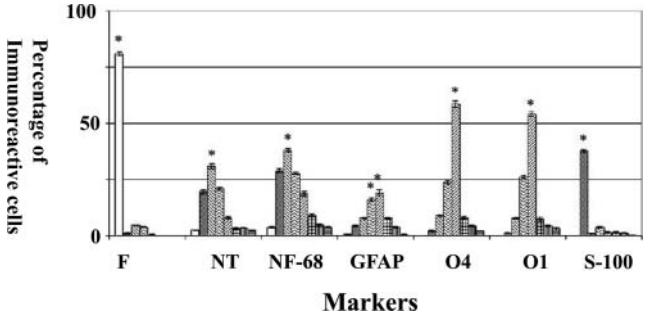


FIGURE 9. The percentage of the immunoreactivity of undifferentiated bone marrow stromal (negative control) and induced cells, using the bone marrow stromal cells (BMSCs) (fibronectin: F), neuroblast (nestin and neurofilament 68: NT and NF-68, respectively), astrocyte (glial fibrillary acidic protein: GFAP), oligodendrocyte lineage (O4 and O1), and Schwann cell (S-100) markers to evaluate the differentiation of the induced cells. A histogram represents the percentages of immunoreactive cells of the above markers to the undifferentiated BMSCs (solid white color column), preinduced cells [using dimethyl sulfoxide (DMS)] followed by retinoic acid (RA), as preinducers), then induced cells [using platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and heregulin (HRG), as inducers] followed by Forskolin (FSK) treatment (solid gray color), or different doses of triiodothyronine (0, 5, 10, 50, 100, and 200 ng/ml: downward cross hatch, wavy hatch, upward cross hatch, square cross hatch, vertical lines, and horizontal lines patterns, respectively). *Indicates that there was a statistically significant difference with other groups in the same labeling.

was downregulated at PP-2. This finding is in accordance with the finding of other investigators [54]. NeuroD expression was not detected in the undifferentiated BMSCs, confirming the results of others [55, 56], but Woodbury et al. [57] reported very low levels of NeuroD gene expressed in BMSCs. NeuroD gene was detected at PP-2 using DMSO-RA, where O4, O1, and GFAP expression increased while NeuroD gene expression was downregulated, agreed with findings of others [57–60].

## CONCLUSION

The transdifferentiation of BMSCs into oligodendrocyte phenotype can be achieved by preinduction of the cells by DMSO followed by RA then induction of the preinduced cells using bFGF-PDGF-HRG followed by T3 (10 ng/ml). The transdifferentiated BMSCs can be a potential source for transplantation of OLCs in CNS disorders.

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