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Comparing the Effect of Uniaxial Cyclic Mechanical Stimulation and Chemical Factors on Myogenin and Myh2 Expression in Mouse Embryonic and Bone Marrow Derived Mesenchymal Stem Cells

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Abstract: **Background:** Environmental factors affect stem cell differentiation. In addition to chemical factors, mechanical signals have been suggested to enhance myogenic differentiation of stem cells. Therefore, this study was undertaken to illustrate and compare the effect of chemical and mechanical stimuli on Myogenin (MyoG) and Myosin heavy chani 2 (Myh2) expression of mouse bone marrow-derived mesenchymal stem cells (BMSCs) and embryonic stem cells (ESCs).
Methods: After isolation and expansion of BMSCs and generation of embryoid bodies and spontaneous differentiation of ESCs, cells were examined in 4 groups: (1) control group: untreated cells; (2) chemical group: cells incubated in myogenic medium (5-azacythidine and horse serum for BMSCs, dimethyl sulfoxide (DMSO) and horse serum for ESCs) for 5 days; (3) mechanical group: cells exposed to uniaxial cyclic strain (8%, 1 Hz, 24 h) and (4) chemical + mechanical group: cells incubated in myogenic medium for 4 days and then exposed to uniaxial cyclic strain. Real-time PCR was used to examine the expression of MyoG and Myh2 as specific myogenic markers.
Results: suggested that mechanical loading, as a single factor, could elevate MyoG and Myh2 expression. Combining chemical with mechanical factor increases expression and there was no significant difference in MyoG expression of ESCs- and

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MSCs-chemical + mechanical groups; however, Myh2 expression was significantly higher in ESCs-mechanical group than that in the same group of MSCs.

Keywords: Embryonic stem cell, Mechanical loading, Mesenchymal stem cell, Myogenic differentiation, Uniaxial cyclic stretch.

1 Introduction

Tissue engineering, which deals with replacement, repair or regeneration of organs and tissues, is a promising approach in medical sciences [1]. Stem cells are essential for tissue engineering applications due to their self-regenerating and differentiation into multiple somatic cell types including skeletal muscle cells [2]. Over the past decade studies, have suggested that stem cells can be a suitable cell source for cell-based therapies, which are novel methods for creating new tissues and organs [3]. There is a considerable interest in skeletal muscle regeneration in sports medicine field for treatment of severe sport injuries and muscular dystrophies [4], possible ablation of mitochondrial myopathies [5] and for recovery from disuse muscle atrophy. Stem cells, which are differentiating into one or more connective tissue phenotypes, are utilized through cell-based tissue engineering intended for repairing tissues [1].

Stem cells are classified into embryonic and adult cell types [6], which are biologically different based on their origin and differentiation potential. Embryonic stem cells (ESCs) are isolated from the inner blastocyst mass. These cells are pluripotent and capable of differentiating into several cell types of three germ layers (endoderm, mesoderm and ectoderm). However, ethical issues surrounding ESCs have limited their utilization and culture in stem cell researches. On the other hand, mesenehymal stem cells (MSCs) are easily available in adult tissues, such as bone marrow, adipose and blood cells. MSCs are multipotent cells with potential of differentiating into multiple cellular phenotypes such as skeletal muscle cells. Also, there is no concern about ethical issues or immunologic rejections in tissue engineering realm [7].

In addition to the findings of a past decade studies indicating the role of "biochemical" environment on differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) [8-10] , recent evidences [11, 12] show that "biomechanical" cues affect cell functions. Although mechanical loadings can direct BMSCs proliferation and differentiation into myogenic [13, 14], osteogenic [15] or chondrogenic [16] lineages, these outcomes depend on other factors such as substrate type or soluble biochemical environment rather than just mechanical signals [17].

Recent studies indicate the role of several muscle-specific genes responsible for muscle development and differentiation [18, 19]. These molecules, called myo-

genic regulatory factors, cause stem cells to express muscle - specific markers. Myogenin (MyoG), which has been reported to play major roles in terminal differentiation of myoblasts, is essential for development of functional skeletal muscle in mice [19]. Mice lacking both copies of MyoG (homozygous null) suffer from perinatal lethality due to the lack of mature secondary skeletal muscle fibers throughout the body. MyoG expression is associated with expression of the fast (type II) myosin heavy chain isoform (Myh2), which has the slowest contraction speed of the fast Myh isoforms [20].

Previous studies have shown that application of mechanical stretch to myocyte induces cell proliferation and affects the muscle structural protein Myhs [21-23]. Mouse myoblast cells (C2C12) were subjected to cyclic strain (15% , 1 Hz, 12-24 h) and monitored for changes in expression of skeletal muscle - specific genes [24]. Similarly, it has been suggested that mechanical stretch (8% strain, 1 Hz) plays an important role in regulating the proliferative capacity of MSCs and their growth [25]. Based on these reports, it is now clear that application of mechanical stress on myocyte not only promotes cell proliferation but also changes the cell characteristics [19]. Since muscle - specific genes are involved in qualitative and quantitative changes in the muscle after exposure to mechanical stress, there might be an increase in the expression of the myogenic regulatory factors, especially MyoG, in response to overloading [26].

Therefore, this study was designed to examine and compare the effect of cyclic stretch, as the mechanical stimuli, on both ESCs and BMSCs by evaluating the expression of myogenic regulatory factors, MyoG and Myh2.

2 Materials and methods

2.1 Isolation of BMSCs (Animal study)

All animal experiments were performed according to the guidelines of Ethics Committee of Pasteur Institute of Iran. Male BALB/C mice (n = 5), 6 weeks old, were sacrificed by cervical dislocation and the tips of their femurs and tibiae were removed. The bone marrow was flushed out of tibias and femurs using alpha modified Eagle's medium (αMEM; Gibco, NY, USA) containing 15% fetal bovine serum (FBS; Sigma, NY, USA), 2 mm L-glutamine (Gibco, NY, USA), 100 U/ml penicillin (Sigma, NY, USA) and 100 U/ml streptomycin (Sigma, NY, USA). After centrifugation at 500 rcf for 5 min, the pellet was suspended in fresh αMEM containing 15% FBS, 2 mm L-glutamine, 100 U/ml penicillin and 100 U/ml streptomycin [27, 28].

2.2 Cultivation of MSCs

Following isolation of MSCs, the culture medium consisting of αMEM (15% FBS and 2 mm L-glutamine) was replaced after 3 days. When primary cultures became nearly confluent, in order to passage the cells, the culture was treated with 0.5 ml of 0.025% Trypsin (Sigma, NY, USA) containing 0.01% EDTA (Sigma, NY, USA) at room temperature for 5 minutes. After 3 passages, the potency and the immunophenotype of isolated MSCs were evaluated using differentiation potential assay and flowcytometry analysis [27, 28]. The cells were then used in the experiments.

2.3 Cultivation of ESCs

Undifferentiated mouse (ESC) line, Royan C4 (Royan Institute, Tehran, Iran), was cultured in plastic flasks (Orange, Belgium) coated with gelatin 0.1% (Gibco, NY, USA). The cell was kept in a humidified atmosphere of 5% CO_2 at 37˚C in Dulbecco's modification of Eagle medium (DMEM; Gibco, NY, USA) supplemented with 2.0 mM L-glutamine, 0.1 mM non-essential amino acids (Gibco, NY, USA), 0.1 mM 2-mercaptoethanol (Merck, NY, USA), 5 mg/ml insulin transferrin selenium (Gibco, NY, USA), 1,000 U/ml (10 ng/ml) mouse leukemia inhibitory factor (Sigma, NY, USA) and 10% FBS [29, 30].

2.4 Generation of embryoid bodies (EBs)

Hanging drop method was used to generate EBs. Briefly, after trypsinizing, ESCs (n=800) per 10 μl of the culture medium (without leukemia inhibitory factor) were aggregated in hanging drops for 2 days, followed by suspension culture in plates for 2 days. Each 10 μl drop of 4-day-old aggregates (approximately 40 EBs) was then cultured on silicon membrane coated with collagen type 1 [31]. Cultured ESCs were then used in the experiments.

2.5 Immunophenotype characterization of MSCs

Surface antigens CD90.1 and CD44 as positive markers and CD45 as a negative marker were evaluated on BMSCs by flowcytometry assay. The cells were detached with trypsin/EDTA and counted. About 5×10^5 cells were divided into 3 aliquots in each test tube. Sample tubes were centrifuged and the cells were incubated in darkness at room temperature for 45 min. The centrifugation was carried out with the following antibodies; Fluorescence isothiocyanate-conjugated rat anti–mouse CD45, CD90.1 , CD44 and Fluorescence isothiocyanate-conjugated rat IgG2bk isotype control (2 μg/ml, all from BD Bioscience, san Diego, CA), which were diluted in 100 μl phosphate buffered saline containing 5% FBS . Gating was set using unstained cells and in each analysis, at least 15,000 events were counted.

Finally, the resulting data was analyzed using Flow Cytometry Analysis Software (version 7.6.4).

2.6 Differentiation potential assays of BMSCs

The potential of the isolated cells to differentiate into osteogenic and adipogenic lineages was examined. For osteogenesis, cultured BMSCs were incubated in an osteogenic medium containing DMEM supplemented with 10 mM β-glycerol phosphate, 50 μg/ml ascorbate-2-phosphate and 0.1 μM dexamethasone (all from Sigma, NY, USA). The medium was replaced twice a week. After three weeks, the cells were fixed with methanol at room temperature for 10 min and then stained with Alizarin red S (pH 4) at room temperature for 5 minutes.

For adipogenesis, subconfluent BMSCs were incubated in adipogenic medium consisting of DMEM supplemented with 1 μM dexamethasone, 500 μM isobutyl methyl xanthine, 60 μM indomethacin and 5 μg/ml insulin (all from Sigma, NY, USA). The adipogenic medium was changed twice a week. After 10 days, the cells were fixed in methanol for 45 minutes and lipid-rich vacuoles were stained with Oil Red O (Sigma, NY, USA) [32].

For Chondrogenesis, pellet of BMSCs were pelleted and cultured in chondrogenic medium including DMEM supplemented with 100 nM dexamethasone, 50 μM ascorbic acid-2 phosphate (Sigma, NY, USA), 1mM sodium pyruvate (Gibco, NY, USA) 10 ng/ml TGF β1 (transforming growth factor- β 1; R&D Systems, MN, USA) and 1% ITS-Premix (BD Biosciences, CA, USA). After 28 days, nodules were fixed in 10% formalin (Sigma, NY, USA), sectioned and consequently stained by Alician Blue (Sigma, NY, USA) and finally observed under optical microscope.

2.7 Application of mechanical strain

MSCs or EBs were cultured in the center of silicon membranes recoated with collagen type I (Sigma, NY, USA) solution (0.8 mg/ml in 0.2% acetic acid). After 48 h, the cells reached? 70% confluence and then subjected to cyclic uniaxial strain at a rate of 1 Hz with 8% elongation for 24 h using a custom-built device described earlier by Haghighipour et al. in 2007 [33]. The experiments were performed within 5 days on 4 test groups: chemical, mechanical, chemical + mechanical and control groups. In chemical group, the cells were incubated in myogenic differentiation medium containing 3 μM 5-azacytine (Sigma, NY, USA) for MSCs, 1% dimethyl sulfoxide (ICN Biomedicals, NY, USA) for ESCs and 5% horse serum (Gibco, NY, USA) for each of MSCs and ESCs. In mechanical group the cells were only stimulated mechanically. In chemical + mechanical group the cells were incubated in myogenic medium and exposed to mechanical strain on the 4^{th} day. Finally, in control group, the stem cells were cultured on silicon membrane without any chemical

or mechanical treatment.

In order to provide identical culture conditions, chemical and control groups were assembled into a device on the 4^{th} day, without any mechanical stimulation. At the end of mechanical test period, the membranes in all groups were returned to the standard culture conditions.

2.8 RNA extraction and real-time PCR analysis of gene expression

Immediately after completion of the experiments on the 5^{th} day, total RNA was extracted using the RNeasy mini kit plus (Qiagen, MD, USA) according to the manufacturer's instructions, quantified and stored in RNase-free water at -80 °C. cDNA was synthesized using QuantiTect® Reverse Transcription kit (Qiagen, MD, USA) according to manufacturer's instructions. SYBER green-based real-time PCR primers were designed to span exon/intron and exon/exon junctions using primer express software (version 2.5). The sequences of primers have been shown in Table 1.

Table 1: The sequences of primers used in real-time PCR.

Primers	Sequences
Myh2	Fw 5′- TGCTGCTGATCACCACGAAC-3′
	Re 5′-GCACTATCAGTGGCCATCAGC -3′
MyoG	Fw 5′-CCAACCCAGGAGATCATTTGC- 3′
	Re 5'-TTGGGCATGGTTTCGTCTG-3′
TBP	Fw 5′-AAGGGAGAATCATGGACCAGAAC-3′
	Re 5′- GGTGTTCTGAATAGGCTGTGGAG-3′
TBP, TATA binding protein	

Two-step real-time PCR was performed using SYBR® Premix (ABI, USA) and LightCycler® (Roche, Germany). Expression level of each target gene was normalized to the reference gene, TATA binding protein, and the resulting data were expressed as a ratio of the control. The comparative threshold cycle (CT) method (using formula $2^{-\Delta\Delta CT}$) was used to analyze the obtained data, which calculate CT using the following equations:

1. ΔCT = CT of target gene – CT of housekeeping gene (Normalization)

2. ΔΔCT = ΔCT of sample – ΔCT of calibrator (control groups).

2.9 Statistical analysis

All data were expressed as means ± SD of three separate experiments. Statistical significance was evaluated by one-way ANOVA and student's *t*-test (SPSS software ver. 17). A *P* value <0.01 was regarded as statistically significant.

3 Results

3.1 Characterization of MSCs

The isolated MSCs at passage 3 were analyzed for cell surface antigens by flowcytometry. Green curves explain the expression level of antibodies in cells and blue regions explain isotype control. The vertical axes of graphs indicate the count of cells using in flowcytometry. The horizontal axes indicate a threshold. A threshold is the lowest signal intensity value an event can have for it to be recorded by cytometer. FL1-H and FL4-H are the channels on a flowcyotometer. The antibodies are labeled with FITC can be detected using the specific filter in the FL channels. Results revealed that 1.2 % of MSCs exhibited positive staining for anti-CD45 (green) compared to an isotype control (blue) (Fig.1A). Flow cytometry results showed the presence of enriched (98.87% for CD90) and (96.86% for CD 44) MSC population compared to an isotype control (Fig.1B, C).

Isolated MSCs passage at 3 was differentiated into osteogenic and adipogenic lineages. In osteogenic cultures, mineralized colonies, which were stained with Alizarin red S, were observed after 21 days (Fig. 2A). In adipogenic cultures after 10 days, the potential of adipogenic differentiation was demonstrated as intercellular lipid vacuoles, which were stained with Oil red O, and were observable as red spots (Fig. 2B).

3.2 Real - time PCR analysis of myogenic differentiation

MSCs and ESCs induced toward the skeletal myogenic lineage express myogenic genes. Total RNA prepared from mouse skeletal muscle was analyzed as a positive control. Serially diluted mouse skeletal muscle was tested by real-time PCR assay and a standard curve of the CT values was obtained. Amplification of the correct product was confirmed by using the mouse skeletal muscle as a positive control.

3.2.1 Myogenic differentiation of MSCs

Based on real-time PCR results, chemical + mechanical group showed significantly higher expressions of both MyoG (Fig. 3A) and Myh2 (Fig. 3A) compared to mechanical and chemical differentiation groups *($P<0.01$).* Moreover, there was a significant difference in expression of MyoG and Myh2 among mechanical, chem-

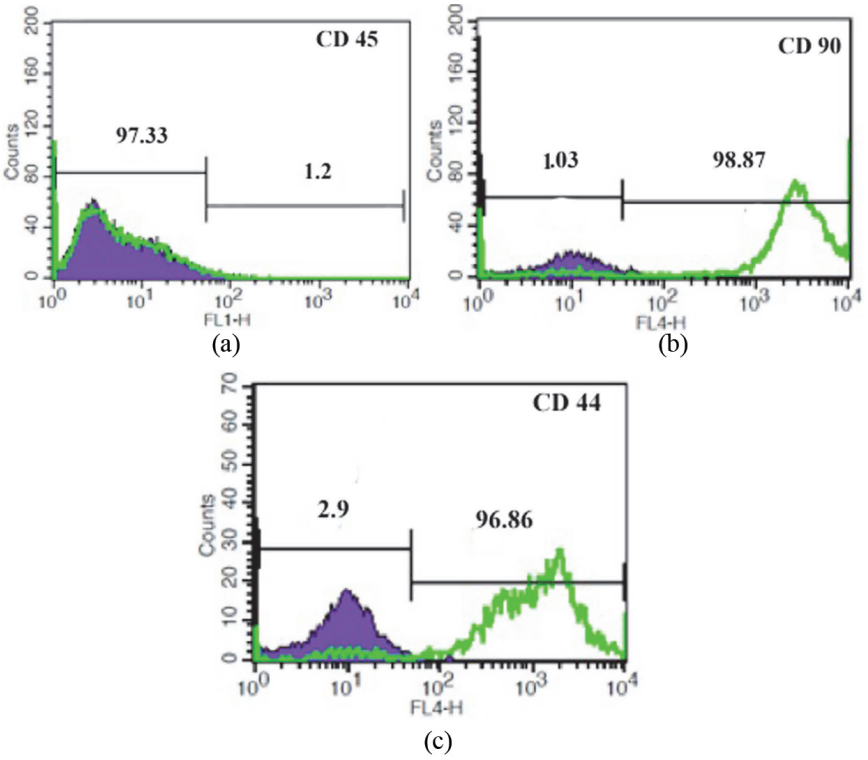

Figure 1: A) Flowcytometry shows 1.2 % of cells exhibited positive staining for anti-CD45 (leukocyte common antigen) (green) compared to an isotype control (blue). B) and C) Flow cytometry show the presence of enriched (98.87% for CD90) and (96.86% for CD 44) BMSC population (green) compared to an isotype control (blue).

ical and control groups ($P < 0.01$). These results indicate that cyclic axial stretch affects the differentiation process of MSCs into skeletal muscle cells and could be used as a single differentiation factor.

3.2.2 Myogenic differentiation of ESCs

Real-time PCR demonstrated that MyoG expression (Fig. 3B) was increased significantly in chemical + mechanical differentiation group ($P <0.01$) as compared to other test groups.

In addition, real-time PCR revealed the highest expression of Myh2 (Fig. 3B) in chemical differentiation group compared to other test groups ($P <0.01$). Furthermore, Myh2 expression in chemical + mechanical differentiation test group was

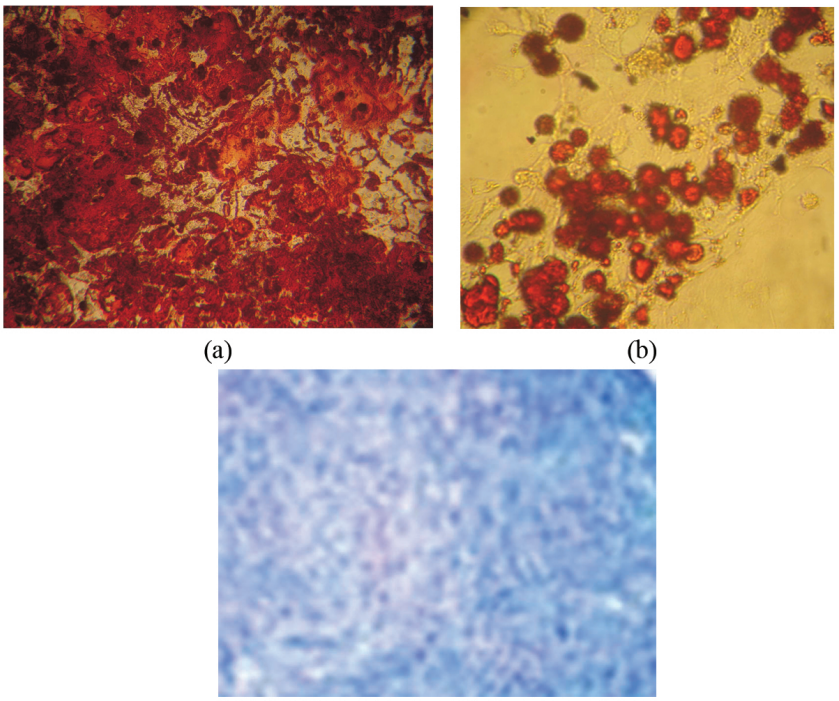

Figure 2: Differentiation potential assays of mouse BMSCs. (A) Osteogenic differentiation and calcium depositions were stained by Alizarin red S, which was appeared as dark red spots in the plate. In some places, thickness of mineralization does not allow the light to pass through the sample when using an inverted microscope (400×). (B) Adipogenic differentiation and adipose droplets were observed after the cells were stained with Oil red O (inverted microscope, 400×).

significantly higher compare to mechanical test group (P <0.01).

Expression of MyoG and Myh2 in mechanical group compared to control group (P <0.01) suggested that mechanical loading affected the differentiation of ESCs into skeletal muscle cells and could be a single differentiation factor (Fig. 3B).

3.2.3 Comparison of myogenic differentiation between MSCs and ESCs

Comparison of real-time PCR results between MSCs and ESCs test groups showed that ESCs-chemical group had significantly higher expression of MyoG compared to the MSCs-chemical group (P <0.01). The expression level of MyoG was increased significantly in MSCs-mechanical group than that in the same group of

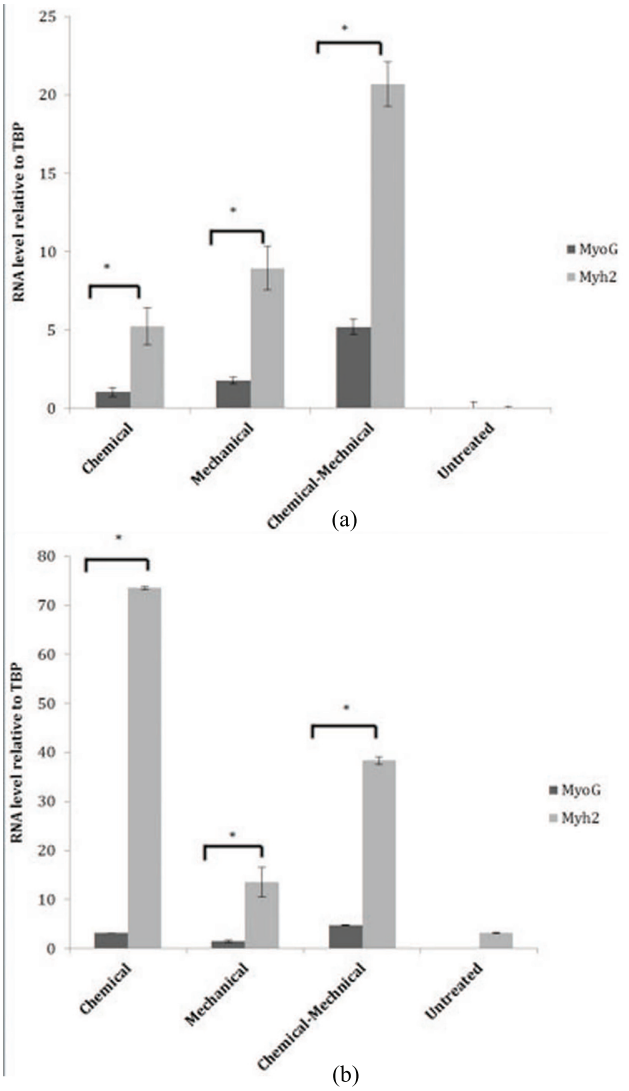

Figure 3: Real-time PCR analysis of myogenic differentiation of (A) MSCs and (B) ESCs. (A) the expression of MyoG in MSC experimental groups was increased 1 $\pm$ 0.3, 1.7 $\pm$ 0.2, 5.1 $\pm$ 0.0.5 folds.Also Myh2 expression was increased 5.2 $\pm$1.2, 8.94 $\pm$ 1.4, 20.7 $\pm$ 1.4 folds in chemical, mechanical and chemical + mechanical groups, respectively as compared to negative control group (* $P < 0.01$). (B) the expression of MyoG in ESCs experimental groups was increased 3.1 $\pm$ 0.05, 1.5 $\pm$ 0.2, 4.7 $\pm$ 0.06 folds Also Myh2 expression was increased 73.5 $\pm$ 0.3, 13.5 $\pm$ 3, 38.3 $\pm$ 0.8 in chemical, mechanical and chemical mechanical groups, respectively as compared to negative control group *(* P< 0.01)*.

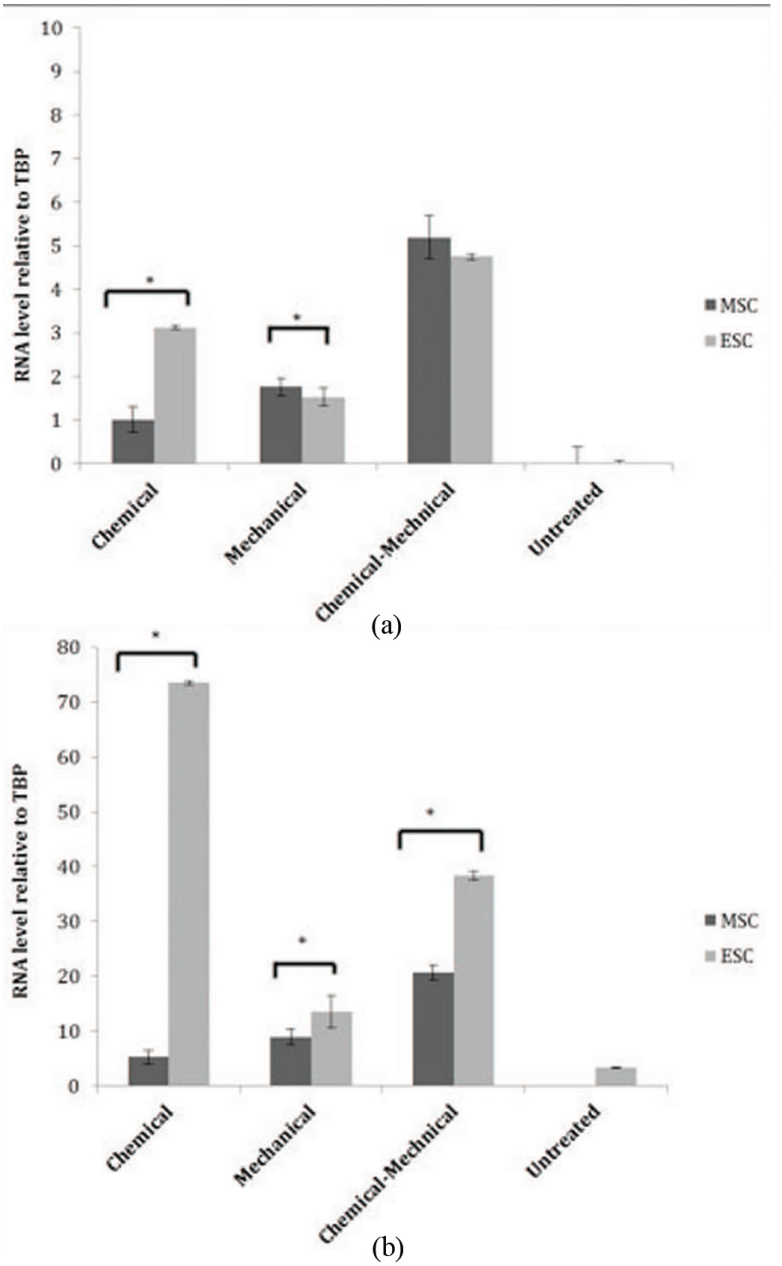

Figure 4: Comparison of (A) MyoG and (B) Myh2 in ESCs and MSCs experimental groups (* $P < 0.01$).

ESCs) $P < 0.01$). In MyoG expression, no significant difference was observed between chemical – mechanical differentiation groups of ESCs and MSCs (Fig. 4A).

Comparison of real-time PCR data of MSCs and ESCs test groups revealed that Myh2 expression was higher in ESCs-mechanical and chemical groups compared to that in the same groups of MSCs ($P < 0.01$). Also, the expression of Myh2 in ESCs mechanical group was significantly higher than that in the same group of MSCs)$P < 0.01$) (Fig. 4B).

4 Discussion

Myogenesis is a complicated series of events. This process starts when multipotent precursor cells proliferate to myoblasts, which subsequently differentiate and fuse into multinuclear myotubes and then myofibrs. Skeletal myogenesis is predominantly regulated by muscle transcription factors such as MyoG and Myh2. Furthermore, signal transduction pathways, gene expression and protein synthesis are influenced by various signals from adjacent milieu, among which load-induced stimulation signals have been newly identified [24]. It has also been demonstrated that when MSCs and ESCs are cultured in suitable conditions for myogenic differentiation, MyoG and Myh2 are expressed [17, 31].

In this study, MSCs and EBs separately were affected either by skeletal myogenic medium ,cyclic uniaxial stretch (8% strain, 1 Hz,24 h) and a combined treatment from the 4th day of chemical induction. The cyclic duration was selected 24h because the expression of MyoG and subsequently Myh2 continued until 24 h after the stretching and decrease thereafter [18]. In consistent with other studies the stretches more than 15% led to an injury to myoblast, in this regard Schultz et al. has pointed to maintain mechanical stretch levels fewer than 10% (34), for this reason the strain which used in this research was 8%. MyoG and thereafter Myh2 expression respond immediately to the 8% strain [25].

Skeletal muscle specific gene expression in the all of the experimental groups was quantified by mRNA levels of MyoG [26], muscle regulatory factors included, in addition Myh2 [36] as the typical skeletal muscle marker of terminal differentiation. The results were compared to negative control.

These transcription factors are known to be of great importance when myoblasts proliferate and differentiate. Furthermore, a recent investigation illustrated that MyoG mRNA level is more associated with alterations in Myh isoform composition than changes in muscle mass [20].

In the chemically test group, addition of 5- azacytidin, horse serum for MSCs differentiated group and DMSO and horse serum for ESCs differentiated group resulted in statistically significant increase of the expression of MyoG and Myh2 ,

according to previous studies using same media of induction of skeletal muscle differentiation.

Although, real-time PCR for ESCs indicated the highest expression of Myh2 in chemical differentiation test groups compared to other test groups. The comparison of results between MSCs and ESCs of test groups showed that expression of MyoG in ESCs-chemical group was significantly higher than MSCs-chemical group. In consistent with Barbet et al. [37] research, our results showed that the expression of Myh2 as a mesoderm sarcomeric muscle is higher in ESCs (included untreated ESCs).

5-azacytidine is known as a super physiological inducer of myogenic transformation. There is a relation between DNA methylation and beginning of myogenesis. 5-azacytidine inhibits DNA methyltransferase by demitilation. Consequently, promote expression of skeletal muscle specific transcription factores [38]. Reports have shown that mouse ESCs were differentiated with 1% DMSO promotes skeletal myogenesis, but not cardiogenesis. When ESCs were induced with 1% DMSO, MyoD, first muscle-specific transcription factor, was expressed high level whose expression promotes MyoG expression [39, 40]. The peak of expression of MyoG is whitin 5 days [41].

In the mechanically test group, expression of MyoG and Myh2 was significantly higher in mechanical group of MSCs compared to that in the same groups of ESCs. Recent studies confirm that resistance exercise induces a transient increase in mRNA levels of MyoG and Myh2 in skeletal muscle. This finding implies that these transcription factors are regulated at the transcriptional level and they might be involved in the regulation of ?ber-type transitions or hypertrophy [26, 42].

MyoG is the important muscle-specific transcriptions factor whose expression indicates the commitment of precursor cells to the myogenic differentiation. Thereafter, the transcriptional regulation of myogenesis progresses and eventually myosin heavy chain isomers such as Myh2 differentiation are expressed [34]. In consistent with other studies the expression of MyoG and subsequently Myh2 continued until 24 h after the stretching [18].

In agreement to Grounds *et al.*, [6] we founded that mechanical loading affects the differentiation of ESCs and MSCs into skeletal muscle cells and could be a single differentiating factor on its own without any axillary assistance. Therefore to mimic *in vivo* mechanical stimulation, skeletal muscle tissue engineering strategies have been advised to maintain mechanical stretch levels fewer than 10%. Stretches more than 15% cause an injury to myotubes [43].

In the Chemical + mechanical test group, combination of chemical factors with mechanical loading (cyclic stretch) further increases the differentiation of ESCs

and MSCs into skeletal muscle cells. Although there was no significant difference in expression of MyoG between the chemical + mechanical differentiation groups of ESCs and MSCs, a significant difference in Myh2 expression between these groups was observed. In the current study, it is assumed that the interaction of biochemical factors and mechanical loading has an effect on initiating specific signaling pathways during differentiation process of stem cells in agreement to previous studies using both factors for skeletal myocyte differentiation [44, 45] . The expression of Myh2 is downregulated by activation of TGFb1/Activin/Nodal pathway. Treatment with TGF-β1 decreases the level of Myh2 expression. Activation of TGFb1/Activin/ Nodal pathway by mechanical strain mimics inhibition of ESC differentiation through TGFb1 ligands [46] Otherwise DMSO inhibits this pathway lead to activate the Myh2 expression. Cross talk between DMSO as a chemical factor and mechanical signal may occur in this pathway. However the effect of chemical factor was higher than mechanical stimulation, therefore the level of Myh2 expression in mechanical chemical group was higher than mechanical group and less than chemical one [47].

It is also possible that mechanical stimulus have potential to regulate and synergize with biochemical signal induced by chemical factors in order to promote activation of interacellular pathway like rab5 or mechano growth factor (MGF) to raise the expression of skeletal myogenic differentiation markers [43, 45]. Mechanical stress induces differentiation of mesenchymal progenitor cells specifically from the bon marrow in vitro even in the absence of chemical factors [48-50].

5 Conclusion

The findings in this study are significant in terms of comparison when MSCs and ESCs differentiate into skeletal muscle lineage. Particularly, the results in this investigation showed that both mechanical stimulation (cyclic strain) and biochemical factors were sufficient to induce in the differentiation of MSCs and ESCs.

Further research is necessary to precisely examine other cyclic uniaxial regimen in order to find the best condition with the maximal similarity to in vivo so as to use differentiated stem cells in muscle regeneration to treat injured, diseased or hypertrophied skeletal muscle tissue.

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