



# Vitamin E and selenium improve mesenchymal stem cell conditioned media immunomodulatory effects

Fereshteh Ghasemi<sup>1</sup>, Majid Khoshmirsafa<sup>1</sup>, Elahe Safari<sup>1</sup>, Marzieh Asgari<sup>1</sup>, Mehdi Alemrajabi<sup>2,3</sup>, Shahrzad Nojehdehi<sup>4,5</sup>, Samane Khorrami<sup>1</sup>

<sup>1</sup>Department of Medical Immunology, School of Medicine, Iran University of Medical Science, Tehran, Iran; <sup>2</sup>Department of General Surgery, School of Medicine, Iran University of Medical Sciences, Tehran, Iran; <sup>3</sup>Firoozgar Clinical Research Development Center (FCRDC), Tehran, Iran;

<sup>4</sup>Department of Biology, Islamic Azad University Central Tehran Branch, Tehran, Iran; <sup>5</sup>Stem Cell Technology Research Center, Tehran, Iran

*Contributions:* (I) Conception and design: F Ghasemi, S Khorrami; (II) Administrative support: F Ghasemi, S Nojehdehi; (III) Provision of study materials or patients: F Ghasemi, E Safari, M Alemrajabi; (IV) Collection and assembly of data: F Ghasemi, M Asgari; (V) Data analysis and interpretation: F Ghasemi, M Khoshmirsafa; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Samane Khorrami. Department of Medical Immunology, School of Medicine, Iran University of Medical Science, Tehran, Iran. Email: khorami.s@iums.ac.ir.

**Background:** Mesenchymal stem cells (MSCs) with immunoregulatory properties affect immune systems. Many studies showed that antioxidants such as vitamin E (Vit E) and selenium (Se) could improve stem cells survival. This study aims to investigate the effects of MSC conditioned media (CM) treated with Vit E and Se on immune cells.

**Methods:** MSCs were isolated and cultured with Vit E and Se. Immature dendritic cells (DCs) and peripheral blood mononuclear cells (PBMCs) were cultured with MSC CM treated with Vit E and Se. The expression of HLA-DR, CD86, CD40, and CD83 on mature DC were evaluated. DC supernatant and PBMCs supernatant was collected for the study of TGF- $\beta$ , IL-10, and IL-12. PBMCs evaluated for the expression of T-bet, GATA3, ROR $\gamma$ t, and FOXP3.

**Results:** MSC CM increased CD40 on myeloid DC (mDC). CD40 has been decreased in DC treated with MSC (Vit E) and MSC (Se) CM. HLA-DR expression on DCs and IL-12 level were significantly reduced in MSC (Vit E) CM. IL-10 concentration increased in DCs treated with MSC (Vit E) and MSC (Se) CM. Treatment of PBMCs with MSC CM decreased IL-10 level, FOXP3, and ROR $\gamma$ t expression. On the other hand, the MSC (Vit E) CM and MSC (Se) CM decreased the IL-10 level and increased IL-12, T-bet, and ROR $\gamma$ t.

**Conclusions:** According to the results, the treatment of MSC with Vit E and Se enhanced the ability of MSCs to inhibit DCs and improved immunomodulatory effects. Concerning the effect of MSC on PBMC, it seems that it increased ROR $\gamma$ t expression through monocytes.

**Keywords:** Mesenchymal stem cell (MSC); dendritic cell (DC); vitamin E (Vit E); selenium (Se)

Received: 10 February 2020; Accepted: 24 March 2021; Published: 07 May 2021.

doi: 10.21037/sci-2020-008

View this article at: <http://dx.doi.org/10.21037/sci-2020-008>

## Introduction

Mesenchymal stem cells (MSCs) are multipotent and have the ability to self-renewing and differentiating into different lineages. These cells can be isolated from many tissues such as bone marrow, adipose tissue, and placenta (1). MSCs show immunoregulatory properties and affect the immune

system. MSCs prevent differentiation of monocytes into dendritic cells (DCs). They also have inhibitory effects on T and B lymphocytes (2).

Vitamin E (Vit E) is a term for a group of fat-soluble compounds that have divided into tocopherol and tocotrienol. Vit E has antioxidant properties and protects

cell membranes against the oxidation-reduction (redox) reactions (3). In different studies have been reported Vit E can increase antibody production and resistant to viral diseases in the elderly (4).

Selenium (Se) is an essential micronutrient that can affect immune response. The effects of Se mainly due to participation in selenoproteins (5). In patients with HIV1, Se deficiency leads to a decrease in T CD4<sup>+</sup> frequency and the cytotoxicity effects of the NK cells. Se supplementation also reduces inflammation. Some studies showed relationship between serum Se levels and the risk of autoimmune diseases (6,7).

DCs play an essential role in initiating acquired and innate immunity. However, they could induce both tolerance and activation of the immune response (8). Besides DC there are other cells such as T cell, B cells, NK, and monocyte which are involved in immune responses. As previously stated MSCs with immunoregulatory properties can affect the function of these cells in various ways (2).

Over the past decade, several studies have been performed on the effects of antioxidants such as Vit E and Se on MSCs. However, very few studies have been carried out about the effects of antioxidant on the immunoregulatory characteristics of MSCs. In this study, the effect of MSC conditioned media (CM) treated with Vit E and Se on DC surface markers such as HLA-DR, CD86, CD40, and CD83 and also IL-12, IL-10 and TGF- $\beta$  levels and T-bet, GATA3, ROR $\gamma$ t, and FOXP3 expression levels in peripheral blood mononuclear cells (PBMCs) were studied. We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/sci-2020-008>).

## Methods

### *MSC isolation and culture*

Adipose tissue was isolated from patients undergoing surgery and washed three times with PBS containing penicillin/streptomycin (Gibco, USA). Adipose tissue was digested with collagenase type IV (Sigma-Aldrich, USA) and incubated for 30 minutes at 37 °C. Cell suspension was passed from 0.7 micron filter and were cultured in DMEM medium (Gibco, USA) containing 10% FBS (Gibco, USA), 1% penicillin/streptomycin and 1% L-Glutamine (Gibco, USA). To examine the ability of differentiation of MSCs to osteocytes 10,000 cells and for adipocytes, 20,000 cells in 1 mL of DMEM medium with 10% FBS were seeded in four-well plates. After 48 hours, the medium was replaced

with a differentiated medium. After 3 weeks, to determine the differentiation of these cells, wells were washed with PBS and stained with Alizarin Red (Sigma-Aldrich, USA) (and Oil Red O (Sigma-Aldrich, USA) (Figure S1). Also, MSCs surface markers were investigated using flow cytometry. MSCs were isolated with trypsin and washed with PBS and then, they were suspended in PBS containing 3% BSA. After addition of antibody, expression of CD105, CD90, CD45, CD34 and CD73 (BioLegend, USA) were investigated by flow cytometry (Figure S2) (9,10).

### *Monocyte-derived DCs*

PBMCs were isolated from the whole blood of three healthy individuals by Ficoll (Germany, inno-train). For monocyte isolation  $1.5 \times 10^6$  PBMCs were seeded in T25 flask and incubated at 37 °C for 3 hours. After that, suspended cells were removed by washing with PBS and RPMI. Adherence cells were cultured in RPMI medium (Gibco, USA) containing 10% FBS and 1% penicillin/streptomycin plus 20 ng/mL IL-4 (PeproTech, UK) and 50 ng/mL GM-CSF (PeproTech, UK). On day 5, LPS (Sigma, USA) with a concentration of 50 ng/mL and the CM of the MSCs treated with Vit E and Se alone and in combination were added to culture media. On day seven, supernatant and cells were collected for further experiments (Figure S3) (11,12). To determine the maturation of DCs and expression of CD1a, CD83, CD86, CD40, and HLA-DR cells (BioLegend, USA) were isolated with cold PBS-EDTA and washed with staining solution. In each group, the appropriate amount of antibody was added to the cell suspension then, cells were incubated for 45 minutes at 4 °C. After washing with PBS cells were analyzed by flow cytometry (13).

### *PBMC isolation*

PBMCs were isolated from whole blood. A total of  $5 \times 10^5$  cells per well were seeded in six-well plates. To each well, 1 mL of complete RPMI medium and 1 mL of the CM of MSCs treated with Vit E and Se was added. The supernatant was replaced and collected every 48 hours. After 7 days, cells were collected for further investigation. They were stored at -70 °C until used.

### *Cell viability assay*

To determine the appropriate and non-toxic concentration of Vit E (alpha-tocopherol, Sigma, USA) and Se

**Table 1** Primer sequences used for RT-PCR

Primer	Function(s)	Sequence
H-ROR $\gamma$ t-F	Cell cycle	5'-GAAGTGGTGCTGGTTAGGATGTG-3'
H-ROR $\gamma$ t-R	Cell cycle	5'-GCCACCGTATTTGCCTTCAA-3'
H-GATA3-F	Lymphocyte/hemato stem cell	5'-GAGACAGAGCGAGCAACG-3'
H-GATA3-R	Lymphocyte/hemato stem cell	5'-CTCGGGTCACCTGGGTAG-3'
H-T-bet-F	Cell cycle	5'-TGCTCCAGTCCCTCCATAAGTAC-3'
H-T-bet-R	Cell cycle	5'-TCTGGCTCTCCGTCGTTTAC-3'
H-FOXP3-F	Hematopoietic	5'-TGGCATCATCCGACAAGG-3'
H-FOXP3-R	Hematopoietic	5'-AGGAACTCTGGGAATGTGC-3'
H-Beta2M-F	Internal control	5'-ATGCCTGCCGTGTGAAC-3'
H-Beta2M-R	Internal control	5'-ATCTTCAAACCTCCATGATG-3'

RT-PCR, real time-polymerase chain reaction; F, forward; R, reverse.

(selenomethionine, Sigma, USA), MSCs were seeded in a 96-well plate. After 24 hours, Vit E and Se were added to the medium at different concentrations. 48 hours later, the MTT (Sigma-Aldrich, USA) diluted with DMEM added to wells. After 4 hours in 37 °C Incubator, 100  $\mu$ L DMSO (Sigma-Aldrich, USA), was added to each well and read at 570 nm wavelengths (14).

#### **Enzyme-linked immunosorbent assay (ELISA)**

To detection, the concentration of cytokines, IL-10 (Mabtech, Sweden), IL-12 (Mabtech, Sweden) and TGF- $\beta$  (Invitrogen, USA) kits were used. The supernatant was collected from mature DCs and PBMCs treated with MSC (Vit E) CM, MSC (Se) CM and MSC (Vit E + Se) CM after 48 hours. Finally, ELISA plates were read by ELISA reader.

#### **Real time-polymerase chain reaction (RT-PCR)**

PBMCs were cultured with different groups of MSC CM. After 7 days RNA was isolated from PBMCs with RNA ex plus (Qiagen, Germany). The concentration of RNA was determined by Nanodrop spectrophotometry. Eventually, cDNA (Takara, Japan) was synthesized and Real-time qPCR reactions were carried out using the SYBR Green PCR Master Mix (Biofact, Korea) (Table 1).

#### **Ethical statement**

This study was approved by the ethics board of Iran

University of Medical Sciences (IR.IUMS.FMD.REC 1396.9411127004) and informed consent was taken from all individual participants. Furthermore, this work was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

#### **Statistical analysis**

In the first step, quantitative data were analyzed for normal distribution by the normalization test. The quantitative data presented in this study were non-parametric. For statistical analysis and comparison of the results of the data in the studied groups, Mann-Whitney (between two groups) and Kruskal-Wallis (more than two groups) were used.

## **Results**

#### **Determining the optimal concentration of Vit E and Se by MTT assay**

The appropriate concentration of Vit E and Se was determined using MTT assay. According to MTT test results, concentrations of 22  $\mu$ M for Vit E (P=0.004), 250  $\mu$ M for Se (P=0.009), and 22  $\mu$ M Vit E with 300  $\mu$ M Se combined (P>0.99) were used (Figure S4).

#### **Evaluation of DC maturation treated with MSC (Vit E and Se) CMs**

The expression of CD40 in DC + MSC CM was

significantly higher compared with DC group ( $P=0.002$ ). The expression of CD40 in DC + MSC (Vit E) CM and DC + MSC (Se) CM was significantly decreased in comparison to DC + MSC CM ( $P=0.002$ ). Also, the expression of HLA-DR was reduced only in DC + MSC (Vit E) CM compared with DC + MSC CM ( $P=0.002$ ). The expression of CD83 and CD86 among the groups were not different (Figure 1).

#### ***Evaluation of IL-10, IL-12, and TGF- $\beta$ in the supernatant of myeloid DCs (mDCs) treated with MSC (Vit E and Se) CMs***

IL-10 level was significantly increased in DC + MSC (Vit E) CM, DC + MSC (Se) CM and DC + MSC (Vit E + Se) CM compared with DC + MSC CM ( $P<0.001$ ), although the highest increase was related to DC + MSC (Vit E) CM. In relation to IL-12 level, there was only a significant reduction in DC + MSC (Vit E) CM in comparison to DC + MSC CM ( $P=0.002$ ) and TGF- $\beta$  concentration was reduced only in DC + MSC (Se) CM compared with DC + MSC CM ( $P=0.03$ ) (Figure 2).

#### ***Evaluation IL-10, IL-12, and TGF- $\beta$ in the supernatant of PBMC treated with MSCs (Vit E and Se) CM***

The concentration of IL-10 in PBMC + MSC CM was significantly higher than PBMC ( $P=0.007$ ). The level of IL-10 in DC + MSC (Vit E) CM ( $P=0.01$ ) and DC + MSC (Se) CM ( $P=0.004$ ) in comparison with PBMC + MSC CM was significantly decreased, while the level of IL-12. In DC + MSC (Vit E) CM, DC + MSC (Se) CM and DC + MSC (Vit E + Se) CM has been significantly increased compared with PBMC + MSC CM ( $P=0.02$ ). Level of TGF- $\beta$  in DC + MSC (Se) CM was decreased than PBMC + MSC CM, although this was not significant (Figure 3).

#### ***The expression of T-bet, ROR $\gamma$ t, GATA3 and FOXP3 in PBMC treated with MSCs (Vit E and Se) CM***

The results showed that the expression of T-bet in PBMC + MSC CM was significantly decreased compared with PBMC ( $P=0.002$ ) while ROR $\gamma$ t ( $P=0.04$ ) and FOXP3 ( $P=0.004$ ) expression have been significantly increased. The expression levels of T-bet in PBMC + MSC (Vit E) CM and PBMC + MSC (Vit E + Se) CM were increased compared with PBMC + MSC CM ( $P=0.001$ ) while ROR $\gamma$ t expression was increased in PBMC + MSC (Vit E) CM ( $P=0.04$ ) and PBMC + MSC (Se) CM ( $P=0.007$ ). FOXP3 expression was increased in

PBMC + MSC (Vit E + Se) CM in comparison with PBMC + MSC CM ( $P=0.004$ ). The expression of GATA3 was not significantly different among groups (Figure 4).

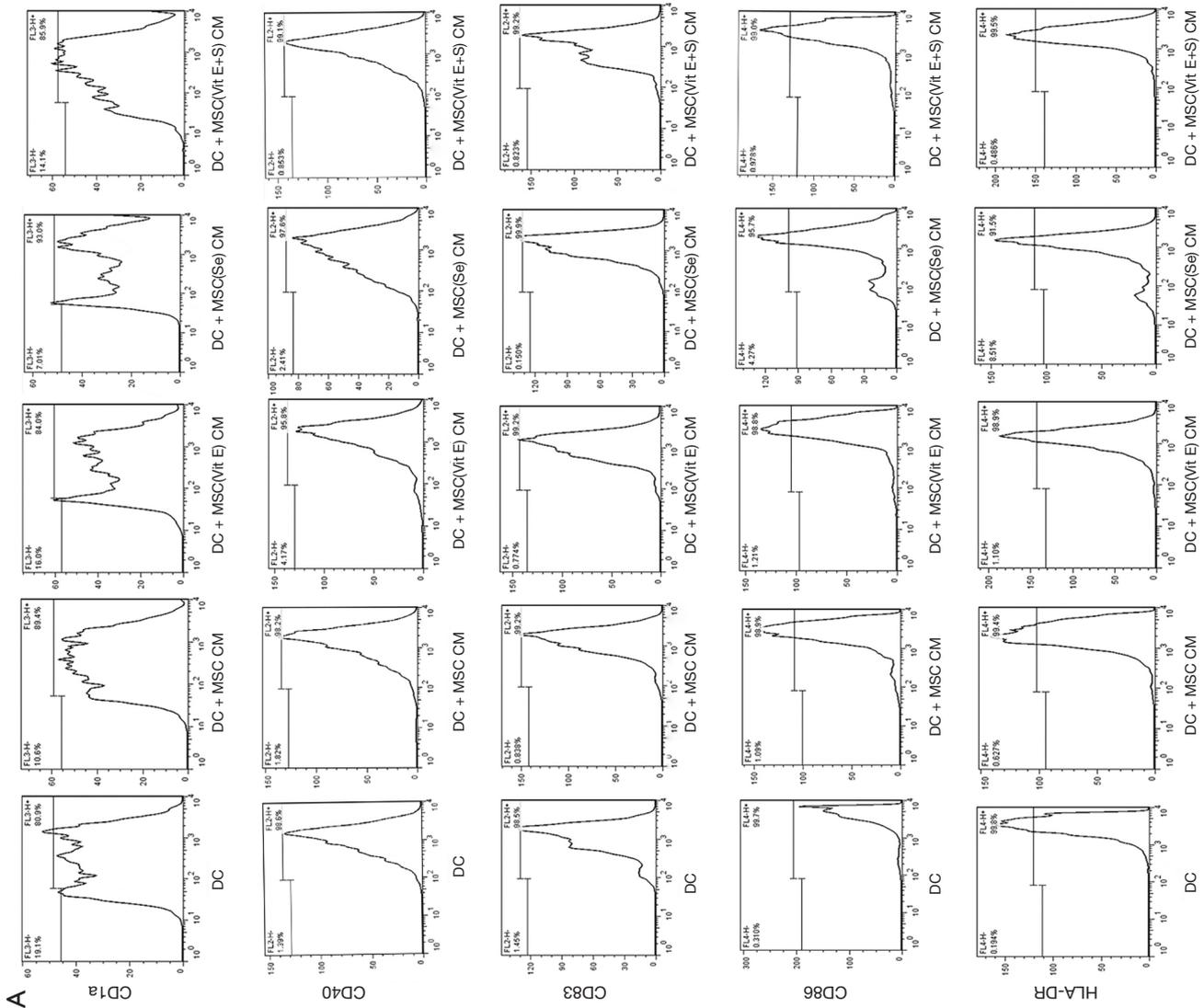
## **Discussion**

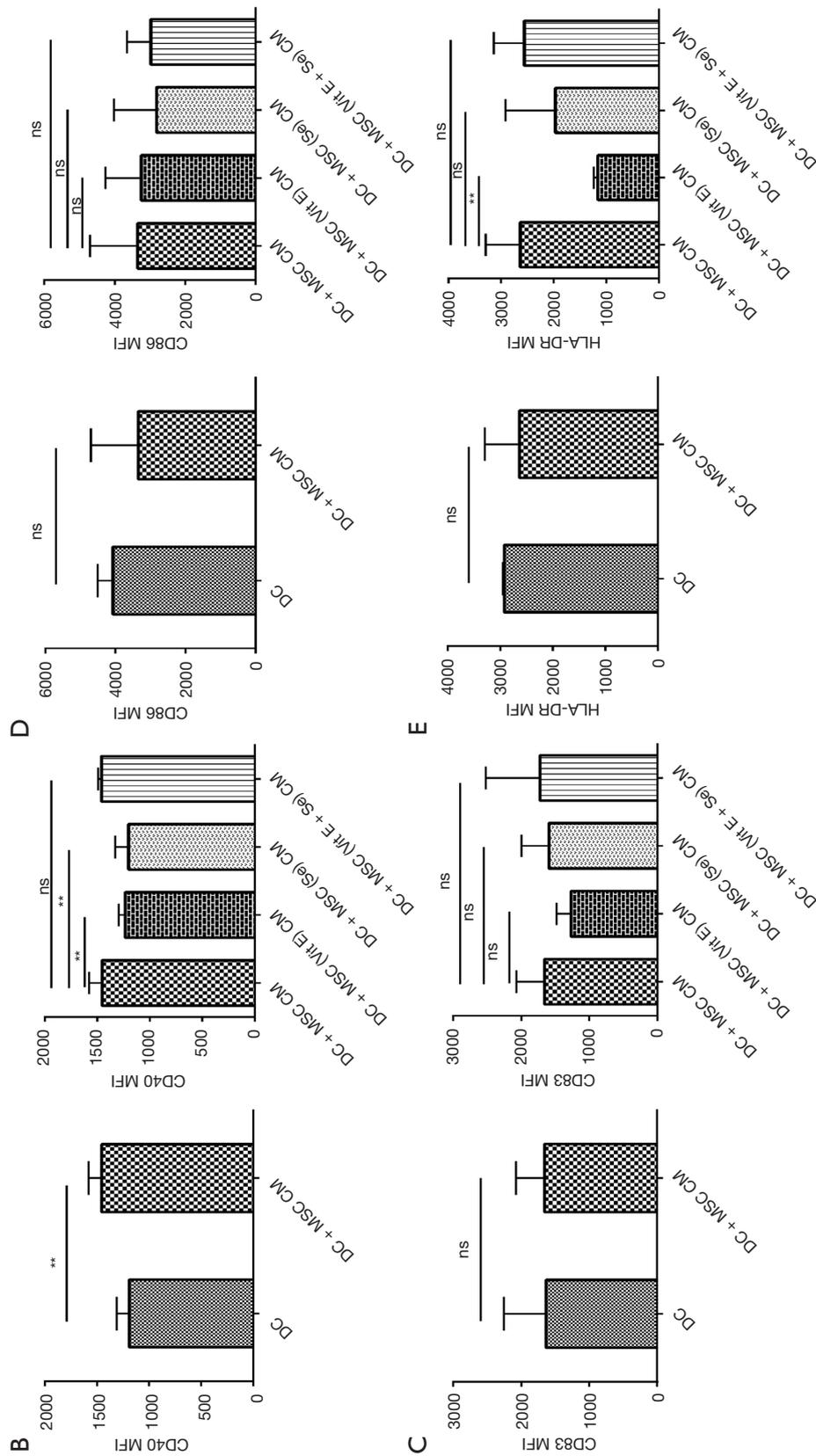
According to the obtained results, MSC CM increases the expression of CD40 in mDCs, but there was no significant difference in the expression of CD83, CD86, and HLA-DR. In the same way, the results of Spaggiari and Zhang studies showed that the effect of MSC on DCs is time-dependent. It was also reported that DCs co-cultured with MSCs on the 5th day of differentiation would not reduce CD80, CD83, CD86, and HLA-DR frequency of DCs and also, there was no significant difference in the concentration of IL-10, IL-12 and TGF- $\beta$  between the two groups. Zhang *et al.* showed that the levels of IL-10 and IL-12 in DC cultured on the 5th day with LPS and MSC CM, were not significantly different (15,16). van den Berk *et al.* reported that umbilical cord blood MSCs didn't prevent DC maturation and increase the expression of CD80 and CD83 and IL-12 level (17). Our results showed that in DC + MSC (Vit E) CM and DC + MSC (Se) CM, expression of CD40 was reduced but, this inhibitory effect was not seen in the DC + MSC (Vit E + Se) CM.

The expression of HLA-DR in the DC + MSC (Vit E) CM showed a significant decrease compared with DC + MSC CM, but in the other groups, there was no difference. In the present study, the level of IL-10 was significantly increased in DC + MSC (Vit E) CM and DC + MSC (Se) CM and DC + MSC (Vit E + Se) CM in comparison to the control group. Also, IL-12 level decreased significantly in the DC + MSC (Vit E) CM compared with the control group. According to the results, it seems that Vit E and Se increasing MSC survival and stability and thus improved the immunomodulatory effect of MSCs on DC (18).

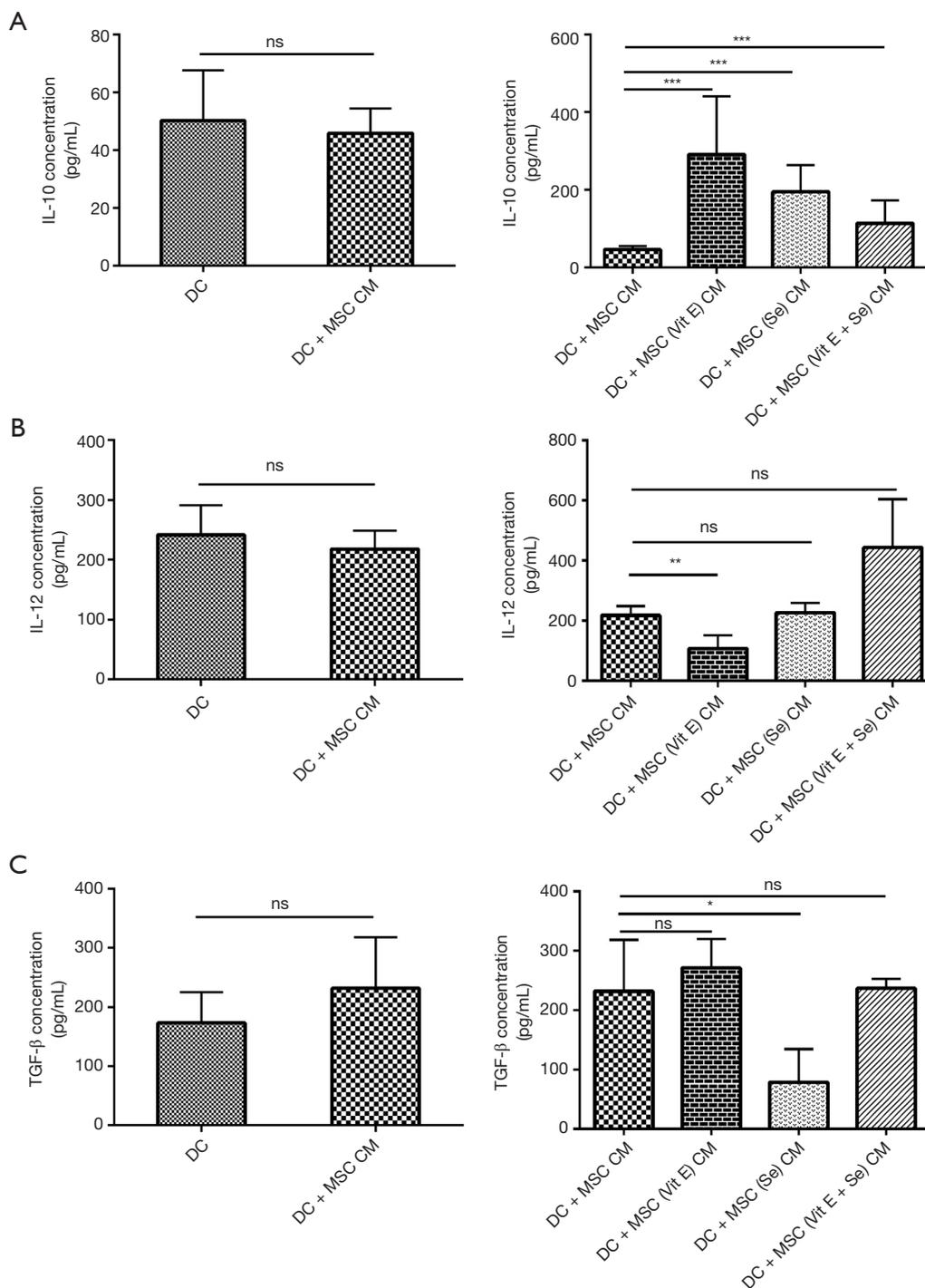
LPS activates NF- $\kappa$ B and cause DC maturation. Prevent expression of NF- $\kappa$ B inhibit DC maturation (19). Several studies showed that MSC CM in osteoarthritis model increase inhibitory- $\kappa$ -B- $\alpha$  (I $\kappa$ B $\alpha$ ) and inhibit expression of NF- $\kappa$ B. Besides, reactive oxygen species (ROS) lead to the activation of NF- $\kappa$ B, and many antioxidants, especially Vit E, have the ability to prevent the activation of NF- $\kappa$ B through this way and can prevent DC maturation (20,21).

Regarding the results, it seems that the MSC CM treated with Vit E and Se improve the immunoregulatory effects of MSC and they could better decrease DC maturation markers and IL-12 level and increase IL-10 concentration.

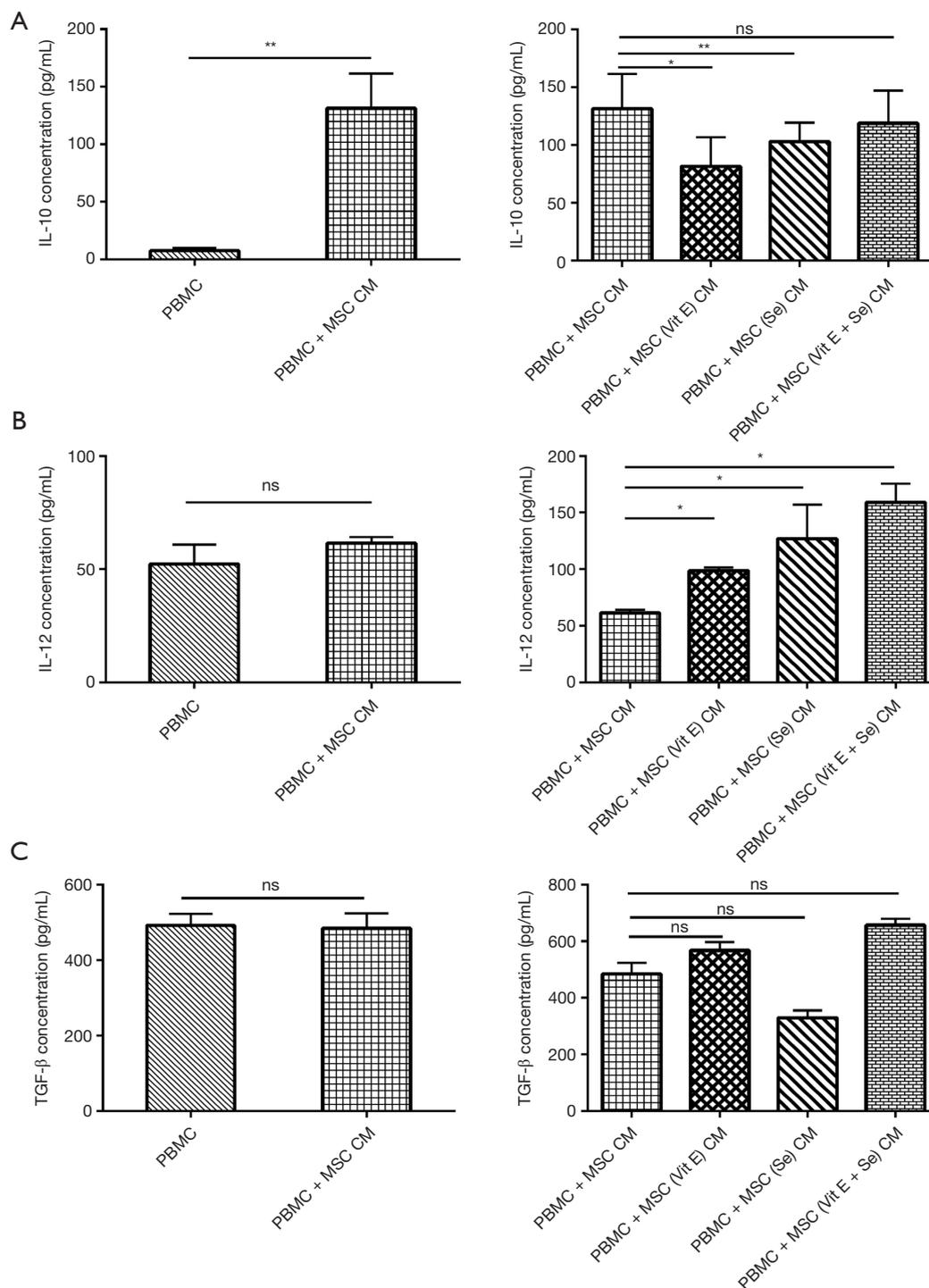




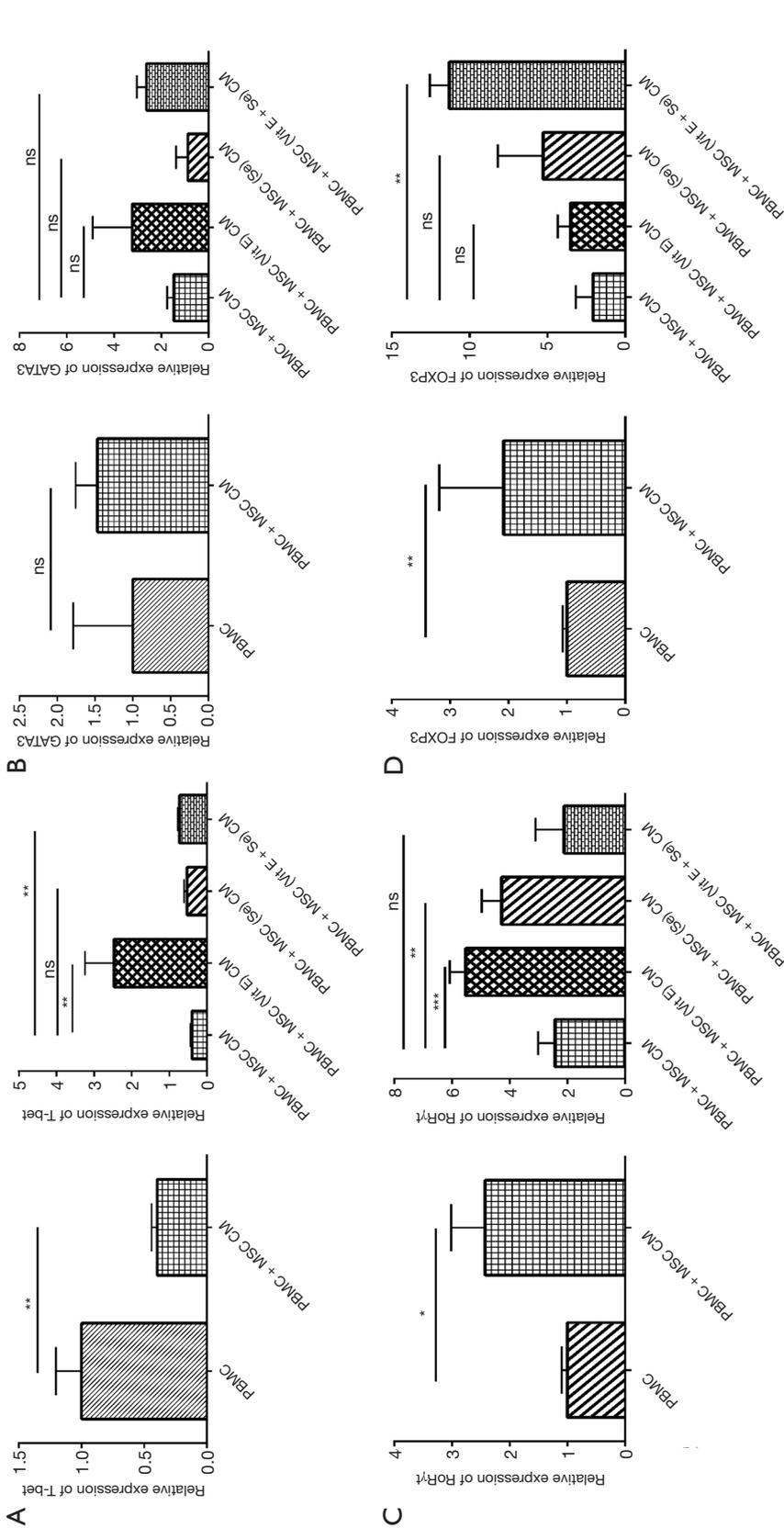
**Figure 1** Mature DC surface marker treated with the different groups of MSC CM. (A) The histogram showed that the treatment of DCs with different groups of MSC CM did not change the percentage of DCs surface markers, so in the next step MFI used to compare surface markers between groups. (B) The expression of CD40 in DCs treated with MSC CM increased significantly. The treatment of DCs with MSC (Vit E) CM and MSC (Se) CM significantly suppressed the expression of CD40. There was no significant difference in MSC (Vit E + Se) CM. (C,D) There was no difference in expression of CD83 and CD86 between the groups. (E) The expression of HLA-DR in DC treated with MSC (Vit E) CM significantly decreased. There was no significant difference among the other groups. \*\*, P<0.01. DC, dendritic cell; MSC, mesenchymal stem cell; CM, conditioned media; Vit E, vitamin E; Se, selenium.



**Figure 2** Effects of the different groups of MSC CM on IL-10, IL-12 and TGF- $\beta$  production from mDC. (A) There was no significant difference between the levels of IL-10 in DC treated with MSC CM. IL-10 level in treated DC with MSC (Vit E) CM, MSC (Se) CM and MSC (Vit E + Se) CM groups were significantly increased. (B) The level of IL-12 in DC treated with MSC (Vit E) CM is significantly reduced. There was no significant difference among the other groups. (C) TGF- $\beta$  concentration decreased in DC treated with the MSC (Se) CM. In other groups, TGF- $\beta$  level was not significantly different. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . MSC, mesenchymal stem cell; CM, conditioned media; mDC, myeloid dendritic cell; Vit E, vitamin E; Se, selenium.



**Figure 3** Effects of the different groups of MSC CM on IL-10, IL-12 and TGF- $\beta$  concentration in PBMCs. (A) IL-10 concentration in PBMC treated with MSC CM significantly increased. The treatment of PBMC with MSC (Vit E) CM and MSC (Se) CM suppresses the production of these cytokines. (B) The concentration of IL-12 in the PBMC treated with MSC CM was not significantly different. Treatment of PBMC with MSC (Vit E) CM, MSC (Se) CM and MSC (Vit E + Se) CM increased the level of this cytokine. (C) There was no difference in TGF- $\beta$  level among the different groups. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . MSC, mesenchymal stem cell; CM, conditioned media; PBMC, peripheral blood mononuclear cell; Vit E, vitamin E; Se, selenium.



**Figure 4** Effects of the different groups of MSC CM on T-bet, GATA3, RORγt and FOXP3 expression in PBMCs. (A) Treatment PBMC with MSC CM suppresses the expression of T-bet. T-bet expression in PBMC treated with MSC (Vit E) CM and MSC (Vit E + Se) CM increased. (B) There was no difference in GATA3 expression between different groups. (C) RORγt expression has significantly increased in PBMC treated with MSC CM, MSC (Vit E) CM and MSC (Se) CM. The expression of this gene was not significantly different in MSC (Vit E + Se) CM. (D) Treatment of PBMC with MSC and MSC (Vit E + Se) CM increase FOXP3 expression. There was no significant difference between the other groups. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001. MSC, mesenchymal stem cell; CM, conditioned media; PBMC, peripheral blood mononuclear cell; Vit E, vitamin E; Se, selenium.

In addition, there might be free Vit E and Se in MSC CM, in which case the two antioxidants will also prevent DC maturation by inhibiting NF- $\kappa$ B.

In the next step of this study, results showed that the levels of IL-10 in PBMC + MSC CM compared with the PBMCs group significantly increased. In this regard, Kim *et al.* reported that MSCs derived from human adipose tissue show an inhibitory effect on the proliferation of PBMCs (22).

In the PBMCs + MSC (Vit E) CM and PBMCs + MSC (Se) CM, the IL-10 level was significantly reduced compared with the PBMCs treated with MSC CM. IL-12 level was significantly increased in PBMC + MSC (Vit E) CM and PBMC + MSC (Se) CM. The study of Hernández *et al.* showed that in PBMCs treated with Vit E IL-10 and IL-4 concentration reduce, and the expression of the T-bet increase (23). On the other hand, Wassall *et al.* showed that Vit E has no specific effect on cytokine responses in people with PBMCs (24).

Daeian *et al.* found that Se supplementation does not have any effect on the production of proinflammatory cytokines in the peripheral blood of patients undergoing hematopoietic stem cell transplantation (HSCT) (25).

The expression levels of GATA3, FOXP3, ROR $\gamma$ t, and T-bet in PBMCs treated with different groups of MSCs CM was studied. T-bet and ROR $\gamma$ t expression in the PBMC + MSC (Vit E) CM was significantly increased compared with the PBMC + MSC CM. There was no significant difference in the expression of GATA3 gene between the two groups. The treatment of PBMC + MSC (Se) CM suppressed the expression of ROR $\gamma$ t. The expression of FOXP3 increased significantly in PBMC + MSC (Vit E + Se) CM.

Kay *et al.* reported MSC CM in patients with RA increased the levels of IL-10 and FOXP3 in T cells (26). Agree with our results, Ivanova-Todorova *et al.* found that the MSCs isolated from the adipose tissue increase the expression of FOXP3 and the concentration of IL-10 (27).

Rozenberg *et al.* reported MSCs suppress T cells activity via the PD-1 pathway. Luz-Crawford *et al.* observed that MSCs regulate Th1 and Th17 response. The MSC CM through PGE2 increased the differentiation of Th17 cells (28,29).

Adolfsson *et al.* reported that Vit E increase the proliferation of T cells in the mouse (30). Besides, Zingg *et al.* showed that alpha-tocopherol activates many genes in a dose-dependent manner and increase the proliferation and survival of T cells compared to other Vit E isoforms (31).

As previously mentioned, Se increase the survival of stem cells. Limited studies have been evaluated the

immunomodulatory effects of Se-treated MSCs. Sang *et al.* observed that sodium selenite reduce ROR $\gamma$ t and T-bet expression levels accompanied with increased frequency of Treg in colitis mice (32). Dustan *et al.* stated that there is no relationship between IL-10 produce from lymphocyte and Se levels (33). The results are controversial about the effects of Se on immune system responses.

## Conclusions

Finally, regarding the results of this study and previous studies, in some cases, such as increasing the expression of ROR $\gamma$ t as well as FOXP3 in the group where these two substances were combined, treatment of MSC with Vit E and Se increased immunomodulatory effects of MSCs and These cells could decrease IL-12 level and reduce the expression of some cell markers of mature DCs. However, the results are contradictory because in the PBMC treated with MSC (Vit E) CM and MSC (Se) CM, IL-10 concentration decreased, IL-12 level and Th1/Treg ratio increased. According to previous studies, the MSC treated with these two antioxidants, might have caused changes in cytokine concentration and gene expression in the PBMC, probably due to its effect on monocytes. Considering the fact that several factors influence the effect of MSC on cells and not all of them studied in this study, a better understanding of the effect of Vit E and Se on MSC requires more studies.

## Acknowledgments

We would like to acknowledge Stem Cell Technology Research Center for their supports.

*Funding:* None.

## Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at <http://dx.doi.org/10.21037/sci-2020-008>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/sci-2020-008>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the study in ensuring that questions related to

the accuracy or reliability of any part of the investigation are appropriately considered and resolved. This study was approved by the ethics board of Iran University of Medical Sciences (IR.IUMS.FMD.REC 1396.9411127004) and informed consent was taken from all individual participants. Furthermore, this work was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

*Open Access Statement:* This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

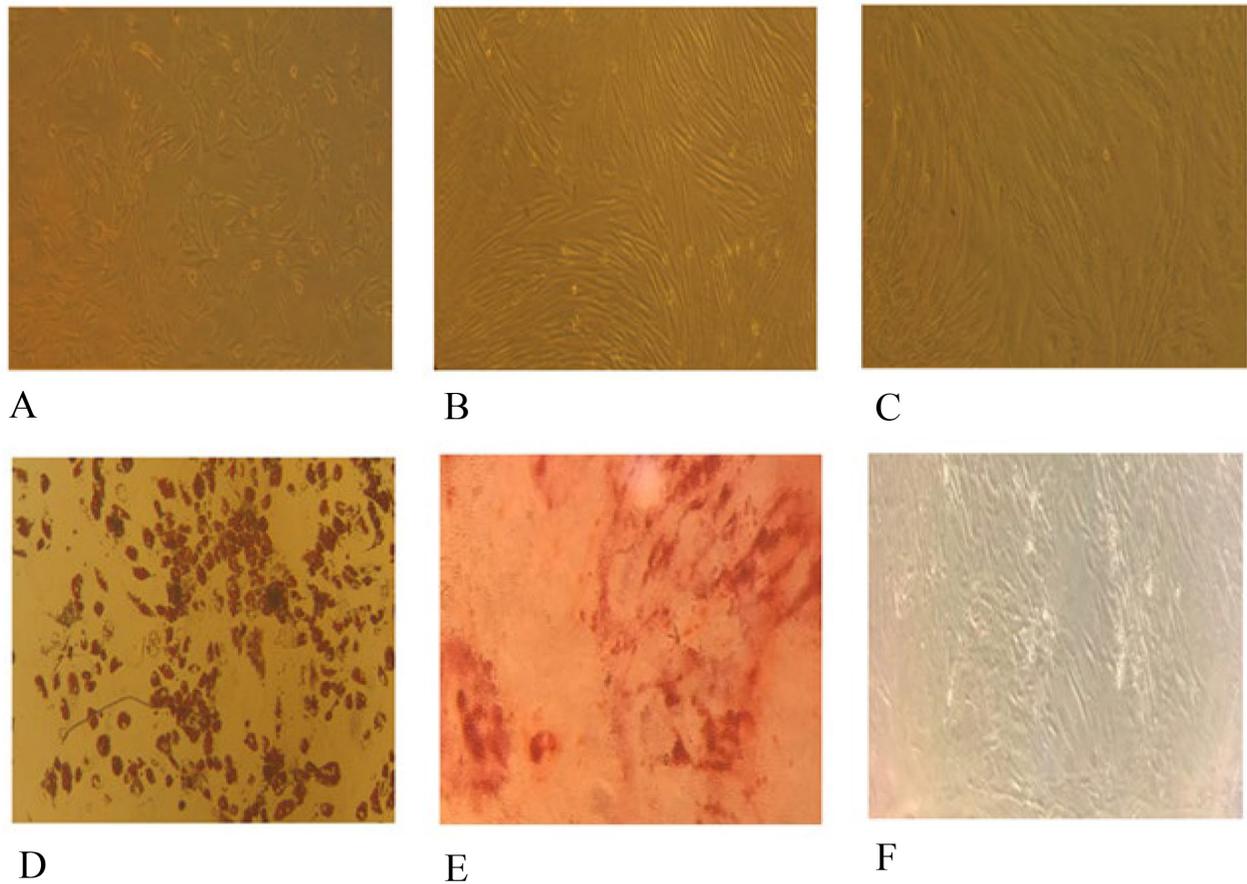
## References

1. Almalki SG, Agrawal DK. Key transcription factors in the differentiation of mesenchymal stem cells. *Differentiation* 2016;92:41-51.
2. Zhao Q, Ren H, Han Z. Mesenchymal stem cells: immunomodulatory capability and clinical potential in immune diseases. *Journal of Cellular Immunotherapy* 2016;2:3-20.
3. Cardenas E, Ghosh R. Vitamin E: a dark horse at the crossroad of cancer management. *Biochem Pharmacol* 2013;86:845-52.
4. Rizvi S, Raza ST, Ahmed F, et al. The role of vitamin e in human health and some diseases. *Sultan Qaboos Univ Med J* 2014;14:e157-65.
5. Hoffmann PR, Jourdan-Le Saux C, Hoffmann FW, et al. A role for dietary selenium and selenoproteins in allergic airway inflammation. *J Immunol* 2007;179:3258-67.
6. Huang Z, Rose AH, Hoffmann PR. The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 2012;16:705-43.
7. Hoffmann PR, Berry MJ. The influence of selenium on immune responses. *Mol Nutr Food Res* 2008;52:1273-80.
8. Stockwin LH, McGonagle D, Martin IG, et al. Dendritic cells: immunological sentinels with a central role in health and disease. *Immunol Cell Biol* 2000;78:91-102.
9. Zhu M, Heydarkhan-Hagvall S, Hedrick M, et al. Manual isolation of adipose-derived stem cells from human lipoaspirates. *J Vis Exp* 2013;(79):e50585.
10. Ge X, Leow SC, Sathiakumar D, et al. Isolation and culture of human adipose-derived stem cells from subcutaneous and visceral white adipose tissue compartments. *Bio Protoc* 2016;6:e2027.
11. Delirezh N, Shojaeefar E, Parvin P, et al. Comparison the effects of two monocyte isolation methods, plastic adherence and magnetic activated cell sorting methods, on phagocytic activity of generated dendritic cells. *Cell J* 2013;15:218-23.
12. Bie Y, Xu Q, Zhang Z. Isolation of dendritic cells from umbilical cord blood using magnetic activated cell sorting or adherence. *Oncol Lett* 2015;10:67-70.
13. El-Sahrigy SA, Mohamed NA, Talkhan HA, et al. Comparison between magnetic activated cell sorted monocytes and monocyte adherence techniques for in vitro generation of immature dendritic cells: an Egyptian trial. *Cent Eur J Immunol* 2015;40:18-24.
14. Zhou H, Li D, Shi C, et al. Effects of Exendin-4 on bone marrow mesenchymal stem cell proliferation, migration and apoptosis in vitro. *Scientific Reports* 2015;5:12898.
15. Spaggiari GM, Abdelrazik H, Becchetti F, et al. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood* 2009;113:6576-83.
16. Zhang Y, Ge XH, Guo XJ, et al. Bone Marrow Mesenchymal Stem Cells Inhibit the Function of Dendritic Cells by Secreting Galectin-1. *Biomed Res Int* 2017;2017:3248605.
17. van den Berk LC, Roelofs H, Huijs T, et al. Cord blood mesenchymal stem cells propel human dendritic cells to an intermediate maturation state and boost interleukin-12 production by mature dendritic cells. *Immunology* 2009;128:564-72.
18. Jung S, Panchalingam KM, Rosenberg L, et al. Ex vivo expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells Int* 2012;2012:123030.
19. Rescigno M, Martino M, Sutherland CL, et al. Dendritic cell survival and maturation are regulated by different signaling pathways. *J Exp Med* 1998;188:2175-80.
20. van Buul GM, Villafuertes E, Bos PK, et al. Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture. *Osteoarthritis Cartilage* 2012;20:1186-96.
21. Tang J, Cui W, Song F, et al. Effects of mesenchymal stem cells on interleukin-1 $\beta$ -treated chondrocytes and cartilage in a rat osteoarthritic model. *Mol Med Rep*

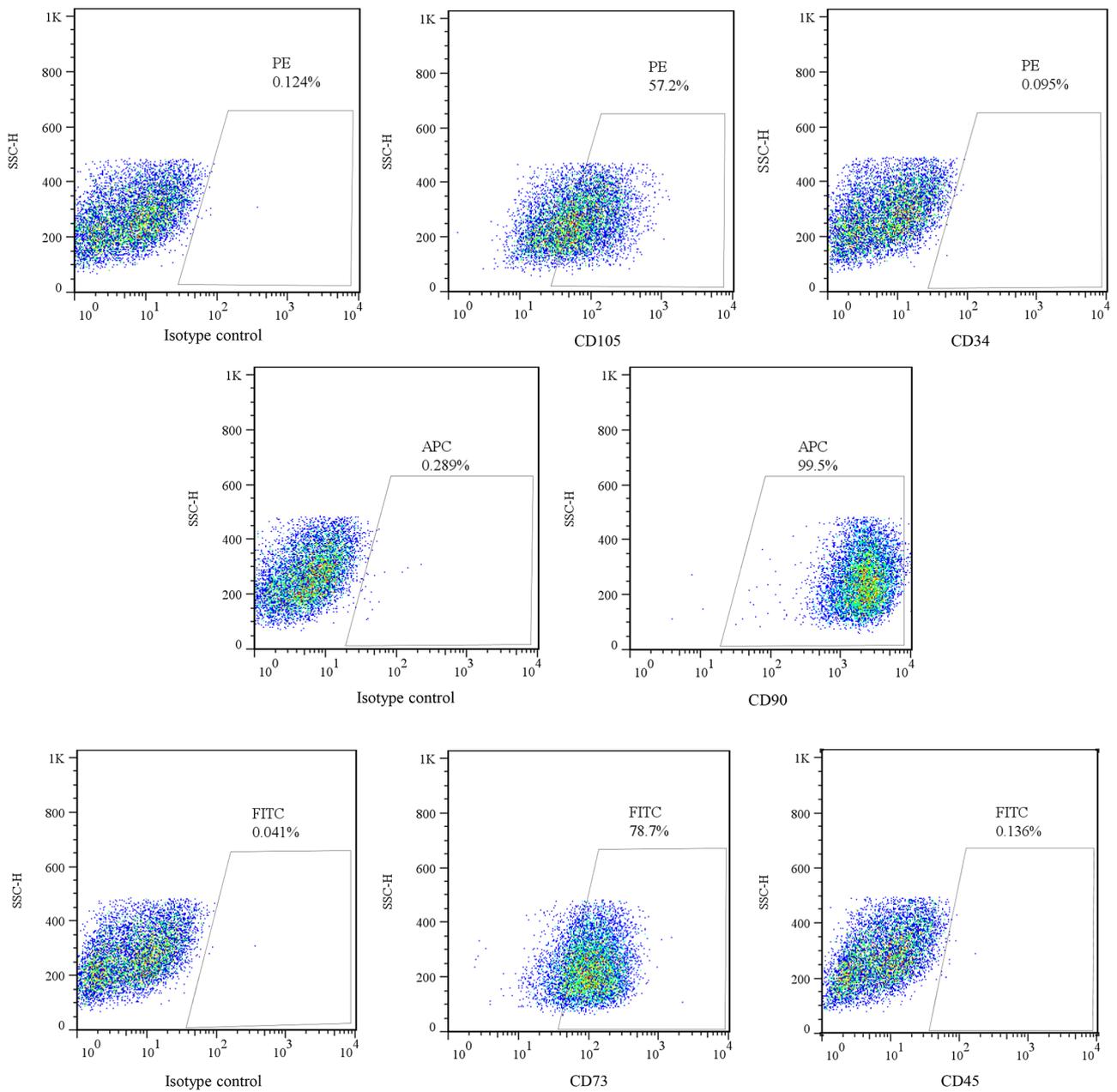
- 2015;12:1753-60.
22. Kim JH, Lee YT, Hong JM, et al. Suppression of in vitro murine T cell proliferation by human adipose tissue-derived mesenchymal stem cells is dependent mainly on cyclooxygenase-2 expression. *Anat Cell Biol* 2013;46:262-71.
  23. Hernández J, Soto-Canevett E, Pinelli-Saavedra A, et al. In vitro effect of vitamin E on lectin-stimulated porcine peripheral blood mononuclear cells. *Vet Immunol Immunopathol* 2009;131:9-16.
  24. Wassall HJ, Devereux G, Seaton A, et al. Complex effects of vitamin E and vitamin C supplementation on in vitro neonatal mononuclear cell responses to allergens. *Nutrients* 2013;5:3337-51.
  25. Daeian N, Radfar M, Jahangard-Rafsanjani Z, et al. Selenium supplementation in patients undergoing hematopoietic stem cell transplantation: effects on pro-inflammatory cytokines levels. *Daru* 2014;22:51.
  26. Kay AG, Long G, Tyler G, et al. Mesenchymal stem cell-conditioned medium reduces disease severity and immune responses in inflammatory arthritis. *Sci Rep* 2017;7:18019.
  27. Ivanova-Todorova E, Bochev I, Dimitrov R, et al. Conditioned medium from adipose tissue-derived mesenchymal stem cells induces CD4+FOXP3+ cells and increases IL-10 secretion. *J Biomed Biotechnol* 2012;2012:295167.
  28. Luz-Crawford P, Noel D, Fernandez X, et al. Mesenchymal stem cells repress Th17 molecular program through the PD-1 pathway. *PLoS One* 2012;7:e45272.
  29. Rozenberg A, Rezk A, Boivin MN, et al. Human mesenchymal stem cells impact Th17 and Th1 responses through a prostaglandin E2 and myeloid-dependent mechanism. *Stem Cells Transl Med* 2016;5:1506-14.
  30. Adolfsson O, Huber BT, Meydani SN. Vitamin E-enhanced IL-2 production in old mice: naive but not memory T cells show increased cell division cycling and IL-2-producing capacity. *J Immunol* 2001;167:3809-17.
  31. Zingg JM, Han SN, Pang E, et al. In vivo regulation of gene transcription by alpha- and gamma-tocopherol in murine T lymphocytes. *Arch Biochem Biophys* 2013;538:111-9.
  32. Sang LX, Chang B, Zhu JF, et al. Sodium selenite ameliorates dextran sulfate sodium-induced chronic colitis in mice by decreasing Th1, Th17, and gammadeltaT and increasing CD4(+)CD25(+) regulatory T-cell responses. *World J Gastroenterol* 2017;23:3850-63.
  33. Broome CS, McArdle F, Kyle JA, et al. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *Am J Clin Nutr* 2004;80:154-62.

doi: 10.21037/sci-2020-008

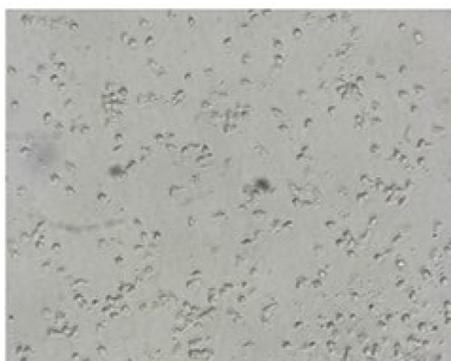
**Cite this article as:** Ghasemi F, Khoshmirsafa M, Safari E, Asgari M, Alemrajabi M, Nojehdehi S, Khorrami S. Vitamin E and selenium improve mesenchymal stem cell conditioned media immunomodulatory effects. *Stem Cell Investig* 2021;8:9.



**Figure S1** MSCs derived from human adipose tissue. (A) MSCs at passage zero ( $\times 20$ ). (B) MSCs at the 2<sup>nd</sup> passage ( $\times 20$ ). (C) MSCs at the 3<sup>rd</sup> passage ( $\times 20$ ). Spindle-shaped and fibroblasts-like MSCs were observed under an inverted microscope [(A,B,C) unstained MSCs]. Differentiation of MSCs into adipocyte and osteocytes. (D) MSC cultured with human adipocyte differentiation medium for 21 days and stained with Oil Red O ( $\times 40$ ). (E) And for osteocytes differentiation calcium deposition stained with Alizarin Red S ( $\times 40$ ). (F) While there was no change of the color in MSCs in the control group ( $\times 40$ ; unstained MSCs). MSC, mesenchymal stem cell.



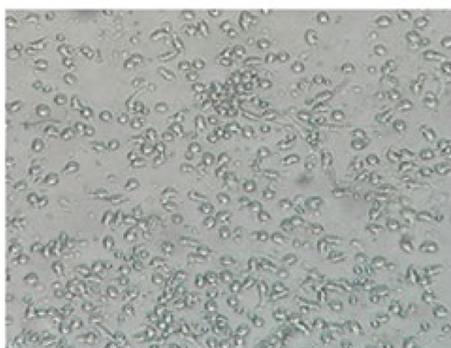
**Figure S2** Human adipose tissue-derived MSCs surface markers. MSCs were lacked expression of CD34 and CD45 but they expressed CD105, CD90 and CD73. MSC, mesenchymal stem cell.



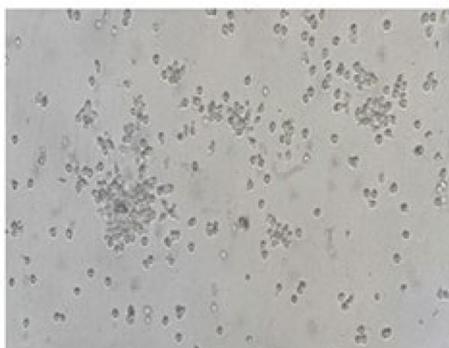
A



B

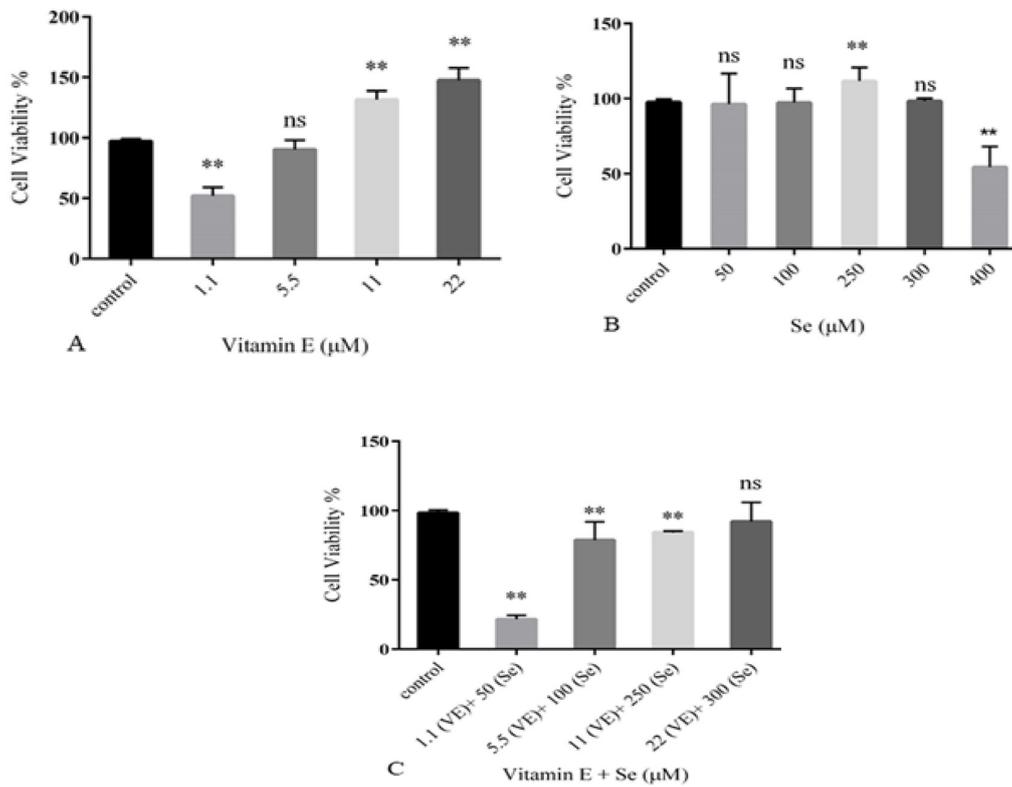


C



D

**Figure S3** Monocyte-derived DCs (unstained PBMC and DC). (A) Monocytes isolated from PBMCs in first day ( $\times 20$ ). (B) Monocyte cultured with IL-4 and GM-CSF in 3<sup>rd</sup> day ( $\times 20$ ). (C) Immature DC in 5<sup>th</sup> day ( $\times 40$ ). (D) Mature DC cultured with LPS in 7<sup>th</sup> day ( $\times 20$ ). DC, dendritic cell; PBMC, peripheral blood mononuclear cell.



**Figure S4** Cell viability assay. (A) The proliferation of MSCs treated with Vit E at the concentration of 22 μM has significantly increased. (B) Se significant increased the proliferation of MSCs at 250 μM concentration. (C) Vit E and Se did not decrease the viability of MSCs at concentrations of 300 μM and 22 Mm. \*\*, P<0.01. MSC, mesenchymal stem cell; Vit E, vitamin E; Se, selenium.