Investigation of FLK-1 Gene Expression in Differentiated Mesenchymal Stem Cells, Exposed to Chemical, Mechanical and Chemical-mechanical Factors, in order to Study the Differentiation and its Stability

E Hasanzadeh [1], GH Amoabediny [1,2], *N Haghighipour [3], N Amirzadeh [4], J Mohammadnejad [1], N Gholami [1]

[1]Department of Life Sciences Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran.
[2] Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran.
[3]National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran.
[4] Blood Research Center, High Institute for Education and Research in Transfusion Medicine, Tehran, Iran.

Background: Mesenchymal stem cells (MSCs) are multipotent cells, capable of differentiating into different cell lines.They can sense their surrounding biochemical and biophysical factors, which play major roles in their differentiation toward different phenotypes. Therefore, the exposure of these cells to endothelial growth factor (VEGF) as well as hemodynamic biomechanical forces, which act on endothelial cells in vivo, may direct MSCs toward vascular covering endothelial cells. The aim of this study, is to investigate the effects of chemical, mechanical and chemical-mechanical factors on the differentiation of mesenchymal stem cells and to examine the stability of this in vitro differentiation.
Materials and Method: Human Subcutaneous adipose-derived mesenchymal stem cells were cultured in DMEM containing 20% FBS. They were characterized in passage 2, using flow cytometery technique and then were used in experiments. Mesenchymal stem cells were cultured in tubular silicone scaffolds, and then exposed to endothelial growth factor (VEGE), shear stress or combination of these two signals. In order to examine the differentiation, the expression level of FLK-1, as an endothelial specific gene, was studied. For studying the stability of this differentiation, FLK-1 mRNA levels were quantified using Real-Time PCR, upon completion of experiments, as well as on the $5^{th}$and 10^{th} days after the tests.
Results: According to the results of Real-Time PCR, immediately upon the completion of tests, mRNA level of FLK-1 was higher in the mechanical group (5.44), compared to the chemical (1.18) and chemical-mechanical (2.65) ones. On the 5^{th} and 10^{th} days after the experiments,
FLK-1 expression levels were higher in chemical-mechanical group (6.34 and 7.56 respectively), compared to the chemical (2.18 and 0.99, respectively), and mechanical (2.96 and 3.92, respectively) ones.
Conclusion: The findings of this study demonstrate that not only FLK-1 gene expression is stable over time, but also cells exposed to the chemical and mechanical cues, simultaneously, show the highest expression of this gene. The increase in FLK-1 expression over time implies the enhanced compatibility of differentiated cells with each other and their surrounding environment, which may be probably due to the higher sensitivity of FLK-1 gene expression to the combination of chemical and mechanical signals.

Keywords: Differentiation, Shear stress, Stem cell, Stability, VEGF.

Poster Presentation

***Corresponding Author:** N Haghighipour, National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran.
Email:haghighipour@pasteur.ac.ir.