

## Studying the effect of low-power red laser irradiation on markers of viability and migration of umbilical cord endothelial cells

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### Abstract

With the increasing advancement of stem cell-based therapeutic methods and various applied technologies such as low-level laser therapy, as a safe technique with extraordinary therapeutic potential, the use of this method has opened a new window in medicine. This study also investigates the effects of this radiation on markers of survival and migration of umbilical cord endothelial cells. Umbilical cord endothelial cells were obtained from the Biopajouh Afza Laboratory and were cultured *in vitro* under appropriate conditions and culture medium. Cell viability was measured 24 and 48 hours after laser irradiation using the MTT method. To examine cell migration, a wounding method using a sampler and photography of the cell migration trajectory were used. Image analysis was performed using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego, CA, USA). Two-way analysis of variance (ANOVA) was used to analyze the data, and Tukey's test was used to compare the means. The values were calculated as mean  $\pm$  standard deviation and the difference was considered significant at a probability level of less than 5%. Comparison of the percentage of viability of different samples between the two time points of 24 and 48 hours showed that there was no statistically significant difference in the comparative times ( $P < 0.05$ ) and at both times the percentage of cell viability in the group treated with a wavelength of 660 nm was significantly different from the control group ( $P < 0.0001$ ). The results of photographing the migration of endothelial cells at times 0, 24 and 48 hours showed that the highest cell migration occurred after 48 hours and in the sample treated with a laser with a wavelength of 660 nm. The results

of the present study indicate that stimulation of cells with low-power laser increases the growth, viability and migration of vascular endothelial cells.

### Introduction:

Endothelial cells (ECs) form the innermost lining of blood vessels and play an important role in maintaining homeostasis, or the stability and stability of blood vessels (1). In addition to their important role in maintaining the health and function of the circulatory system, endothelial cells play a role in various aspects of the body's health. One of their most important roles is that they regulate blood flow, affecting the activity of various muscles and organs (1,2). These cells are found on the inner surface of umbilical cord blood vessels and are similar to endothelial cells found in adult blood vessels. One of the important features of these cells is their high proliferation capacity, which makes them easily cultured in the laboratory and used for research and therapeutic purposes (3, 4). Umbilical cord endothelial cells have the ability to differentiate into various types of cells, including smooth muscle cells and stem cells. In addition, umbilical cord endothelial cells secrete growth factors that can be effective in tissue growth and repair, especially in the treatment of cardiovascular diseases such as atherosclerosis and stroke. All of these features make umbilical cord endothelial cells play a very important role in tissue repair, vascular tissue engineering, medical research, and the development of new treatments for various diseases, including cardiovascular diseases (4, 5). The migration of these cells is crucial for the formation and regeneration of tissues, the prevention of infection and wounds, and the maintenance of the health and function of body systems. On the other hand, the survival and proper function of these cells in culture or after transplantation into the body is essential for the engineering of new tissues such as epithelial tissues (6, 7). Markers of survival and migration of umbilical cord epithelial cells play a very important role in various scientific and medical fields. These markers are molecules whose expression is associated with cell survival and migration. These markers indicate the activity and ability of cells to migrate to other parts of the body and can help in the diagnosis and prediction of cancer metastasis, the selection of appropriate treatment methods, and the assessment of treatment success (8, 9). Low-level red laser radiation is an advanced and

effective method in the treatment of many medical problems. This type of radiation, with its specific wavelength, is easily absorbed by body tissues and can be effective in relieving pain, reducing inflammation, accelerating wound healing, skin rejuvenation, reducing hair loss, and even treating vascular diseases. The advantages of this method include its non-invasiveness, safety when used correctly, and cost-effectiveness (7, 10). Teschon et al. (11) investigated the effect of laser photobiomodulation on wound healing and found that this treatment method caused faster wound healing by stimulating cell migration and collagen deposition by osteoblasts. The results of the study by Goeriters et al. (12) indicated a significant effect of low-level laser irradiation on increasing the proliferation and differentiation of apical papilla stem cells. Also, the study by Lee et al. (10) showed that low-level laser irradiation caused the proliferation and migration of human umbilical vascular endothelial cells, resulting in an increase in the angiogenesis process. This study also investigated the effects of this irradiation on markers of survival and migration of umbilical cord endothelial cells, which will provide more information about the biological effects and mechanism of action of this irradiation in this field..

#### Research Method:

##### Sample Preparation

Umbilical cord endothelial cells were obtained from the Bio-Pajouh Afza Laboratory and were cultured in appropriate laboratory conditions and culture medium. Umbilical cord endothelial cells were cultured in DMEM culture medium and after the cell density at the bottom of the flask reached 80-90%, the cells were divided into three groups: control, 660 nm irradiation, and 980 nm irradiation.

##### Determination of cell viability

The MTT method was used to examine cell viability 24 and 48 hours after laser irradiation. In this method, cell viability is measured by measuring their metabolism. To perform this test, the control and treatment groups were cultured in MTT culture medium (USA, Sigma) (at a concentration of 5 mg/L). The plate containing cells and MTT was kept in a 37°C,

5% CO<sub>2</sub> incubator for 4 hours, away from light. Then, 125 µL of phosphate buffer solution and 25 µL of dimethyl sulfoxide (DMSO) were added to each sample. The amount of formazan produced by living cells was measured by absorption at 570 nm using an ELISA plate Reader, model 3200, manufactured by Averyance, USA. It should be noted that living cells are able to convert MTT to formazan, while dead cells are unable to make this transformation. Therefore, the survival and viability of each sample from the test group was compared with the control sample. The percentage of cell viability was calculated using the following formula:

Growth formula =  $100 \times [(T-T_0)/(C-T_0)]$   
 T: optical absorption of the sample, C: optical absorption of the control, T<sub>0</sub>: absorption at time zero.(13)

##### Cell migration study

To study cell migration, 20,000 cells from each of the treatment and control groups were cultured in a 48-well plate. After the cells reached 80% confluence, a groove was created in the middle of each well using a 200 µL sterile sampler tip. After that, the cells were photographed at 0, 6, 12, 24, and 48 hours after the scratch, and the rate of cell migration towards the scratch in each group was measured using NIH Image J software.

After data collection, analysis was performed using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego, CA, USA). Two-way analysis of variance (ANOVA) was used to analyze the data, and Tukey's test was used to compare the means. The values were calculated as mean ± standard deviation and the difference was considered significant at a probability level of less than 5%.

##### Findings:

###### Effect of laser on endothelial cell viability

Cell viability was examined at two times, 24 and 48 hours, in the control and treated samples at two wavelengths of 660 and 980 nm, and the percentage of cell viability is shown in Figure 1. Based on the findings, cells at a wavelength of 660 nm showed the highest percentage of viability ( $P<0.05$ ) compared to the control group.

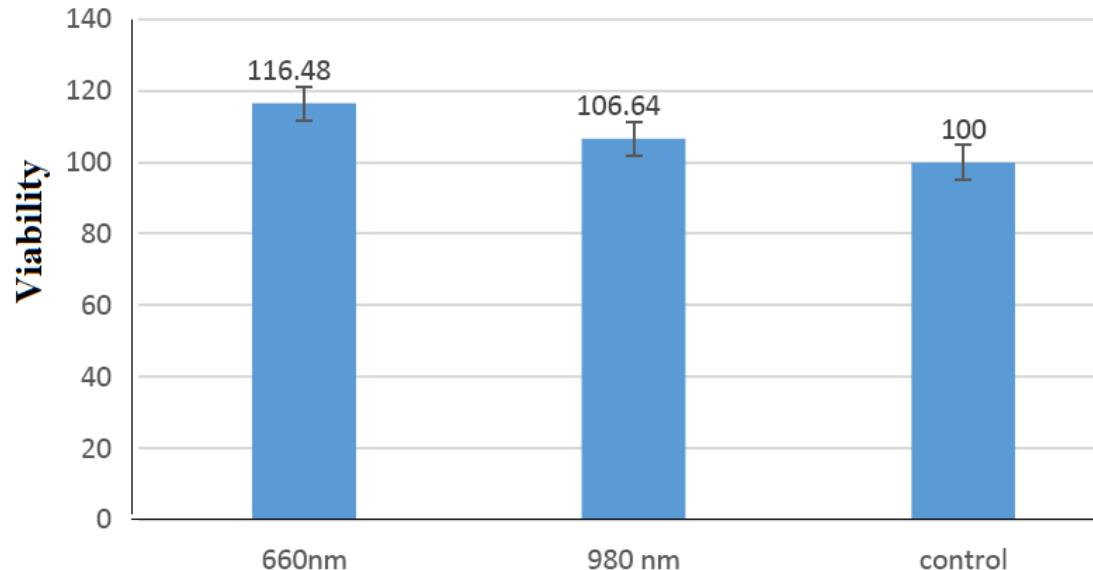


Figure 1- Percentage of endothelial cell viability in control and treated groups at wavelengths of 660 and 980 nm in 24 hours

The results showed that after 48 hours, the highest percentage of viability was observed in cells treated at a wavelength of 660 nm (Figure

2), which showed a statistically significant difference with the control group and cells treated at a wavelength of 980 nm ( $P<0.005$ ).

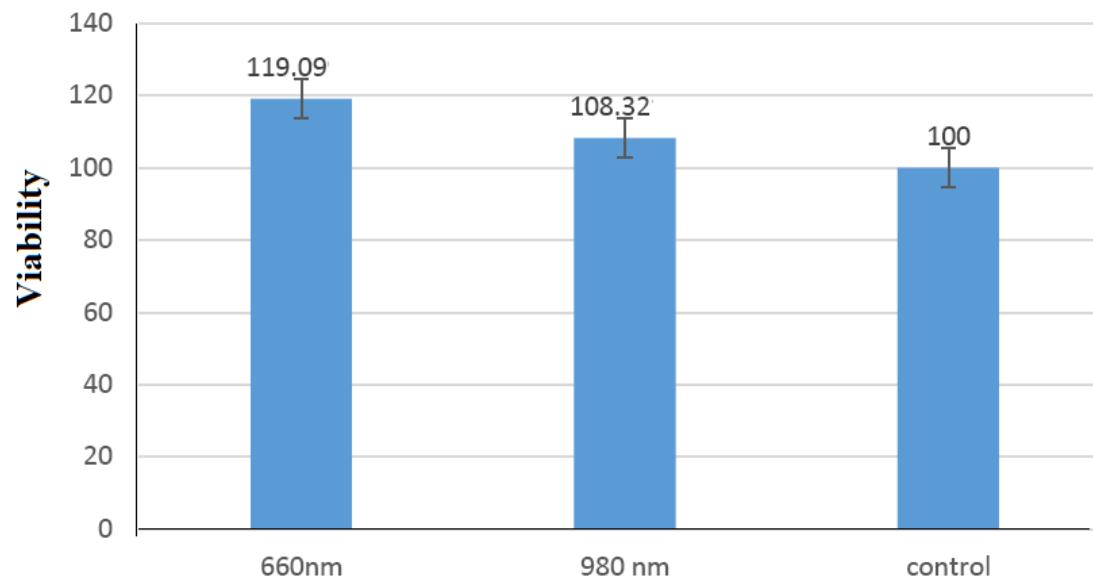


Figure 2- Percentage of endothelial cell viability in control and treated groups at wavelengths of 660 and 980 nm for 48 hours

Comparison of the percentage of viability of different samples between two times of 24 and 48 hours showed that there was no statistically significant difference in the comparative times

with each other ( $P<0.05$ ) and at both times the percentage of cell viability in the group treated with a wavelength of 660 nm was significantly different from the control group ( $P<0.0001$ ).

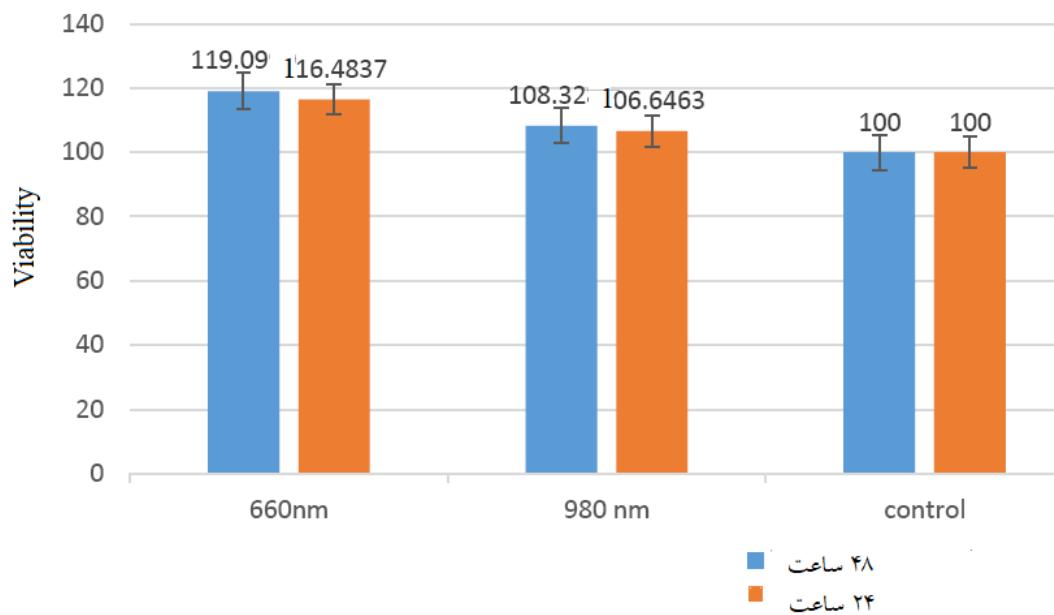
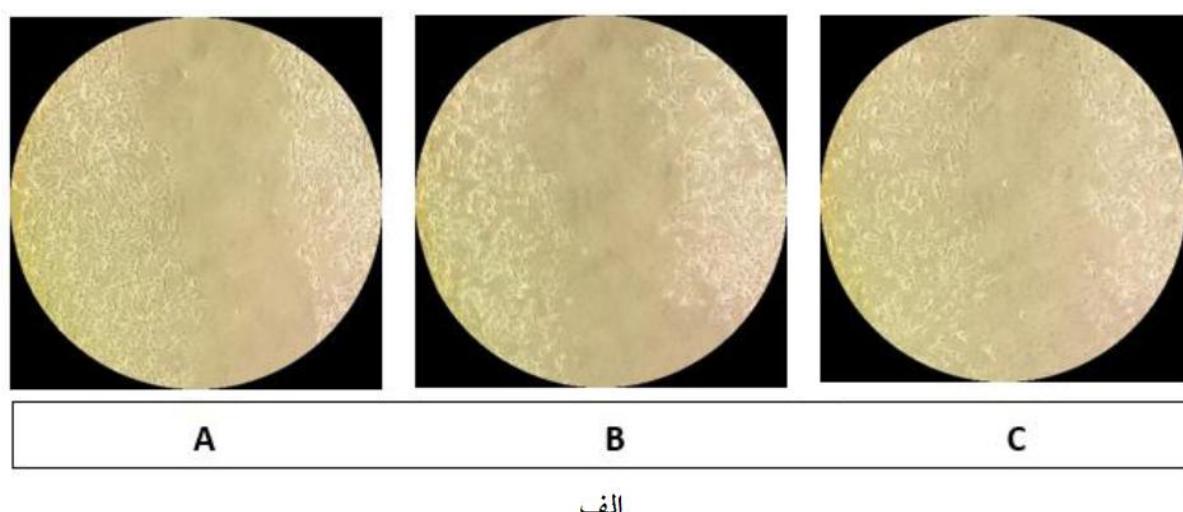


Figure 3 - Comparison of the percentage of endothelial cell viability in the control groups, treated at a wavelength of 660 nm and 980 nm at 24 and 48 hours.

#### Effect of laser on endothelial cell migration

In this experiment, the effect of laser on endothelial cell migration was studied using an in vitro model. After a wound is created between a dense layer of endothelial cells, the cells migrate from the wound edges and repair the wound. The cell migration test was examined at two times, 24 and 48 hours, and in

three groups: control, treated with a wavelength of 660 nm, and treated with a wavelength of 980 nm. The results of photographing endothelial cell migration at times 0, 24, and 48 hours are shown in Figure 4. As can be seen in the figure, the highest amount of cell migration occurred in the sample treated with a laser with a wavelength of 660 nm.



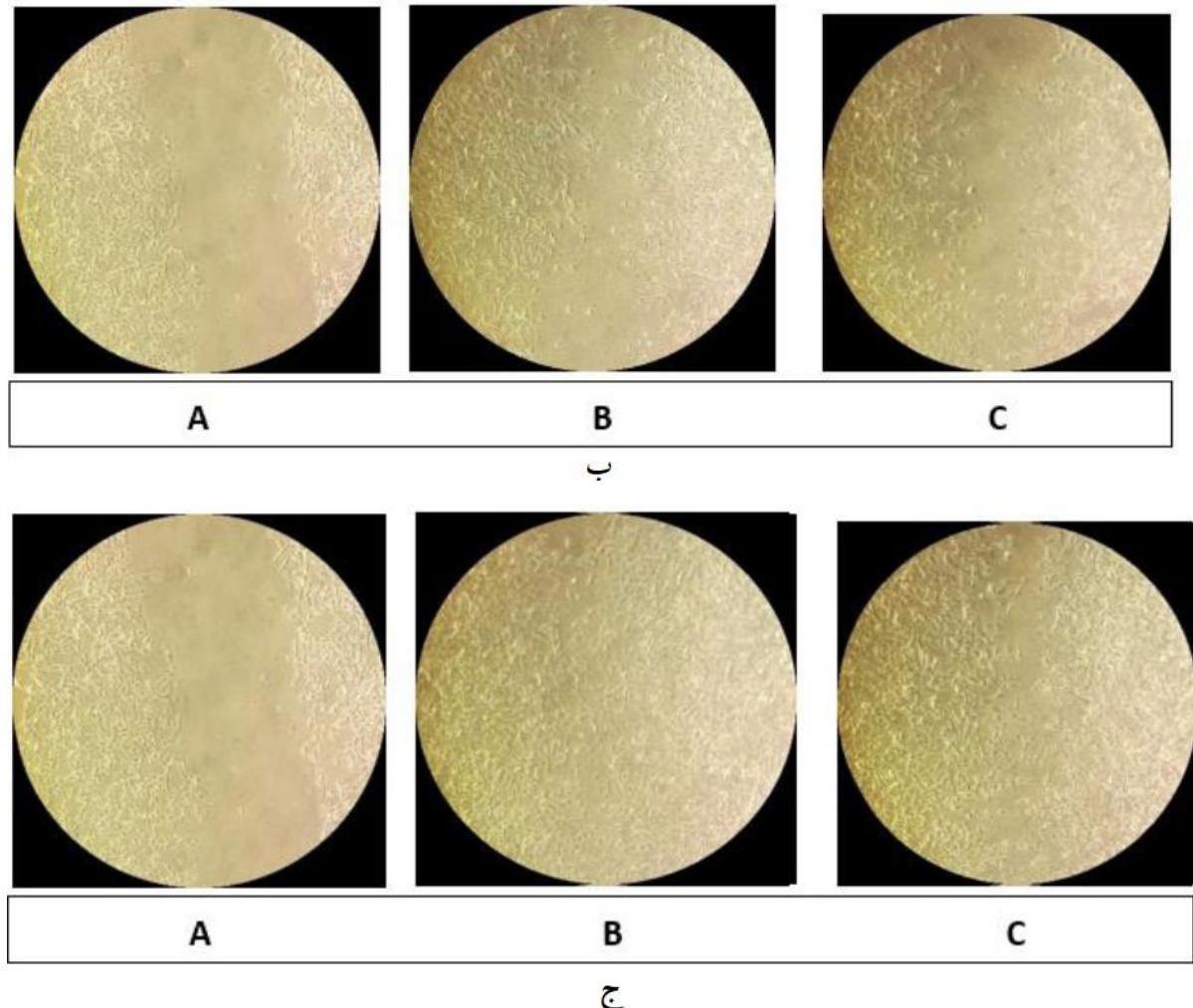


Figure 4- Migration of endothelial cells towards wound healing A) Time zero, B) 24 hours, C) 48 hours (A: control, B: 660 nm, C: 980 nm)

#### Discussion

In the present study, two factors of survival and migration of umbilical cord endothelial cells were investigated. The samples were treated with low-power laser radiation with two wavelengths  $\lambda = 660$  nm and  $\lambda = 980$  nm. Cell migration is a very key method in many biological processes and how molecular, physical and chemical aspects can affect cell movements. The factors examined in this study included the wavelength of low-power radiation and the time after treatment. According to the results of the present study, the shorter the wavelength of radiation, the less endothelial cell migration and the greater the cell survival. In the cell migration protocol, according to previous studies, lasers affect cell growth, proliferation and death. In this study, after experiments, the results confirmed that laser radiation reduces the migration and proliferation of vascular endothelial cells, and

its effectiveness depends on its potency and protects against restenosis by inhibiting the proliferation and migration of vessels. In the study by Ebrahimi et al., the combined treatment of dendritic nanocurcumin and low-level laser therapy was investigated, and the results showed that this treatment method promoted the proliferation and migration of mouse embryonic fibroblasts and changed the levels of TGF- $\beta$ , VEGF, TNF- $\alpha$  and IL-6, which are involved in the wound healing process. The results also showed that simultaneous exposure to antioxidants and low-level laser increased the proliferation and migration of fibroblast cells by regulating growth factor expression levels and shortening the inflammatory phase by modulating cytokines (14). This study is consistent with the current study and indicates that low-level laser stimulates cell migration. Gueriters et al. also investigated the effect of low-level laser

irradiation on the proliferation and differentiation of apical papilla stem cells. The findings of this study also showed that low-level laser increases cell migration and cell viability, and this property can be used in wound healing (12). According to the results of the study by Li et al., low-level laser therapy induces human umbilical vein endothelial cell proliferation, migration, and angiogenesis by activating the PI3K/Akt signaling pathway (10). In this study, Zhang also showed that the initial inflammatory response of laser-damaged skin in mice and the promotion of its proliferation and angiogenesis, which in turn promotes wound healing, were enhanced by low-level laser irradiation (3). Teschon et al. also showed that laser photobiomodulation at 519 nm improves wound healing mainly by stimulating cell migration and collagen deposition by osteoblasts.(11)

#### Conclusion

Thus, regenerative medicine and tissue engineering, by using a combination of modern treatments such as stem cells and low-energy laser therapy, can improve the quality of life of patients by providing healthy and functional tissues and organs.

#### Resources:

- 1- Sumpio, B.E., J.T. Riley, and A. Dardik, Cells in focus: endothelial cell. *The international journal of biochemistry & cell biology*, 2002: 34(12); 1508-1512.
- 2- Sattler S, Kennedy-Lydon T, editors. *The immunology of cardiovascular homeostasis and pathology*. Cham, Switzerland: Springer International Publishing; 2017: 71-91.
- 3- Zhang X, D.P., Chen Y, Lin Z, Zhao X, Xie H., Human umbilical cord mesenchymal stem cell-derived exosomes combined with gelatin methacryloyl hydrogel to promote fractional laser injury wound healing. *International Wound Journal*, 2023: 20(10); 4040-9.
- 4- Medina-Leyte DJ, D.-P.M., Mercado I, Villarreal-Molina MT, Jacobo-Albavera L. , Use of human umbilical vein endothelial cells (HUVEC) as a model to study cardiovascular disease: A review. *Applied Sciences*, 2020 : 10(3);938.
- 5- Saleh R ,R.H., Short review on human umbilical cord lining epithelial cells and their

potential clinical applications. *Stem cell research & therapy*. 2017: 8(1); 1-9.

6- Ebrahiminaseri, A., et al., Combination treatment of dendrosomal nanocurcumin and low-level laser therapy develops proliferation and migration of mouse embryonic fibroblasts and alter TGF- $\beta$ , VEGF, TNF- $\alpha$  and IL-6 expressions involved in wound healing process. *PloS one*, 2021: 16(5); e0247098.

7- Ahrabi, B., et al., The effect of photobiomodulation therapy on the differentiation, proliferation, and migration of the mesenchymal stem cell: a review. *Journal of lasers in medical sciences*. 2019: 10(Suppl 1); S96.

8- Saleh R, R.H., Short review on human umbilical cord lining epithelial cells and their potential clinical applications. *Stem cell research & therapy*. 2017: 8(1);1-9.

9- Lim IJ, P.T., Epithelial and mesenchymal stem cells from the umbilical cord lining membrane. *Cell transplantation*. 2014: 23(4-5); 497-503.

10- Li, Y ,et al., Low-level laser therapy induces human umbilical vascular endothelial cell proliferation, migration and tube formation through activating the PI3K/Akt signaling pathway. *Microvascular Research*. 2020: 129; 103959.

11- Tschon M, I.-P.S., Cepollaro S, Checchi L, Fini M. , Photobiomodulation with low-level diode laser promotes osteoblast migration in an in vitro micro wound model. *Journal of biomedical optics*. 2015: 20(7) ;078002.-

12- Góralczyk K, S.J., Gryko Ł ,Fisz J, Rośc D., Low-level laser irradiation modifies the effect of hyperglycemia on adhesion molecule levels. *Lasers in medical science*. 2018: 33; 1521-6.

13- Paydayesh A., Soltani S., Dadkhah A. Preparation and evaluation of polyvinyl alcohol hydrogels with zinc oxide nanoparticles as a drug controlled release agent for a hydrophilic drug. *Journal of Polymer Engineering*. 2023: 43.

14- Ebrahiminaseri, A., et al., Combination treatment of dendrosomal nanocurcumin and low-level laser therapy develops proliferation and migration of mouse embryonic fibroblasts and alter TGF- $\beta$ , VEGF, TNF- $\alpha$  and IL-6 expressions involved in wound healing process. *PloS one*, 2021: 16(5); e0247098