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THE EFFECT OF PULSED PHOTOBIOMODULATION ON PROLIFERATION AND MIGRATION OF HUMAN MESENCHYMAL STEM CELLS IN VITRO

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Summary

Aim. To experimentally study the effect of light of different ranges on the proliferation and migration of mesenchymal stem cells of human MSCs and to select its optimal parameters for treatment.
Materials and methods. The experiment was conducted in vitro on 260 cultures of human MSCs isolated from peripheral blood by magnetic separation. Cells were treated with pulsed LED light: 475 nm, 516 nm, 635 nm or left unstimulated. All LED devices had a peak radiation intensity of 80 mW/cm2. The average radiation intensity reached 40 mW/cm2. Irradiation was carried out at room temperature for 10 minutes at a distance of 2 cm from the cells. Cells embedded in a 2D fibrin gel matrix to study cell proliferation and a 3D fibrin gel matrix to study cell migration were studied. Hereby, cells used for 2D experiments were stimulated on day 0, while cells embedded in 3D arrays were stimulated on day 0 and then every 24 h until quantification. The effect of different wavelengths on both proliferation and cellular metabolic activity of MSCs from peripheral blood was evaluated after initial light treatment at 24 hours, 48 hours and 72 hours.
Results. During the first 48 hours after stimulation, cells proliferated in all studied groups (stimulated and non-stimulated). At the same time, there were no significant differences between the groups at 24 hours and 48 hours. For 2D experiments, cells were stimulated only on day 0, whereas for 3D experiments, stimulation was performed every 24 h. Quantification of cells migrating into the surrounding fibrin gel matrix showed that red and green light stimulation significantly affected 3D migration after 4 days. Irradiation with blue light had no significant effect on migration.
Conclusions. Thus, exposure of MSCs to red and green light increases cell proliferation in 2D and 3D environments, while exposure to blue light decreases their metabolic activity. In our opinion, this fact should be used to modernize laboratory equipment and improve treatment regimens for patients using MSCs. Further research on the influence of light on the differentiation of MSCs is promising.

Keywords: **pulse irradiation, green light, mesenchymal stem cells, stimulation of regeneration**

INTRODUCTION

In recent decades, regenerative medicine (RM), the main task of which is to restore the structure and function of organs and tissues lost as a result of certain diseases or injuries, has been increasingly used in clinical practice. The human organism can regenerate at the level of a cell layer consisting of one or, perhaps, several types of closely related cells. This property is used in medicine to regenerate skin epithelium, in case of bone fractures, tendon and muscle ruptures, nerve trunk damage, etc. This property is also used in medicine. However, after damage to several layers of tissues (for example, skin epithelium and the underlying dermis) or disruption of the structure of tissues and organs always remain scars, which is a clear indication of incomplete regeneration. In such injuries, specific treatment methods must be applied for complete recovery. The arsenal of such methods constitutes the applied part of RM, and the indications for their application constitute the main feature of the RM field, which distinguishes it from other fields of medicine. Two main directions can be distinguished within RM: creation and use of structural carriers [1].

Foreign clinical practice has convincingly shown that autologous mesenchymal stem cells (MSC) of patients (especially elderly or suffering from degenerative diseases) have, as a rule, significantly reduced proliferative potential [2], and allogeneic cells often cause transplant rejection reaction [3]. The fundamental danger of SC transformation during prolonged cultivation was also revealed. Thus, when adipose tissue SCs were cultured for more than two months (>20 passages), transformed cells were detected in 50 % of samples [4]. When SCs were injected into a wound, a significant part of them died after 24 hours due to unfavourable microenvironment [5]. At the same time, for the effective therapy of SCs, it is necessary to have a sufficient amount of them often in the shortest possible time.

As an additional highly effective physical stimulant of SC treatment, irradiation with an electromagnetic field of visible red and infrared ranges is well established [6, 7]. In a study by Barboza C. A. et al. [8] on adipose tissue MSCs it was shown that their proliferation on the first day was slowed down by 20 % in comparison with the control (at the same time there were 32 % more cells in the experiment at the zero point. Fukuhara E. et al. [9] also noted a similar effect in their studies, which the authors explain by the arrest of cells in the G2/M phase of the cell cycle on the first day after irradiation. There are several reviews summarizing the effects of light exposure on cells in the red and near-infrared regions. Some of them mainly discuss the mechanisms of light action on proliferation and apoptosis [10-12]. However, the works devoted to the effect on SC proliferation when exposed to pulsed light of other ranges are single [13].

AIM

To experimentally study the effect of pulsed light of different ranges on the proliferation, and migration of human MSCs and to choose its optimal parameters for treatment.

MATERIALS AND METHODS

This section describes the in vitro experiment conducted on 260 human MSC cultures isolated from peripheral blood by magnetic separation, containing 6.0 × 106 cells. The LED lamps used for light therapy were provided by Pharma Complex Solutions, the official distributor of the German company MEDlight, Herford. Specifically, Treviolux and SmartComfort lamps were used.

The cells were exposed to pulsed LED light of different wavelengths, including blue (475 nm), green (516 nm), red (635 nm), or no stimulation (control). All LED devices had a peak irradiance of 80 mW/cm2, which was measured using a USB 2000 spectrometer (Ocean Optics, Florida, USA).

Simultaneously, we fulfilled the essential prerequisites to achieve a favourable impact on cell proliferation [14-16]. The cells were placed in Petri dishes containing a phosphate buffer during irradiation, and they were in a relatively inactive state, either functionally or due to unfavourable conditions (taken from patients with diabetes mellitus).

The study design included 20 cultures of MSCs from patients with diabetes mellitus as the control group (non-irradiated cultures). Blood was collected from a peripheral vein of the patient. MSCs were obtained from human peripheral blood using the immunomagnetic separation method, which employs paramagnetic polystyrene microparticles coated with monoclonal antibodies to specific antigens. This method was chosen for its high purity range of 90-99 % and minimal cell loss. The AtoMACS 3 magnetic separator was used with monoclonal antibodies, following the instructions.

The cultures under study (20 in each series) were irradiated with red (λ=635 nm), blue (λ=475 nm), and green (λ=516 nm) light. An average radiation intensity of 40 mW/cm2 was achieved, considering a pulse frequency of 50 % and a repetition rate of 2.5 Hz. The daily dose provided was 24 J/cm2. Illumination was performed at room temperature for 10 minutes, 2 cm away from the cells (fig. 1).

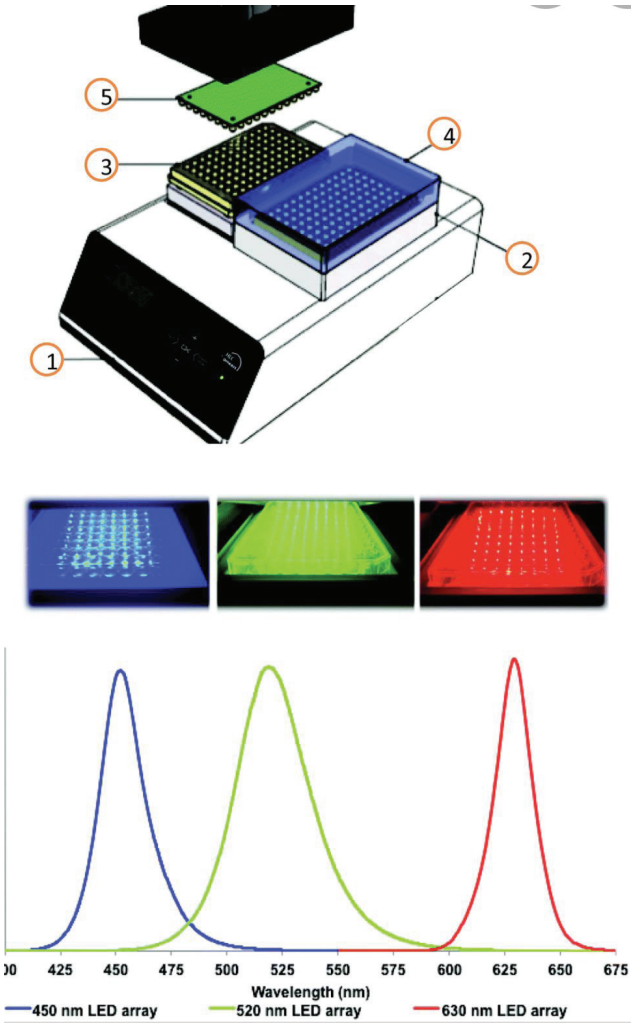

Figure 1. A schematic representation of the experiment.

To study cell proliferation and migration, cells were embedded in 2D and 3D fibrin gel matrices. The cells used for 2D experiments were stimulated on day 0, while the cells embedded in 3D matrices were stimulated on day 0 and then every 24 hours until quantification (fig. 2).

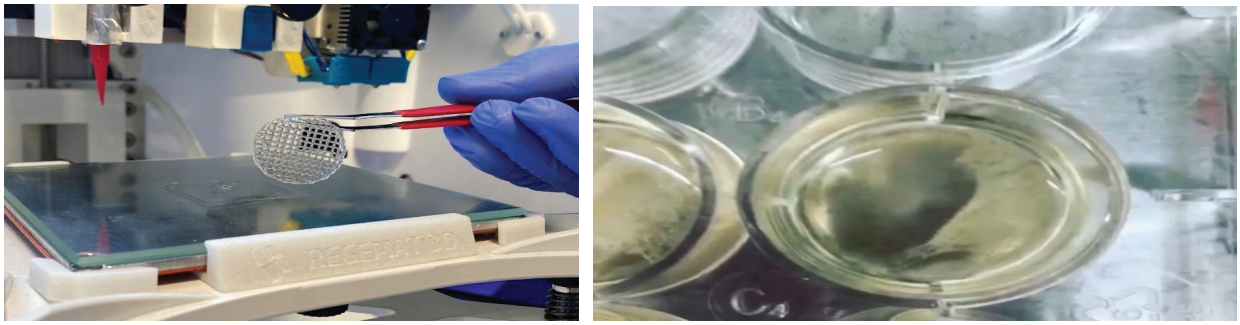

Figure 2. Cells embedded in a 2D matrix.

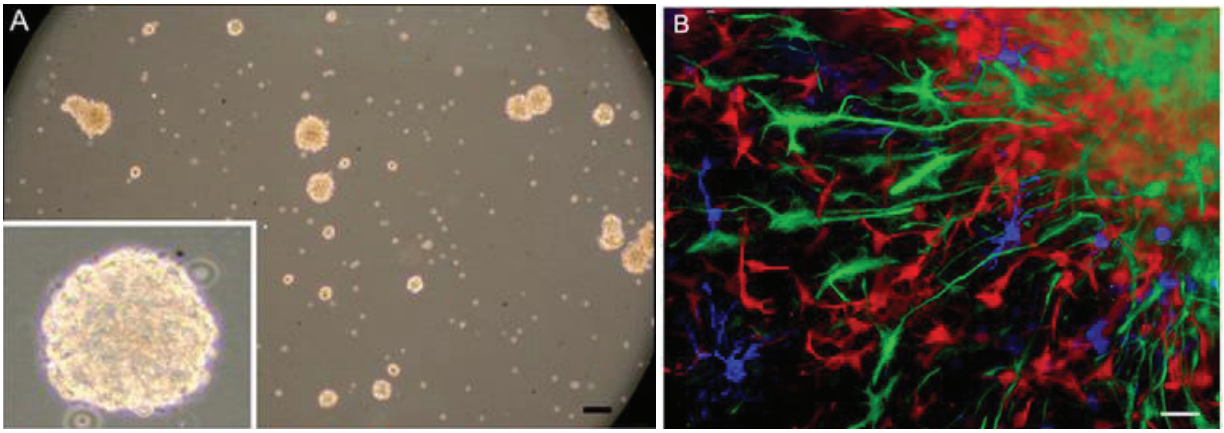

Figure 3. 3D cell migration 4 days after green light irradiation.

Quantitative evaluation, representing changes in form factor, was performed 4 days after treatment of cell cultures. The following results were obtained: the area occupied by cells increased significantly after treatment with red (~43 %) and green (~47 %) light. Here, a 10 % decrease in form factor was observed in all groups (fig. 4).

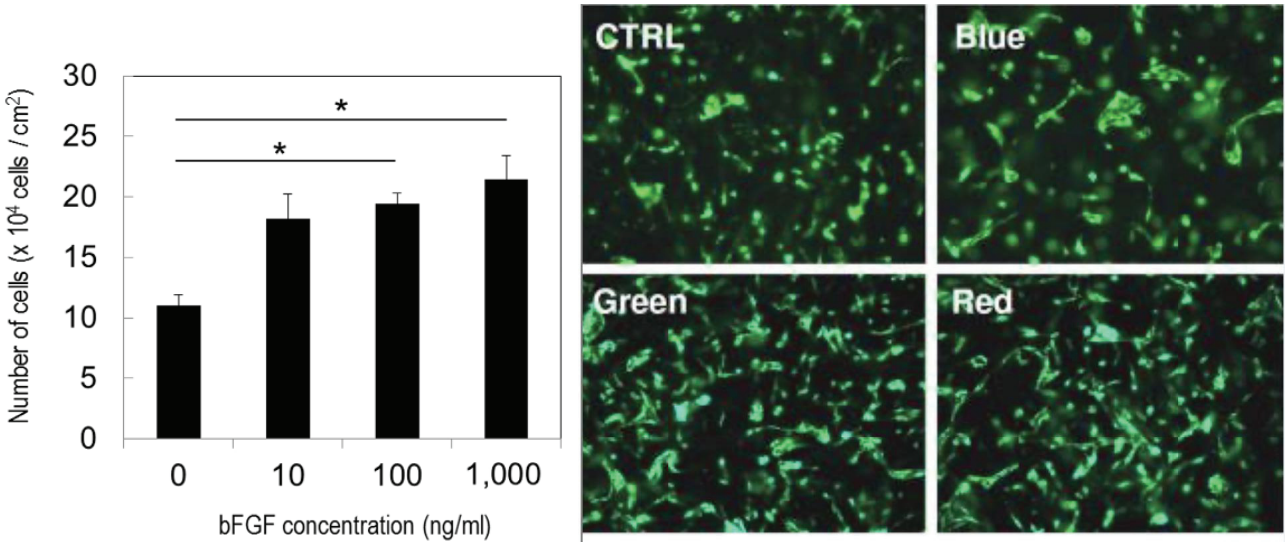

Figure 4. Effect of irradiation of cultures with light of different range on MSC proliferation after culturing at concentrations of 0, 10, 100 and 1000 ng / ml * $p < 0.05$; significant difference between the studied groups.

At 7 days after culture treatment, this trend was maintained for cell cultures exposed to green and red illumination (respectively, the form factor decreased by about 15 % in the same groups).

As for the study of metabolic activity of cells, it was found that 72 hours after stimulation with light of different ranges, there was a decrease in it in all groups treated with light compared to the control, which reached statistical significance after irradiation with blue light.

DISCUSSION

Despite numerous studies examining the effect of wavelength on human MSCs [17-18], which indicate that this radiation parameter does not significantly affect MSC proliferation, our data suggest that the optimal values are $\lambda = 516$ nm and $\lambda = 635$ nm. Treatment with light of a wavelength of 475 nm leads to a decrease in the metabolic activity of MSCs. The absorption spectra of molecules are always significantly blurred, which explains the presence of at least four absorption peaks in the red and infrared regions and one in the violet in cytochrome c oxidase. Additionally, other photoreceptors in the respiratory chain, such as NADH-dehydrogenase, absorb in the blue region [11], and cytochromes b and c1 absorb in the green region [19, 20]. The mitochondrial respiratory chain can be activated by different wavelengths. However, this can lead to nonspecific effects since the process of mitochondrial respiration itself is nonspecific. The effect of light on MSCs is also nonspecific, as confirmed by the fact that light of the same wavelength can lead to an acceleration of either proliferation or differentiation, depending on the initial state of the cells [21-23, 25]. However, clinical studies have shown differences in patient exposure to light of different wavelengths. For instance, when treating wounds, it is preferable to use blue light due to its bactericidal and analgesic effects, as well as green light for its pronounced anti-edematous effect during the exudation phase. In the proliferative phase of wound healing, the use of red light is recommended due to its great regenerative effect. The presence of a specific action at the organismal level may indicate the possibility of a corresponding action at the cellular level. Several significant photoacceptors have been discovered outside mitochondria, such as opsins [23], cryptochromes [24], and NADP(H)-oxidase [25]. Additionally, there are many little-studied photoacceptors that can lead to specific reactions when activated.

CONCLUSIONS

Irradiation of MSCs with red and green light has been found to increase cell proliferation in both 2D and 3D environments. Conversely, irradiation with blue light has been shown to reduce their metabolic activity. These findings suggest a potential for modernizing laboratory equipment and improving treatment regimens for patients using MSCs.

Prospects of further research: further studies on the effect of light on MSC differentiation are promising.

FUNDING AND CONFLICT OF INTEREST

The authors declare that they have no actual or potential conflict of interest. The article was funded at the authors' own expense.

COMPLIANCE WITH ETHICAL REQUIREMENTS

All ethical norms are abided. All agreements for the procedures from the patients were received.

REFERENCES

1. Atala A., Lanza R., Thomson J. A., Nerem R. M. (2008). Principles of regenerative medicine. USA: AcademicPressElsevier Inc.
2. Dawoud L.E., Hegazy E. M., Galhom R. A., Youssef M. M. (2022). Photobiomodulation therapy upregulates the growth kinetics and multilineage differentiation potential of human dental pulp stem cells-an in vitro. Study. Lasers Med Sci., 37(3), 1993-2003. https://doi.org/10.1007/s10103-021-03461-4
3. Wang L., Wu F., Liu C., Song Y., Guo J., Yang Y., Qiu Y. (2019). Low-level laser irradiation modulates the proliferation and the osteogenic differentiation of bone marrow mesenchymal stem cells under healthy and inflammatory condition. LasersMedSci, 34(1), 169-178. https://doi.org/10.1007/s10103-018-2673-8
4. Mikami R., Mizutani K., Aoki A., Tamura Y., Aoki K., Izumi Y. (2018). Low-level ultrahigh-frequency and ultrashort-pulse blue laser irradiation enhances osteoblast extracellular calcification by upregulating proliferation and differentiation via transient receptor potential vanilloid 1. LasersSurgMed, 50(4), 340-352. https://doi.org/10.1002/lsm.22775
5. Zhang H., Hou J. F., Shen Y., Wang W., Wei Y. J., Hu S. (2010). Low level laser irradiation precondition to create friendly milieu of infarcted myocardium and enhance early survival of transplanted bone marrow cells. J. CellMol. Med., 14, 1975-1987.

6. Bourouni I., Kyriakidou K., Fourmousis I., Vrotsos I. A., Karoussis I. K. (2021). Low level laser therapy with an 810-nm diode laser affects the proliferation and differentiation of premature osteoblasts and human gingival fibroblasts in vitro. J LasersMedSci, 12: e33. https://doi.org/10.1002/lsm.22775
7. M Abdelgawad L., Salah N., Sabry D., Abdelgwad M. (2021) Efficacy of Photobiomodulation and Vitamin D on Odontogenic Activity of Human Dental Pulp Stem Cells. J LasersMedSci., 24,12, e30. https://doi.org/10.34172/jlms.2021.30. eCollection 2021.
8. Barboza C.A., Ginani F., Soares D. M., Henriques A. C., Freitas R. A. (2014). Low-level laser irradiation induces in vitro proliferation of mesenchymalstem cells. Einstein., 12, 75-81.
9. Fukuhara E., Goto T., Matayoshi T., Kobayashi S., Takahashi T. (2006) Optimal low-energy laser irradiation causes temporal G2/M arreston rat calvarial osteoblasts. Calcif. TissueInt., 79, 443-50.
10. Gao X., Xing D. (2009). Molecular mechanisms of cell proliferation induced by low power laser irradiation. J. Biomed. Sci. 16, 4. https://doi.org/10.1186/1423-0127-16-4
11. Peplow P.V., Chung T. Y., Ryan B., Baxter G. D. (2011). Laser photobiomodulation of gene expression and release of growth factors and cytokines from cells in culture: a review of human and animal studies. Photomed, Laser Surg., 29, 285-304.
12. Eroglu B., Genova E., Zhang Q., Su Y., Shi X., Isales C., Eroglu A. (2021). Photobiomodulation has rejuvenating effects on aged bone marrow mesenchymal stem cells. Sci Rep.,11(1),13067. https://doi.org/10.1038/s41598-021-92584-3
13. Stroh A., Tsai H. C., Wang L. P.. (2011). Tracking stem cell differentiation in the setting of automated optogenetic stimulation. Stem Cells., 29, 78-88.
14. AlGhamdi K.M., Kumar A., Moussa N. A. (2012). Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med. Sci., 27, 237-49.
15. Peplow P.V., Chung T. Y., Baxter G. D. (2010). Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. Photomed. LaserSurg., 28 (Suppl. 1), S3-40.
16. Reddy G.K. (2004). Photobiological basis and clinical role of low-intensity lasers in biology and medicine. J. Clin. Laser Med. Surg., 22, 141-50.
17. Abrahamse H., Houreld N. N., Muller S., Ndlovu L. (2010). Flutnce and wavelength of low intensity laser irradiation affect activity and proliferation of human adipose derived stem cells. Medical Technology SA., 24, 9-14.
18. Anders J.J., Romanczyk T. B., Ilev I. K. (2008). Light supports neurite outgrowth of human neural progenitor cells In Vitro: the role of P2Y receptors. IEEE J. Selected Topics in Quantum Electronics.,14, 118-25.
19. Berg J.M., Tymoczko J. L., Stryer L. (2002). Biochemistry. 5th Ed. New York: W. H. Freeman & Co.
20. Kassak P., Przygodzki T., Habodaszova D., Bryszewska M., Sikurova L. (2005). Mitochondrial alterations induced by532 nm laser irradiation.Gen. Physiol. Biophys, 24, 209-20.
21. Bai, J, Li, L, Kou, N, Bai, Y, Zhang, Y. (2021). Low level laser therapy promotes bone regeneration by coupling angiogenesis and osteogenesis. Stem Cell Res Ther. Aug 3, 12(1), 432. https://doi.org/10.1186/s13287-021-02493-5
22. Monici, M., Cialdai, F., Ranaldi, F. (2013). Effect of IR laser on myoblasts: a proteomic study. Mol. Biosyst., 9, 1147-61.
23. Ong, W. K., Chen, H. F., Tsai, C. T., Fu, Y. J., Wong, Y. S., Yen, D. J., Chang, T. H., Huang, H. D., Lee, O. K., Chien, S., & Ho, J. H. (2013). The activation of directional stem cell motility by green light-emitting diode irradiation. Biomaterials, 34(8), 1911-1920. https://doi.org/10.1016/j.biomaterials.2012.11.065
24. Kushibiki, T., & Awazu, K. (2009). Blue laser irradiation enhances extracellular calcification of primary mesenchymal stem cells. Photomedicine and laser surgery, 27(3), 493-498. https://doi.org/10.1089/pho.2008.2343
25. Karu, T. I. Low-power laser therapy. In: Biomedical photonics handbook. (2003). (T. Vo-Dinh, ed.). CRC Press: BocaRaton, 1-25.

Резюме

ВПЛИВ ІМПУЛЬСНОГО ВИПРОМІНЮВАННЯ РІЗНИХ ДІАПАЗОНІВ НА ПРОЛІФЕРАЦІЮ ТА МІГРАЦІЮ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН ЛЮДИНИ IN VITRO

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Мета. Метою даної роботи було експериментальне вивчення впливу світла різних діапазонів на проліферацію та міграцію мезенхімальних стовбурових клітин МСК людини та вибір її оптимальних параметрів для лікування.
Матеріали та методи. Експеримент проводився in vitro на 260 культурах МСК людини, виділених з периферичної крові методом магнітної сепарації. Клітини оброблялися імпульсним світлодіодним світлом: 475 нм, 516 нм, 635 нм або залишалися нестимульованими. Усі світлодіодні пристрої мали пікову інтенсивність випромінювання 80 мВт/см2. Середня інтенсивність випромінювання сягала 40 мВт/см2. Опромінення проводилося за кімнатної температури протягом 10 хвилин на відстані 2 см від клітин. Досліджували клітини, вбудовані в 2D-матриці фібринового гелю для вивчення проліферації клітин та 3D-матриці фібринового гелю для вивчення міграції клітин. При цьому клітини, що використовуються для 2D-експериментів, стимулювалися на 0-й день, тоді як клітини, вбудовані в 3D-матриці, стимулювалися на 0-й день, а потім кожні 24 години до кількісної оцінки. Вплив різних довжин хвиль як на проліферацію, так і на клітинну метаболічну активність МСК з периферичної крові, було оцінено після початкової світлообробки при 24 годинах, 48 годинах і 72 годинах.
Результати. Протягом перших 48 годин після стимуляції клітини розмножувалися у всіх досліджуваних групах (стимульованих та нестимульованих). При цьому у терміни 24 години та 48 годин не було суттєвих відмінностей між групами. Для 2D-експериментів клітини стимулювалися лише на 0-й день, тоді як для 3D-експериментів стимуляція виконувалася кожні 24 години. Кількісна оцінка клітин, які мігрують в навколишню матрицю фібринового гелю, показала, що стимуляція червоним і зеленим світлом суттєво вплинули на 3D-міграцію через 4 дні. Опромінення синім світлом не чинило суттєвого ефекту на міграцію.
Висновки. Таким чином, опромінення МСК червоним і зеленим світлом збільшує поширення клітин у середовищі 2D і 3D, в той час як опромінення синім світлом знижує їх метаболічну активність. Цей факт, на наш погляд, доцільно використовувати з метою модернізації лабораторного обладнання та удосконалення схем лікування пацієнтів із використанням МСК. Перспективними є подальші дослідження щодо світлового впливу на диференціювання МСК.

Ключові слова: **імпульсне опромінення, зелене світло, мезенхімальні стовбурові клітини, стимуляція регенерації**