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Effect of Low-Level Laser Irradiation on the Proliferation of Human Chondrocytes: An In Vitro Study

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Abstract

Introduction: Chondrocytes are the major cell in hyaline cartilage playing a crucial role in maintaining the mechanical resilience of the tissue. We assessed the effect of an 808nm diode laser on the proliferation of human chondrocytes.

Methods: This study was conducted on human chondrocytes in vitro. The cells were divided into 5 cases and one control group. The cells were irradiated by low-level laser 808 nm, with energy levels of 1, 2, 3 J/cm² (0.2 W, for 5, 10, and 15 seconds), 4 J/cm² (0.3 W,13 s), and 5 J/cm² (0.4 W,12 s). The culture was incubated for 24 hours. The MTT assay was performed to determine the cell viability. After 72 hours of incubation, the procedure was reperformed to assess the effect of incubation duration. The cell viability in terms of incubation duration and irradiation parameters were investigated by a two-way ANOVA test. Pairwise comparisons were performed using the Bonferroni test.

Results: In the 72-hour incubation group, cell viability in the group that received 5 J/cm² energy was significantly lower than that in the groups receiving 1 J/cm², 2 J/cm² and 4 J/cm² irradiation. The variables of time ($P=0.001$) and energy level ($P=0.024$) had significant effects on the cell viability of the samples. In the 24-hour incubation groups, no significant difference in cell viability was observed.

Conclusion: The diode 808 nm Low-level laser irradiation (LLLI) at doses of 5 J/cm² and less did not show a significant increase in the proliferation of chondrocytes (regardless of incubation time). However, the best survival rate of chondrocytes was observed in the group of 4 J/cm² with 72-hour incubation.

Keywords: Diode 808 nm; Low-level laser; Human chondrocytes; Proliferation.

Introduction

Cartilage is a durable and strong form of supporting connective tissue characterized by an extracellular matrix (ECM) containing glycosaminoglycans and proteoglycans that interact with collagen and elastic fibers.[1]

The temporomandibular joint is a joint capable of hinge and sliding movements along with the bone component covered by and connected to the fibrous capsule. Condylar cartilage is also a proliferative layer of dividing cells that acts as precursor cells of cartilage growth.[2] Cartilage lacks vascular nutrition, and chondrocytes are fed by the diffusion of nutrients from the capillaries of the surrounding connective tissue (perichondrium). Like tissue cells without blood vessels, chondrocytes have low metabolic activity and chondrocyte respiration is done at low oxygen pressure. These cells mainly metabolize glucose through anaerobic glycolysis. When injured during childhood, the damaged cartilage regenerates slowly and often incompletely, which is mainly dependent on the activity of perichondrium cells. In the site of damage, perichondrium repairs cause a scar of dense connective tissue instead of making new cartilage. The low ability to repair or regenerate cartilage is partially caused by the absence of blood vessels in this tissue and its low metabolic activity.[1]

Temporomandibular disorders (TMDs) are a group of musculoskeletal disorders that affect the temporomandibular joints and muscles of mastication.[3] Meanwhile, damage to the articular disc, such as disc perforation (DP) or retrodiscal tissue, occurs in the late

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stages of TMD and mostly in joints with previous disc disorder (anterior, medial anterior, or anterior lateral disc dislocation) or at advanced stages of TMJ dysfunction and degenerative changes.[4]

Some methods have been performed to restore the cartilage structure and stimulate the inherent capabilities of the tissue, such as surgical techniques, tissue engineering treatments,[5] electrotherapy, ultrasound, or laser therapy.[6] Despite providing much information to replace the lost cartilage by recent methods, the problem of cartilage repair remains unsolved. Autologous chondrocyte implantation (ACI) is also useful in this regard, but it causes complications in the donor site.[7,8] Recently, the use of mesenchymal stem cells in cartilage repair has increased.[9]

Among the physical therapy methods, low-level laser therapy has received attention due to its simple application, short treatment duration, and few contraindications. Some prospective clinical trials have been conducted to determine the effects of photobiomodulation (PBM) on the treatment of TMD. At the same time, some authors reported the superiority of PBM over placebo and others observed no significant difference between PBM and placebo.[6]

Optimal proliferation of differentiated chondrocytes can be achieved through the modulation of various factors. One of the easy approaches in this regard is laser irradiation. Low doses of laser irradiation stimulate biological processes by increasing cytoplasmic Ca^{2+}. At higher doses, a large amount of Ca^{2+} is released and causes a severe depletion of cellular energy and the process of cellular metabolism is compromised. Low-level laser irradiation (LLLI) by the He-Ne laser effectively regulates the differentiation and proliferation of chondrocytes in the culture medium.[10] This can be used as a conservative method for treating the injured disk of the temporomandibular joint.

The main advantage of the proliferation of chondrocytes by a laser is to create reactions that cause cartilage defects to be filled with hyaline-like tissue. The basic biological mechanism of these reactions is the stimulation of the differentiation of existing immature stem cells and the increase in the accumulation of ECM components by hyaline cartilage chondrocytes. According to the advanced concepts, most of the reactions may occur through the changes that occur in the cartilage ECM under laser irradiation.[11] Probably the most important feature of laser therapy is the engagement and activation of the intrinsic mechanisms of cartilage repair.[5]

Cultured chondrocytes treated with LLLI are a promising cell source for repairing cartilage lesions in vivo.[10]

However, considering the lack of investigation about the effects of an 808 nm diode laser on the proliferation of human chondrocytes and the necessity of in vitro evaluation before clinical applications, the present study was conducted to determine the effects of low-power 808 nm diode laser irradiation on human chondrocyte in vitro.

Materials and Methods

The present study is an experimental laboratory study. First, the cell line of human chondrocyte was obtained from the Pasteur Institute cell bank (NCBI: C620). This stable cell line can express cartilage morphology, chondrocyte specific collagens, collagens IX, II, and XI, and continuous proliferation (more than 80 passages).[12]

The cell survival rate was evaluated using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). To culture the cells, first, the culture medium (DMEM: Dulbecco's modified eagle medium) was prepared according to the factory instructions and finally, the culture medium was sterilized by a filter with a pore diameter of 0.2 µm. To complete the culture medium, first, 100 IU/mL of penicillin (USA, Gibco) and 100 µg/mL streptomycin (Gibco, USA) were added to the sterilized DMEM culture medium. Also, 10% fetal bovine serum (FBS) (Gibco, USA) was added to the culture medium under sterile conditions. The cell culture flask obtained from Pasteur Institute containing the human chondrocyte line after culture in the DMEM was kept in an incubator (Memmert, Germany) containing 5% CO_2 along with 95% humidity at 37 °C. After the cells reached 75%-85% confluency, the cells were detached by trypsin and EDTA and passaged. After reaching the required number in the fifth passage, the cells were used for the preparation of cell suspension. In case of bacterial contamination of the cell culture or contamination with other microorganisms, the sample was removed from the research.

Next, the suspension cells were counted and then divided into equal numbers at the bottom of the wells. After 24 hours of the initial culture of cells, the wells were divided into 5 groups, and in each group, there were three duplicate wells, with each well, being individually exposed to the diode 808 nm low-power laser in a non-contact form with a distance of 1 mm and based on a continuous mode (Figure 1). The parameters used for laser irradiation were energies of 1, 2, 3, 4, and 5 joules with powers of 0.2 and 0.4 W for 5, 10, 12, 13, and 15 seconds, and the irradiation surface was equal to 1 cm^2.[10]

The irradiation in groups 1-5 was as follows:

- Group 1: 1 J/cm^2 energy (0.2 W and 5 s)
- Group 2: 2 J/cm^2 energy (0.2 W and 10 s)
- Group 3: 3 J/cm^2 energy (0.2 W and 15 s)
- Group 4: 4 J/cm^2 energy (0.3 W and 13 s)
- Group 5: 5 J/cm^2 energy (0.4 W and 12 s)

For 24 hours, the plates were incubated in an incubator at 37 °C and 5% CO_2. All the stages were done under sterile conditions and a Class II hood. All the above

Figure 1. Low-Power Laser Irradiation in a Non-contact Mode

procedures were repeated for the second time. The only difference was the incubation time, which means the plates were incubated for 72 hours to assess the effect of the incubation period after the irradiation.

For the MTT assay, 5 mg/mL of tetrazolium bromide salt (Sigma, USA) was prepared in phosphate buffered saline (PBS), and then it was sterilized using a filter with a pore diameter of 0.2 micrometers and kept in a freezer at -20 °C. The samples were diluted at the ratio of 1:10 in DMEM during the test.

Then, the MTT salt solution, which was diluted in the culture medium at a ratio of 1:10, was added to each well and incubated for 1.5 hours under culture conditions (98% humidity, 37 °C temperature, and 5% CO_2). Next, the supernatant was removed and 200 microliters of DMSO (USA, Sigma) was added to each well to dissolve the formed formazan crystals and the emergence of a purple color. The view of formazan crystals in the control and laser groups is presented in Figure 2.

After mixing several times, finally, the optical absorbance of the produced color was read using the ELISA Reader (Austria, 2020 Anthos) at 570 nm and with the reference filter at 620 nm. To calculate cell viability, the light absorbance of the test group was divided by the light absorbance of the control group, and the number obtained was multiplied by 100 as follows:

$$cell\ viability = \frac{light\ absorbance\ of\ the\ test\ group}{light\ absorbance\ of\ the\ control\ group} \times 100$$

After calculating the cell viability, its average was checked for each studied group and the results of the groups were compared with each other. All the above stages were repeated for the 72-hour group.[12,13]

Data were analyzed using Statistical Package for Social Sciences: SPSS version 25. For this purpose, the mean and standard deviation of the percentage of human chondrocyte cells in different groups of low-power diode laser irradiation at two times of 24 hours and 72 hours of incubation were calculated and reported in comparison with the samples of the control group without laser irradiation. At first, the condition of the data following the normal distribution was checked and verified using the Kolmogorov-Smirnov test.

The effects of laser irradiation parameters and incubation time duration of culture samples on cell viability were investigated using a two-way ANOVA test. According to the significant results of two-way ANOVA, pairwise comparisons of irradiation groups at 24-hour and 72-hour incubation times were performed using the Bonferroni test.

The first-type error in the present research was determined at the limit of 0.05 ($\alpha = 0.05$).

Results

The cell viability data related to different groups of LLLI were first examined to determine whether the data followed a normal distribution, and according to the results of the Kolmogorov-Smirnov test, it was found that these data had a normal distribution ($P > 0.05$). Of course, in group 1 (laser irradiation at 1 J/cm^2, power of 0.2 W, and time period of 5 seconds), normal data distribution was not confirmed. Considering these results, parametric tests were used for statistical analysis. Table 1 shows the mean and standard deviation of cell viability at different laser irradiation times and groups.

The Bonferroni test for different energy levels of laser irradiation in the group of 24-hour incubation showed no significant difference in terms of cell viability in different groups (Table 2).

According to the test results for different energy levels of laser irradiation in the 72-hour group incubation, significant differences were observed in terms of cell viability in groups 1-5 ($P = 0.04$), 2 and 5 ($P = 0.03$), and 4-5 ($P = 0.01$). In pairwise comparison of other groups, no significant difference was observed in terms of differences in cell viability in the studied groups (Table 3).

According to the results of pairwise comparison of different groups of laser irradiation (different energy levels) with different incubation durations, significant differences were observed in groups 1 ($P = 0.01$), 2 ($P = 0.007$), and 4 ($P = 0.01$) in terms of cell viability. However, these differences in the 3rd and 5th groups did not show significant results (Table 4).

Discussion

Considering the limited ability of chondrocytes to proliferate, many studies have investigated different methods to improve chondrocyte proliferation. This

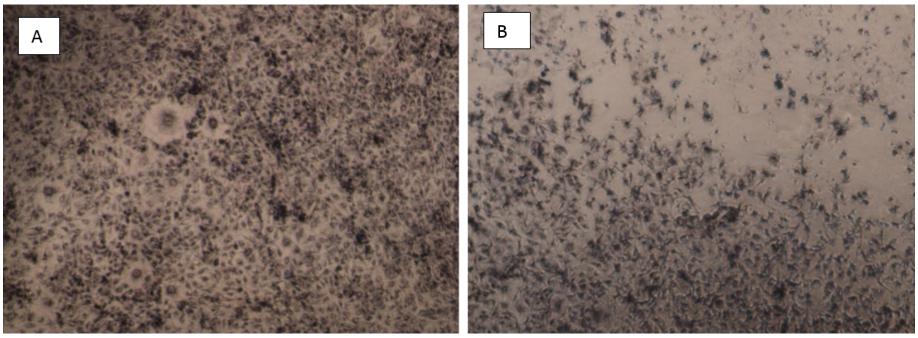

Figure 2. Formazan Crystals in the Control (A) and Laser (B) Groups

Table 1. Mean and Standard Deviation of Cell Viability at Different Times and Groups of Laser Radiation

Group	Time (h)	M	SD	Upper Limit-Lower Limit
Group 1: 1 J/cm² energy (0.2 W and 5 s)	24	0.8	0.2	69-90%
	72	0.98	0.03	88-108%
Group 2: J/cm² energy (0.2 W and 10 s)	24	0.79	0.06	68-89%
	72	0.99	0.11	89-109%
Group 3: 3 J/cm² energy (0.2 W and 15 s)	24	0.93	0.07	82-101%
	72	0.94	0.11	83-104%
Group 4: 4 J/cm² energy (0.3 W and 13 s)	24	0.82	0.12	72-92%
	72	1/01	0.11	91-111%
Group 5: 5 J/cm² energy (0.4 W and 12 s)	24	0.78	0.13	68-88%
	72	0.77	0.07	66-86%

Table 2. Results of Pairwise Comparisons of Laser Irradiation Groups for 24 Hours in Terms of Cell Viability

Group	Group	M	SD	*P* Value
1	2	0.01	0.071	1.0
	3	-0.13	0.071	0.74
	4	-0.022	0.071	1.0
	5	0.018	0.071	1.0
2	3	-0.14	0.071	0.553
	4	-0.032	0.071	1.0
	5	0.008	0.071	1.0
3	4	0.108	0.071	1.0
	5	0.148	0.071	0.433
4	5	0.04	0.071	1.0

Table 3. Results of Pairwise Comparison of Laser Irradiation Groups for 72 Hours in Terms of Cell Viability

Group	Group	M	SD	*P* Value
1	2	-0.008	0.071	1.0
	3	0.044	0.071	1.0
	4	-0.03	0.071	1.0
	5	0.218	0.071	0.04
2	3	0.052	0.071	1.0
	4	-0.022	0.071	1.0
	5	0.226	0.071	0.03
3	4	-0.074	0.071	1.0
	5	0.174	0.071	0.19
4	5	0.248	0.071	0.01

Table 4. Results of Pairwise Comparison of Different Groups of Laser Radiation After 24 and 72 Hours in Terms of Cell Viability

Group	Time	M	SD	*P* Value
1	24 and 72 hours	-0.184	0.071	0.01
2	24 and 72 hours	-0.202	0.071	0.007
3	24 and 72 hours	-0.01	0.071	0.89
4	24 and 72 hours	-0.192	0.071	0.01
5	24 and 72 hours	0.016	0.071	0.82

study also investigated the effect of low-level 808 diode laser radiation, which has many other clinical uses, on the proliferation of human chondrocytes.

The low-power red or near-infrared laser can prevent cell apoptosis and improve cell proliferation. Minar et al investigated the effect of diode 808 nm indium gallium arsenide (InGaAs) laser on the proliferation and synthesis of glycosaminoglycans (sGAG) in rabbit articular cartilage cells and concluded that although the diode 808 nm InGaAs laser with output power of 1.0 W cannot stimulate cell proliferation, it has no inhibitory effect on cell function and metabolism, and in contrast, it can increase the activity of cell secretion and sGAG deposition.[14]

As mentioned earlier, the most important reason for the loss of cell viability of chondrocytes is the low blood flow of cartilage tissue, and the effectiveness of the laser in cartilage repair is due to its effects on the tissue (e.g. promoting angiogenesis) which leads to the improvement of cartilage tissue repair. Even some previous studies have attributed the results such as the growth of the lower jaw or the treatment of temporomandibular disorders to the proliferation of chondrocytes.[3,15,16] Given that the present study was conducted on human chondrocytes in vitro, the study results cannot be compared with previous studies that have investigated the effects of lasers on cartilage tissue.

A low-level laser has proven to have effects on tissue and cell treatments. In many studies, it was attempted to identify the optimal irradiation intensity and parameters to achieve the maximum biological stimulation of the target cells in terms of wavelength, irradiation power, and irradiation time. At the same time, the range of low-level laser energy density to increase proliferation in different cells is equal to 0.4-5.20 J/cm² and its visible spectrum is from 600 to 700 nm.[17] In the present study, we used a 1-5 J/cm² diode low-level laser at powers of 0.2 and 0.4 W for 5, 10, 15, 12, and 13 seconds to induce the proliferation of chondrocytes. These parameters were within the range of previous reports to increase the proliferation of chondrocytes.[10] Based on previous evidence, different effects of this amount of energy have been observed on tissue and chondrocytes. Adjusting the pattern of heat distribution in the tissues after laser irradiation leads to the induction of controlled physical effects. Exposure has to be within a reasonable range; more or less than the necessary exposure can cause significant effects on clinical results.[17] Laser energy in low ranges has stimulated articular cartilage repair.[18,19] In this regard, in a study conducted on the effects of Nd:YAG and Ho:YAG, a positive correlation was shown between tissue metabolism and synovial cells at low irradiation energies.[20] In a study by Karic, LLLI led to the differentiation of stem cells into chondrocytes and fibroblasts.[21] Yang et al reported that by 3.58 J/cm² irradiation for 5 minutes, the survival rate of chondrocytes was significantly higher than the control group. In contrast to the results of the above studies, the results of this study revealed that the survival rate of chondrocytes after laser irradiation was not significantly higher compared to the control group in any of the groups under irradiation of 1 to 5 J/cm². Considering the effect of irradiation time on cell vitality,[10] one possible reason for the difference between the results of this study and the results of Yang and colleagues' study can be due to the difference in irradiation time.

To evaluate the survival rate of the cells after laser irradiation, chondrocytes in each group were irradiated only once by the diode laser but with different energy levels in each group. Given that the biochemical effects of LLLI are cumulative, it is expected that the effect of the laser on the tissue will increase by repeating the irradiation over several days to increase the life of the chondrocyte. In confirmation of this, Yang et al showed that chondrocytes in the groups that were exposed to laser irradiation several times had a higher proliferation rate.[10] In the present study, given that the proliferation rate of chondrocytes in the 72-hour group was higher than that in the 24-hour group, it is expected that the effect of the laser on the proliferation of chondrocytes will significantly increase by repeating the exposure and evaluating the effects during longer periods.

The differences in the results of our study compared to Yang and colleagues' study can also be attributed to the environmental conditions of the chondrocytes, in that the first one was in vitro but the latter one was on the intercellular matrix of cartilage tissue. Chondrocytes in the cartilage tissue are in a condition with relatively less oxygen,[1] and generally, old chondrocytes of cartilages have few organelles but have many free ribosomes. These cells are relatively inactive and have a low metabolism rate but can be reactivated by increasing protein synthesis.[22] Thus, it is possible that laser irradiation could have a more significant effect on relatively inactive chondrocytes of cartilage tissue in comparison to chondrocytes in an ideal culture medium of an in vitro environment.

So far, some evidence has been presented about the effect of low-power lasers on chondrocytes. Spivak and Holden showed that as a result of LLLI, the synthesis of the intercellular matrix in cartilage increased.[23,24] Bayat et al and Ebert et al also stated that the change in metabolism and ultrastructural structures was the cause of the effect of lasers on the growth and survival of chondrocytes.[25,26] Sakata et al mentioned that the survival of chondrocytes after LLLI happens due to the reduction of inflammatory cytokines.[27] Karic et al showed that diode laser irradiation can differentiate stem cells into fibroblasts and chondrocytes.[21] Some studies also showed the effect of lasers on chondrocytes through such pieces of evidence as mandible growth or cartilage epiphysis changes.[28,29]

LLLI regulates the differentiation and proliferation of chondrocytes in the culture medium and it also improves cell viability and differentiation.[10] On the other hand, LLLI stimulates cell proliferation through the induction of regular proteins in cell cycle, moreover the ability to induce in vitro cell differentiation following laser irradiation has also been reported.[30] LLLI can reduce the manifestations of inflammatory cytokines in normal human joint chondrocytes through the regulation of NF-κB, so it can be used for cartilage repair treatment.[27] Therefore, laser irradiation has also been shown to be useful for the treatment of a degenerative TMJ disc.[21]

Mo et al, in a study on the rate of survival and regeneration of chondrocytes after cartilage deformation following diode 1460 nm laser irradiation, showed that in the low-power treatment group (0.3 and 0.5 W), all chondrocytes regenerated in 4 weeks, but in the treatment group 1 W, chondrocytes failed to regenerate during the same time.[31]

In the present study, the laser showed a negative impact on chondrocyte proliferation in short term (less than 24 hours) and led to a reduction in the number of cells in all groups, but after that a gradual increase in the number of cells happened. This positive change may occur either due to the proliferation ability of the cells in the culture medium or delayed effects of the laser.[21] To prove these two hypotheses, studies with more samples and more extended time should be conducted. Therefore, with the highest energy used in this study (5 joules), the lowest cell

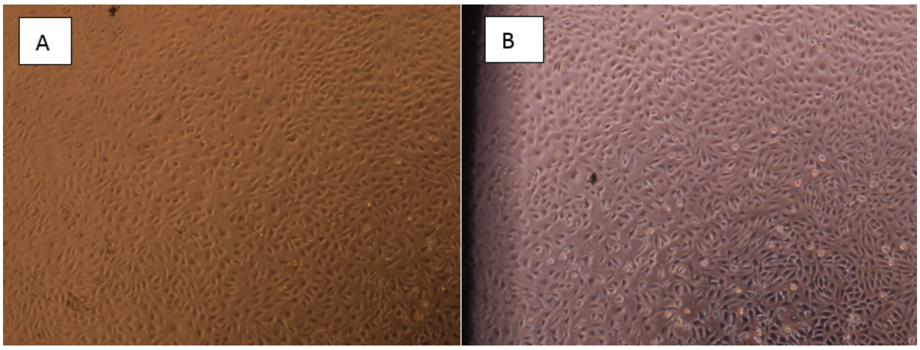

Figure 3. Chondrocytes Before (A) and After (B) Laser Irradiation in Group 4 (4 J/cm²)

viability was recorded for the 24-hour group, while no statistically significant difference was observed in the 24-hour cell viability between the rest of the studied groups with irradiation doses less than 5 joules.

In regard to cell viability, in the 72-hour incubation group, group 4 showed the highest cell viability in a way that the number of cells increased compared to the control group. Although this difference was not statistically significant, it seems that the irradiation protocol of 4 J/cm² (0.3 W, 13 seconds) is more effective than the others in terms of the cell viability of human chondrocytes (Figure 3). Also, the lowest cell viability was observed in group 5; considering the 24-hour cell viability of this group, this result was predictable. Spivak et al investigated the effects of Nd:YAG laser irradiation on adult articular cartilage in vitro and showed no significant difference in terms of cell count or morphology between the control and experimental group samples after irradiation,[24] which is inconsistent with other study results.

Conclusion

In general, regardless of the incubation time, the diode 808 nm LLLI at doses of 5 J/cm² and less did not show a significant increase in the proliferation of chondrocytes. However, the irradiation regime of 4 J/cm² of the 72-hour incubation group showed the best effect on the survival rate of chondrocytes among all the groups.

Authors' Contribution

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Methodology: Fahimeh Anbari, Hamidreza Khalighi, Maryam Baharvand.
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Visualization: Fahimeh Anbari, Maryam Baharvand, Hamidreza Khalighi, Sahba Khosousi sani.
Writing–original draft: Fahimeh Anbari, Sahba Khosousi sani, Mani Sharaki, Mohammad Khosousi sani.
Writing–review & editing: Fahimeh Anbari, Mani Sharaki.

Competing Interests

None declared.

Ethical Approval

The research protocol was reviewed and approved by the Medical Research Ethics Committee of Shahid Beheshti University of Medical Sciences (ethics committee code: IR.SBMU.DRC.REC.1400.086).

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References

1. Mescher AL. Junqueira's Basic Histology: Text and Atlas. 13th ed. New York: McGraw-Hill Medical; 2013.
2. Nanci A. Ten Cate's Oral Histology Development, Structure and Function. Elsevier Health Sciences; 2017.
3. Zwiri A, Alrawashdeh MA, Khan M, Ahmad W, Kassim NK, Ahmed Asif J, et al. Effectiveness of the laser application in temporomandibular joint disorder: a systematic review of 1172 patients. Pain Res Manag. 2020;2020:5971032. doi: 10.1155/2020/5971032.
4. Machon V, Levorova J, Hirjak D, Drahos M, Foltan R. Temporomandibular joint disc perforation: a retrospective study. Int J Oral Maxillofac Surg. 2017;46(11):1411-6. doi: 10.1016/j.ijom.2017.05.008.
5. Sobol E, Shekhter A, Guller A, Baum O, Baskov A. Laser-induced regeneration of cartilage. J Biomed Opt. 2011;16(8):080902. doi: 10.1117/1.3614565.
6. Xu GZ, Jia J, Jin L, Li JH, Wang ZY, Cao DY. Low-level laser therapy for temporomandibular disorders: a systematic review with meta-analysis. Pain Res Manag. 2018;2018:4230583. doi: 10.1155/2018/4230583.
7. Reddy S, Pedowitz DI, Parekh SG, Sennett BJ, Okereke E. The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. Am J Sports Med. 2007;35(1):80-5. doi: 10.1177/0363546506290986.
8. Bexkens R, Ogink PT, Doornberg JN, Kerkhoffs G, Eygendaal D, Oh LS, et al. Donor-site morbidity after osteochondral autologous transplantation for osteochondritis dissecans of

the capitellum: a systematic review and meta-analysis. Knee Surg Sports Traumatol Arthrosc. 2017;25(7):2237-46. doi: 10.1007/s00167-017-4516-8.

9. Borakati A, Mafi R, Mafi P, Khan WS. A systematic review and meta-analysis of clinical trials of mesenchymal stem cell therapy for cartilage repair. Curr Stem Cell Res Ther. 2018;13(3):215-25. doi: 10.2174/1574888x12666170915120620.
10. Yang X, Liu TC, Liu S, Zhu W, Li H, Liang P, et al. Promoted viability and differentiated phenotype of cultured chondrocytes with low-level laser irradiation potentiate efficacious cells for therapeutics. Front Bioeng Biotechnol. 2020;8:468. doi: 10.3389/fbioe.2020.00468.
11. Bjordal JM, Couppé C, Chow RT, Tunér J, Ljunggren EA. A systematic review of low-level laser therapy with location-specific doses for pain from chronic joint disorders. Aust J Physiother. 2003;49(2):107-16. doi: 10.1016/s0004-9514(14)60127-6.
12. Goldring MB, Birkhead JR, Suen LF, Yamin R, Mizuno S, Glowacki J, et al. Interleukin-1 beta-modulated gene expression in immortalized human chondrocytes. J Clin Invest. 1994;94(6):2307-16. doi: 10.1172/jci117595.
13. Adolphe M, Thenet-Gauci S, Demignot S. Chondrocyte culture: a target system to evaluate: pharmacotoxicological effects of drugs. In: In Vitro Methods in Pharmaceutical Research. Academic Press; 1997. p. 181-207.
14. Minar M, Hwang YW, Choi SH, Kim G. In vitro effect of 808-nm diode laser on proliferation and glycosaminoglycan synthesis of rabbit articular chondrocytes. J Vet Clin. 2015;32(4):295-300. doi: 10.17555/jvc.2015.08.32.4.295.
15. El-Bialy T, Alhadlaq A, Felemban N, Yeung J, Ebrahim A, Hassan AH. The effect of light-emitting diode and laser on mandibular growth in rats. Angle Orthod. 2015;85(2):233-8. doi: 10.2319/030914-170.1.
16. Tam SY, Tam VC, Ramkumar S, Khaw ML, Law HK, Lee SW. Review on the cellular mechanisms of low-level laser therapy use in oncology. Front Oncol. 2020;10:1255. doi: 10.3389/fonc.2020.01255.
17. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci. 2012;27(1):237-49. doi: 10.1007/s10103-011-0885-2.
18. Dew DK, Supik L, Darrow CR 2nd, Price GF. Tissue repair using lasers: a review. Orthopedics. 1993;16(5):581-7. doi: 10.3928/0147-7447-19930501-11.
19. Schultz RJ, Krishnamurthy S, Thelmo W, Rodriguez JE, Harvey G. Effects of varying intensities of laser energy on articular cartilage: a preliminary study. Lasers Surg Med. 1985;5(6):577-88. doi: 10.1002/lsm.1900050606.
20. Vangsness CT Jr, Ghaderi B. A literature review of lasers and articular cartilage. Orthopedics. 1993;16(5):593-8. doi: 10.3928/0147-7447-19930501-13.
21. Karic V, Chandran R, Abrahamse H. Laser-induced differentiation of human adipose-derived stem cells to temporomandibular joint disc cells. Lasers Surg Med. 2021;53(4):567-77. doi: 10.1002/lsm.23332.
22. Poole CA. Articular cartilage chondrons: form, function and failure. J Anat. 1997;191(Pt 1):1-13. doi: 10.1046/j.1469-7580.1997.19110001.x.
23. Holden PK, Li C, Da Costa V, Sun CH, Bryant SV, Gardiner DM, et al. The effects of laser irradiation of cartilage on chondrocyte gene expression and the collagen matrix. Lasers Surg Med. 2009;41(7):487-91. doi: 10.1002/lsm.20795.
24. Spivak JM, Grande DA, Ben-Yishay A, Menche DS, Pitman MI. The effect of low-level Nd:YAG laser energy on adult articular cartilage in vitro. Arthroscopy. 1992;8(1):36-43. doi: 10.1016/0749-8063(92)90133-v.
25. Bayat M, Ansari E, Gholami N, Bayat A. Effect of low-level helium-neon laser therapy on histological and ultrastructural features of immobilized rabbit articular cartilage. J Photochem Photobiol B. 2007;87(2):81-7. doi: 10.1016/j.jphotobiol.2007.02.002.
26. Ebert DW, Bertone AL, Roberts C. Effect of irradiation with a low-intensity diode laser on the metabolism of equine articular cartilage in vitro. Am J Vet Res. 1998;59(12):1613-8.
27. Sakata S, Kunimatsu R, Tsuka Y, Nakatani A, Gunji H, Yanoshita M, et al. High-frequency near-infrared diode laser irradiation suppresses IL-1β-induced inflammatory cytokine expression and NF-κB signaling pathways in human primary chondrocytes. Lasers Med Sci. 2022;37(2):1193-201. doi: 10.1007/s10103-021-03371-5.
28. Seifi M, Maghzi A, Gutknecht N, Mir M, Asna-Ashari M. The effect of 904 nm low-level laser on condylar growth in rats. Lasers Med Sci. 2010;25(1):61-5. doi: 10.1007/s10103-009-0651-x.
29. Cressoni MD, Dib Giusti HH, Pião AC, de Paiva Carvalho RL, Anaruma CA, Casarotto RA. Effect of GaAlAs laser irradiation on the epiphyseal cartilage of rats. Photomed Laser Surg. 2010;28(4):527-32. doi: 10.1089/pho.2009.2572.
30. Torricelli P, Giavaresi G, Fini M, Guzzardella GA, Morrone G, Carpi A, et al. Laser biostimulation of cartilage: in vitro evaluation. Biomed Pharmacother. 2001;55(2):117-20. doi: 10.1016/s0753-3322(00)00025-1.
31. Mo JH, Kim JS, Lee JW, Chung PS, Chung YJ. Viability and regeneration of chondrocytes after laser cartilage reshaping using 1,460 nm diode laser. Clin Exp Otorhinolaryngol. 2013;6(2):82-9. doi: 10.3342/ceo.2013.6.2.82.