



Enhanced Therapeutic Efficacy of Gold Nanoparticle-Enhanced Laser Therapy for Oral Cancer: A Promising Photothermal Approach

Fatemeh Sobhanifar¹, Fatemeh Tavakoli², Hosein Eslami¹, Elahe Dalir Abdolahinia³, Farzaneh Pakdel^{1*}, Paria Motahari¹, Sana Seyyedshariatdoust¹, Farshad Barzegar¹, Nasrin Valizadeh⁴, Bahare Jafari⁵

¹Department of Oral and Maxillofacial Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

²Oral and Maxillofacial Medicine Department, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

³Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran

⁴Chemistry Department, Sciences Faculty, Azarbaijan Shahid Madani University, Tabriz, Iran

⁵School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

*Correspondence to

Farzaneh Pakdel,
Email: farzaneh_pakdel@yahoo.com

Received: January 7, 2024

Accepted: July 17, 2024

Published: September 24, 2024



Abstract

Introduction: Laser therapy employs a concentrated and slender light beam to eliminate or eradicate cancerous cells and pre-cancerous abnormalities. The specific wavelength of 808 nm light is preferentially absorbed by tumor cells compared to healthy cells. This study aimed to assess the combined therapeutic impact of laser and gold, given that gold exhibits photothermal properties when exposed to laser radiation.

Methods: In this in vitro study, two cell lines, namely healthy HuGu cells (human gingival fibroblast cells) and head and neck cancer cells (HN-5), were obtained from the Pasteur Institute. The effect of the laser diode with a density of 3 J/cm² and wavelength of 808 nm on the proliferation and the survival rate of oral squamous cell carcinoma (HN-5) and human gingival fibroblast (HUGU) was assessed in 60 seconds. MTT assay, DAPI test, and trypan blue staining were used to determine the growth and proliferation of HN-5 and HUGU cell lines.

Results: Findings showed that the laser diode along with gold decreased the rate of proliferation and survival cells in HN-5 compared to healthy cells. The changes in the cell population treated with gold and laser diode 808 were insignificant.

Conclusion: Findings reveal that using a low-power laser can effectively inhibit the growth of oral cancer cells. It seems that photothermal therapy is a novel approach to oral cancer therapy.

Keywords: Laser therapy; Oral squamous cell carcinoma; Diode laser; Cancer cells.

Introduction

Head and neck cancer (HNC) is the seventh most common cancer globally, and approximately 90% of HNCs are squamous cell carcinoma, which arises from the epithelial lining of the oral cavity, pharynx, and larynx.¹ The overall incidence of HNC continues to rise, with a predicted 30% increase annually by 2030.^{1,2} In the early stages, the basic treatment for HNC includes surgery, radiotherapy, or a combination of surgery and radiotherapy, with a good recovery rate, although they often induce functional and aesthetic disturbances simultaneously.³

Recently, with advances in medicine and dentistry, the application of lasers has significantly progressed. A photothermal mechanism is the most important laser-tissue interaction in medical applications. Photothermal interaction represents a type of interaction in which the temperature increases locally. These thermal effects are

created by both continuous and pulsed lasers. At the microscopic level, the origin of thermal effects in the tissue is the absorption of rays by tissue molecules and the conversion of this absorbed energy into heat.^{4,5}

The diode laser has been used in dental treatments in various fields. Various wavelengths of this laser are available, from 600 to 1000 nm.⁶ Low lasers are used to enhance tissue repair, reduce pain, eliminate edema, and even improve the function of damaged nerve tissue. The therapeutic effects of this method are achieved by photochemical reactions that change the permeability of the cell membrane, followed by an increase in the synthesis of mRNA and cell proliferation.^{5,7}

Thomson and colleagues' review on interventional laser surgery for premalignant disorders examined the outcomes of laser treatments performed on patients with potentially malignant disorders (PMDs) and found

that 75% of patients were disease-free, indicating the effectiveness of the intervention in eliminating the disease. However, it is important to note that unexpected HNC was identified in 12% of cases, suggesting that there may be limitations to the effectiveness of laser surgery in preventing the development of cancer. Additionally, 4.8% of cases progressed to malignancy, indicating that some patients may still experience disease progression despite the intervention.⁸

Photothermal treatment with gold nanoparticles is one of the methods for cancer treatment. Gold nanoparticles have many advantages, making them suitable for the photothermal treatment of cancer, including: (1) they can be administered into the local tumor area while minimizing non-specific distribution; (2) they can be activated via near-infrared (NIR) laser light, creating the ability to penetrate deep into biological tissues; and (3) they can be modulated to create multifaceted cancer PTT and drug delivery systems.⁹ The gold nanoparticle-mediated thermal ablation of tumors following NIR light exposure is superior to conventional techniques since it is minimally invasive and easy to apply and has a great capacity to destroy malignant lesions in critical areas where surgical removal is not possible. Radiofrequency techniques or other thermal destructive methods cannot differentiate between the tumor and the surrounding normal tissue, and they often affect the healthy tissue as well.^{10,11} Photothermal treatment employing gold nanoparticles (NIR laser radiation that excites the electrons of different atomic levels and sub-levels to the ground state by emitting energy in the form of heat) can destroy cancerous cells and cause selective necrosis at cellular levels.^{9,2-19}

Since previous studies have not investigated and compared the effect of different types of laser treatment on HNC, especially on the proliferation of HNC cells treated with gold nanoparticles, the present study investigated the effect of low-level 808-nm diode laser and gold nanoparticles on the proliferation of HNC cells.

Materials and Methods

Cell Culture

In this in vitro study, the investigated cells included two cell lines: healthy HuGu cell (human gum fibroblast cells) and the head and neck cancer (HN-5), obtained from the Pasteur Institute. The cells were removed from the nitrogen tank (-196 °C) and cultured in DMEM (Dulbecco's Modified Eagle Medium) culture medium containing 100-U/mL streptomycin/penicillin and 10% Fetal bovine serum (FBS). After three passages, the cells entered a logarithmic phase and were ready for the following analysis.

Gold Nanoparticle

The synthesis of gold nanoparticles (AuNPs) followed

the Turkevich method.²⁰ In summary, a boiling solution of HAuCl₄·3H₂O (0.25 mM) was prepared, and then 12.5 mL of trisodium citrate (40 mM) was rapidly added to it. As a result, the solution's color transitioned from yellow to dark red. The mixture was allowed to boil for 10 minutes, after which the heater was turned off, and stirring continued for an additional 15 minutes to obtain the final AuNPs.

Cells Treated in Different Groups

Then the cells were placed in the wells of the 96-well culture medium (Falcon Biosciences, Milano, Italy) with a cross-sectional area of 0.32 cm². After incubation for 24 hours, each of the healthy and cancerous cells was investigated in four treatments: (1) no intervention, (2) gold nanoparticles with different concentrations, (3) 808-nm diode laser irradiation, and (4) gold nanoparticles and then 808-nm diode laser irradiation.

Laser Radiation Condition

In the laser radiation group, the cells cultured in the osteogenic medium were exposed to diode 808-nm irradiation (Mustang 2000, Russia) with a density of 3 J/cm². Laser irradiation was carried out twice with a time interval of 48 hours for 60 seconds each time. A row was left empty between each row of the cultured ones to prevent unwanted irradiation. FDA guidelines were observed for employee safety in laser applications.

In the gold nanoparticle group, cultured cells were treated with different concentrations of gold nanoparticles (0.3, 0.7, 1, and 1.5 mg/mL). After 24 hours, the cell proliferation rate was counted by trypan blue.

In the group of gold nanoparticles and under laser irradiation, the cultured cells were treated with different concentrations of gold nanoparticles (0.3, 0.7, 1, and 1.5 mg/mL), and after 48 hours, they were irradiated twice for 60 seconds with a time interval of 48 hours.

Cell culturing and cell counting methods were used to evaluate the proliferation rate. The number of living cells was counted four times before any intervention and 24, 48, and 72 hours after the intervention by the MTT survival method.

Cell Nuclei in Healthy and Cancer Cell Lines by DAPI

Changes in the number of cell nuclei in the samples were investigated by the 4',6-diamidino-2-phenylindole (DAPI) test method on two cell lines: healthy cells HuGu and cancerous cells Hn5. First, the samples were washed with normal saline and then fixed with 4% paraformaldehyde. The fixed samples were stained with DAPI dye. The images were examined under an Olympus BX53-F fluorescent microscope.

The Number of Cells Viable by Trypan Blue

HN-5 and HUGU were cultured by 10⁴ cell density in the

96-well plate. After 48 hours of cell treatment, 50 μL of cell suspensions of each well were transferred to a new vial, and an equal volume of trypan blue dye 0.4% was added. Then, 20 μL of suspension was poured into the Neubauer counting chamber. Under converted microscopy, the number of living/dead cells was counted. Living cells were considered to have an intact cell membrane that did not allow certain dyes, such as trypan blue, to enter the cell, while dead cells did not have such characteristics. Finally, the counted number of cell viability was divided into total counted cells, and the obtained number was multiplied by 100. The whole process is shown in Figure 1.

Statistical Section

Descriptive methods (percentages and frequencies) were used to report the data. Proliferation and survival were compared by Mann-Whitney and Kruskal-Wallis tests. SPSS 24 was used for data analysis. $P < 0.05$ was considered the significance level.

Results

AuNP Characterization

The UV-Vis analysis was performed by using the Cytation™ 5 cell imaging (BioTek, Winooski, USA). Dynamic light scattering (DLS) was carried out by the Nanotracer wave (Microtrac, Pennsylvania, USA). The synthesized AuNPs

were characterized by UV-Vis spectroscopy. As shown in Figure 2a, the characteristic absorption peak was observed at 520 nm, which indicates the AuNPs were successfully synthesized. Moreover, the AuNPs size distribution was also investigated by using DLS (Figure 2b). The average diameter of AuNPs was 24.97 nm.

Proliferation Alteration in HUGU and HN-5 Cell Lines After Treatments

Proliferation results showed a significant difference between the four intervention groups in both cancerous and healthy cells. The two groups of diode laser and combined use of laser and nanoparticles showed the lowest proliferation rate in cancerous cells. The application of gold nanoparticles resulted in a significant decrease in proliferation than the group without intervention (Table 1).

In the healthy group, the lowest proliferation rate was observed in the group using gold nanoparticles. Moreover, the proliferation rate was similar in the laser group and the group with the combined application of laser and nanoparticles (Table 1).

A comparison of the results between the healthy and cancerous cells showed that with no therapeutic intervention, the number of proliferating cells in cancerous cells was significantly higher than in healthy

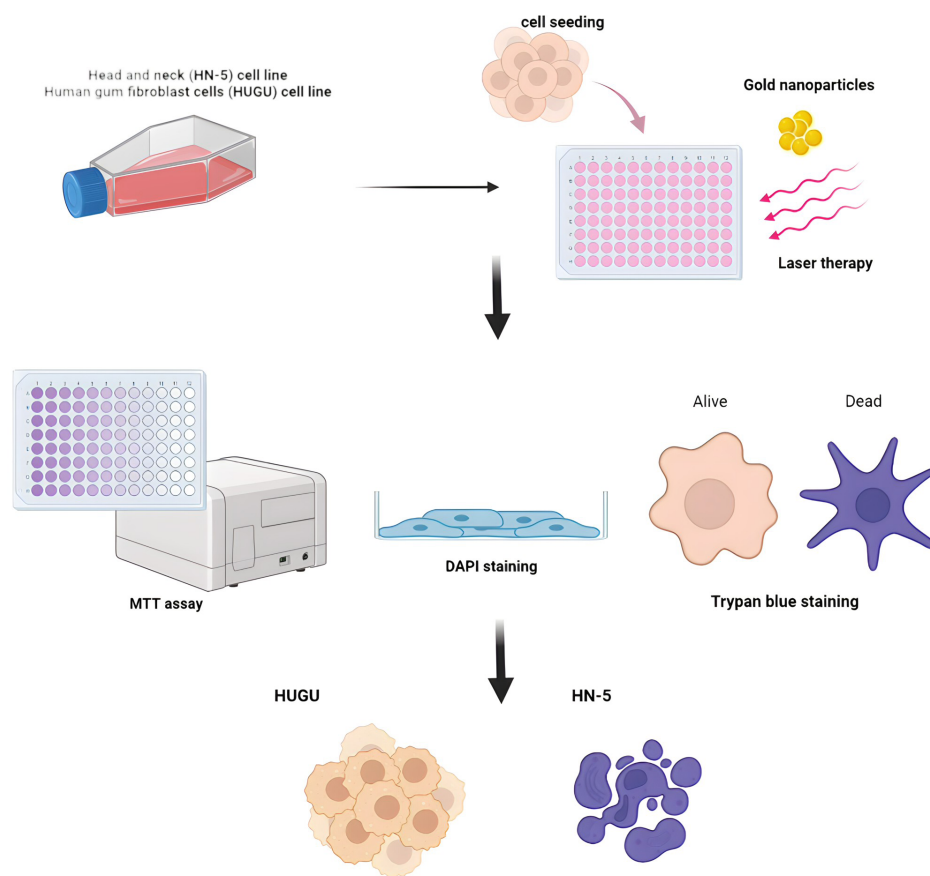


Figure 1. Cell Treating

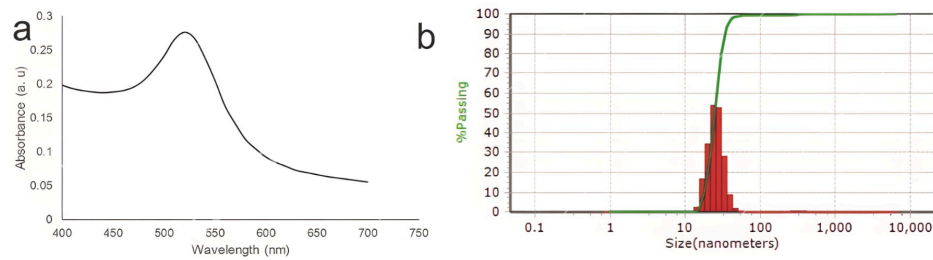


Figure 2. Characterization of AuNPs. (a) The UV-Vis absorbance spectrum of AuNPs; (b) The DLS analysis of AuNPs. AuNPs: Au nanoparticles, DLS: Dynamic light scattering

Table 1. Comparison of Mean Proliferation and Cell Survival Percentages Between Cancerous and Healthy Cells With Four Intervention Methods

		HN-5		HUGU		P Value ^a
		Mean	SD	Mean	SD	
Proliferation	Control	152.67	2.52	70.67	1.53	<0.001
	Nanoparticle	93.33	3.06	61.33	0.57	<0.001
	Diode	78.67	2.52	76.67	1.53	0.305
	Nanoparticle+ Diode	72.67	0.58	75.67	1.53	0.033
	P value ^b	<0.001		<0.001		
Viability%	Control	97.15	1.05	92.45	0.8	0.004
	Nanoparticle	88.93	0.41	78.49	0.99	<0.001
	Diode	88.99	0.44	89.13	0.6	0.755
	Nanoparticle+ Diode	64.68	2.79	74.86	1.83	0.006
	P value ^b	<0.001		<0.001		

^a Mann-Whitney, ^b Kruskal-Wallis.

cells. In the intervention with gold nanoparticles, proliferation in cancerous cells was significantly higher than in healthy cells. Diode laser irradiation resulted in similar proliferation in cancerous and healthy cells. The combined application of diode laser and gold nanoparticles gave rise to a lower proliferation of cancerous cells than healthy cells (Table 1).

Rate of Cell Survival in HN-5 and HUGU Cell Lines After Treatments

The survival test results showed a significant difference between the four intervention groups in both cancerous and healthy cells. In cancerous cells, the lowest survival rate was observed in the combined application of laser and nanoparticles. Applying only gold nanoparticles or laser irradiation resulted in a similar survival rate (Table 2). In the healthy group, the lowest proliferation rate was observed in the group with combined laser and nanoparticles. Furthermore, the survival rate of the nanoparticles group was lower than the laser group (Table 2).

According to the results obtained from the study, a comparative diagram of the proliferation rate (Figure 3) and survival rate (Figure 4) can be drawn.

Discussion

In the present study, the application of laser and gold nanoparticles, both individually and in combination (simultaneously), resulted in a more significant reduction in the proliferation of cancerous and healthy cells compared to no intervention. The results showed that the diode laser was more effective than gold nanoparticles in reducing proliferation in cancerous cells. The laser effect was so slight that adding gold nanoparticles along with the laser did not significantly reduce proliferation than the diode laser alone. However, the opposite was true in healthy cells, and gold nanoparticles were more effective in reducing proliferation than the laser. The results even indicated that laser radiation combined with gold nanoparticles increased proliferation more than nanoparticles alone.

The results showed that the application of laser and gold nanoparticles, both alone and in combination, caused a significant decrease in survival in both cancerous and healthy cells. The diode laser and gold nanoparticles had similar performance in cancerous cells, and both interventions had a similar effect on reducing survival. The combined application of two agents (diode laser and gold nanoparticles) also significantly reduced the survival rate compared to their individual applications. In other words,

Table 2. Two-by-Two Comparisons of Treatment Methods in Terms of Cell Proliferation and Survival Between Cancerous and Healthy Cells

	(I)	(J)	HN-5		HUGU	
			Mean Difference	P Value ^a	Mean Difference	P Value ^a
			(I-J)		(I-J)	
Proliferation	Control	Nanoparticle	59.333	<0.001	9.333	<0.001
	Control	Diode	74	<0.001	-6	0.004
	Control	Nanoparticle+Diode	80	<0.001	-5	0.011
	Nanoparticle	Diode	14.667	<0.001	-15.33	<0.001
	Nanoparticle	Nanoparticle+Diode	20.666	<0.001	-14.33	<0.001
	Diode	Nanoparticle+Diode	6	0.057	1	0.822
Viability	Control	Nanoparticle	8.22	0.001	13.96	<0.001
	Control	Diode	8.16	0.001	3.32	0.032
	Control	Nanoparticle+Diode	32.47	<0.001	17.59	<0.001
	Nanoparticle	Diode	-0.06	1	-10.64	<0.001
	Nanoparticle	Nanoparticle+Diode	24.25	<0.001	3.63	0.02
	Diode	Nanoparticle+Diode	24.31	<0.001	14.27	<0.001

^a Mann-Whitney.

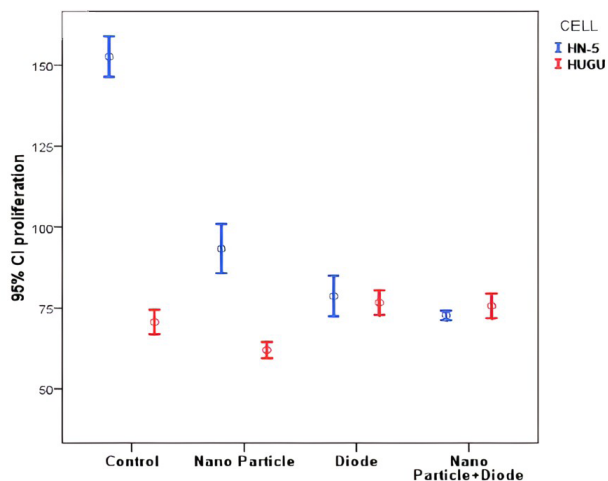


Figure 3. Comparison of the Number of Proliferating Cells Between Cancerous and Healthy Cells With Four Intervention Methods

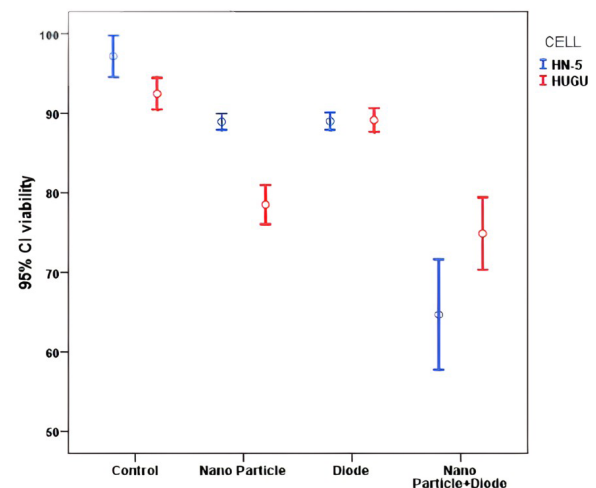


Figure 4. Comparison of Cell Survival Between Cancerous and Healthy Cells With Four Intervention Methods

in cancerous cells, the best performance in reducing the survival rate was in the simultaneous treatment of laser and nanoparticles. In healthy cells, the performance of nanoparticles in reducing survival was higher than the laser, and combining laser and nanoparticles did not show a significant effect on survival.

In animal research on mice, Dykman and Khlebtsov showed that injecting gold nanotubes modified with polyethylene glycol and laser irradiation (808 nm, 2 J/cm², 5 minutes) for 5 minutes destroyed the tumor after 72 hours. Consistent with the present study's results (in reducing the survival of cancerous cells), the performance of the combined application of nanoparticles and laser was more effective than their individual application.¹⁷ Liao et al indicated that exposing gold nanorods modified with alginate (GNR@Alg-Cys) to a diode laser (808 nm

with 2-W power) caused hyperthermia on cells and tumors, resulting in cell apoptosis and inhibition of tumor growth.¹⁸

Abdelatif Abdelaziz et al showed that adding gold nanoparticles increased the detection of squamous cell carcinoma of the tongue, especially at 635-nm laser irradiation, with 94% sensitivity, 87% specificity, and 91% overall accuracy.^{19,20}

In the present study, the combined application of laser and nanoparticles resulted in the greatest reduction in the survival of cancerous cells. Consistent with the present study, Maksimov et al, in a simulation and in vitro computer test, demonstrated effective photodestruction of cancerous cells by local injection of plasmon-resonant gold nanoparticles followed by continuous-wave (CW) semiconductor laser irradiation at 808 nm.²¹

Previous studies have reported the effect of laser on inhibiting cancerous cells. Disadvantages include the lack of selectivity and the need for a high-power laser to effectively induce tumor cell death despite its advantages. Del Vecchio et al reported that PBM had a beneficial role in treating cancer patients.²² da Silva et al, in a review study, examined the effects of low-level laser therapy on cancerous cells and reported that photobiomodulation therapy could be applied to cancer lesions to reduce the proliferation of cells.²³ Shirazian et al showed that laser irradiation at zero and 24 hours significantly inhibited oral cancer cell proliferation, especially at 660 nm/80 mW and 808 nm/200 mW.²⁴

In the present study, an 808-nm diode laser was used with a density of 3 J/cm² and two irradiations with a time interval of 48 hours, each time for 60 seconds. The results showed that the effectiveness of the diode laser in reducing the proliferation of cancerous cells was more than that of gold nanoparticles. However, this issue did not hold in healthy cells.

Thomson et al performed laser surgery on 590 patients with oral malignant disorders and indicated that interventional laser surgery facilitated definitive diagnosis and treatment and enabled early detection of Oral squamous cell carcinoma (OSCC). However, it did not stop carcinogenesis.⁸

Although studying cancerous cells under laboratory culture environments cannot exhibit the complex environment in the progressing host tumors, it is the simplest and safest method to investigate cellular processes such as progression and invasion.

Adah et al (oral cancerous cells) and de Castro et al (oral cancerous cells) reported opposite results and showed that low-level lasers increased the proliferation of malignant cells. According to the results of the above studies, low-level laser therapy promoted cell proliferation and invasion. The biostimulatory effect of low-level laser therapy induced an adverse increase in tumor progression in neoplastic diseases since malignant cells are more sensitive to proliferative stimuli.^{25,26}

Although Martins et al reported that photobiomodulation did not affect oral squamous cell carcinoma, as their study aimed to examine the effect of photobiomodulation treatment on the biology and behavior of head and neck squamous cell carcinoma cell lines, it was observed that in vitro administration of photobiomodulation (660-nm diode laser with a power of 100 mW) for squamous cell carcinoma of the head and neck did not change the behavior of tumor cells (cell migration, survival, and oncogenically transformed cancer stem cell (CSC) percentage).²⁷ Differences in the intensity and time of irradiation and the type of cancerous cells examined account for different results.

Bamps et al reported that tumor cells could be stimulated in a low-level laser radiation field. This study

aimed to stimulate the proliferation of head and neck squamous cell cancerous cells with low-level laser therapy. It was reported that low-level laser therapy increased cell proliferation dose-dependently in HNC cells. These researchers showed that low-power laser therapy should be applied cautiously in treating oropharyngeal mucositis in head and neck squamous cell cancer patients since the tumor cells can be stimulated in the low-level laser radiation field.²⁸

The present study showed that the effect of nanoparticles on both proliferation and survival in healthy cells was more significant than the laser. Laser irradiation combined with gold nanoparticles increased proliferation compared to the application of gold nanoparticles alone. The performance of nanoparticles in reducing survival was also more significant than the laser. However, in cancerous cells, the effect of diode laser on reducing proliferation was greater than gold nanoparticles. However, the laser and gold nanoparticles exhibited similar effects on reducing survival, and the combined application resulted in a significant reduction in cell survival than their individual application. Gold nanoparticles have shown considerable promise as light-to-heat converters, local field enhancers, drug carriers, and radiation sensitizers to effectively destroy malignant tumors.

Conclusion

The findings of the current study reveal that using a low-power laser with an energy density of 3 J/cm² at a wavelength of 808 nm can effectively inhibit the growth of oral cancer cells. In addition, the study found that gold nanoparticles had a stronger impact on healthy cell survival and proliferation compared to laser treatment alone. The combination of gold nanoparticles and laser treatment was particularly effective in reducing cancer cell survival. This suggests that gold nanoparticles could be a promising option for cancer therapy.

Moreover, the duration of laser exposure proved crucial to its efficacy. Specifically, when the laser was applied at 48-hour intervals, it demonstrated a significant inhibitory effect on cell proliferation compared to other time points under investigation. Further research is required to explore this aspect more comprehensively.

Acknowledgments

The authors thank the Vice-Chancellor of Tabriz University of Medical Science for supporting this research. This manuscript is based on the thesis written by Dr. Fatemeh Sobhanifar.

Authors' Contribution

Conceptualization: Hosein Eslami, Farzaneh Pakdel.

Data curation: Fatemeh Sobhanifar.

Formal analysis: Elahe Dalir Abdolahinia, Paria Motahari.

Investigation: Fatemeh Sobhanifar.

Methodology: Farzaneh Pakdel.

Project administration: Fatemeh Sobhanifar.

Resources: Sana Seyyedshariatdoust, Farshad Barzegar.

Software: Fatemeh Tavakoli.

Supervision: Hosein Esлами, Nasrin Valizadeh.

Validation: Fatemeh Tavakoli.

Visualization: Nasrin Valizadeh.

Writing—original draft: Fatemeh Sobhanifar.

Writing—review & editing: Fatemeh Tavakoli, Bahare Jafari.

Competing Interests

The authors have no conflict of interest to declare.

Ethical Approval

The study has been approved by the ethical committee of Tabriz University of Medical Science by the ethical code of IR.TBZMED.VCR.REC.1401.223.

Funding

There is no funding to declare.

References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-49. doi: [10.3322/caac.21660](https://doi.org/10.3322/caac.21660).
- Johnson DE, Burtneš B, Leemans CR, Lui VW, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nat Rev Dis Primers.* 2020;6(1):92. doi: [10.1038/s41572-020-00224-3](https://doi.org/10.1038/s41572-020-00224-3).
- Wang K, Tepper JE. Radiation therapy-associated toxicity: etiology, management, and prevention. *CA Cancer J Clin.* 2021;71(5):437-54. doi: [10.3322/caac.21689](https://doi.org/10.3322/caac.21689).
- Azadgoli B, Baker RY. Laser applications in surgery. *Ann Transl Med.* 2016;4(23):452. doi: [10.21037/atm.2016.11.51](https://doi.org/10.21037/atm.2016.11.51).
- Ren Y, Yan Y, Qi H. Photothermal conversion and transfer in photothermal therapy: from macroscale to nanoscale. *Adv Colloid Interface Sci.* 2022;308:102753. doi: [10.1016/j.cis.2022.102753](https://doi.org/10.1016/j.cis.2022.102753).
- Wyman A, Duffy S, Sweetland HM, Sharp F, Rogers K. Preliminary evaluation of a new high power diode laser. *Lasers Surg Med.* 1992;12(5):506-9. doi: [10.1002/lsm.1900120509](https://doi.org/10.1002/lsm.1900120509).
- Moskvin SV. Low-level laser therapy and light energy. *Photobiomodul Photomed Laser Surg.* 2019;37(5):267-8. doi: [10.1089/photob.2019.4622](https://doi.org/10.1089/photob.2019.4622).
- Thomson PJ, Goodson ML, Cocks K, Turner JE. Interventional laser surgery for oral potentially malignant disorders: a longitudinal patient cohort study. *Int J Oral Maxillofac Surg.* 2017;46(3):337-42. doi: [10.1016/j.ijom.2016.11.001](https://doi.org/10.1016/j.ijom.2016.11.001).
- Akhter S, Ahmad MZ, Ahmad FJ, Storm G, Kok RJ. Gold nanoparticles in theranostic oncology: current state-of-the-art. *Expert Opin Drug Deliv.* 2012;9(10):1225-43. doi: [10.1517/17425247.2012.716824](https://doi.org/10.1517/17425247.2012.716824).
- Vigderman L, Zubarev ER. Therapeutic platforms based on gold nanoparticles and their covalent conjugates with drug molecules. *Adv Drug Deliv Rev.* 2013;65(5):663-76. doi: [10.1016/j.addr.2012.05.004](https://doi.org/10.1016/j.addr.2012.05.004).
- Dykman L, Khlebtsov N. Gold nanoparticles in biomedical applications: recent advances and perspectives. *Chem Soc Rev.* 2012;41(6):2256-82. doi: [10.1039/c1cs15166e](https://doi.org/10.1039/c1cs15166e).
- Iancu C, Ilie IR, Georgescu CE, Ilie R, Biris AR, Mocan T, et al. Applications of nanomaterials in cell stem therapies and the onset of nanomedicine. *Part Sci Technol.* 2009;27(6):562-74. doi: [10.1080/02726350903328985](https://doi.org/10.1080/02726350903328985).
- Ilie I, Ilie R, Mocan T, Bartos D, Mocan L. Influence of nanomaterials on stem cell differentiation: designing an appropriate nanobiointerface. *Int J Nanomedicine.* 2012;7:2211-25. doi: [10.2147/ijn.s29975](https://doi.org/10.2147/ijn.s29975).
- Abdelhamid S, Saleh H, Abdelhamid M, Gohar A, Youssef T. Laser-induced modifications of gold nanoparticles and their cytotoxic effect. *J Biomed Opt.* 2012;17(6):068001. doi: [10.1117/1.jbo.17.6.068001](https://doi.org/10.1117/1.jbo.17.6.068001).
- Huo S, Ma H, Huang K, Liu J, Wei T, Jin S, et al. Superior penetration and retention behavior of 50 nm gold nanoparticles in tumors. *Cancer Res.* 2013;73(1):319-30. doi: [10.1158/0008-5472.can-12-2071](https://doi.org/10.1158/0008-5472.can-12-2071).
- Jain S, Hirst DG, O'Sullivan JM. Gold nanoparticles as novel agents for cancer therapy. *Br J Radiol.* 2012;85(1010):101-13. doi: [10.1259/bjr/59448833](https://doi.org/10.1259/bjr/59448833).
- Dykman LA, Khlebtsov NG. Gold nanoparticles in biology and medicine: recent advances and prospects. *Acta Naturae.* 2011;3(2):34-55.
- Liao YT, Liu CH, Chin Y, Chen SY, Liu SH, Hsu YC, et al. Biocompatible and multifunctional gold nanorods for effective photothermal therapy of oral squamous cell carcinoma. *J Mater Chem B.* 2019;7(28):4451-60. doi: [10.1039/c9tb00574a](https://doi.org/10.1039/c9tb00574a).
- Abdelatif Abdelaziz A, Saafan AM, Abdelgawad LM. Phototherapy with gold nanoparticles and a diode laser for oral squamous cell carcinoma of the tongue in rats. *Ann Rom Soc Cell Biol.* 2021;25(6):7570-85.
- Emami T, Madani R, Golchinfar F, Shoushtary A, Amini SM. Comparison of gold nanoparticle conjugated secondary antibody with non-gold secondary antibody in an ELISA kit model. *Monoclon Antib Immunodiagn Immunother.* 2015;34(5):366-70. doi: [10.1089/mab.2015.0021](https://doi.org/10.1089/mab.2015.0021).
- Maksimova IL, Akchurin GG, Khlebtsov BN, Terentyuk GS, Akchurin GG, Ermolaev IA, et al. Near-infrared laser photothermal therapy of cancer by using gold nanoparticles: computer simulations and experiment. *Med Laser Appl.* 2007;22(3):199-206. doi: [10.1016/j.mla.2007.09.004](https://doi.org/10.1016/j.mla.2007.09.004).
- Del Vecchio A, Tenore G, Luzi MC, Palaia G, Mohsen A, Pergolini D, et al. Laser photobiomodulation (PBM)—a possible new frontier for the treatment of oral cancer: a review of in vitro and in vivo studies. *Healthcare.* 2021;9(2):134. doi: [10.3390/healthcare9020134](https://doi.org/10.3390/healthcare9020134).
- da Silva JL, Silva-de-Oliveira AF, Andraus RA, Maia LP. Effects of low-level laser therapy in cancer cells—a systematic review of the literature. *Lasers Med Sci.* 2020;35(3):523-9. doi: [10.1007/s10103-019-02824-2](https://doi.org/10.1007/s10103-019-02824-2).
- Shirzian S, Keykha E, Pourshahidi S, Ebrahimi H. Effects of 660 nm and 810 nm low-power diode laser on proliferation and invasion of oral cancer cells in cell culture media. *Photochem Photobiol.* 2021;97(3):618-26. doi: [10.1111/php.13351](https://doi.org/10.1111/php.13351).
- Adah O. The Effects of Genistein, Thymoquinone, Epigallocatechin-3-Gallate, 5-Fluorouracil, and Low-Level Laser Therapy on FaDu Hypopharyngeal and Laryngeal Carcinoma Cells [dissertation]. University of Mississippi; 2016.
- de Castro JL, Pinheiro AL, Werneck CE, Soares CP. The effect of laser therapy on the proliferation of oral KB carcinoma cells: an in vitro study. *Photomed Laser Surg.* 2005;23(6):586-9. doi: [10.1089/pho.2005.23.586](https://doi.org/10.1089/pho.2005.23.586).
- Martins MD, Silveira FM, Webber LP, Wagner VP, Martins MA, Squarize CH, et al. The impact of photobiomodulation therapy on the biology and behavior of head and neck squamous cell carcinomas cell lines. *J Photochem Photobiol B.* 2020;209:111924. doi: [10.1016/j.jphotobiol.2020.111924](https://doi.org/10.1016/j.jphotobiol.2020.111924).
- Bamps M, Dok R, Nuyts S. Low-level laser therapy stimulates proliferation in head and neck squamous cell carcinoma cells. *Front Oncol.* 2018;8:343. doi: [10.3389/fonc.2018.00343](https://doi.org/10.3389/fonc.2018.00343).