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Modulating Inflammation and Enhancing Cell Viability: A Study on 980 nm and 660 nm Laser Irradiation in Synovial Cells

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Abstract

Introduction: Photobiomodulation (PBM) is a non-invasive approach known to influence cellular activity and reduce inflammation. This study investigated the comparative effects of 980 nm and 660 nm PBM on human synovial cells (K4IM), focusing on cell viability and the expression of inflammatory mediators within the TRPV4/PI3K/AKT/mTOR signaling pathway.

Methods: Human synovial cells were irradiated with 980 nm or 660 nm lasers for 30, 60, or 75 seconds using a Ploon device (continuous wave, 500 mW, 1 cm^2). Cell viability was measured using the MTT assay. Gene expression levels of TRPV4, PI3K, AKT, mTOR, IL6, IL8, and IL10 were quantified via Real-Time PCR following RNA extraction and cDNA synthesis using commercial kits. Statistical analyses were performed using SPSS 22 and Excel 2021.

Results: Both wavelengths significantly enhanced cell viability, with the strongest effect observed at 60 seconds ($P<0.001$). A slight, non-significant increase occurred at 30 seconds, while 75 seconds produced a marked improvement compared to 30 seconds ($P<0.001$). PBM at both wavelengths upregulated TRPV4, PI3K, AKT, mTOR, IL8, and IL10, while downregulating IL6. AKT expression was significantly elevated across all treated groups ($P<0.0001$). The 660 nm wavelength demonstrated comparable efficacy to 980 nm.

Conclusion: PBM at 660 nm and 980 nm enhances synovial cell viability and modulates inflammation through activation of the TRPV4/PI3K/AKT/mTOR pathway. These findings highlight the importance of wavelength and exposure duration in optimizing PBM therapeutic protocols.

Keywords: Low-level laser therapy, 980 nm, Synovial inflammation, Cytokine modulation, TRPV4/PI3K/AKT/mTOR pathway

Introduction

Inflammation is a complex and essential biological response that plays a critical role in protecting the body against infections and injuries.[1] Over time, the conceptual understanding of inflammation has evolved from its rudimentary depiction in ancient medical texts to a sophisticated framework grounded in molecular and cellular biology. Seminal observations by Virchow and others established inflammation not merely as a clinical symptom but as a fundamental pathological process. This tightly regulated response is designed to restore tissue integrity and maintain physiological homeostasis following injury or infection.[2] However, when dysregulated and sustained chronically, inflammation can become a major contributor to disease pathogenesis. Chronic inflammation, often triggered by persistent infections, autoimmune dysfunctions, or environmental stressors, is now recognized as a key etiological factor in a wide range of conditions, including cardiovascular diseases, type 2 diabetes, various malignancies, and autoimmune disorders such as rheumatoid arthritis.[3, 4] These associations highlight the central role of chronic inflammation in disease progression and underscore the urgent need to elucidate its underlying mechanisms to facilitate the discovery of novel therapeutic targets.[5]

Among the earliest tissues investigated in the context of chronic inflammation were synovial membranes, particularly in relation to rheumatoid arthritis. Synovial inflammation characterized by the activation of synovial cells and the release of pro-inflammatory mediators represents a defining feature of inflammatory joint disorders. The human synovial cell line K4IM has become a valuable in vitro model for elucidating the molecular mechanisms underlying joint inflammation.[6] These cells are capable of producing a broad spectrum of inflammatory mediators, including cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), as well as chemokines, growth factors, and

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matrix metalloproteinases. These mediators were first implicated in cartilage degradation in studies from the late 20th century, which demonstrated their involvement in extracellular matrix breakdown and joint destruction.[7] Furthermore, lipid-derived signaling molecules, such as prostaglandins and leukotrienes, serve to amplify pro-inflammatory cascades and nociceptive transmission, thereby exacerbating disease severity and intensifying clinical symptoms. Oxidative stress, driven by the generation of reactive oxygen and nitrogen species by activated immune cells, compounds the complexity of this process by inducing direct tissue damage and perpetuating the inflammatory cycle.[8]

The study of intracellular signaling networks in inflammation has expanded dramatically over the past two decades. These networks translate extracellular cues into targeted molecular responses that govern gene expression, cytokine production, and immune cell behavior. Among these, the TRPV4/PI3K/AKT/mTOR signaling axis has emerged as a central regulator of inflammation and immune function. TRPV4, identified in the early 2000s as a mechanosensitive ion channel, modulates calcium signaling and cellular stress responses. Its downstream effectors PI3K, AKT, and mTOR were initially characterized in cancer biology but have since been implicated in inflammatory regulation. Unusual modulation of this signaling axis has been mechanistically connected to the onset and progression of a wide spectrum of inflammatory pathologies, positioning it as a compelling molecular target for therapeutic intervention and drug development.[9-11]

Over the past several decades, the medical landscape has increasingly embraced non-invasive therapeutic modalities. Among these, low-level laser therapy (LLLT), now commonly referred to as photobiomodulation (PBM), has gained attention for its ability to modulate inflammatory signaling networks without inducing thermal damage. The therapeutic use of light dates back to the pioneering work of Endre Mester in the 1960s, who first observed accelerated wound healing in laser-treated rats. Since then, PBM has evolved into a scientifically grounded technique that employs specific wavelengths to stimulate cellular functions. Photobiomodulation is postulated to exert therapeutic effects by recalibrating intracellular signaling pathways that coordinate inflammatory modulation, oxidative homeostasis, and tissue regeneration. Through its interaction with key molecular networks, PBM may facilitate the resolution of pathophysiological disruptions associated with chronic injury and inflammation. Notably, photobiomodulation using a wavelength of 980 nm has demonstrated potential in regulating critical inflammatory pathways, including the TRPV4/PI3K/AKT/mTOR axis, thereby mitigating inflammation and enhancing tissue viability.[12-14]

Emerging preclinical insights underscore the therapeutic promise of PBM in regulating chronic inflammation, with reported effects including enhanced cellular resilience, balanced cytokine secretion, and attenuation of pro-inflammatory molecular circuits.[15] However, despite decades of research, the mechanistic landscape remains largely unresolved. Gaps persist in our understanding of intracellular signaling dynamics and gene-level interactions driving PBM's efficacy. Moreover, the lack of standardized irradiation protocols covering dosage, spectral properties, and exposure parameters has hindered clinical harmonization. These uncertainties collectively emphasize the necessity for rigorous experimental validation and expansive clinical studies to authenticate PBM's safety and therapeutic utility across diverse inflammatory models.[16,17]

Human synovial cells, particularly the K4IM cell line, represent a well-established and extensively characterized model system for exploring the effects of PBM on inflammatory processes. Derived from synovial tissues, these cells exhibit a remarkable capacity to produce and secrete a diverse array of inflammatory mediators. Notably, they retain their phenotypic characteristics under in vitro conditions, rendering them an optimal model for investigating the molecular and cellular mechanisms underlying synovial inflammation. The utilization of K4IM cells in this study enables a controlled and systematic analysis of the effects of 980 nm photobiomodulation on cellular viability and inflammatory responses, with a specific emphasis on the TRPV4/PI3K/AKT/mTOR signaling pathway.[18] This study endeavors to bridge existing knowledge gaps by examining the therapeutic potential of 980 nm and 660 nm PBM in modulating inflammatory responses within human synovial cells. Specifically, the research aims to assess the comparative impact of these wavelengths on cellular viability and the expression of critical inflammatory mediators, including cytokines and signaling molecules implicated in inflammation. By integrating cellular viability assays with detailed gene expression analyses, this investigation seeks to elucidate the molecular and cellular mechanisms underlying the effects of 980 nm and 660 nm PBM. Ultimately, the findings are anticipated to contribute to the development of innovative and effective therapeutic strategies for managing chronic inflammatory diseases, while providing a robust foundation for optimizing laser irradiation parameters for clinical applications.

Materials and Methods

Cell Culture Conditions

The human synovial cell line (K4IM), obtained from the Pasteur Institute of Iran, was employed as a model system to investigate synovial inflammation. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Kimia Pars Gene Co., Tehran, Iran), supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, USA), 1% penicillin-streptomycin (Sigma-Aldrich, Germany), and 1% L-glutamine (Razi Vaccine and Serum Research Institute, Iran). Cultures were maintained at 37°C in a humidified incubator with 5% CO_2.

To ensure optimal growth and minimize cellular stress, the cells were passaged upon reaching 70–80%

confluency. Prior to passaging, all reagents including DMEM, FBS, phosphate-buffered saline (PBS) (Kimia Pars Gene Co., Iran), and Trypsin/EDTA (1x) (Bio-Idea, Iran) were equilibrated to room temperature. The culture medium was aspirated, and residual serum was removed by rinsing the cells with PBS. Trypsin/EDTA was added to facilitate the detachment of adherent cells, followed by incubation at 37°C for 3–5 minutes. Cellular detachment was promoted by gentle tapping of the flask, after which the suspension was neutralized with DMEM containing 10% FBS.

The resulting cell suspension was centrifuged at 1500 rpm for 90 seconds at 25°C using a Hettich Rotina 380 centrifuge (Germany). The pellet was resuspended in a fresh medium and seeded into new T-25 cm^2 flasks (SPL Life Sciences, South Korea), with 4 mL of the culture medium added to each flask. Cultures were maintained under standard incubation conditions, and the medium was replaced every three days to support sustained cell growth.

Treatment and Preparation of Experimental Samples

On the first day of the experiment, blank control samples containing only the cell culture medium were prepared to serve as negative controls. Prior to the treatment, the morphology of cells in all wells was examined under a microscope to ensure cellular integrity and consistent confluency. The cell culture medium in all wells was replaced with fresh medium, and the concentrations of the experimental compounds were adjusted accordingly. The 96-well plate containing the treated samples and controls was incubated under standard culture conditions (37°C, 5% CO_2, 96% humidity) for a period of 24 to 48 hours to allow the cells to respond to the treatments.

Laser Irradiation Protocol

Low-level laser therapy (LLLT), also referred to as photobiomodulation (PBM), is a non-invasive therapeutic modality that utilizes low-intensity laser light to modulate cellular activity without inducing thermal damage. This technique has gained substantial traction in biomedical research due to its capacity to promote tissue regeneration, attenuate inflammation, and enhance cellular resilience across a variety of pathological contexts. In the present study, two distinct wavelengths 980 nm and 660 nm were selected based on their documented efficacy in penetrating biological tissues and modulating intracellular signaling pathways relevant to inflammation and cellular viability. The laser source employed was a PIoon diode laser system (manufactured in China), configured to operate in continuous wave (CW) mode. The device delivered a calibrated power output of 500 milliwatts (mW), with a beam spot size of 1 cm^2, resulting in a power density (irradiance) of 500 mW/cm^2. This configuration aligns with established photobiomodulation protocols, which typically recommend power densities below 1000 mW/cm^2 to avoid photothermal effects while ensuring biological efficacy.

Laser exposure durations were set at 30, 60, and 75 seconds, corresponding to energy densities of 15 J/cm^2, 30 J/cm^2, and 37.5 J/cm^2, respectively. These fluence values fall within the therapeutic window commonly cited in the PBM literature, where energy densities between 4–50 J/cm^2 have been shown to elicit beneficial cellular responses without inducing cytotoxicity. The laser beam was delivered perpendicularly to the culture surface at a fixed distance, ensuring uniform irradiation across the monolayer of human synovial cells (K4IM line).

To control for non-specific thermal effects, ambient temperature was continuously monitored using a digital infrared thermometer (Beurer FT 90, Germany) before and after each irradiation session. No significant temperature elevation was detected across all exposure durations, confirming the absence of photothermal artifacts. Additionally, a sham irradiation control group was included, in which cells were subjected to identical handling and positioning under the laser device with the power source disabled. This ensured that any observed biological effects could be attributed specifically to photobiomodulation rather than mechanical or environmental factors. The rationale for selecting 980 nm and 660 nm wavelengths stems from their differential tissue penetration and chromophore absorption profiles. The 660 nm wavelength, situated in the red spectrum, is known for its interaction with cytochrome c oxidase and its efficacy in superficial tissue modulation. In contrast, the 980 nm wavelength, located in the near-infrared range, penetrates deeper into tissues and has been associated with the modulation of calcium signaling and mitochondrial activity.

All laser parameters including wavelength, power output, beam size, exposure time, and mode of operation were meticulously calibrated to reflect clinically relevant dosimetry standards. This methodological rigor, combined with thermal monitoring and sham controls, ensures reproducibility and enhances the translational potential of the findings, contributing to the broader effort to standardize PBM protocols for therapeutic applications.

MTT Assay for Cellular Viability Analysis

The MTT assay was employed to evaluate the impact of 980 nm laser irradiation on the viability of human synovial cells (K4IM), leveraging its ability to measure metabolic activity as a proxy for cell viability. Adherent cells were detached using Trypsin-EDTA (Bio-Idea, Iran), neutralized with a complete culture medium (DMEM from Kimia Pars Gene Co., Iran, supplemented with 10% FBS from Gibco, USA), and centrifuged using a Hettich Rotina 380 centrifuge (Germany) to collect a homogeneous cell suspension.

The cells were counted using a Neubauer hemocytometer (Marienfeld, Germany) and seeded into a 96-well plate (SPL Life Sciences, South Korea) at a density of 10,000 cells per well. A 24-hour incubation period at 37°C in a humidified atmosphere with 5%

CO_2 was allowed for cell attachment and stabilization. Following incubation, the cells were exposed to 980 nm laser irradiation for durations of 30, 60, or 75 seconds using a PIoon diode laser system (China). The MTT assay was then performed by adding 20 µL of MTT reagent (Sigma-Aldrich, Germany) at a concentration of 5 mg/mL to each well. Metabolically active cells reduced the MTT reagent to insoluble formazan crystals during a four-hour incubation at 37°C. Formazan crystals were solubilized using 100 µL of dimethyl sulfoxide (DMSO; Merck, Germany) or isopropanol (Kimia Pars Gene Co., Iran), and optical density was measured at 570 nm using an ELx800 microplate reader (BioTek Instruments, USA). Cell viability was calculated as a percentage relative to untreated controls, providing quantitative insights into the biological effects of 980 nm laser irradiation on synovial cell metabolism and survival. This methodology ensured precise assessment of cellular responses, offering reproducible data to support the therapeutic potential of low-level laser therapy in inflammatory conditions.

RNA Isolation and Quantitative Assessment of Inflammatory Gene Expression

To elucidate transcriptomic alterations induced by 980 nm low-level laser irradiation, human synoviocytes (K4IM) were cultured under both treated and control conditions. Total RNA was extracted using a modified RNX-Plus protocol (SinaClon, Iran), incorporating phase separation with chloroform, isopropanol-induced precipitation, and ethanol purification. RNA integrity was assessed by 1% agarose gel electrophoresis, and concentration was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

Genomic DNA contamination was eliminated using DNase I (SinaClon, Iran), followed by enzyme inactivation with EDTA and heat treatment. Complementary DNA (cDNA) synthesis was performed using the Fermentas First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Each reaction contained random hexamer primers, dNTPs, RNasin (SinaClon, Iran), and reverse transcriptase, executed under optimized thermocycling conditions. Synthesized cDNA was stored at −20°C for downstream analysis. Quantitative real-time PCR (qPCR) was conducted to evaluate the transcriptional dynamics of inflammation-associated genes, including TRPV4, PI3K, AKT, mTOR, IL6, IL8, and IL10, with GAPDH serving as the internal normalization control. Reactions were prepared in a final volume of 20 microliters, comprising SYBR Green Master Mix (Hi-SYBr Master Mix, HiMedia Laboratories, India), gene-specific forward and reverse primers (designed and synthesized by Bioneer, South Korea), cDNA template, and nuclease-free water (SinaClon, Iran). Amplification was performed using a Rotor-Gene Q real-time PCR system (QIAGEN, Germany), with cycling conditions and reagent compositions detailed in Tables 1 and 2. Melt curve analysis was included to confirm amplification specificity. This approach enabled sensitive fluorescence-based quantification of target transcripts across treated and control groups, providing robust insights into the molecular effects of photobiomodulation.

Table 1. Primer sequences used for quantitative real-time PCR (qRT-PCR). This table lists the forward and reverse primer sequences designed for target genes involved in inflammation (IL6, IL8, IL10), signaling pathways (AKT, PI3K, mTOR, TRPV4), and the h

Gene	Primer Type	Sequence
mTOR	Forward	AGCATCGGATGCTTAGGAGTGG
	Reverse	CAGCCAGTCATCTTTGGAGACC
IL6	Forward	AGACAGCCACTCACCTCTTCAG
	Reverse	TTCTGCCAGTGCCTCTTTGCTG
IL10	Forward	TCTCCGAGATGCCTTCAGCAGA
	Reverse	TCAGACAAGGCTTGGCAACCCA
IL8	Forward	GAGAGTGATTGAGAGTGGACCAC
	Reverse	CACAACCCTCTGCACCCAGTTT
GAPDH	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA
AKT	Forward	TGGACTACCTGCACTCGGAGAA
	Reverse	GTGCCGCAAAAGGTCTTCATGG
PI3K	Forward	GAAGCACCTGAATAGGCAAGTCG
	Reverse	GAGCATCCATGAAATCTGGTCGC
TRPV4	Forward	TCACTCTCACCGCCTACTACCA
	Reverse	CCCAGTGAAGAGCGTAATGACC

Table 2. Reaction components and volumes used for qRT-PCR. This table outlines the composition of each 20 µL qRT-PCR reaction, including SYBR Green Master Mix, gene-specific primers, cDNA template, and RNase-free water. All reactions were performed under standard

Component	Volume per Reaction (µL)
SYBR GREEN Master Mix	10
Forward Primer	1
Reverse Primer	1
cDNA	1
RNase-Free Water	7

Statistical Analysis

The data obtained using the ΔΔCT method, also known as the Livak method, were analyzed with GraphPad Prism 5 for detailed statistical evaluation. Statistical analyses were performed using t-tests and one-way ANOVA to identify significant differences between experimental groups. A significance level of 95% was considered, and *P*-values less than 0.05 ($P<0.05$) were deemed statistically significant. Additionally, the correlation between changes in gene expression was assessed using Pearson's correlation coefficient, calculated through GraphPad Prism 5. Pearson's correlation coefficient provides a robust statistical tool to determine the type and strength of the relationship between two quantitative variables. A positive correlation coefficient indicates that an increase in one parameter is associated with an increase in the other, while a decrease in one parameter corresponds to a decrease in the other, as observed in the examined experimental conditions. Results were reported as the mean ± standard deviation (SD) to ensure clarity,

precision, and reproducibility in data presentation.

Result

Effects of 980 and 660 nm Low-Level Laser Irradiation on the Viability of Human Synovial Cells (K4IM): An MTT Assay Analysis

To evaluate the impact of low-level laser therapy (photobiomodulation) at 980 nm and 660 nm on cellular viability, human synovial cells (K4IM) were subjected to laser irradiation for three durations: 30, 60, and 75 seconds. The MTT assay was employed to assess the metabolic activity of cells as an indicator of their viability, with apoptosis induction being a key focus. The results of this analysis are depicted in Figure 1.

As shown in the upper graph, which represents the 980 nm irradiation results, a slight increase in cell viability was observed after 30 seconds of laser exposure; however, this increase was not statistically significant. Interestingly, the 60-second irradiation demonstrated the most pronounced and statistically significant enhancement of cell viability compared to the control group (P-Value < 0.001), highlighting this duration as the optimal exposure time for promoting cell survival. Furthermore, laser exposure for 75 seconds resulted in a significant increase in cell viability compared to the 30-second group (P-Value < 0.001), albeit slightly lower than the 60-second group.

For 660 nm laser exposure, the lower graph indicates that the 60-second duration produced the highest cell viability. Both 30- and 75-second durations showed a reduction in cell survival compared to the 60-second exposure, with statistical significance observed (P-Value = 0.01). This demonstrates that, similar to 980 nm, the 60-second irradiation is the optimal duration for enhancing cell viability, while the 30- and 75-second durations are less effective.

Comparison of Inflammatory Gene Expression Levels (TRPV4, PI3K, AKT, mTOR, IL6, IL8, IL10) Before and After 980 nm Low-Level Laser Therapy Using Real-Time PCR

The expression levels of key inflammatory genes (TRPV4, PI3K, AKT, mTOR, IL6, IL8, and IL10) were analyzed in human synovial cells (K4IM) before and after treatment with 980 nm low-level laser therapy (photobiomodulation). Gene expression was quantified using the Real-Time PCR technique, with results reported as cycle threshold (Ct) values, representing the point at which fluorescence surpasses the defined threshold. This analysis revealed significant differences in the expression of the targeted genes post-treatment, reflecting the modulatory effects of 980 nm photobiomodulation on inflammatory pathways. The results suggest that laser exposure induces differential regulation of pro- and anti-inflammatory genes, highlighting its therapeutic potential in reducing inflammation and supporting tissue health.

Figure 2 illustrates that treatment with 980 nm and 660 nm photobiomodulation elevated the expression of inflammatory genes TRPV4, PI3K, AKT, mTOR, IL8, and IL10 in K4IM synoviocytes versus controls. Both wavelengths modulated the TRPV4/PI3K/AKT/mTOR signaling cascade, with 980 nm notably reducing IL6 and significantly upregulating AKT (P < 0.0001), indicating an anti-inflammatory shift. Similarly, 660 nm irradiation increased PI3K, AKT, mTOR, and IL-8 expression (P = 0.001), affirming its regulatory potential. These findings highlight the therapeutic relevance of both wavelengths in modulating inflammation and enhancing

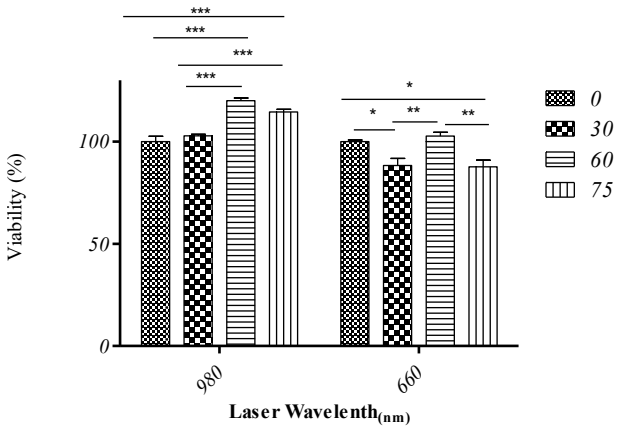

Figure 1. MTT Analysis of Human Synovial Cell Line (K4IM) Following 980 nm and 660 nm Laser Irradiation at 30, 60, and 75 Seconds. Data for three durations of irradiation: 30, 60, and 75 seconds. Statistical significance between treated groups and the control group is denoted by symbols (*, **, and ***), representing P-Value < 0.05, P-Value < 0.01, and P-Value < 0.001, respectively.

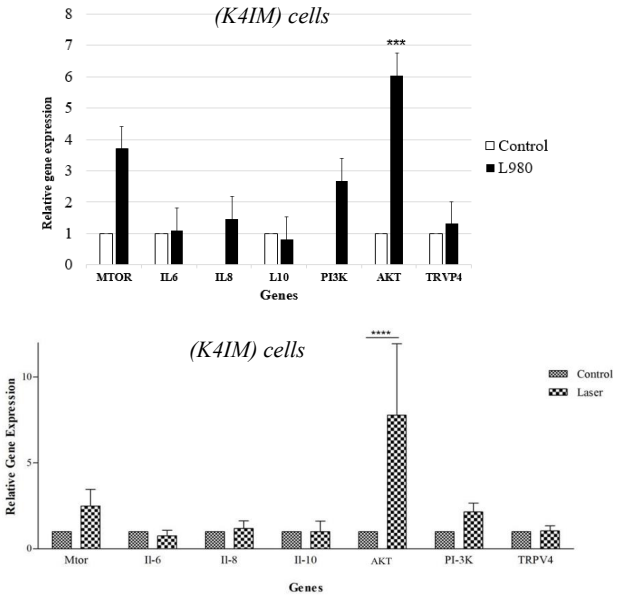

Figure 2. Comparative Expression Levels of Inflammatory Genes (TRPV4, PI3K, AKT, MTOR, IL6, IL8, IL10) in Human Synovial Cells (K4IM) Following Low-Level Laser Treatment. The upper graph represents the gene expression levels after 980 nm laser treatment, while the lower graph corresponds to the results obtained after 660 nm laser treatment.
The bar chart illustrates the relative expression of inflammatory genes (TRPV4, PI3K, AKT, MTOR, IL6, IL8, and IL10) in K4IM cells before and after treatment with 980 nm low-level laser therapy. Statistical significance is denoted by symbols (*, **, ***, ****), corresponding to P-Value thresholds of < 0.05, < 0.01, < 0.001, and < 0.0001, respectively. The results emphasize the extent of modulation in gene expression levels across the analyzed groups, indicating the therapeutic potential of the laser in regulating inflammatory pathways.

synovial cell function.

Discussion

This study examined the wavelength-specific effects of 980 nm and 660 nm photobiomodulation on K4IM synovial cells, assessing cell viability, inflammatory gene expression, and intracellular signaling. Both wavelengths improved survival and modulated cytokine profiles, with 980 nm inducing an anti-inflammatory shift via TRPV4/PI3K/AKT/mTOR and 660 nm similarly upregulating PI3K and AKT. These findings support the therapeutic potential of non-invasive PBM for mitigating synovial inflammation in RA and OA.

Enhanced Cell Viability

MTT assay findings revealed significant viability enhancement in K4IM synoviocytes exposed to 980 nm and 660 nm photobiomodulation, each exhibiting distinct temporal efficacy profiles. These wavelength-dependent kinetic patterns underscore the importance of time-sensitive dosing strategies to optimize therapeutic performance. For 980 nm photobiomodulation, the peak effect occurred at 60 seconds of irradiation ($P<0.001$). Viability at 75 seconds was significantly greater than that at 30 seconds ($P<0.001$), aligning with the biphasic dose-response relationship described by T Fan et al. (2024), which underscores the significance of optimizing irradiation parameters to maximize therapeutic consequence. Similarly, for 660 nm photobiomodulation, cell viability was enhanced at the 60-second duration, while 30- and 75-second exposures resulted in statistically significant reductions in viability (P-Value = 0.05). The observed outcomes emphasize the critical role of wavelength-specific exposure timing in maximizing the biological responsiveness of photobiomodulation therapy. The strategic calibration of irradiation duration enhances reproducibility and therapeutic consistency across experimental contexts.[19]

Supporting this, prior investigations including the study by H. Wu et al. (2012) revealed enhanced cell viability in murine tibialis anterior muscle tissue following 808 nm photobiomodulation, attributed to attenuated oxidative stress and suppression of apoptotic pathways.[20] Similarly, Fallahi et al. (2023) showed enhanced ATP production and cell survival in adipose-derived stem cells treated with 980 nm photobiomodulation, further validating the relevance of this wavelength. YS lu et al. (2023) reported similar findings in a pulmonary ischemia-reperfusion injury model, where 660 nm photobiomodulation improved mitochondrial metabolism and reduced oxidative damage. These observations are consistent with our findings, highlighting the conserved mechanisms by which photobiomodulation enhances cellular viability across various models and cell types.[21,22]

Interestingly, studies by J Robijin et al. (2022) and JF Algorri (2021) underscore the critical influence of laser parameters particularly wavelength and energy density on the biological efficacy of photobiomodulation. Bjordal et al. demonstrated that 904 nm irradiation enhanced tendon cell survival through the suppression of prostaglandin E2 synthesis, a key pro-inflammatory and pain-associated mediator. However, Alves et al. reported enhanced fibroblast viability with 780 nm photobiomodulation. These studies underline the importance of optimizing photobiomodulation protocols to achieve consistent results, a consideration supported by the time-dependent effects observed in our study.[23,24]

Modulation of Inflammatory Gene Expression

Real-time PCR analysis indicates that 980 nm and 660 nm photobiomodulation can modulate inflammatory gene expression in K4IM cells. For 980 nm, TRPV4, PI3K, AKT, and mTOR were upregulated, while IL-6 was suppressed and IL-10 enhanced, indicating immune homeostasis regulation and anti-inflammatory potential ($P<0.0001$). Similarly, 660 nm exposure increased IL-6, IL-8, and IL-10, confirming dual modulatory and reparative effects.

These findings are consistent with previous studies. N Yamauchi et al. (2022) reported reduced IL-6 levels in rheumatoid synoviocytes following 810 nm photobiomodulation, attributing the effect to NF-κB inhibition.[25] Similarly, K He et al. (2022) demonstrated a decrease in IL-6 and an increase in IL-10 in a murine tendinitis model treated with 780 nm photobiomodulation.[26] Meng et al. (2020) highlighted the role of TRPV4 in mediating anti-inflammatory effects via PI3K/AKT/mTOR signaling, aligning with the observations of our study.[27] The ability of photobiomodulation to recalibrate cytokine balance, as observed here, underscores its therapeutic potential for inflammatory joint diseases.

Additionally, Nambi et al. (2011) demonstrated reduced IL-6 levels following photobiomodulation in a murine knee inflammation model, associating the effect with decreased inflammatory cell infiltration.[28] Similar findings were reported by JH Ryo et al. (2023) and Bomfim et al. (2024) in studies of synovial and joint inflammation. These studies reinforce the broader applicability of photobiomodulation in reducing inflammation across various tissues and conditions.[29,30]

Activation of Signaling Pathways

Both 980 nm and 660 nm photobiomodulation activated the trpv4/pi3k/akt/mtor signaling cascade, as evidenced by the significant upregulation of these genes. TRPV4 stimulation facilitates calcium entry, initiating PI3K/AKT/mTOR pathway activation central to synovial cell survival and immunometabolic regulation. Following 980 nm photobiomodulation, AKT expression was markedly elevated ($P<0.0001$), with 660 nm exposure exhibiting comparable signaling enhancement.

Li et al. (2022) demonstrated that 808 nm photobiomodulation enhanced antioxidant responses and reduced inflammation via PI3K/AKT activation, consistent with our findings.[31] Similarly, Y Huang et al. (2010) linked mTOR upregulation following

photobiomodulation in a mouse traumatic brain injury model to improved neural recovery. These studies, along with those by PR Arany et al. (2022) and Salman et al. (2023), emphasize the versatility of photobiomodulation in modulating key signaling pathways across diverse cellular contexts.[32, 33]

Interestingly, AC Bahr et al. (2024) reported opposing effects in MC3T3-E1 cells, where 808 nm photobiomodulation inhibited PI3K/AKT/mTOR to promote autophagy. This contrast highlights the context-dependent nature of photobiomodulation effects, influenced by factors such as cell type, irradiation parameters, and disease state. In this research, the followed activation of this pathway aligns with its clear roles in promoting cell survival and reducing inflammation.[34]

Photobiomodulation Mechanisms

The effects of 980 nm and 660 nm photobiomodulation stem from photon absorption by mitochondria, leading to enhanced ATP and ROS generation and triggering downstream signaling cascades. These pathways drive TRPV4 overactivation and cytokine modulation via NF-κB inhibition (IL-6 suppression) and STAT3 activation (IL-10 elevation), resulting in anti-inflammatory and pro-survival cellular responses, as detailed by YE Rongan (2024) and H Saito (2024).[15, 35]

In our study, this mechanism likely underpins the observed activation of TRPV4, facilitating calcium influx and subsequent pathway activation. The dual modulation of cytokines, with IL-6 suppression mediated by NF-κB inhibition and IL-10 elevation via STAT3 activation, reflects the complexity of photobiomodulation-induced signaling. These processes contribute to the anti-inflammatory and pro-survival effects observed here.

Extracellular Matrix Properties and Their Influence on Photobiomodulation

Although the present study focused on intracellular signaling and cytokine modulation in K4IM synoviocytes, it is imperative to contextualize these findings within the framework of extracellular matrix (ECM) biology. The ECM is a critical determinant of cellular behavior, serving not only as a structural scaffold but also as a dynamic regulator of mechanotransduction, biochemical signaling, and irradiation response. Recent evidence indicates that the physical and optical properties of the ECM, particularly elastic stiffness and transparency, can significantly modulate cellular responses to both ionizing and non-ionizing radiation.

Klabukov et al. (2025) demonstrated that ECM stiffness directly influences the mechanical sensitivity of cells, thereby altering downstream signaling cascades in response to photonic stimulation. In the context of photobiomodulation (PBM), increased matrix stiffness may attenuate or amplify mechanosensitive pathways such as TRPV4 and PI3K/AKT/mTOR, which were shown to be activated in our study. Furthermore, the optical transparency of the ECM affects photon propagation and energy distribution, particularly at near-infrared wavelengths such as 980 nm. This property may influence the depth of penetration and uniformity of irradiation, thereby impacting the efficacy of PBM in vivo.

Additionally, the biochemical composition of the ECM, including collagen density, proteoglycan content, and hyaluronic acid concentration, can modulate cytokine diffusion and sequestration, potentially altering the inflammatory milieu and cellular responsiveness to PBM. These considerations underscore the necessity of incorporating ECM parameters into future experimental designs to enhance translational relevance and mechanistic clarity.

Physiological Relevance of the Cellular Model

The use of K4IM synoviocytes in this study provides a controlled and reproducible platform for investigating PBM-induced molecular changes. However, the limitations inherent to immortalized cell lines must be acknowledged. While K4IM cells retain key phenotypic characteristics of synovial fibroblasts, including cytokine responsiveness and TRPV4 expression, they do not fully recapitulate the complex cellular and extracellular architecture of native synovial tissue.

In vivo, synovial inflammation involves intricate interactions among fibroblasts, macrophages, endothelial cells, and ECM, all of which contribute to the pathophysiology of rheumatoid arthritis (RA) and osteoarthritis (OA). The absence of these components in monolayer cultures may limit the extrapolation of findings to clinical contexts. Moreover, the mechanical and biochemical cues provided by the native ECM are absent in 2D culture systems, potentially affecting the activation thresholds and signaling dynamics of pathways such as PI3K/AKT/mTOR.

To address these limitations, future studies should employ three-dimensional culture systems, organotypic explants, or co-culture models that better mimic the in vivo microenvironment. Such approaches would allow for a more physiologically relevant assessment of PBM effects, particularly in relation to ECM remodeling, Mechan transduction, and immune cell crosstalk.

Clinical Implications and Future Directions

The findings of this study suggest potential relevance for managing inflammatory conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA). By enhancing cell viability and modulating inflammatory pathways, both 980 nm and 660 nm photobiomodulation demonstrate promising biological effects in vitro. Previous studies by SY Tam et al. (2020) and Landucci et al. (2016) have highlighted the clinical utility of photobiomodulation in reducing musculoskeletal pain and joint inflammation, which aligns with our observations. However, it is important to note that our results are limited to in vitro conditions and do not include disease model induction, such as TNF-α stimulation.[36,37]

Therefore, while the therapeutic implications are

encouraging, they remain speculative until validated in disease-specific models and in vivo systems. Future research should incorporate inflammatory stimuli and clinically relevant models to better assess the efficacy and translational potential of 980 nm and 660 nm wavelengths. Additionally, the wavelength- and time-dependent effects observed here underscore the importance of optimizing irradiation parameters for tailored therapeutic applications.

Conclusion

This study highlights the therapeutic potential of 980 nm and 660 nm photobiomodulation in K4IM synovial cells by enhancing viability and modulating inflammation via TRPV4/PI3K/AKT/mTOR activation. 980 nm suppressed IL-6 and increased IL-10, promoting anti-inflammatory effects; 660 nm elevated IL-8 and IL-10, supporting repair and immune modulation. These results support both 660 and 980 wavelengths as efficient non-invasive treatments for joint inflammation. Further research is needed to clarify mechanisms, evaluate long-term outcomes, and optimize clinical protocols.

Authors' Contribution

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Competing Interests

The authors declare that they have no conflict of interest.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

Not applicable

Data Availability of Statement

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study

Ethical Approval

This study was conducted in full accordance with institutional and national ethical guidelines for research involving human-derived cell lines. All experimental procedures, including the handling and irradiation of human synovial cells (K4IM), were reviewed and approved by the Ethics Committee of the Islamic Azad University under the approval numbers IR.IAU.D.REC.1403.103 and IR.IAU.D.REC.1403.086. The study adhered to the principles of the Declaration of Helsinki, and all laboratory protocols were performed following established biosafety and ethical standards to ensure the integrity, safety, and ethical compliance of the research process.

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