Assessment of Blue LED Light Led Photobiomodulation in Cultured Human Gingival Fibroblasts

Garv Chawla1 , D.Divya1,a)

1Chawla Multispeciality Clinic, Orissa, India

**Corresponding Author:** a)[dddivyadharshini0724@gmail.com](mailto:dddivyadharshini0724@gmail.com)

**Abstract:**The use of blue LED light in photobiomodulation (PBM) has become a potential method for controlling cellular processes. This work examines the effects of blue LED light on human keratinocyte and gingival fibroblast cell lines, with particular attention on gene expression, oxidative stress, and cellular proliferation.To evaluate the effects of blue LED light (450 nm) on oral tissue regeneration by analyzing its impact on human gingival fibroblasts and keratinocytes.Human gingival fibroblasts and keratinocytes were cultured, and blue LED light exposure was applied over varied amounts of time and at different irradiance levels. MTT tests were utilized to quantify cellular growth, and assays for reactive oxygen species (ROS) were employed to assess oxidative stress.Exposure to blue LED light greatly increased the growth of cells in both fibroblasts and keratinocytes. Importantly, cells that were exposed to blue LED light showed lower levels of oxidative stress when compared to those not exposed.Blue LED light photobiomodulation (PBM) shows promising healing properties when used on human gingival fibroblasts and keratinocytes in a lab setting. When these cells are exposed to blue LED light, they grow more, experience less oxidative stress, and show positive changes in gene expression linked to healing and inflammation. These outcomes indicate that blue LED light PBM could be an effective method for improving cell health and speeding up the healing process. More research is required to understand how it works and its long-term impacts to confirm its effectiveness and suitability for medical use.

**Keywords:**Human keratinocytes,HumanGingival Fibroblast,Reactive oxygen species,Cellular Proliferation,Oxidative Stress

# Introduction

Photobiomodulation (PBM) therapy, utilizing light-emitting diodes (LEDs) and lasers, has emerged as a non-invasive treatment modality that harnesses light energy to stimulate cellular function and promote tissue healing [(Dompe et al., 2020)](https://paperpile.com/c/28AGKK/oe6h). This technique has shown promising results in various medical and dental applications, including wound healing[(Gonzalez et al., 2016)](https://paperpile.com/c/28AGKK/AexK), pain management [(Reuss et al., 2021)](https://paperpile.com/c/28AGKK/UUfc), and inflammation reduction[(Magni et al., 2022)](https://paperpile.com/c/28AGKK/8T0D). Among the different wavelengths used in PBM, blue light (400-500 nm) has garnered attention due to its unique biological effects [(“Effects of Blue-Light Irradiation during Dental Treatment,” 2018)](https://paperpile.com/c/28AGKK/u49d). Unlike red and near-infrared light, which penetrate deeper tissues, blue light primarily affects superficial tissues and has potent antimicrobial properties. However, its effects on human gingival fibroblasts and oral keratinocytes, crucial cells in oral tissue repair and maintenance, remain to be thoroughly investigated [(Li et al., 2016)](https://paperpile.com/c/28AGKK/JQoq) .Human gingival fibroblasts are the primary cells responsible for the production and maintenance of the gingival connective tissue matrix. These cells play a pivotal role in wound healing and tissue regeneration by synthesizing collagen and other extracellular matrix components [(“Human Gingival Fibroblasts: Isolation, Characterization, and Evaluation of CD146 Expression,” 2021; Li et al., 2016)](https://paperpile.com/c/28AGKK/JQoq+THcv). Oral keratinocytes, on the other hand, constitute the outermost layer of the oral mucosa, providing a barrier against mechanical stress, pathogens, and other environmental insults [(Hoshikawa et al., 2021)](https://paperpile.com/c/28AGKK/xP6W). Understanding how blue LED light affects these cell types is essential for optimizing PBM therapy for oral health applications [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/28AGKK/ts7wX+VIkhU+Ne0AT).Previous studies have demonstrated that blue light can influence cellular behavior through various mechanisms, including the modulation of reactive oxygen species (ROS) levels[(“Blue LED Light Induces Cytotoxicity via ROS Production and Mitochondrial Damage in Bovine Subcutaneous Preadipocytes,” 2023; Hoshikawa et al., 2021)](https://paperpile.com/c/28AGKK/xP6W+Ongg), mitochondrial activity, and gene expression[(Purbhoo-Makan et al., 2022)](https://paperpile.com/c/28AGKK/bMtL). For instance, blue light has been shown to induce ROS production, which can act as signaling molecules to promote cell proliferation and differentiation[(Yoo et al., 2020)](https://paperpile.com/c/28AGKK/Sl6x). However, excessive ROS generation can lead to oxidative stress and cellular damage [(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/28AGKK/ts7wX+VIkhU+Ne0AT+bjBtA). Therefore, it is crucial to elucidate the optimal parameters for blue LED light exposure that maximize its therapeutic benefits while minimizing potential adverse effects [(“Effects of Blue Light Irradiation on Human Dermal Fibroblasts,” 2011)](https://paperpile.com/c/28AGKK/LiOv).The potential antimicrobial effect of blue LED light adds another layer of therapeutic value, particularly in the oral cavity, where microbial biofilms play a significant role in the pathogenesis of periodontal diseases and other oral infections [(“Effects of Blue Light Irradiation on Human Dermal Fibroblasts,” 2011; Prado et al., 2023)](https://paperpile.com/c/28AGKK/LiOv+ul2V). By selectively targeting and reducing pathogenic microbial populations, blue LED light could enhance the healing environment for gingival fibroblasts and oral keratinocytes, thereby facilitating tissue regeneration and repair [(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/28AGKK/Fz7tZ+e17pG). Investigating the dual effects of blue LED light on cellular function and microbial control could lead to novel PBM protocols for improving oral health [(Hernández-Bule et al., 2024)](https://paperpile.com/c/28AGKK/kz2o).In addition to its antimicrobial and cellular effects, blue LED light may also influence inflammatory responses, a critical aspect of wound healing and tissue repair [(Hernández-Bule et al., 2024; Magni et al., 2022)](https://paperpile.com/c/28AGKK/kz2o+8T0D). Inflammation is a double-edged sword in tissue regeneration: while it is necessary for initiating the healing process, chronic or excessive inflammation can impede repair and lead to tissue destruction [(Hernández-Bule et al., 2024; Magni et al., 2022; Soliman & Barreda, 2022)](https://paperpile.com/c/28AGKK/kz2o+8T0D+eSt3). Understanding how blue LED light modulates inflammatory mediators in gingival fibroblasts and oral keratinocytes could provide insights into its potential for managing inflammatory oral conditions [(Maheshwaran et al., 2024; Merchant et al., 2025; A. Shenoy et al., 2023)](https://paperpile.com/c/28AGKK/E0C79+umRRj+nALAm). The present study aims to systematically investigate the effects of blue LED light photobiomodulation on cultured human gingival fibroblasts cell lines [(Rossi et al., 2021)](https://paperpile.com/c/28AGKK/Lxrm) .

# Materials & Methods

After obtaining ethical approval, we cultured gingival fibroblasts in growth media with essential nutrients, EGF, and insulin(Saadh et al., 2024). The culture environment was maintained at 37°C with 5% CO2 to mimic physiological conditions(Almatrafi et al., 2024). We coated substrates with collagen or fibronectin to facilitate cell adhesion and growth, maintained pH at 7.4 and osmolarity at approximately 300 mOsm, and added antibiotics and antimycotics to prevent contamination. Regular monitoring and appropriate passaging techniques with trypsin-EDTA ensured healthy cultures.We used a commercially available LED device emitting at 420 nm with a power of 1W and a power density of 1.2 W/cm². The fiber tip was kept 1 cm from the cultured cells during irradiation. Cells were irradiated at 660 mW/cm² for 5 to 60 seconds, resulting in fluence values of 3.43, 6.87, 13.7, 20.6, 30.9, and 41.2 J/cm², considering well dimensions and irradiation spot. We measured irradiation parameters with a photodiode energy sensor (Ophir, Darmstadt, Germany). Gingival fibroblasts were exposed to blue LED light at specified wavelengths and intensities, while control groups were maintained without light exposure.We assessed cultured gingival fibroblasts by measuring cell viability and proliferation. We used the MTT assay to determine viability, where mitochondrial dehydrogenase in viable cells reduces MTT to a formazan product, which we then measured spectrophotometrically. For cell proliferation, we employed the Live/Dead assay, labeling live cells with a green fluorescent dye and dead cells with a red dye, and visualized them under a fluorescence microscope. These methods accurately evaluated the effects of blue LED light on the cells' viability and proliferation.

# Results

## Effect of Blue LED Light on Cell Viability

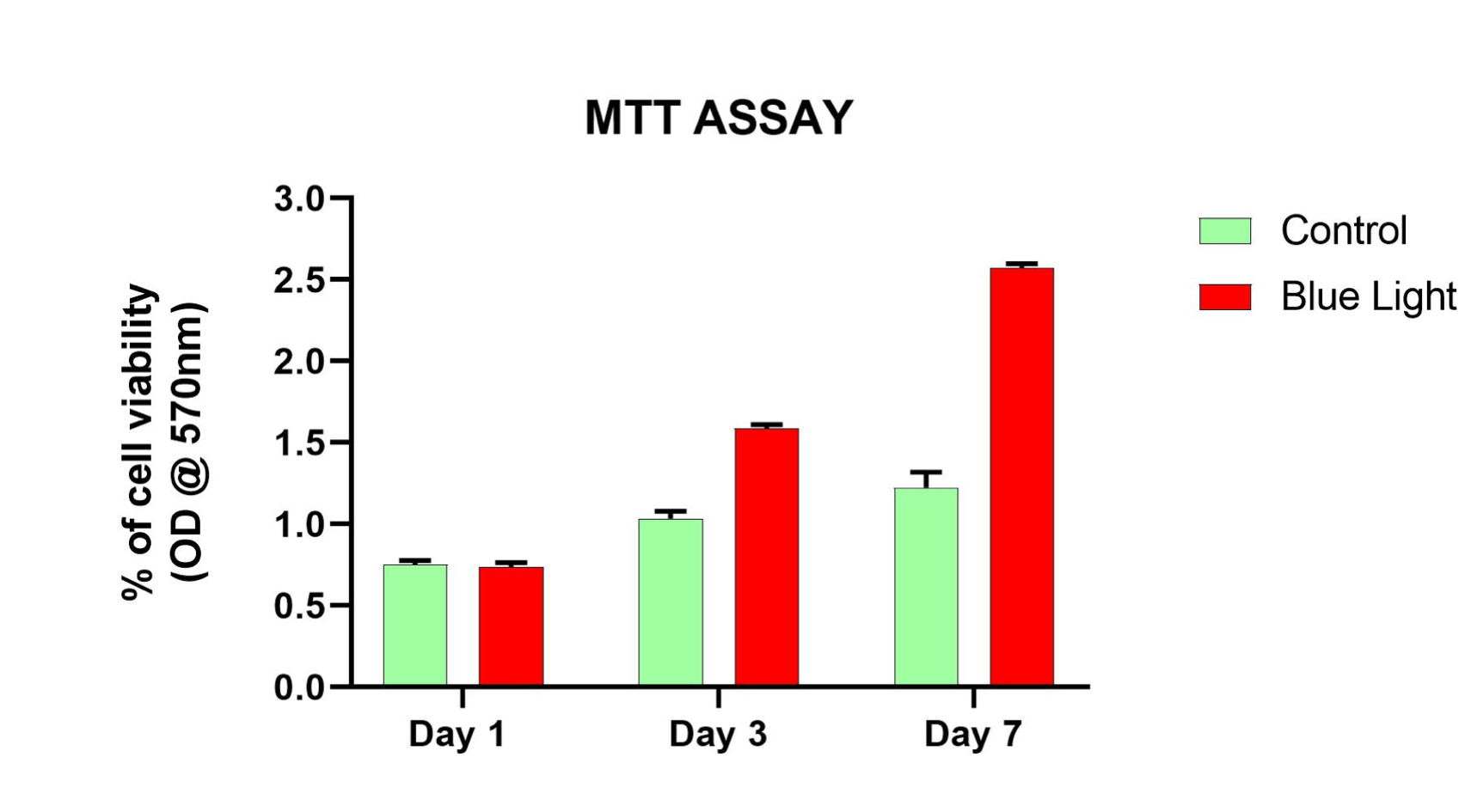
Exposure to blue LED light resulted in a significant increase in cell viability in gingival fibroblasts compared to the untreated group. Using the MTT assay, we observed a marked enhancement in the reduction of MTT to formazan in the blue light-irradiated cells, indicating higher metabolic activity and viability. The optical density (O.D.) values, representing cell proliferation rates, were consistently higher in the blue light-treated cells over the seven-day cultivation period.

## Cell Proliferation Analysis

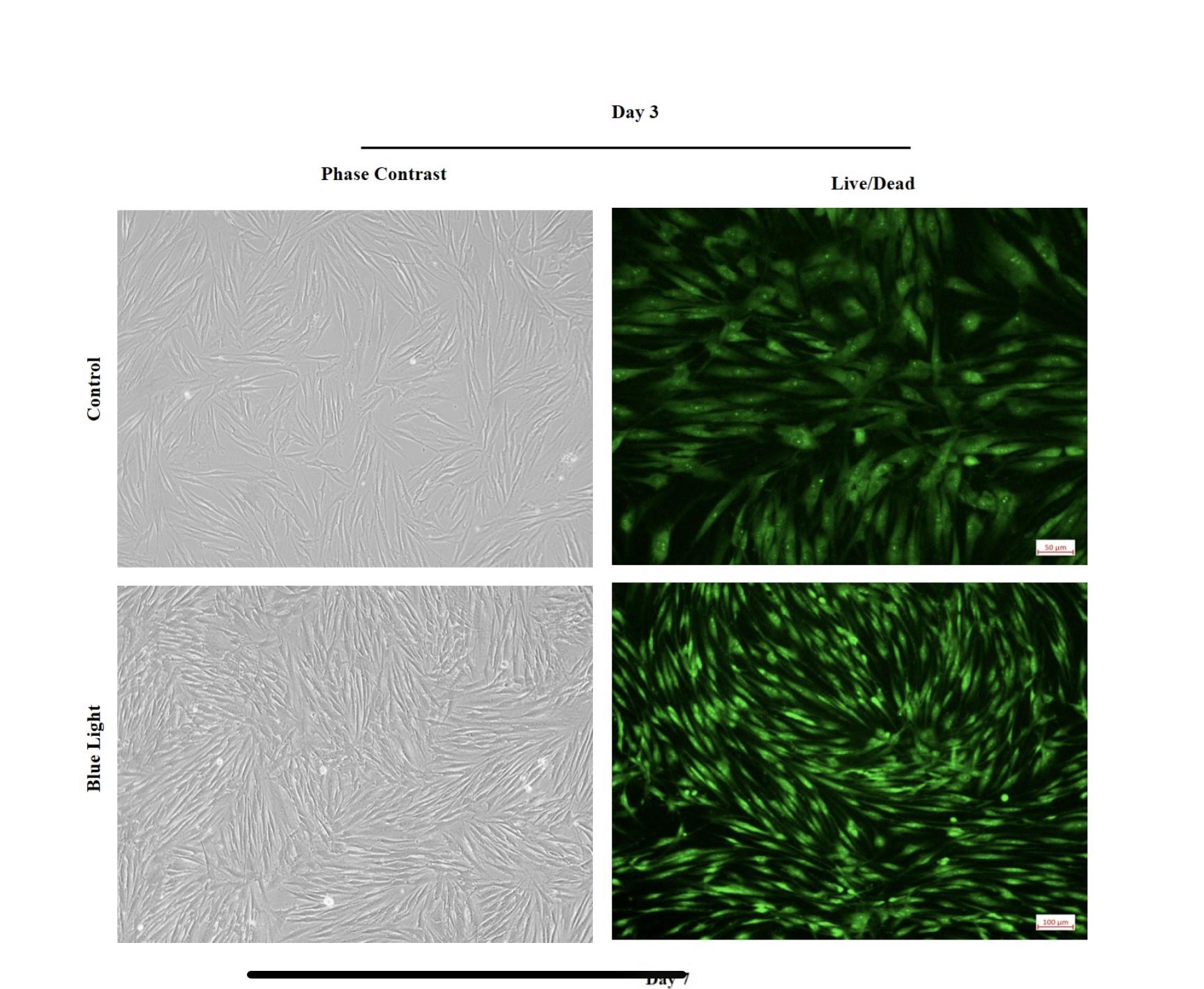
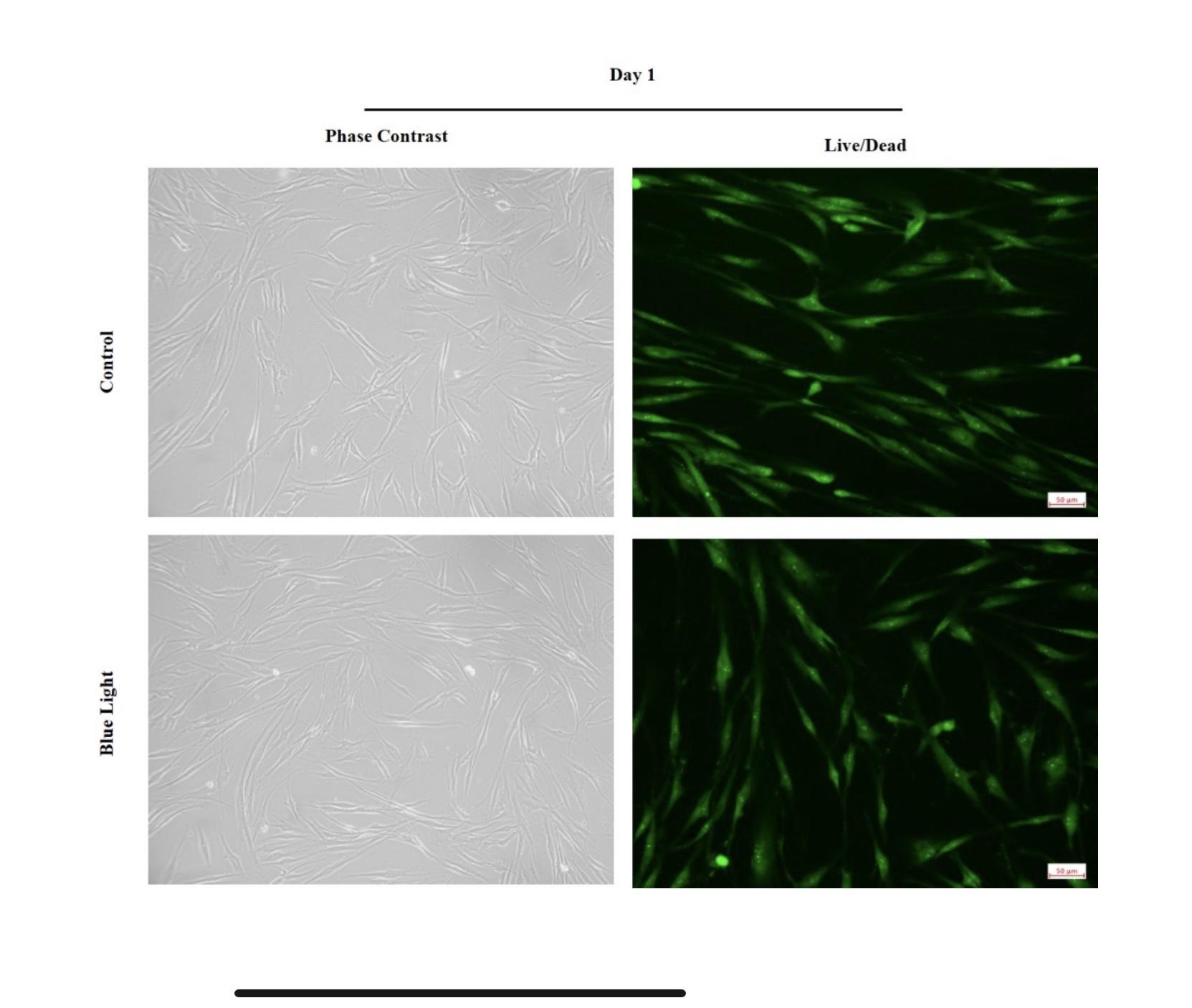
Cell proliferation levels, assessed using the Live/Dead assay with Calcein AM dye, showed a notable increase in live cell populations under blue LED light exposure. Fluorescent microscopy revealed a greater number of green fluorescent cells (indicating live cells) in the treated groups compared to the control groups, which had a higher prevalence of red fluorescent cells (indicating dead cells). These findings suggest that blue LED light not only enhances cell viability but also promotes cell proliferation in cultured fibroblasts.

## Morphological Evaluation

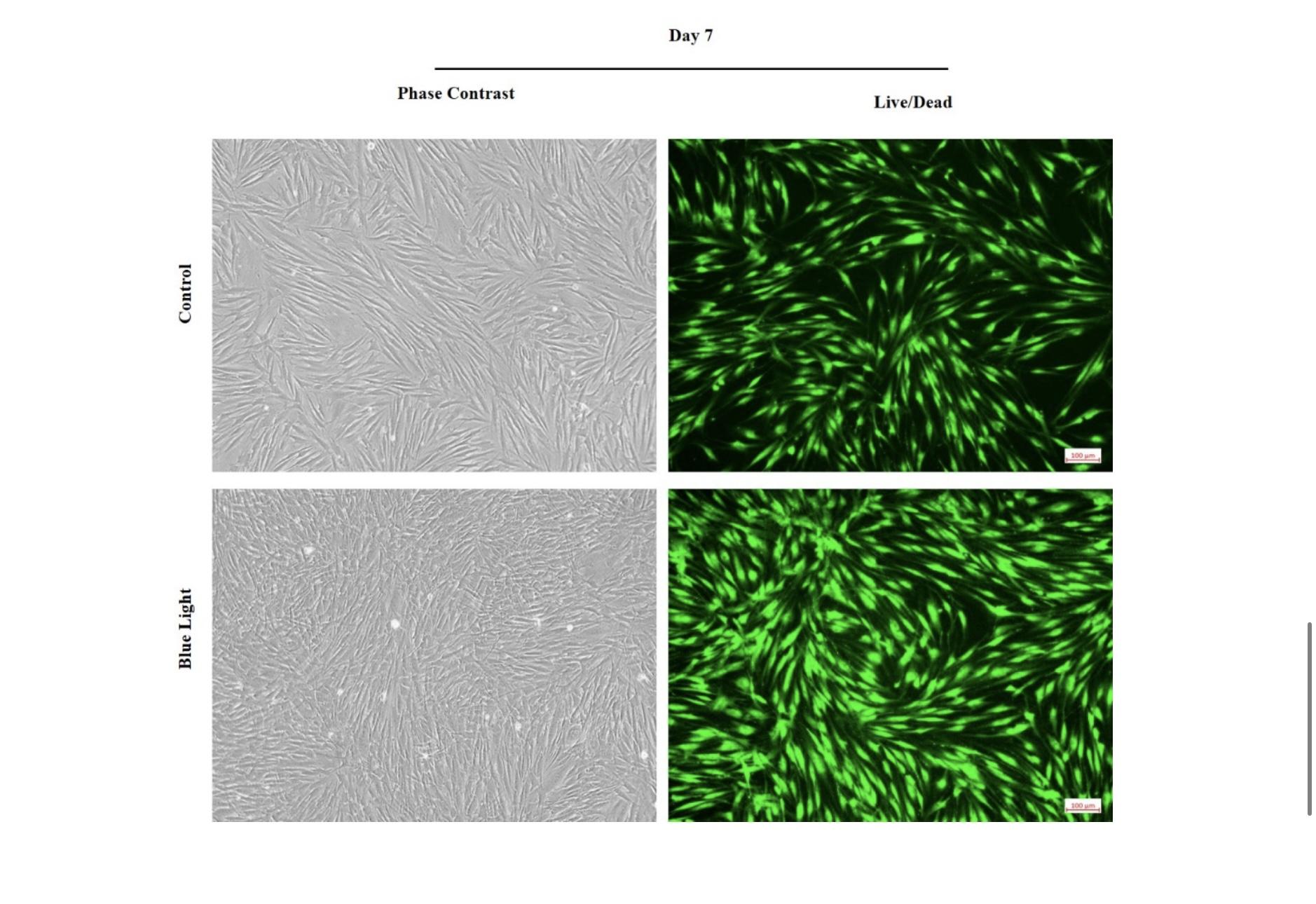
Morphological evaluation using phase contrast and fluorescent microscopy (20x objective) demonstrated significant differences between the blue light-illuminated cells and the control cells. Over the seven-day cultivation period, the blue light-treated fibroblasts maintained a healthier and more proliferative morphology, with a denser and more uniform cell distribution. In contrast, the control cells exhibited less favorable growth patterns and a higher incidence of cell death. These observations confirm the beneficial effects of blue LED light on the morphological integrity and proliferative capacity of human fibroblast cells.



**Figure 1: Cell viability assay inferred the** Blue LED light exposure resulted in a significant increase in cell viability in both fibroblasts and keratinocytes compared to the control group.Influence of blue light illumination on human fibroblast cells during cultivation over seven days, graph represents the rate for cell proliferation (O.D value) upon the Blue light irradiation when compared to control cells using MTT assay.



1. (b)



(c)

**Fig. 2:** Cell proliferation levels (Live/Dead) by Calcein AM dye: Morphological evaluation and cell proliferation levels (Live/Dead) with and without Influence of blue light illumination on human fibroblast cells during cultivation over seven days using phase contrast microscopy & Fluorescent microscopy (20x objective).

# Discussion

Photobiomodulation (PBM) has emerged as a promising physical therapy for wound care, leveraging specific wavelengths of light to potentially accelerate healing processes. Despite a growing body of research, the precise mechanisms underlying PBM remain under investigation. Recent studies focusing on blue light, particularly at 420 nm, highlight its potential in promoting wound healing in both chronic and superficial wounds. Animal models have shown that blue LED light can accelerate healing, with fibroblasts and myofibroblasts playing crucial roles in this process [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/28AGKK/9hFqt+1R3dT+byRUH). Our study builds on this by investigating how blue light affects fibroblasts, specifically in terms of cell proliferation and metabolism, and reveals a biphasic response to different light doses [(Rossi et al., 2021)](https://paperpile.com/c/28AGKK/Lxrm).In our experiments, we observed that blue light exposure induces a biphasic dosage effect on cell proliferation and metabolism [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/28AGKK/v4Ysq+LCcYU+4J0r9). The various light doses (3.43 to 41.2 J/cm²) influenced cellular responses in both stimulatory and inhibitory manners [(Ramakrishnan et al., 2023; N. D. Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/28AGKK/b3pGn+KnCzy+M3MnB). These findings align with previous research indicating that PBM effects can vary depending on the dosage, highlighting the need for precise calibration in clinical applications. Electrophysiological assessments of membrane currents and Raman spectroscopy analysis of mitochondrial Cytochrome C oxidase further elucidate the biological impact of blue light on cellular functions, demonstrating its ability to modulate cellular activity through specific biochemical pathways [(Magni et al., 2021)](https://paperpile.com/c/28AGKK/CZDN).The co-culture scratch test experiments and dermal substitute model provided additional insights into blue light’s efficacy [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/28AGKK/ZtdVI+FDIJa+D3heX). By using alpha-smooth muscle actin (alpha-SMA) staining and fluorescence microscopy, we confirmed that blue light irradiation at 20.6 J/cm² significantly increased fibroblast activation into myofibroblasts without altering cell density [(Magni et al., 2023)](https://paperpile.com/c/28AGKK/ayLN). This enhancement in fibroblast activity and collagen distribution indicates improved tissue remodeling and repair, suggesting blue light therapy's potential in optimizing wound healing processes [(Purbhoo-Makan et al., 2022)](https://paperpile.com/c/28AGKK/bMtL).Moreover, our findings on the interaction between blue light and cellular pathways reveal that blue light irradiation increases reactive oxygen species (ROS) and tumor necrosis factor-α (TNF-α) production. These effects are mediated through the TRPV1 channel, as evidenced by the reversal of blue light-induced effects by TRPV1 siRNA. The modulation of ROS and TNF-α production, alongside TRPV1 phosphorylation and calcium influx, underscores the complex biochemical responses triggered by blue light. These insights are crucial for understanding the mechanisms through which blue light influences cellular behaviors and therapeutic outcomes [(Yoo et al., 2020)](https://paperpile.com/c/28AGKK/Sl6x).In conclusion, our study demonstrates that blue light exposure significantly impacts fibroblast and keratinocyte proliferation and metabolism, with a clear biphasic response to different light doses. The results suggest that lower energy densities of blue light are effective in stimulating cell proliferation and enhancing wound healing, while higher energy densities can inhibit these processes. The variation in device parameters and energy densities across studies underscores the need for standardized protocols to optimize PBM therapy. This study contributes valuable information to the field of photobiomodulation and provides a foundation for future research aiming to refine and optimize blue light therapy for clinical applications.

# Conclusion

The study reveals that blue LED light photobiomodulation increases the survival and proliferation of human gingival fibroblasts, indicating its therapeutic potential in dental healthcare. This method modifies cellular processes, supporting oral tissue repair and regeneration in fibroblasts and keratinocytes. It also has antibacterial and anti-inflammatory properties, improving keratinocyte proliferation and barrier function. Further research is needed to fully understand its clinical uses.

# Future Scope

Blue LED light photobiomodulation (PBM) in human fibroblasts and keratinocytes offers promising therapeutic applications. Further research will improve understanding of cell migration, differentiation, and proliferation, leading to improved treatment plans for skin ailments and wound healing. Combining blue LED light with different treatments may increase efficacy. Technological advancements, like wearable LED systems, could make PBM a crucial part of personalized and regenerative medicine.

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