THE EFFECT OF 660NM LIGHT-EMITTING DIODE IRRADIATION ON HUMAN GINGIVAL FIBROBLAST CELL PROLIFERATION

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by

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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OUTLINE

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Abstract

Light therapy has become a common treatment modality in various medical fields, including dermatology, dentistry, and cosmetics. This study aimed to evaluate the effect of 660 nm ligh-emitting diode on the proliferation and viability of human gingival fibroblasts in vitro. The cells were irradiated with 660nm light-emitting diode for 60 and 120 seconds 24-hours post-seeding. After 24- and 48-hours irradiation, cell count, and viability were determined. The results then were analysed using a one-way ANOVA test ($p < 0.05$). The findings revealed a significant increase in cell proliferation rate and viability in the irradiated groups compared to the control group, with the 120 seconds irradiation group showing the highest enhancement at both time points. These results suggest that 660 nm LED light can effectively promote the proliferation and viability of human gingival fibroblasts, highlighting its potential as a valuable tool in regenerative dentistry and periodontal therapy.

KESAN SINARAN DIOD PEMANTAPAN CAHAYA 660NM TERHADAP PEMBIMBANGAN SEL FIBROBLAST GINGIVAL MANUSIA

Abstrak

Terapi cahaya telah menjadi kaedah rawatan biasa dalam pelbagai bidang perubatan, termasuk dermatologi, pergigian, dan kosmetik. Kajian ini bertujuan untuk menilai kesan diod pemancar cahaya 660 nm terhadap percambahan dan daya maju fibroblas gingival manusia secara in vitro. Sel-sel telah disinari dengan diod pemancar cahaya 660nm selama 60 dan 120 saat 24 jam selepas pembenihan. Selepas penyinaran 24 dan 48 jam, kiraan sel, dan daya maju ditentukan. Keputusan kemudiannya dianalisis menggunakan ujian ANOVA sehala ($p < 0.05$). Penemuan menunjukkan peningkatan ketara dalam kadar percambahan sel dan daya maju dalam kumpulan yang disinari berbanding dengan kumpulan kawalan, dengan kumpulan penyinaran 120 saat menunjukkan peningkatan tertinggi pada kedua-dua titik masa. Keputusan ini menunjukkan bahawa cahaya LED 660 nm boleh menggalakkan percambahan dan daya maju fibroblas gingiva manusia secara berkesan, menonjolkan potensinya sebagai alat yang berharga dalam pergigian regeneratif dan terapi periodontal.

Introduction

1.1 Photobiomodulation (Light Therapy)

Light-emitting diode (LED) light therapy is a non-invasive treatment that enters the outer layer to improve the tissue and cell regeneration and repair. Modern light therapy (phototherapy) had a marked uptake in use in medicine in Scandinavia, America, and Australia from 1903, following the pioneering work of Niels Finsen in the late 19th century, which culminated in Dr Finsen receiving the Nobel Prize for Medicine for the treatment of tuberculosis scarring with ultraviolet (UV) light, and treatment of smallpox scarring with red light (Liebert & Kiat, 2021). Nowadays, dermatologists and dentists commonly use LED light therapy to treat a range of skin and oral problems. During the therapy, commonly will combine LED light irradiation with other treatments, such as creams, ointments, and facials in skin treatment and scaling and root planning in oral treatment, to get the best results in multiple treatment.

There are multiple type of LED light therapy by using different wavelength that correspond to different visible colours such as blue light, yellow light, and red light.

Blue light (400 to 420nm wavelength) and yellow light (560 to 590nm wavelength) therapy are commonly used in skin therapy, the features of blue and yellow light are that their light can only effect the uppermost and upper layer of skin, that can effectively destroy acne-causing bacteria *(P. acnes)* and triggers the lymphatic system to remove toxins from the exposed area. Therefore, yellow light therapy can increase blood circulation to improves wound healing, increases the skin's ability to retain moisture, and keeps the skin hydrated and amplifies your skincare regimen. However, the defect of blue and yellow therapy is that, single light therapy cannot get the best and

rapid result on skin therapy, commonly will combine used with red light therapy (Scott et al., 2019).

Figure 1.1: The usage of yellow light therapy on Dermatology/Cosmetology

Reference: https://palmyraskinclinic.com.au/skin_services/healite-yellow-light-therapy/

Red light (620 to 700nm wavelength) therapy can travel further to the deepest layer of skin, which can effectively reduce inflammation and stimulate collagen and elastin production to increase antiaging properties, improve skin tone, and reduce wrinkles. Based on the features of red light therapy, the usage of red light therapy not only limitations in skin therapy but can also applied to dentistry to treat chronic gingivitis and expedite the regeneration of hard and soft tissue in extraction sockets and periodontal defects (Takeuchi et al., 2023). While even red light therapy is generally safe when used correctly, it can cause skin irritation or blistering if used for too long or at too high an intensity. Also, directly observe the red LED light will damage the eyes, so it's important to wear protective goggles or isolate the light during experiment.

Figure 1.2: The usage of red light therapy on Dentistry

Refenrence: https://redlightman.com/blog/red-light-therapy-improves-all-oral-health/

1.2 Low Level Light Therapy (LLLT)

In this research, 660nm LED light will be used to irradiate human gingival fibroblast (HGnF) cell to observe how LED light irradiation to effect on HGnF cell proliferation and the cell morphology. Low-level light therapy (LLLT) refers to the wavelength between 600 - 1070nm and the output power between 1 - 1000 mW LED light irradiation therapy, which can be used to treat a multitude of conditions that require stimulation of healing, relief of pain and inflammation, can also improve the tissue regeneration and repair. During the light irradiation, the photons are absorbed by mitochondrial chromophores in skin cell and induce electron transport, adenosine triphosphate (ATP) nitric oxide release, blood flow, reactive oxygen species increase and diverse signaling pathways get activated. Stem cells can be activated allowing increased tissue repair and healing (Cheng et al., 2021).

LLLT is a non-invasiveness treatment method that can give more sense of safe to each patient compare to common invasiveness treatment method, which can minimise the chances of allergic reaction and lower risk of infection, also non-invasiveness method are painless and provide immediate clinical response that give much easier reproducibility.

1.3 Periodontitis

Chronic inflammatory processes in the oral mucosa and periodontitis are common disorders caused by microflora and microbial biofilms. These factors activate both the innate and adaptive immune systems, leading to the production of pro-inflammatory cytokines. Cytokines are known to play a crucial role in the pathogenesis of gingivitis and periodontitis and have been proposed as biomarkers for diagnosis and follow-up of these diseases. They can activate immune and stromal cells, leading to local inflammation and tissue damage. This damage can include destruction of the periodontal ligaments, gingiva, and alveolar bone (Neurath & Kesting, 2024).

Healthy gums are firm and fit snugly around teeth, but for periodontitis patient, the gum will show as swollen or puffy with bright red, dark red or dark purple colours. When patients try to touch the gums will feel pain and with bleeding in some seriously cases. Also, patient can find pus between teeth and gums and might let to loose teeth or loss of teeth (Neurath & Kesting, 2024).

Dental plaque is an oral biofilm of microorganisms that continuously grows on the surfaces of teeth. This plaque plays an important role in both oral health and the development of diseases such as gingivitis and periodontitis. Subsequent research has demonstrated that periodontitis is characterized by an imbalance between the types of

microorganisms present in a person's natural microflora in the oral cavity. This dysbiosis has been identified as a crucial factor in driving local inflammation in periodontitis. In 2020, a new theory on the etiology of periodontitis was formulated, which was designated the "Inflammation-Mediated-Polymicrobial-Emergence and Dysbiotic-Exacerbation" (IMPEDE) model. In this context, it was proposed that inflammation represents the consequence of the dysbiotic events that occur in the disease, which drive the transition from oral health to periodontitis (Neurath & Kesting, 2024).

The periodontium is the collagenous structure that surrounds and attaches the teeth to the underlying alveolar bone, allowing for adequate esthetics and tooth function. There is a stepwise transition from non-inflamed gum and healthy periodontal tissue to progressive periodontitis. In the initial stages of periodontal disease, the soft tissues of the gum are affected. However, advanced periodontal disease not only affects the gums but also the bone structures supporting the teeth, leading to local inflammation that can affect the surrounding bone. Finally, periodontitis can progress to an advanced or severe stage, which is characterized by local abscesses, marked inflammation, and progressive bone loss (Neurath & Kesting, 2024).

Figure 1.3: The difference between Normal tooth and Periodontitis

Reference:https://austinlaserdentist.com/periodontitis-stages-symptoms-and-treatments-2/

The current method of treatment are scaling and root planning to remove dental plaque from the surface of the roots of the tooth, during the treatment, periodontist makes a small incisions in the infection gum and elevated back the gum tissue to exposing the roots for more effective scaling and root planning. Since periodontitis often causes bone loss, the process will lead to significant pain before the gum tissue is put back in place after suturing. After the surgery, it might cause secondary infection, bleeding with long lasting dull pain. Hence, people look for a low risk and more safety method to alternative the surgery, which is light therapy.

1.4 Objective

1.4.1 General Objective

- To evaluate the effect of 660nm LED irradiation on human gingival fibroblast (HGnF) cell line

1.4.2 Specific Objective

- To determine the effect of 660nm LED irradiation on cell viability of human gingival fibroblast (HGnF) cell by using trypan blue staining
- To determine the human gingival fibroblast (HGnF) cell morphology after 660 nm LED irradiation by using crystal voilet staining

Review of Literature

2.1 Research of Literature

In the literature by Tripodi et al (2021), they used 0.5, 1, 5 and 5.5 J/cm2 of 600nm-1070nm light to irradiate human dermal fibroblasts in vitro to observe the effects. Based on the data on the proliferation rate and activity of dermal fibroblasts, it can be concluded that these light doses can improve the proliferation and activity of human dermal fibroblasts. At the same time, they conducted experiments with stronger doses of light, but the conclusions reached were different. Light with 10 J/cm2 decreased cell proliferation rate and activity, but a higher dose, 30 J/cm2, increased cell growth. Additionally, they measured cell migration, proliferation, and hypertrophy by assessing wound convergence through a scratch wound assay, which supported their point that low-level light irradiation can successfully increase cell migration and proliferation. The data supports the efficacy of low-level light therapy and also provides many different angles to interpret the effects of light therapy on in vitro cell lines (Tripodi et al. 2021).

In the literature by Sterczała et al. (2021), they used a human gingival fibroblast cell line as the cell sample for the in vitro illumination experiment and used different wavelengths of laser light for control experiments during the experiment, which 405nm, 465nm, and 635nm light. In their results, human gingival fibroblasts in the three cell control groups all experienced varying degrees of cell proliferation after being irradiated with light of corresponding wavelengths, and an increase in cell activity and intracellular ATP energy could be observed (Sterczała et al. 2021). MTT assay was used to detect the cell survival rate by measuring the mitochondrial activity (MA) of the tested cells. After blue laser light (405nm and 465nm) irradiation, a statistically

significant decrease in mitochondrial activity was observed 24 hours after the exposure in the irradiated subgroup and increased back to normal after 48 hours. However, in red light (635nm) irradiation after 24 hours, there was an increase in the MA for all energy densities but no statistically significant differences in relation to the control group. After 48 h, the mitochondrial activity reached the highest value for an energy density of 64 J/cm2 (122.1%). Red light is more effective than blue light, which can more effectively stimulate cell proliferation and ATP energy production (Sterczała et al. 2021).

Furthermore, in the literature of Yoon et al (2023), they used 660nm wavelength LED light to measure its effect on astrocyte migration by using two different migration assays (scratch assay and transwell assay) and collect the result after 24 and 48 hours incubation. Astrocytes are star-shaped glial cells found in the brain and spinal cord. They possess many functions, including biochemical control of endothelial cells that form the blood-brain barrier, providing nutrients to neural tissue, maintaining extracellular ion balance, regulating cerebral blood flow (CBF), and regulating cerebral blood flow (CBF). It plays an important role in repairing damage and glial scar formation in the brain and spinal cord due to infection or physical injury (Yoon et al. 2023).

At 24 hours after the scratch assay, the wound areas were smaller in the 660-nm LED exposure group (6 and 12 J/cm^2) than in the control and 18 J/cm^2 group, and the differences were significant. At 48 h after the scratch test, there was no difference between the control and 660-nm LED exposure groups, because the gaps were filled by cells in all groups. The result showed a significant difference in astrocyte migration following PBM irradiation that could increase the astrocyte migration by altering the cell morphology and DBN expression pattern (Yoon et al. 2023).

2.2 Difference between LED and Laser Light Therapy

Both laser and LED therapies rely on delivering sufficient energy to target tissues to initiate a photochemical process known as photobiomodulation (PBM). PBM is a non-thermal process that involves endogenous chromophores, triggering photophysical and photochemical events across various biological scales. These processes include, but are not limited to, pain and inflammation relief, immune modulation, and the promotion of wound healing and tissue regeneration. Both light sources operate through the same mechanism and are typically produced using diode technology. In therapeutic applications, lasers and LEDs generally emit similar wavelengths, usually within the red or near-infrared spectrum. Studies have demonstrated that these wavelengths are effective in reducing pain and inflammation (Dompe et al., 2020).

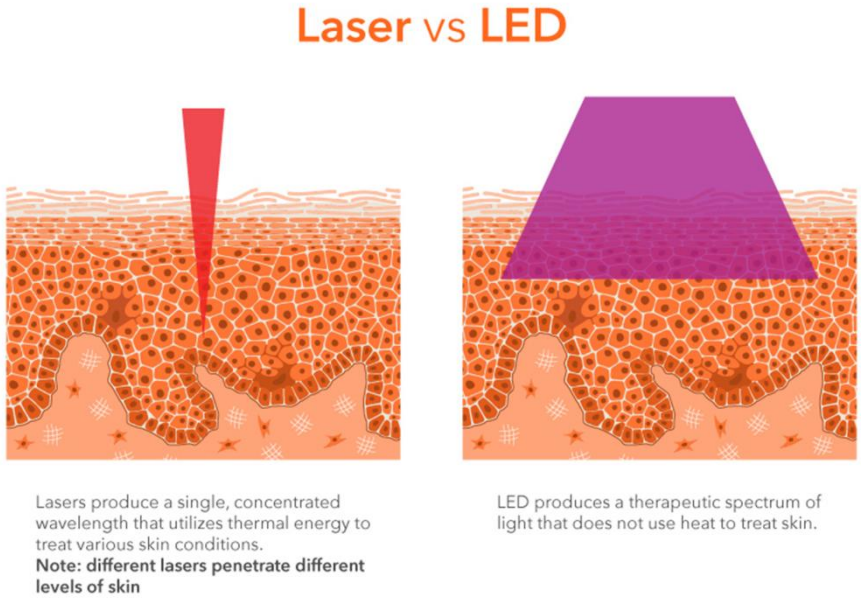

Figure 2.1: Laser light VS LED light (https://drdennisgross.com/blogs/skincare-blog/led-light-therapy-vs-laser-facials)

Significant differences exist between laser and LED light therapies, particularly in the power output, wavelength specificity, and physical characteristics of the beam produced by the diode. Laser light therapy is unique in that it is monochromatic, coherent, and collimated. These properties make it ideal for many medical applications. Monochromatic light, consisting of a single wavelength, is particularly effective in stimulating chromophores in biological tissues that respond to specific wavelengths. Coherent photons are organized, unlike incoherent photons, and this organization minimizes photon scattering when light interacts with tissue. Additionally, the collimated, columnar beam of a laser focuses energy into a narrow, direct path, which is particularly advantageous for treating deep tissues often located inside the body. In contrast, LED light therapy emits light over a small range of wavelengths, typically around 20 nanometers wide, compared to lasers that emit a highly specific wavelength (about 1 nanometer wide). This broader bandwidth reduces the precision of LEDs in targeting specific tissues. Moreover, LED light is neither collimated nor coherent, making it less effective for penetrating deeper tissues. Finally, LEDs operate at significantly lower power (wattage) than most lasers, which limits their ability to deliver sufficient energy to deeper tissues within a shorter time frame (Dompe et al., 2020).

When targeting deeper tissues with light therapy, wavelength is a key variable that determines the ability of light to penetrate tissue. However, it is not the only determinant of treatment effectiveness. Power is another critical factor in achieving consistent results. Laser light therapy typically delivers much higher power than LED therapy, which significantly enhances its ability to penetrate deeper tissues. This capability is due to the concept of treatment depth, where sufficient photon energy must reach the target tissue to produce a photobiomodulation effect. Since a substantial amount of light energy is lost as it passes through tissue, having a higher initial energy

at the surface increases the likelihood of delivering adequate energy at depth (Dompe et al., 2020).

For superficial applications, such as wound healing, where only a small amount of energy is needed at the surface to achieve therapeutic effects, LEDs are well-suited for this purpose. However, for deeper or more extensive conditions, such as fibromyalgia or chronic low back pain, a greater amount of energy is required to achieve an adequate therapeutic effect (Dompe et al., 2020).

2.3 Light Therapy in Dental Science

Light therapy is widely used in the field of dentistry, especially in non-surgical and post-surgical wound healing and pain relief. At the same time, light therapy's ability to effectively relieve inflammation and promote wound repair also makes it useful in the treatment of some diseases.

For canker sores (aphthous ulcers), is are small, shallow ulcers that occur in the lining of your mouth. A canker sore starts as a white or yellowish mouth sore with a red border. They're usually very small (less than 1 millimeter) but may grow to 1/2 inch to 1 inch in diameter. Canker sores commonly found on tongue, gums, roof of mouth, inside of lip or under tongue. They can be painful and often make eating and talking uncomfortable. There are two types of canker sores: simple canker sores and complex canker sores. Simple canker sores may appear three or four times a year and last up to a week. Compared to somple conker sores, complex canker sores are less common and occur more often in the people who have previously had them (Gasmi Benahmed et al., 2021).

Figure 2.2: Canker sore (aphthous ulcers) (https://www.drgalante.com/preventative-care/canker-sores/)

Canker sores can be cause by nutritional deficiencies in vitamin B-12, zinc, folic acid or iron, and it often found in patient with long term stress or have injury inside of mouth. Also, long term intake acidic foods, such as citrus fruits can be the inducement that lead to canker sores. For complex canker sores, it may develop in people with immune system conditions, including: lupus, Bechet's disease, celiac disease, ulcerative colitis, Crohn's disease and AIDS. In the early stage of canker sore, the common symptoms including one or more painful sores inside of mouth. These ulcers may form on tongue, the inside of lips, inner cheeks or the roof of mouth. The surrounding area will fell burning or tingling sensations, and small, round ulcers that are white, gray or yellow with a red border can be observe. In the severe cases, like delay trearment lead to worsening of condition, patient will show fever, physical sluggishness and swollen lymph nodes (Gasmi Benahmed et al., 2021).

The common treatment method of canker sore may include over-the-counter or prescription products to ease the symptoms. Follow with the medicine, patient may need to used topical anesthetics, such as benzocaine to decrease the severe pain cause by canker sore. Also, for early stage canker sore, patient can used mouth rinses containing

hydrogen peroxide, chlorhexidine or dexamethasone to reduce the symptoms of canker sore. For severe canker sores, patient may need to cauterization (burning the affected tissue). This can sterilize the area, reduce pain and speed up healing. However, the common method will cause severe pain and uncomfortable feeling to the canker sore patients, this may cause young or special-case patients to resist or even avoid treatment, leading to more severe symptoms (Gasmi Benahmed et al., 2021).

Light therapy is a good method that can replace the common method of treating canker sores. Based on the research, most patients who receive phototherapy report significant reductions in pain and functional complications. At the same time, they claimed that wounds healed faster than traditional drug treatments and found no adverse effects on epithelial cells (Rathod et al., 2022).

Herpes simplex virus (HSV), known as herpes, is a common infection that can cause painful blisters or ulcers. It primarily spreads by skin-to-skin contact. It is treatable but not curable. There are two types of herpes simplex virus, type 1 (HSV-1) and type 2 (HSV-2). Type 1 (HSV-1) mostly spreads by oral contact and causes infections in or around the mouth (oral herpes or cold sores). It can also cause genital herpes. Most adults are infected with HSV-1. Type 2 (HSV-2) spreads by sexual contact and causes genital herpes. Most people who infect HSV have no symptoms or only mild symptoms. The infection can cause painful blisters or ulcers that can recur over time. Medicines can reduce symptoms but can't cure the infection.

Figure 2.3: Herpe Simplex Virus (https://contourderm.com/herpes-simplex/)

Common herpes symptoms include pain, recurring blisters or sores, and people newly infected with the virus may experience symptoms such as fever, body aches and swollen lymph nodes. Symptoms when an infection first strikes may be different from symptoms when a recurrence occurs, and symptoms are usually more severe when the infection first occurs than when the infection relapses. If symptoms occur, they usually begin with tingling, itching, or burning near the ulcer. Common oral herpes symptoms include blisters (cold sores) or open sores (ulcers) in or around the mouth or lips. These sores and blisters are often painful. The blisters may burst, ooze fluid, and then crust over. During the first infection, people may experience symptoms such as fever, body aches, sore throat (oral herpes), headache, swollen lymph nodes near the infection, and more. And because it is incurable, patients may experience recurring attacks over time. These attacks usually last shorter and are less severe than the first attack (WHO, 2023).

Drugs are usually used to treat spores. Commonly used antiviral drugs include acyclovir, famciclovir and valacyclovir. Generally speaking, treatment with these drugs works best when given within 24-48 hours of the first outbreak and each recurrence, and symptoms can also be reduced if the patient takes a lower dose of one of these drugs every day (suppressive therapy). Daily treatment is usually recommended for people

whose pain is very severe or comes back frequently, or for those who want to reduce the risk of spreading herpes to others. Ulcers caused by spores can cause severe pain at the infected site, so patients often take medications to relieve ulcer pain, including acetaminophen (acetaminophen), naproxen, or ibuprofen (WHO, 2023). Some herpes simplex infections studies have shown that phototherapy has a positive effect on the healing of herpes simplex infections. In one of the trials, patients received low level light therapy (LLLT) daily for two weeks. Patients who received laser treatment were free of herpes lesions for an average of 37.5 weeks, compared with an average of three weeks for patients who did not receive laser treatment (Rathod et al., 2022).

Furthermore, oral lichen planus is a persistent (chronic) inflammatory condition that affects the mucous membranes in the mouth, often manifesting as white band-like patches, red and swollen tissue, or open sores. These lesions may produce burning, pain, or other discomfort. Oral lichen planus does not spread from person to person, but usually occurs when the immune system begins to attack cells lining the oral mucosa for unknown reasons, resulting in an autoimmune disease. Symptoms are generally controllable, but people with oral lichen planus need regular testing because the affected area may develop into oral cancer. Lesions caused by oral lichen planus usually occur on the inside of the cheeks, but in some cases, they are found in the gums, tongue, palate and other internal parts of the mouth. Lesions caused by oral lichen planus usually appear as lacy, white, raised patches of tissue with red, swollen, tender tissue patches and sores (Popa et al., 2024).

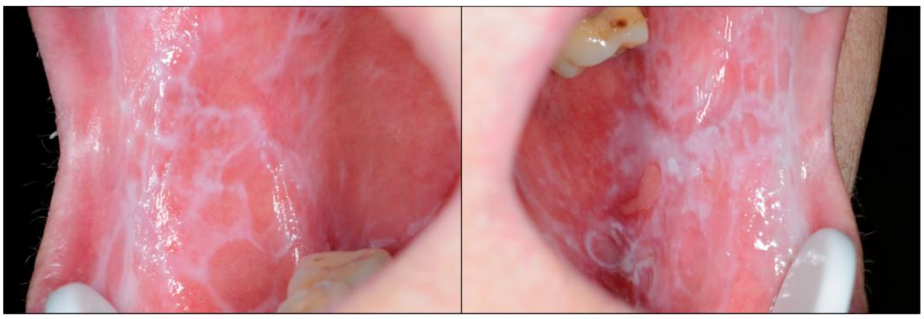

Figure 2.4: Oral Lichen Planus (https://www.mdpi.com/1422-0067/23/21/13099)

In the early stages of oral lichen planus, when white, lacy patches appear on the inside of the cheeks, it may not cause discomfort. However, symptoms associated with redness, swollen patches, and open sores can cause patients to experience burning or pain, sensitivity to hot, acidic, or spicy foods, bleeding and intense irritation from the infected area when brushing, inflammation of the gums (gingivitis), sore, thickened patches on the tongue, and discomfort when speaking, chewing, or swallowing. In severe cases of oral lichen planus, there may be an increased risk of significant and intolerable pain, weight loss or malnutrition in the patient, depression due to stress or anxiety, scarring from erosive lesions, and subsequent Recurrent oral mold or fungal infections can even lead to oral cancer (Popa et al., 2024).

Oral lichen planus is a chronic disease is currently no cure, so treatment focuses on helping heal severe lesions and reducing pain or other discomfort. Corticosteroids can reduce the inflammation associated with oral lichen planus, and the preferred method is by topical application directly to the infected site, such as mouthwash, ointment, or gel applied directly to the mucosa. Corticosteroids are also available by mouth, but they are taken as pills and last for a limited time(Oral Lichen Planus - Diagnosis and Treatment - Mayo Clinic, n.d.). Some oral lichen planus studies have shown that treating oral lichen planus with light therapy can reduces discomfort and

significantly reduces lesion size and light therapy did not show any side effects and was as effective as topical corticosteroids in treating lichen planus. Therefor, the use of an infrared laser significantly increases salivary flow in patients with xerostomia. Based on the research, using 904 nm laser to activate cells in the parotid and submandibular glands has been shown to successfully reduce xerostomia (Rathod et al., 2022).

Therefore, oral mucositis is a severely debilitating condition characterized by erythema, edema, and ulcerations of the oral mucosa. It is a complication of radiation therapy (RT) to the head and neck, chemotherapy, chemoradiotherapy, and hematopoeitic stem cell transplantation (HSCT). The lesions can also break the mucosa barrier resulting in local or systemic infection. In severe cases, this can lead to parenteral nutrition, ultimately leading to poor quality of life. Also, it is a common complication in patients receiving chemotherapy or radiation therapy for cancer.

Figure 2.5: Oral Mucositis, with widespread ulceration, tissue necrosis, and inflammation (https://www.researchgate.net/figure/Oral-Mucositis-Extensive-oral-mucositis-partly-covered-by-a-yellow-fibrin_fig1_355759473)

Oral mucositis is a common complication in patients receiving radiation therapy (RT) to the head and neck, chemotherapy for solid tumors or lymphomas, and high-dose myeloablative chemotherapy before hematopoietic cell transplantation. The incidence of oral mucositis varies with chemotherapy agents. Chemotherapeutic drugs that affect DNA synthesis (S phase), such as 5-fluorouracil, methotrexate, and cytarabine, are associated with a high incidence of oral mucositis. Using light therapy to treat mucositis can significantly reduces oral mucositis and daily mucositis index scores, clinical studies have shown that low-level laser therapy (LLLT) reduces the severity of mucositis in patients receiving chemoradiotherapy before hematopoietic stem cell transplantation. Additionally, it reduces the side effects of xerostomia, reduces pain levels, and improves swallowing compared to patients who do not receive laser treatment (Bell & Kasi, 2022).

Lastly, one of the most prevalent conditions affecting the tooth's attachment system is periodontitis.

Figure 2.6: Periodontitis, with inflamed, receding gums and bleeding (https://www.bodyexpert.online/en/periodontitis)

It is the main reason why elderly people lose their teeth. Obradovic et al. found that when periodontal disease patients were treated with LLLT (670 nm) in addition to traditional periodontal therapy, healing and collagenization improved. Lower-level soft tissue lasers have been shown to have analgesic benefits in the literature. The impact is thought to be due to endorphin production being stimulated or interference with the mediation of pain impulses. Lower-intensity soft tissue lasers have been shown to have analgesic benefits in the literature. The impact has been described in terms of interfering with the transmission of pain impulses and/or stimulating the synthesis of endorphins. The lymph vessel's permeability is reduced by LLLT, which can also stimulate the lymph vessel's collaterals. By increasing phagocytosis and expanding the number and size of lymph vessels, edema may be reduced.

Methodology

3.1 Materials

3.1.1 Human Gingival Fibroblast (HGnF) Cell Culture

- Complete growth media: Alpha-MEM
- Cells in cryovials
- Sterile new tissue culture flask (25cm^2)
- Sterile pipette tips (1000 uL)
- Micropipette
- Inverted microscope

3.1.2 Subculture Cells

- Complete growth media: Alpha-MEM
- Trypsin
- Sterile new tissue culture flask (25cm^2)
- Sterile serological pipette (5ml, 10ml)
- Sterile centrifuge tube (15ml)
- Inverted microscope