FATIH UNIVERSITY

MSc /Ph.D.

THESIS TEMPLATE

Do not forget to delete this page

26.12.2015

T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

INVESTIGATION OF PHOTOBIOSTIMULATORY EFFECT OF 670NM DIODE LASER ON MRC-5 FIBROBLASTS (HUMAN FETAL LUNG) PROLIFERATION

NUHU MUHAMMAD ABUBAKAR

M.Sc. THESIS
BIOMEDICAL ENGINEERING PROGRAMME

THESIS ADVISOR
Assist. Prof. Dr. HAŞİM ÖZGÜR TABAKOĞLU

İSTANBUL, APRIL / 2016

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

670 NM DİYOT LAZERİN MRC-5 (İNSAN FETAL AKCİĞER) FİBROBLAST HÜCRELERİNİN ÇOĞALIMI ÜZERİNDEKİ FOTOBİYOUYARICI ETKİSİNİN ARAŞTIRILMASI

NUHU MUHAMMAD ABUBAKAR

YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
YRD.DOÇ. DR. HAŞİM ÖZGÜR TABAKOĞLU

İSTANBUL, NISAN / 2016

T.C.

FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

Nuhu Muhammad Abubakar, a **M.Sc.** student of Fatih University **Institute of Biomedical Engineering** with student ID 520114017, successfully defended the **thesis/dissertation** entitled "**INVESTIGATION OF PHOTOBIOSTIMULATORY EFFECT OF 670nm DIODE LASER ON MRC-5 FİBROBLASTS (HUMAN FETAL LUNG) PROLIFERATION**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

_____

Assist.Prof. Dr. Haşim Özgür TABAKOĞLU

Thesis Supervisor

Examining Committee Members:

Assist.Prof. Dr. Haşim Özgür TABAKOĞLU _____

Fatih University

Assist.Prof. Dr. Alper YAMAN _____

Yeditepe University

Assist. Prof. Dr. Şükrü OKKESİM _____

Fatih University

It is approved that this thesis has been written in compliance with the formatting rules laid down by the Institute of Biomedical Engineering.

_____

Prof. Dr. Sadık KARA

Director

Date of Submission : 15 March 2016

Date of Defense : 06 April 2016

This work is dedicated to Zainab, my wife, contant companion, and best friend for three years of marriage. She is the loving mother of my children: Lubna (Aisha) and Hidaya (Yahanasu), she has demonstrated incredible patience and understanding with rather painful process of this research while maintaining a most pleasant, cheerful and comforting home while my love to her keep increasing daily, thank Allah the Almighty for your blessings to our marriage.

This study was supported partly by TÜBITAK with the project numbers of 213E011 and 112S625 to Hasim Ozgur Tabakoglu, PhD.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, the Most Merciful. All praises and thanks be to Him Whom we worship and unto Whom we seek refuge. I immensely thanks Almighty Allah for the Mercy and Blessing He bestowed on me by Whose will and assistances I have yet completed this master of science (MSc) research thesis.

My sincire gratitude and appreciation goes to the staffs of Institute of Biomedical Engineering Fatih Uninversity, especially my course and thesis supervisor, Assist. Prof. Dr. Haşim Özgür Tabakoğlu, Head of BME Institute, Professor Sadik Kara, Assist. Prof. Dr. Şukru Okkesim, Assist. Prof. Dr. Halil Ibrahim **Ç**akar, Assist. Prof. Dr. Aydin Albarak, Mahir Balik and other hardworking staffs of the Institute.

I also like to thank my father Malam Muhammad Abubakar and my mother Hajia Yahanasu Ahmad, whom I am grately indebted to them for their expert, sincire, valuable, incredible guidance, encouragements and love extended to me before and during my progam. Also to my siblings including Zainab, Aisha, Jamilu(Islam), Fatima, Idris, Ruqayya, Sumayya, Ammatullah, Hafsa and the last born of the family Abubakar(Abban Umma) and other relatives for their prayers and supports.

Infinite gratitudes and regards goes to the staff of Istanbul University, most especially Prof. Dr. Bedia Çakmakoğlu, Assist. Prof. Dr. Elif Sinem Bireller, Tuğba Kiriş, Tolga Çakmaktaş, and Barış Ertuğrul at DETAE Experimental Medicine for giving me the necessary assistance when and where-ever required. I will not forget to mention Musbahu M. Sani, Abdurrazak Abdulhamid, PhD. Bello Hamza Abdullahi, PhD. Muhammad Nasir Ahmad (Eddy), Jamilu Usman (Lagas), Ali Ahmed Mohamud and Özer Bekir Akin whose assistance helped seeing to the success of this thesis research.

In addition, a special note of thanks to Alh. Abdullahi Ibrahim C.A, Alh. Shahru Haruna Dan Diyma, Alh. Muhammad M. Lawal Bani Mbaka and all who directly or indirectly, have lent their helping hands in this research. I will not forget to thank the entire G9 Group members especially our leader Mukthar D. Ibrahim. Finally, my reserved appreciation goes to Kano State Government of Nigeria under the administration of His Excellency Egnr. Dr. Rabiu Musa Kwankwaso whose ambitious foresight made this studies a reality and also the administration of His Excellency Dr. Abdullahi Umar Ganduje who help in the completion of this program.

April 2016 Nuhu Muhammad ABUBAKAR

TABLE OF CONTENTS

LIST OF SYMBOLS

ƛ	Lambda (Wavelength)
®	Registered sign
™	Trade mark sign
°	Degree sign
µ	Micro sign
α	Alpha sign
β	Beta sign

ABBREVIATIONS

ADP : Adinosine Diphosphate

ADSCs : Adipose-Derived Mesenchymal Stem Cells

ALP : Alkaline Phosphate

ATP : Adenosine Triphosphate

BMSCs : Bone Marrow Mesenchymal Stem Cells

COX : Cytochrome c oxidase

CO_2 : Carbon Dioxide

DMSO : Dimethyl Sulfoxide

DNA : Deoxyribo Nucleic Acid

ECM : Extracellular Matrix

EMEM : Eagle's Minimum Essential Medium

ETC : Electron Transport Chain

FBS : Fetal Bovine Serum

FCS : Fetal Calf Serum

GaAlAs : Gallium Aluminum Arsenide Laser

LED : Light Emitting Diode

LLLI : Low Level Laser Irradiation

LLLT : Low Level Laser Therapy

MRC-5 : Human Fetal Lung Cells

$NADH^+$: Nicotinamide Adenine dinucleotide Reduced

NADPH: Nicotinamide Adenine dinucleotide Phosphate Reduced

NIR : Near Infrared

NO : Nitric Oxide

O_2 : Oxygen

PBS : Phosphate Buffered Saline

RNA : Ribonucleic Acid

ROS : Reactive Oxygen Species

UV : Ultra Violet

XY : Normal Diploid Male

LIST OF FIGURES

LIST OF TABLES

Page

SUMMARY

INVESTIGATION OF PHOTOBIOSTIMULATORY EFFECT OF 670NM DIODE LASER ON MRC-5 FİBROBLASTS (HUMAN FETAL LUNG) PROLIFERATION

Nuhu Muhammad ABUBAKAR

Biomedical Engineering Programme

M.Sc. Thesis

Advisor: Assist. Prof. Dr. Haşim Özgür TABAKOĞLU

Low Level Laser Therapy (LLLT) has been shown to be effective in a variety of medical conditions such as healing processes exerting a positive photobiostimulatory effect due to its ability to promote cell proliferations. In this study, the MRC-5 human fetal lung fibroblast was submitted to three applications of low level laser irradiation (670nm, power 10, 50 and 100 mW with 1.58J/cm^2). WST-1 assay was used to to evaluate cell viability, and growth curves were used to analyaze proliferation at 3, 6 and 24 hours. MRC-5 human lung fibroblast responded to LLLT in power-dependant manner. Higher cells proliferation was observed in the group irradiated with power of 50 mW at 3 and 6 hours. Mean proliferation rate for all treatment groups were found to differ significantly from the control groups with lower mean value ($p>0.05$) from the control than tests. No statistical significant difference ($p>0.05$) was found between compared 10 & 100 mW, 10 & 100 mW and 50 & 100 mW in 3, 6 and 24 hours respectively. Therefore, LLLI stimulate the proliferation of MRC-5 more effectively with 50 mW power especially at 3 hours as evidenced by increase in cell numbers as observed in the growth curves. The biostimulation of MRC-5 fibroblasts using laser therapy may be an important tool in regenerative or healing therapy.

Keywords: LLLT, Cell Proliferation, MRC-5 Fibroblast, Cell Viability, 670nm Diode.

ÖZET

670 NM DİYOT LAZERİN MRC-5 (İNSAN FETAL AKCİĞER) FİBROBLAST HÜCRELERİNİN ÇOĞALİMİ ÜZERİNDEKİ FOTOBİYOUYARİCİ ETKİSİNİN ARASTİRİLMASİ

Nuhu Muhammad ABUBAKAR

Biyomedikal Mühendisliği Programı

Yüksek Lisans Tezi

Danışman: Yrd.Doç. Dr. Haşim Özgür TABAKOĞLU

Düşük Seviye Lazer Terapisinin hücre çoğaltımını sağlayıcı foto-biyo uyarıcı özelliği, bu terapinin iyileşme ile ilgili birçok medikal vakada etkili olduğunu göstermiştir.

Çalışmamızda, MRC-5 insan cenin akciğer fibroblastı deneysel tüm uygulamalarda kullanıldı (3, 6 ve 24 saatlik) Lazer uygulama parametreleri : 670nm, 1.58 J/cm2 ile güç 10, 50 ve 100 mW olarak belirlendi. Hücre canlılığını değerlendirmek için WST-1 analizi kullanıldı. Gelişim eğrileri çoğalmanın 3, 6 ve 24 saatlerde analizi için kullanıldı. MRC-5 insan akciğer fibroblastları DSLT'nin gücüne bağlı olarak tepki verdi. Daha yüksek hücre çoğalması 3 saat 50 mW'lık gücün uygulandığı grupta gözlemlendi. Ortalama çoğalma oranının tüm uygulama grupları için daha düşük ortalama seviyesine sahip kontrol gruplarından ($p>0.05$) istatistiksel olarak farklı olduğu bulgulandı. Sırasıyla 3, 6 ve 21 saatlik 10&100 mW, 10&100 mW ve 50&100 mW uygulamalarıı arasında belirgin istatistiksel bir farklılık ortaya çıkmadı ($p>0.05$). Böylelikle, 50mW güçte özellikle 3 saatlik DSLT uygulaması MRC-5 çoğalmasını sağladığı, çoğalma eğrilerinde hücre sayısında artışın gözlemlenmesiyle tespit edilmiştir. MRC-5 fibroblastların lazer terapiyi kullanarak biyo-uyarımı, doku yenilenmesi veya yara iyileştirme terapilerinde önemli bir araç olarak kullanılabilir.

Anahtar kelimeler: DSLT, Hücre Çoğalması, MRC-5 Fibroblast, Hücre Canlılığı, 670nm Diyot.

CHAPTER 1

1.1 Purpose of the Thesis

Laser (Light amplification by stimulated emission of radiation) is a device that simply generates electromagnetic radiations which are relatively uniform in wavelength, phase, and polarization, which was demonstrated in 1960 by a scientist called Theodore Maiman in the form of a ruby laser [1]. Typically we can define laser as a source of light or as radiation energy [2]. Low Level Laser (LLL) is a inique device that is used as biostimulatory tool which exert its effects on biologic systems on the bases of non-thermal means [3]. This interesting area of investigation was initiated since 1067 by Mester et al. who put their efforts in this regard. They reported non-thermal effects of laser on mouse hair growth [4]. Low level laser irradiation (LLLI) is used in several researches to stimulate different types of cells proliferation and differentiation [4-8]. However, sometimes the photo-biostimulation is not always effective due to the different types of factors that may influence the process [9]. Thefore, for this reason *in vitro* biostimulation depends on laser-related parameters such as wavelength, dose, power and time of irradiation [7, 8, 10], type of cells irradiated [11], and the physiological characteristics of the cells at the time of irradiation [8, 10]. So as a consequence of these factors, the interaction of laser light with cells and tissues can stimulate or inhibit cell proliferation.

The present study aims to evaluate a possible photo-biostimulatory influence of low level laser irradiation on MRC-5 Fibroblasts (Human Fetal Lung) cell line proliferation in a systematic manner. The experiment was designed to access the following individual objectives:

1.2 Objectives:

The individual objectives of this research include the followings:

1. To evaluate cell proliferation rate among the treatment and control groups.
4. To make statistical comparison among the treatment and control groups.

3. To find out the possible best therapeutic mode of 670nm diode low level laser irradions power.

1.3 Motivation

MRC-5-fibroblasts (human fetal lung) are undifferentiated cells with important potential for applications in cell therapy because of their capacity for self-renewal, proliferation, and differentiation into diverse types of specialized cells. The MRC-5 cell line was developed in September 1966 from lung tissue taken from a 14 week fetus aborted for psychiatric reason from a 27 year old physically healthy woman. The cell morphology is fibroblast-like. The karyotype is 46, XY; normal diploid male. Cumulative population doublings to senescence is 42-48. G6PD isoenzyme is type B. Studies have shown that the higher the proliferation of the cells, the greater the regenerative and healing capacity of the tissues where they reside.

According to the authors, *in vitro* studies normally reflect the same conditions as observed in open wounds, i.e., the lack of an optical barrier, and lower doses are, thus, indicated in the case of cells biostimulation.

Health clinicians seek optimal methods of promoting wound healing, however this remains a challenge all over the world. Wound and injuries presents a dilemma of global proportions and attract great clinical interest due to high morbidity attributed to changes in the normal healing process [12]. Economically, chronic wound management has been reached a total cost of 2-4% of the health budget in western countries [12] and that the annual expenditure on wound and injuries related problems in the USA alone exceeds one billion dollars [13]. Complications in non-healing wounds are vast, and patients are at risk of severe pain, septicaemia, hospitalization, and in some cases amputations [14].

Furthermore, these changes may also inflict increased cost to society, and reduce quality of life for a huge number of peoples worldwide. The idea of effective therapeutic strategies and cheap alternatives that might work in the healing process attracts much attention [15]. Indirectly, this research will contribute to the enhancement of the recovery and lead to shorter stay in health care facility (reduced nosocomial infections),

reduced cost of wound care, and enhanced quality of life for patients suffering from wound related problems as well as faster return to routine activities.

Additionally, LLLI has been shown to stimulate the growth, proliferation and differentiation of different types of cells in culture, including keratinocytes, fibroblasts, endothelial cells, myoblasts, and osteoblasts, by exerting positive biomodulatory effects[16, 17]. However, little is known about the effect of laser therapy on MRC-5 because the proliferation of MRC-5 is usually slow and the yield of these cells after first harvest is low, a therapeutic tool that increases their proliferation without causing molecular damage while maintaining their specific characteristics is important for effective clinical application of these cells.

The laser radiation produces many different effects depending on light-beam parameters as well as the tissues that are subjected to irradiation. Despite documented therapeutic effect of low-level laser therapy (LLLT), there is still much controversy and scientific debate over its effectiveness on different cells[18-20].

The variety of procedures and the inconclusive results of the conducted studies mobilized to search for the parameters of laser radiation, which both in vitro and in vivo will result in acceleration of cell proliferation and the expected therapeutic efficacy. The relevance of some irradiation parameters remains occult and represents an important area for further research and repeated treatments are necessary to achieve a positive laser effect in clinical applications. This draws my attention to make a further study in order to optimize irradiation parameters by assessing the MRC-5 Fibroblasts (Human Fetal Lung) proliferations.

CHAPTER 2

2.1 Cells

The living things are composed of cells which exist in different, ranging from single cell organisms that practice free living to those in complex biological organisms like human. All the biological living systems despite the great diversity exhibited between them, remarkably, are composed of the same types of chemical molecules and apply similar principles in metabolism and replication, in higher organisms such as human, they have the ability to organize at the cellular levels [21, 22].

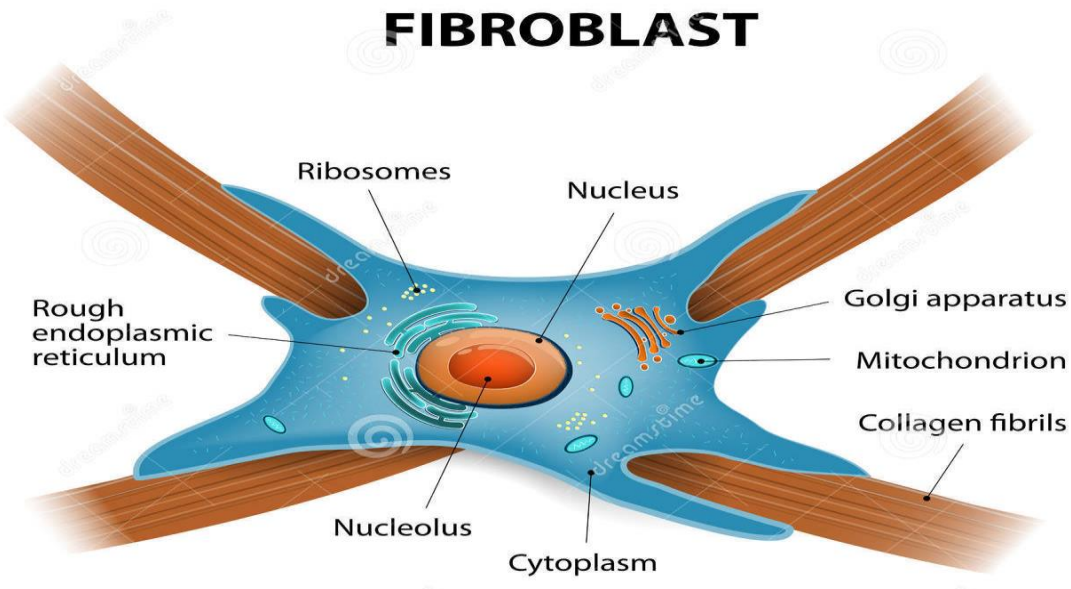


Figure 2.1 Structure of Fibroblast [23]

Regarding the internal structures and organelles of the cell, thus serve as biological system that are essentially an assembly of molecules where water, amino acids, carbohydrates (sugar), fatty acids, and ions account for 75–80% of the matter in cells, the remaining 25–20% cell mass are macromolecules [24].

2.2 Mechanism of Cell Proliferation

Cell proliferation lead to the productions of two daughter cells from single one, this is achieved through cell growth followed by cell division. Cell proliferation as observed in normal tissue is generally restricted to cells that replenish the tissue. Proliferation depends on availability of tissue-type specific growth factors, which are signals, not nutrients. Proliferation requires interaction of transmembrane proteins called integrins with components of the extracellular matrix (ECM) components. Contact inhibition of growth limits division in culture when cells form a contiguous monolayer. For human fibroblasts divide a limited number of times (ca. 50-70 divisions for human cells) before the cells enter a senescent state that maintains metabolic activity but stops all further division [25].

2.2.1 Cell Cycle

Cell division occurs in defined stages, which together comprise the cell cycle. In terms of the genetic material, cells must replicate their chromosomal DNA once every cell cycle and segregate the sister chromatids produced by DNA replication to yield two genetically identical daughter cells [26].

Molecular mechanisms induced by laser radiation involved mitochondrial photo-acceptors and production of Reactive Oxygen Species (ROS) [27–30]. Furthermore, laser radiation might affect cell cycle progression, protein synthesis, cell energy metabolism, proliferation and apoptosis by modulation of specific kinases and phosphatases [28, 31, 32]. The effect of laser radiation on cell growth, cell cycle progression and apoptosis has been widely studied and compared to the effect produced on non-irradiated cells by the use of many different Sources (generally low power lasers), wavelengths (mostly red and NIR radiation), energy doses (from tenths to tens of J/cm^2) and treatment modes.

2.2.2 Stages of Cell Cycle during Cell Proliferations

The cell division cycle is broken up into four stages: G1, S, G2 and M (Figure3). DNA replication occurs during S ("synthesis") phase [26]. DNA packaging, chromosome segregation and cell division (cytokinesis) occur in M (mitosis). S phase and M phase are separated by Gap phases. G1 is the gap between M and S. Cell growth is one of the important events of G1. The transition from G1 to S is the critical control point in the cell cycle. G2 is the gap between S and M, and provides time for proofreading to ensure DNA is properly replicated and packaged prior to cell division. G0 or quiescence occurs when cells exit the cell cycle due to the absence of growth-promoting signals or presence of pro-differentiation signals. The G1, S, and G2 phases comprise interphase, which accounts for most of the time in each cell cycle [26].

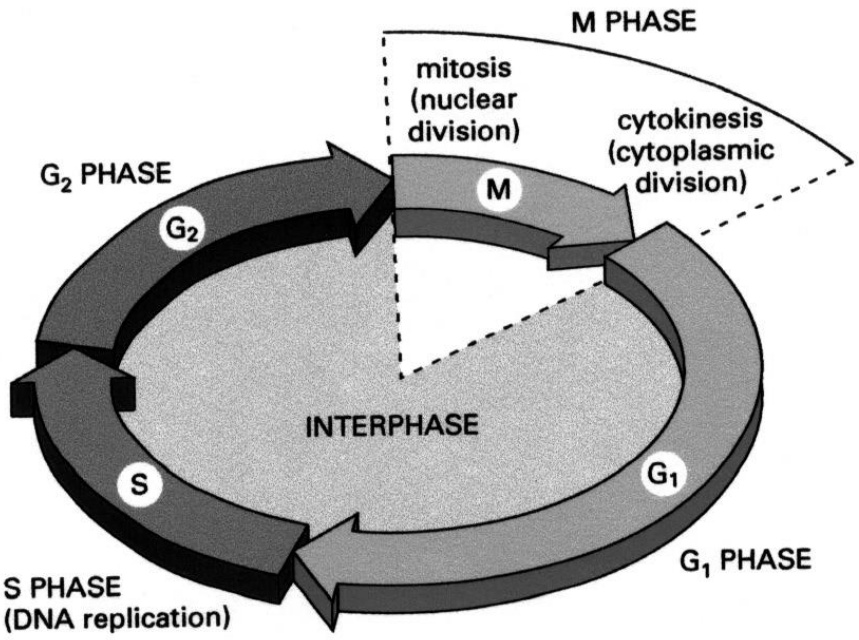


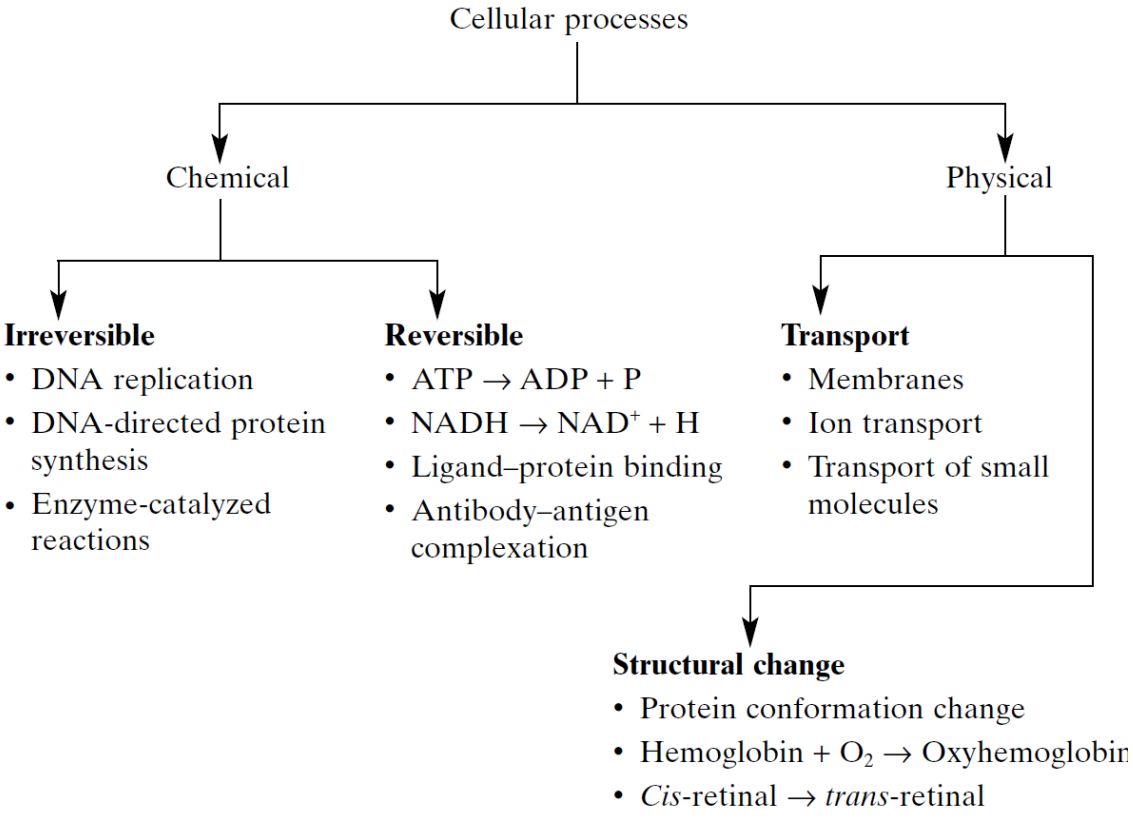
Figure 2.2 Demonstration of cell cycle stage [26]

2.3 Cellular Processes

Physical and chemical changes are those processes that are involved in cellular processes [21]. However, chemical changes can result in permanent products (such as synthesis of protein, replication of DNA) or can be cyclic or reversible (like in conversion of ATP to ADP and back). The type of chemical changes that are taking

place are of highly complex, which are often accelerated by enzymes (proteins) and coenzymes (NADH). Table 2.1 showed different cellular processes that occur in cells [21].

Table 2.1 Tissue, Cellular and Molecular Components that make ineteraction with Light when the light strike them [21]



2.4 Light-Induced Cellular Processes

Cells undergo different photophysical and photochemical processes during light absorption. Some of these processes are amount of cellular constituents fluoresce when excited directly or excited by energy transfer from another constituent [33]. Photochemical processes involving a chemical reaction in the excited state of a cellular constituent are varied [34, 35]. These chemical reactions that occur within the cellular membrane include the followings:

(i) *Photoaddition.* In this process, UV-induced molecular lesions in the structure of DNA, an example of this process is photodimerization of thymine process responsible for.

(**ii) *Photofragmentation***. In photofragmentation reaction, the original molecule when photoexcited, decomposed into smaller chemical fragments by the cleavage of a chemical bond. This type of reaction is very common in biomolecules when exposed to short wavelength UV light. An example is the photofragmentation of riboflavin.

(iii) *Photooxidation*. The main important point here is that, when excited molecule adds an oxygen molecule from the surroundings (a chemical process called oxidation). Photooxidation of cholesterol is one of the examples.

(iv) *Photohydration*. This process involved the creating of lesions in DNA in which an excited molecule adds a water molecule to produce a new product, as occur in uracil.

(v) *Photoisomerization*. This reaction involved the change in geometry or conformation of stereoisomers. This process account for retinal vision is that of 11-*cis*-retinal which upon excitation rotates by 180° around a double bond to produce a geometric isomer, the all *trans*- retinal.

(vi) *Photorearrangement*. In this process of photo-inducing, chemical formula of the molecule does not change, but bonds rearrangements take place as can be illustrated for 7-dehydrocholesterol in skin which upon UV exposure produces vitamin D3 [34, 35].

2.5 LLL Effect on Mitochondrial Cytochrome C Oxidase

Different studies shows mechanism of LLLT effects inevitably involve mitochondria, a main organelle for energy generation and metabolism via oxidative phosphorylation. Low level laser therapy (LLLT) induced processes that confirmed cellular physiologic activities. Many researches revealed the potentials of LLLT to trigger the mitochondrial respiratory chain. Cytochrome c oxidase (Cox), an enzyme that involved in transferring electrons between complexes III and IV of the electron transport chain (ETC) accompanying by near-infrared light stimulation [36, 37, 38]. Absorption spectra of Cox at different oxidation states were similar to that for biological responses to light [39].

This finding result into the proposal of Cox becoming the photoacceptor for the red to NIR range in human cells [39].

Therefore, stimulation of Cox leads to increased transfer of electrons and enhanced regulation of oxidative phosphorylation thus production of more ATP molecules [40, 41]. Recent studies also mentioned that under physiological conditions nitric oxide regulate Cox activity via reversible inhibition of mitochondrial respiration [42].

2.6 LLLT Mechanism of Actions

For Photo-biostimulatory effect of LLLT to occur and to have effect on living biological system, photons must be absorbed by electronic absorption bands of some molecular photoacceptors, or chromophores [43].

The cellular mechanism of LLLT has been linked to monochromatic visible and NIR radiation absorption by components of electron transport chain [44]. Mitochondrion is a core organelle responsible for cellular response to red and NIR light. LLL effects on rat liver mitochondria resulting the enahcement of proton electrochemical potential, increased ATP, NADH, RNA and protein synthesis [45, 46] and elavate membrane potential, and oxygen intake.

Several studies [47-50] support that cellular mechanism of LLLT on the bases of increased mitochondrial oxidative metabolism which caused by excitation of electronic components of the respiratory chain.

There have been four stages of Cellular mechanism of LLLT effect designated as primary, secondary, and tertiary [51, 52]. Generally, Primary reactions involved reactions due to photon absorption while secondary effects is not related to LLLT and their occurrence depended on cell sensitivity which differ from the primary. Tertiary (systemic) effects are affected by both internal and external environment including intracellular interactions as clearly reported why the treatment of one lesion can also stimulate healing in other lesions present [53].

2.6.1 Primary Effect of Light to Cells

Laser beams exert its effect on mitochondria [54] and cell membranes of fibroblasts, keratinocytes and endothelial cells, where chromophores (cytochromes, porphyrins and flavoproteins) absorb and convert the heat energy to chemical kinetic energy [55, 56] within the cell. This changes membrane permeability improves mitochondrial signaling with nucleus and cytosol, nitric oxide formation and elavates oxidative metabolism thereby producing more ATP [44, 54, 57, 58,] which further results in suitsble cell function, cell proliferation, pain relief and healing of wound [44, 55, 56]. Below are some of the proposed assumptions that explain LLLT mechanism which includes:

2.6.1.1 Assumption of Singlet Oxygen ($^1O_2$)

This was based on visible laser light action on RNA synthesis rates in HeLa cells and spectroscopic data for porphyrins and flavins (photo-absorbing molecules which can be reversibly converted to photosensitizers) [59]. The molecular absorption of light is responsible for generation of singlet oxygen $^1O_2$ which leads to stimulation of RNA and DNA synthesis [60, 61].

2.6.1.2 Assumption of Nitric Oxide (NO)

In hypoxic or stressed cells, mitochondrial nitric oxide can bind to Cox, displacing O_2 in competitive mode thus inhibiting respiration especially [62]. And also NO concentration is enhanced under pathological conditions (resulting from activation of NO producing macrophages [63]. It was assumpted that [64, 65] laser irradiation and activation of electron flow in cytochrome c oxidase molecule could reverse the partial inhibition of the catalytic center by NO and thus enhance binding of oxygen and respiratory rate. Increased concentrations of nitric oxide have been reported in cell cultures and animals after LLLT due to its release, from Cox in the mitochondria and from other intracellular stores such as nitrosylated hemoglobin and myoglobin, by the photon energy [66].

2.6.1.3 Redox Properties Change and Superoxide Anion Assumptions

The state of redox in certain chromophores like CuA, CuB or hemes *a* and *a3* in cytochrome c oxidase molecule and the rate of electron flow in the molecule are affected by photo-excitation [36]. It was proposed that low or no cellular response is observed at overall optimal cellular redox potential for the particular growth conditions. A rise in intracellular pH due to irradiation has been measured experimentally both in mammalian cells [67] and in *E. coli* [68].

Reactive oxygen species (ROS) formed as natural by-products of oxygen metabolism, have key roles in cellular signaling [69], regulating synthesis of nucleic acids and protein, activation of progression of cell cycle also and enzymes [70]. In [71] suggested that "activation of the respiratory chain by irradiation would also increase production of superoxide anions, the production of which depends on the metabolic state of the mitochondria" [72].

It was also confirmed that LLLT shifts the overall cellular redox potential in favor of greater oxidation [73] and increased generation of ROS and cell redox activity have been demonstrated [74-76]. These cytosolic changes may consequently induce transcriptional changes (regulation of transcription factors).

2.6.1.4 Local Transient Heating Assumptions

The excited electronic state (by means of light) is converted to heat, as a result causing a local transient rise in the temperature of the absorbing molecules. So this transient increase the temperature of the biomolecules may in turn cause structural or conformational changes and initiate biochemical activities [77].

Remarkably, [78] stated that only one of above reactions occurs upon irradiating and formation of excited electronic states in cells, stating that "it is entirely possible that all the mechanisms proposed above lead to modulation of the redox state of the mitochondria (a shift in the direction of greater oxidation)". However, depending on dose and intensity of the light used, some of these mechanisms may significantly prevail. Experiments with *E. coli* showed that, different mechanisms take place at different laser doses, with photochemical at low doses and thermal at higher doses [53].

2.6.2 Secondary Effect/Reactions or Cellular Signaling

The mentioned primary photoreactions are improved by cellular signaling. A metabolic cascade at cellular level results in various physiological changes including cell membrane permeability [53, 79]. Mitochondrial calcium is released into the cytoplasm thereby changing intracellular calcium levels [80] and thus stimulating cell metabolism and regulation of signaling pathways responsible for processes required for wound repair such as migration of cells, synthesis of RNA and DNA, cell mitosis, secretion of proteins and proliferation of cells [81, 82]. All these events are mediated through cascades of cellular signaling and photosignal transduction [83].

Many researches confirmed that intracellular redox state regulates differents cellular signaling pathways [84, 85]. It is believed that fourteen extracellular stimuli stimulate cellular responses such as proliferation, differentiation, and even apoptosis through cellular signaling pathways. Cellular redox state modulation also affects gene expression through cell signaling mechanisms (via effector molecules like transcription factors, such as nuclear factor kappa B (NF-κB) and activator protein (AP)-1 [84], and phospholipase A2) [86, 87]. Commonly, cell signaling systems are activated by oxidants, while reductants inhibit upstream signaling cascades, with resultant suppression of transcription factors [88]. Despite similarity in cellular signaling, the resultant cellular responses to irradiation may differ due to variation in modes of regulating transcription factors.

An effort was made to enumerate the extent of irradiation effects as dependent on the metabolic status of *E. coli* cells. A correlation was found between the amount of ATP in irradiated cells and the initial amount of ATP in control cells [16]. Thus, variations in the effects of low level laser at the cellular level are explained by the cellular redox state (and pHi) at the time of irradiation.

2.6.3 Tertiary Effect or Response

Here the effects induced in cells are away from site of secondary reactions [53]. Communication between irradiated and non-irradiated cells, occur through amplified levels of cytokines or growth factors [89]. This boosts immune response by activating

T-cells, macrophages and mast cells, with subsequent improved synthesis of endorphins and decline in bradykinin resulting in pain relief [90].

2.6.4 Non-mitochondrial Chromophores as Agent of Metabolic Improvement

Processes that involved in the regulation of redox activities are unabled to occur via the respiratory chain alone. Redox chains containing visible spectra chromophores are the main key structures of regulating metabolic pathways. NADPH-oxidase multi-component enzyme system of phagocytic cells, responsible for non-mitochondrial respiratory burst, is a redox chain that generates ROS in response to microbicidal or other types of activation. Irradiation with 670nm diode laser and other lasres exerted a positive biomodulatory effect on the growth and differentiation of human osteoblasts proliferations as well as others cells [91].

CHAPTER 3

LITERATURE REVIEW

3.0 Low Level Laser Therapy (LLLT)

Low level light/laser therapy (LLLT) is the use of light to promote tissue repair, reduce inflammation, induce painlessness or stimulate cell proliferation etc. Not like many others, LLLT treatments is not an ablating or heating based therapy but rather a photochemical effect which means the light is absorbed and cause a chemical change [92] and is more equivalent to photosynthesis in its mode of action. LLLT also differs from photodynamic therapy (PDT), which employs light indirectly to activate photo-sensitive dyes to produce bactericidal molecules that kill causing disease microbes.

In contrast, LLLT or photo-bio-modulation uses the achievement of light and light alone to directly kindle host cells in order to reduce inflammation, relieve pain and/or stimulate wound healing. The technique termed low level because the optimum levels of energy density delivered is low and is not comparable to other forms of laser therapy as practiced for ablation, cutting, and thermal tissue coagulation [93].

The biological effects of low level laser therapy have been described in a diverse range of cell and tissue culture systems, animal models, and occasionally in humans. Different cells and tissues have been studied, some normal, some with different disease characteristics. At cellular level LLLT causes adjustments biochemically and bio-energetically, leading to metabolic enhancement, proliferation and maturation of cell, amplified quantity of granulation tissue with decreased inflammatory mediators and enhanced healing process [94, 95].

Furthermore, cellular absorption of laser irradiation results in increased cell metabolism with characteristic stimulation of mitochondrial respiratory chain photoreceptors, changes in cellular level of ATP, release of growth factors, and synthesis of collagen [96].

Replication of DNA, proliferation of cells, enhancement of microcirculation and other cellular processes have been shown to occur between the red and IR wavelength. Low

laser irradiation has also increased growth factors, with subsequent angiogenesis, release of cytokine and increased production of cellular matrix [97, 98].

Both in-vitro and in-vivo experimental studies on LLLT have been underway since 1960s, which led to its approval by U.S. Food and Drug Administration as an important method for hastening cell proliferation and wound healing in early 1990s [99-101].

Low power and doses of laser irradiation have been shown to stimulate in vitro proliferation of fibroblasts [102, 103], keratinocytes [104], 16 endothelial cells [105], and lymphocytes [106]. The proposed proliferative mechanisms involve photo-stimulation of mitochondrial processes, which enhance release of growth factor, ultimately leading to proliferation of cells.

This study [107] showed a dose dependent enhancement of human gingival fibroblasts proliferation by low level irradiation. This study also [108] showed that low laser irradiation could activate skeletal muscle satellite cells, enhance their proliferation, inhibit differentiation and regulate synthesis of proteins. Furthermore, modulation of matrix metalloproteinase activity and gene expression in porcine aortic smooth muscle cells were evident [109].

However, there is no uniform result in instruments tested, parameters of the laser used like energy density, duration and frequency of exposure [110]. The statement that LLLT may have clinical utility in speed up wound healing is result from its ability to stimulate cell proliferation, cell motility and angiogenesis, while alleviating the inflammatory response. Several studies report LLL-induced cell proliferation, findings are not uniform, perhaps influenced by the variations in cell lines tested, culture conditions and timing of LLL exposure, and differences in LLL fluence, energy, and density.

There is study like [111] that reported LLL stimulated fibroblast proliferation, but decreased similar changes in human bronchial epithelial cells and in a human oral squamous cell cancer (SCC) cell line. In line with this, another study by [112] reported an increased in proliferation and expression of cyclin D1 in LLL-exposed (3 J/cm^2 to 12 J/cm^2) human keratinocytes. In another study, the same authors studied the effects of LLL (2.05, 3.07 or 6.15 J/cm^2) on oral dysplastic cells or oral SCC cells. Similar to previous reports, they find out that Akt/mTOR/Cyclin D1-mediated modulation of cell

growth, expression modification of proteins associated with progression and invasion of the cell lines, and pAkt, pS6 and cyclin D1 with production of an aggressive Hsp90 isoform [113]. In the research by [114] reported that LLLI (0.5J/cm^2 or 1.0J/cm^2) on cultured human tongue SCC cell lines, higher proliferation was observed in the two laser treated groups after 24 hours of culture with 0.5J/cm^2 being more significant.

One study using protein kinase C's as a replacement endpoint showed relation in cell proliferation and apoptosis, the reaction of human lung adenocarcinoma cells to energy levels of 0.8J/cm^2 or 60J/cm^2 were compared. While lower energy LLLT induced cell proliferation by activating PKCs which showed PKC activity decreased during apoptosis induced by high fluence laser [115].

Similarly study by [116] found that the best photobiostimulatory dose of LLL was 180mJ/cm^2 compared to 420–600 mJ/cm^2 dose bioinhibitory activities. In another study of oral epithelial cells, it was found that in contrast to 20 J/cm^2, 4 J/cm^2 resulted in ROS accumulation which did not caused the damaging of DNA and did not induce genomic instability. By comparing the two energy levels in oral keratinocytes, both energy levels contributed to epithelial cell migration through activation of the mTOR signaling pathway [117].

Low-energy laser irradiation using gallium-arsenide semiconductor laser in the study of histopathological rat submandibular salivary glands revealed a results that mitoses of duct epithelial cells without atypia elevated between one and twenty four hours after irradiation, having a greater increased 24 hours as compared with the control. Mitoses of duct epithelial cells are more effeciant in granular ducts, low in striated ducts and still lower in intercalated ducts [118].

Human cells i.e Human gingival fibroblast that is isolated from gingival connective tissue recieved soft laser irradiations showed a higher proliferation activity. High significant differences were observed at 24 hour after irradiation but decreased at 48 and 74 hours in an energy-dependent manner [119]. The proliferation of collagen fibers promoted by laser radiation was also observed in a study by [120] when assessing the therapeutic effect of GaAlAs laser at 63.2 J/cm^2 on the repair structure of the medial collateral ligament of rats and endothelial cell (EC) proliferation plays a key role in the process of tissue repair.

At different energy fluencies of 1.96, 3.92, and 7.84 J/cm^2 809nm using GaAlAs diode laser showed a significantly increased in the metabolic activity of periodontal ligament fibroblasts (PDLFs) which were incubated for three days [121]. A study by [122] found out an increased in fibroblast proliferation in vitro irradiated with infrared LLL irradiation, which can lead to the indication of possible stimulatory effect on wound healing in vivo. The Chinese hamster ovary cells (CHO K-1) that received LLL (830nm; 10mW; 2J/cm^2) irradiations showed an increased level of cellular division, as proved by examining the intermediary filaments of the cytoskeleton and the chromosomes [123]. And also by [124] studied osteoblasts that recieved LLLI and found that in proliferation phase the number of osteoblast significantly increased 3 and 5 days after LLL irradiation in comparison to the control.

It was observed that the proliferations of fibroblasts are faster than that of endothelial cells in response to laser irradiation. Using 665 and 675 nm light has higher or maximum proliferation than 810 which inhibit the fibroblast proliferations [105]. Another research by [125] demonstrated the effect of LLLT on the proliferation of human osteoblast-like cells which are the cells derived from human mandibular bone were exposed to GaAlAs diode laser at dosages of 1.5 or 3 J/cm^2 which were then seeded on titanium discs. At ninety six hours, they found that LLL irradiation put significantly increased in the cell proliferation.

According to the study by [126], the densities of one and three J/cm^2 of LLLI energies was found to significantly increase the number of mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs) up to 2 and 4 weeks respectively when compared to the control. Another study by [127] assesses the effect of 830nm LLLI on cell proliferation of normal primary osteoblast (MC3T3). The LLLI was performed at 3 different wavelengths and four energies levels of 0.5, 1, 5, and 10 J/cm^2. One day after LLL irradiation, cell proliferation was measured. Osteoblast proliferation and alkaline phosphatase (ALP) activity in the osteoblast line revealed a significantly increased after the irradiation with 830nm laser irradiation at ten J/cm^2.

[128] used a diode-based method to generate a similar wavelength to the He-Ne laser (363 nm). They reported that irradiation at 5 J/cm^2 of adipose derived mesenchymal stem cells resulted in enhanced proliferation, viability and expression of the adhesion

molecule beta-1 integrin as compared to control. [129] found that irradiation of gingival fibroblast with LLL (685 nm) for 140 s at 2 J/cm^2 increased the proliferation as well as the release of bFGF, IGF-1, and IGFBP3 from these cells. Irradiation of bone marrow derived mesenchymal stem cells with 635 nm diode laser (60 mW; 0, 0.5, 1.0, 2.0 and 5.0 J/cm^2) significantly stimulated their proliferation and 0.5 J/cm^2 was found to be the optimal energy density [130].

The research by [131] comfirmed the effect of LLLT as it increased endothelial cell proliferation and migration. Achilles tendon fibroblasts proliferation increased significantly as it irradiated with LLLI, whereas higher laser intensity did not achieve a correspondingly higher cell proliferation effect [74]. In a study of a rat model of disuse atrophy muscle regeneration by LLLI, at the time of the recovery period, rats irradiated daily with (Ga-Al-As laser; 830 nm; 60 mW; total, 180 s). After two-weeks, the percentage of capillaries and fibroblast growth factor levels differed significantly in the laser-treated and the control groups of the muscles. Furthermore, LLL treatment has reasonable effect on the proliferation of satellite cells [132]. The main idea of LLLI to have potentialeffect on cellular proliferation is a wavelength and dosage dependent; however, other important key parameters such as irradiance need to be address [133].

A study by [134] showed that significant increased in the proliferation of Primary fibroblast cell culture from human keloids irradiated with three (3) J LLLI while the 3T3 cell culture revieled no significant difference. Significant decreasein the percentage of the cells was observed in the proliferative phases of the cell cycle that were irradiated with 21 J in the two cell types. These findings support the assumptions that the physiological state of the cells has can have influence to the LLLI results, and high metabolic rate and short cell cycle of 3T3 are not responsive to LLLT.

Human periodontal ligament fibroblasts (PDLFs) received GaAlAs semiconductor diode laser irradiation for ten, twenty and thirty seconds showed an increased in cell proliferation in a time-dependent manner as the cell gradually increased proliferations during 72 hours with no significant difference between treatment and control groups compared within 72 hours, significant increased in PDLFs proliferation was observed between 24 and 48 hours at both 1.97 and 3.94 J/cm^2 energy fluencies [135].

Human osteoblast-like cells seeded on titanium/zirconia surfaces, were irradiated 3 hours later, the activity of mitogen and ALP activity were increased indicating that LLLT stimulates osteogenic cell proliferation [136]. One study of osteoblastic activity in the rats by [137] found that the cellular doubling time in the proliferative stage at 3-7 days was decreased 24 and 48 h after LLLI by the increased growth of the cells in a culture.

For the study conducted by [138] used 810nm, 50 mW diode laser with 2 different energies (1 J/cm^2 and 4 J/cm^2) irradiate human schwann cells (SCs) for 3 consecutive days. Significant decrease in cell proliferation of the control groups was observed compared with treatment groups. On the seven day, there is significant increase in proliferation in both irradiated and control groups with no significant difference between the laser treated groups.

The photobiostimulatory effect of LLLT is obvicious by enhancing production of some cytokines and growth factors. Laser (685 nm, 25 mW, 14.3 mW/cm^2, 140 sec, 2 J/cm^2) was used to irradiate osteoblasts for for 2 consecutive days. The result showed that the irradiated groups revealed higher proliferation, viability, bFGF, IGF-I, and IGFBP3 expressions than the control group. They came to the conclusion that the biostimulatory effect of LLLT has connection with increased in the production of the growth factors [139].

Another research [140] Nd:Yag laser at different powers (100 mJ, 1.5 W and 150 mJ, 2.25 W) was used to irradiated osteoblasts. There were significant increased in the proliferation of treatment groups compared to the control at 7days. After 14 days, there was no effct on cell proliferation.

ALP activity and LLLT effects on cell proliferation of human osteoblast-like cells (Saos-2) treated with different level of zoledronate, the bisphosphonate, were studied by [141]. Different concentrations of zoledronate were used to treate the cells then irradiated with diode laser (808 nm wavelength, 10 s, 0.25 or 0.50 W). The LLLT was observed to increase the ALP activity and proliferation rate of the cells, while zoledronate reduced both the two. LLLT declined the harmful effects of zoledronate and improved cell function and/or proliferation when applied together. In addition, [142]

found that the proliferation of mouse MSCs was significantly improved after irradiation with 635 nm diode laser.

Some researches have been proved thst lasers have been shown to be stimulatory, inhibitory, and or not effective at all. Simply, these observations are due to the experimental set up of the parameters used in the research. One study [143] made an interesting statement that "the magnitude of the laser bio-stimulation effect depends on the physiological state of the cell at the moment of irradiation". Currently, the lack of single well matured and accepted actual explanation of mechanism of low level laser biostimulation complicates the evaluation of the conflicting reports in literature.

There is a study [144] irradiated human umbilical vein endothelial cell (HUVEC) with a 670nm diode laser (power: 10-65 mW cm-^{2}, dose: 2-8 J cm-^{2}) for 6 days. They found a reasonable stimulatory effect of laser irradiation on HUVEC cell proliferation. Doses between 2 and 8 Jcm-2 induced statistically significant cell proliferation. Testing different powes at a constant dose of 8 Jcm-^{2}, 20 and 65 mW cm-^{2} present well pronounced cell proliferation. They came to the conclusion that LLLT influences EC proliferation and this may leads to the increase in angiogenesis and the acceleration of wound healing in vivo.

One study [145] observed that LLLI 670nm and doses of 1.0 and 2.0J/cm^2 exerted a positive biomodulatory effect on the growth and differentiation of human osteoblasts. The best results were obtained with the dose of 1.0J/cm^2, which was in agreement with the present findings showing higher proliferation of BMSCs and ADSCs treated with a dose of 1.0J/cm^2 over period of time.

CHAPTER 4

MATERIALS AND METHODS

4.1 Cell Culture

Human MCR-5 lung fibroblasts (ATCC® CCL171™) cell line which was isolated from lung tissue taken from a 14 week fetus aborted for psychiatric reason from a 27 year old physically healthy woman was cultured and was used in this experiment in accordance with standard sterilization technique and safety rules maintaining recommended strict aseptic conditions under the supervision and approved protocol of ethic commity of İstanbul Üniversitesi at Deneysel Tıp Araştırma Enstitüsü (DETAE).

4.1.1 MRC-5 Cell Thawing Processes

The biological safety cabinet and all equipments were used in strict aseptic conditions. MCR-5 cells were used in this experiment. The cryopreserved vial of MRC-5 cell line that was stored in liquid nitrogen vapour at -80 ^{o}C was placed in 37 ^{o}C water bath for 2 min agitation. The thawed cryomedia with DSMO (Dimethyl Sulfoxide) in the vial was added into 15 ml falcon tube containing 10 ml of EMEM (ATCC-formulated Eagle's Minimum Essential Medium with Earle’s Salts, with L-Glutamine Cat. No.: MEM-A (500 ml)) and centrifuge at 1500 spin for 2 min. The supernatant was discarded and the pellet of the cells was resuspended in 10 ml medium and centrifugation was repeated once more in order to get rid of DMSO. All of the cells in the pellet were seeded with EMEM containing 10% FBS and the next day medium was refreshed in order to get rid of dead cells (Figure 4.1).

Figure 4.1 Seeding cells in the wells and Subculturing steps

4.1.2 Seeding and Subculturing

After seeding of cells into 75cm^2 culture flasks, cells were attached to the surface of flasks (Figure 4.2). When cells became 80-90% confluent, they were subcultured. Before the subculture, EMEM, FBS, PBS and trypsin were warmed in a 37^{o}C water bath. Medium in the flask was removed by a sterile pipette and then 5 ml of calcium and magnesium free Phosphate Buffered Saline (PBS) was added to remove residual medium. After removal of PBS, 4 ml of pre-warmed 0.25% Trypsin/EDTA (GIBCO) was added to the flask and kept at room temperature for 1-2 min. Then cells were observed under invert microscope (Leica Microsystems CMS GmbH P MAX=5W U=5V DC Type 11090137001). When cells were detached from the surface of the flask, 1 ml of FBS added to the flask to inactivate the function of trypsin.

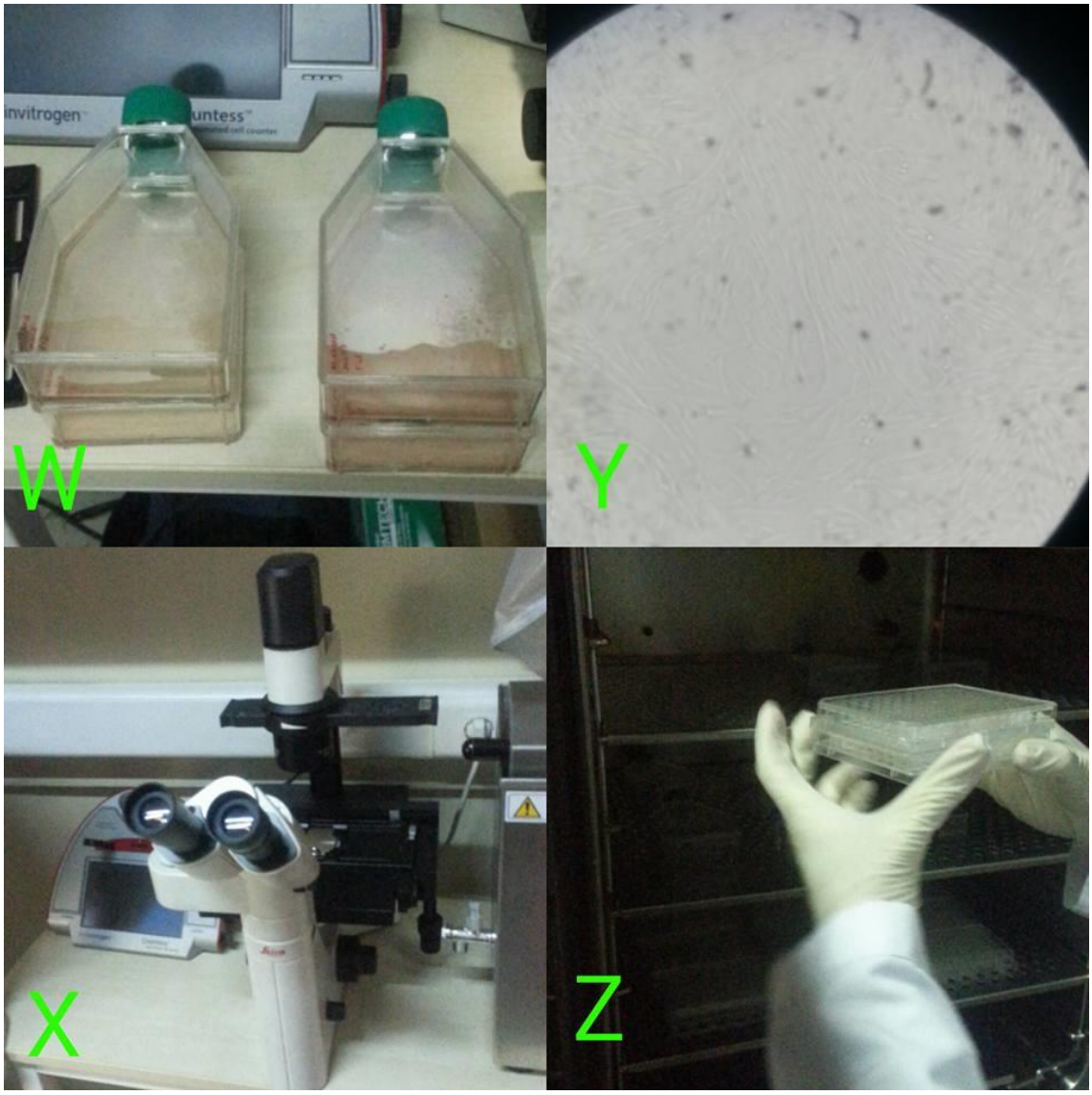


Figure 4.2 W: 75 cm^2 culture flask, X: light Microscope, Y: Growing cell after subcultured under microscope and Z: an incubator that maintained at 5%CO_2, 37^oC with relative humidity

The cells in the flask with trypsin and FBS was transferred into a new 15 ml falcon tube and centrifuged at 1500 rpm for 10 min at room temperature. After centrifugation, supernatant was discarded leaving about 0.5 ml of the cell suspension at the bottom. Pellet was finger mixed and volume was up to 10 ml with EMEM medium in order to remove the remaining any trypsin. Centrifugation step was repeated once more and then cells were counted by hemocytometer. After counting, cells were seeded at density 1 x

10^4 cells/well with 10% FBS containing EMEM for expansion. Subculture of cells was repeated at about 4-5 days intervals.

4.2 Experimental Design and Operations

In this experiment, a laser system set up with three 670 nm emitting diodes, controlled sepaterely was used (Figure 4.3). System speccifications is given in Table 4.1 (ƛ=670nm, 3x400mW 500mw Sony MM Laser Diode 9mm N type 30^{o}C SLD1332V Prophotonix fiber of Fatih University).

Figure 4.3: Laser System: J: Power Meter, K: Laser Beam & Eye protecter and L: Laser system with three 670 nm emitting fiber outputs. M: Laser parameters can be set on the touch-screen.

Table 4.1 Laser Parameters used

Laser Parameters	
Laser	3 Diode Lasers
Wavelength (nm)	670
Wave Emission	CW
Power Output (mW)	10, 50 and 100 (used in experiments) (400 mW max)
Spot Size (cm^2)	0.63
Fluence (J/cm^2)	1.58
Power Density (W/ cm^2)	0.0158, 0.079 and 0.158
Durations (s)	100, 20 and 10 for 10, 50 and 100 mW applications respectively

The MRC-5 cell lines were seeded in 96 well plates (1×10^4 cells / well) in 100 µl medium and incubated in a humid atmosphere with 5% CO_2 at 37^{o}C to settle down overnight. Three 96 well cultured plate seeded with 1×10^4 cells / well designated as plate1, plate2 and plate3 ware used and each plate (Figure 4.4) contains A, B, C and D groups, each with six replicates wells. Group A (Light1+Cells) received LLLI of 10 mW power output for 100s, Group B (Light2+Cells) received 50 mW for 20s, Group C (Light3+Cells) received 100 mW for 10s thus achieving a single dose of $1.58J/cm^2$ each and Group D (Only Cells) served as control group and received no LLLI as showed in Figure 4.4. Plate1, plate2 and plate3 were immediately incubated (in humid atmosphere with 5% CO_2 at 37^{o}C) after the treatment for 3hours, 6hours and 24hours respectively.

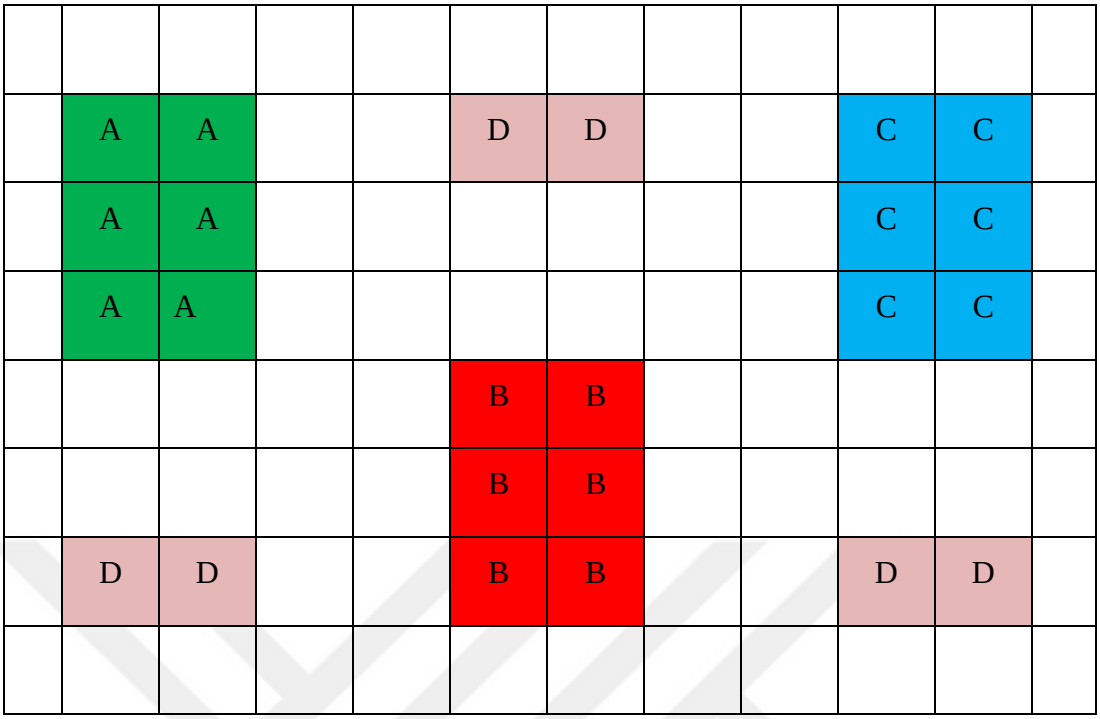


Figure 4.4 Groups distribution on 96 wellplate with empty wells between the groups

The irradiations were done spot by spot to cover the entire cell well area with the tip of the optical fiber positioned above the cultured well during all laser applications and each group was surrounded with empty well to avoid scattering (Figure 4.4). The spot size of the laser beam was measured as 0.63 cm^2 and laser power was checked with an energy meter (S121C Standard Photodiode Power Sensor, Si, 400 - 1100 nm, 500 mW, PM200 Optical Power and Energy Meter Thorlabs). Continuous mode of irradiation was applied and the plates were not covered during the irradiation process which was performed in the room temperature. All the experiments included a control group of cells grown under the same conditions but not irradiated and the parameters were set after thorough review of related literature as reported above.

4.3 Cell Viability

The measurement of cell viability is one of the most important tests in different forms of cell culture, which tests the number of healthy cells in a sample. Among the methods apply to determine cell viability, the reduction of different kinds of tetrazolium salts etc.

WST-1 assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases (Figure 4.5) that lead to an accurate cell prolifearion result. The product formazan produced by WST-1 is soluble which leads to a wider linear range and higher sensitivity with the standard incubation time of WST-1 time is 2 hours. Single addition of the reagents can reflect the effect of the testing materials at different time points on the trend of relative cell viability [145A].

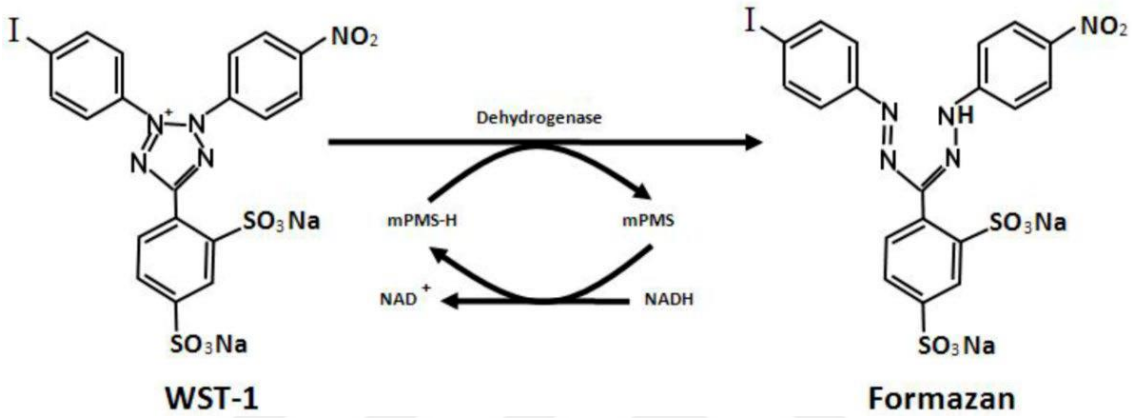


Figure 4.5 Schematic mechanism of the WST-1 reduction [145A]

4.3.1 Application Protocol

WST-1 (Roche) cell proliferation assay was used to determine the number of healthy cells in control and laser treated groups of this experiment. The reagents was taken from its storage (-20 ^{o}C) and thawed for two minutes using water bath. After incubating the laser treated and control groups in 96 well cultured plate designated as plate1, plate2 and plate3 for 3hours, 6hours and 24hours respectively. 10µl of WST-1 reagent mixtures was added to each well plate ($1x10^4$ cell/well) in 100µl of EMEM. The cultured plate was gently shaked for one minute in order to ensure the homogeneousity of the colour and was incubated for two hours at 37^{o}C, 5% CO_2 incubator.

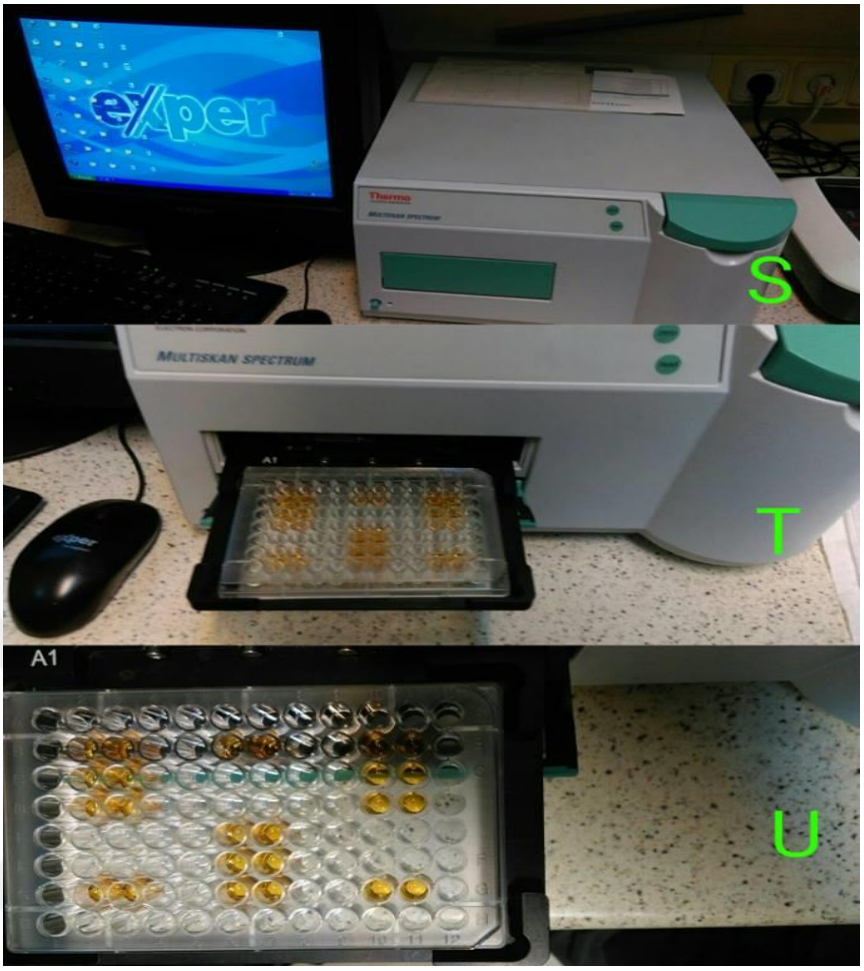


Figure 4.6 Analysis of Cell viability S: Microplate Reader System, T: Side View, U: Top View

After two hours of incubation, the plate was placed in microplate reader (Thermo Electron Corporation, Multiskan Spectrum) (Figure 4.6) to measure the absorbance at 450nm wavelength. The same procedure was done for all plates. The cell viability percentage in each group was calculated by comparison to that of the control group.

4.4 Statistical Analysis

ANOVA (Analysis of variance) and paired comparison analysis followed T-test were employed to test for statistical differences in the set of data at p equal to 5% (p=0.05) level of significance for laser treated and non laser treated groups at three differents incubation hours.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 Results

Below are the graphical illustrations of the results, the lowest proliferation was observed for the control group (no laser treatment) at all studied time frames when compared with irradiated groups.

The comparison of the three laser powers showed a tendency towards higher cell proliferation in the groups treated with 50 mW at 3 and 6 hours, followed by 10 mW in 24 hours (Figure 5.1 to 5.4). Inorder to find out where are the significant differences are, we conducted paired comparison (t-test) of six possible pairs (AxD, BxD, CxD, AxB, AxC and BxC groups). A significant difference in cell proliferation was observed between the control groups and the groups treated with the three power output 10, 50 and 100 mW after 3, 6 and 24 hours respectively with all the powers stimulating cell growth. No significant difference is observed in cell proliferation in the 4 possible pairs compared as AxC in 3 hours, AxD & AxC in 6 hours and finally BxC in 24 hours Groups (Figure 5.5 to 5.10).

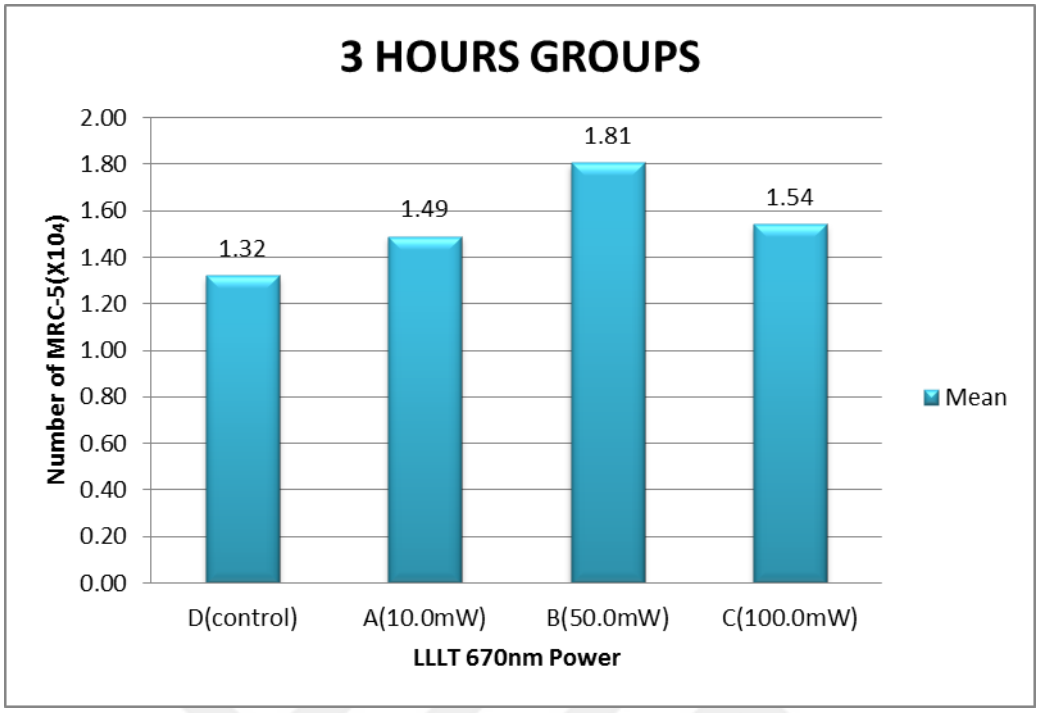


Figure 5.1 Mean proliferation of MRC-5 human fetal lung fibroblasts submitted or not submitted to laser irradiation over 3 hours incubation time

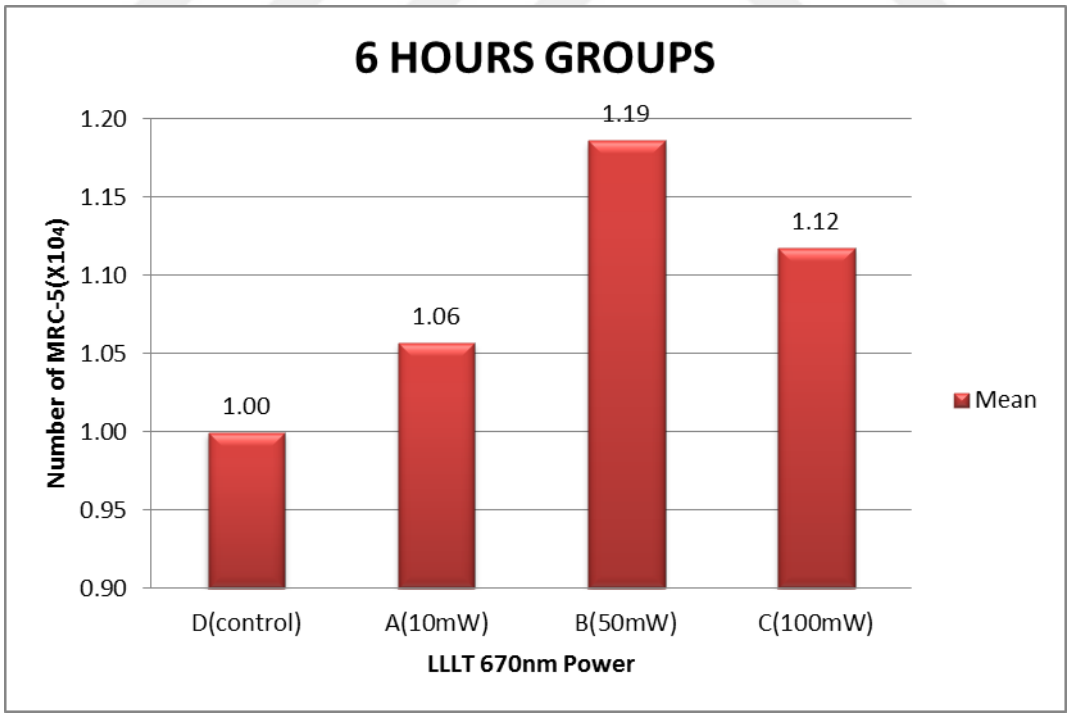


Figure 5.2 Mean proliferation of MRC-5 human fetal lung fibroblasts submitted or not submitted to laser irradiation over 6 hours incubation time.

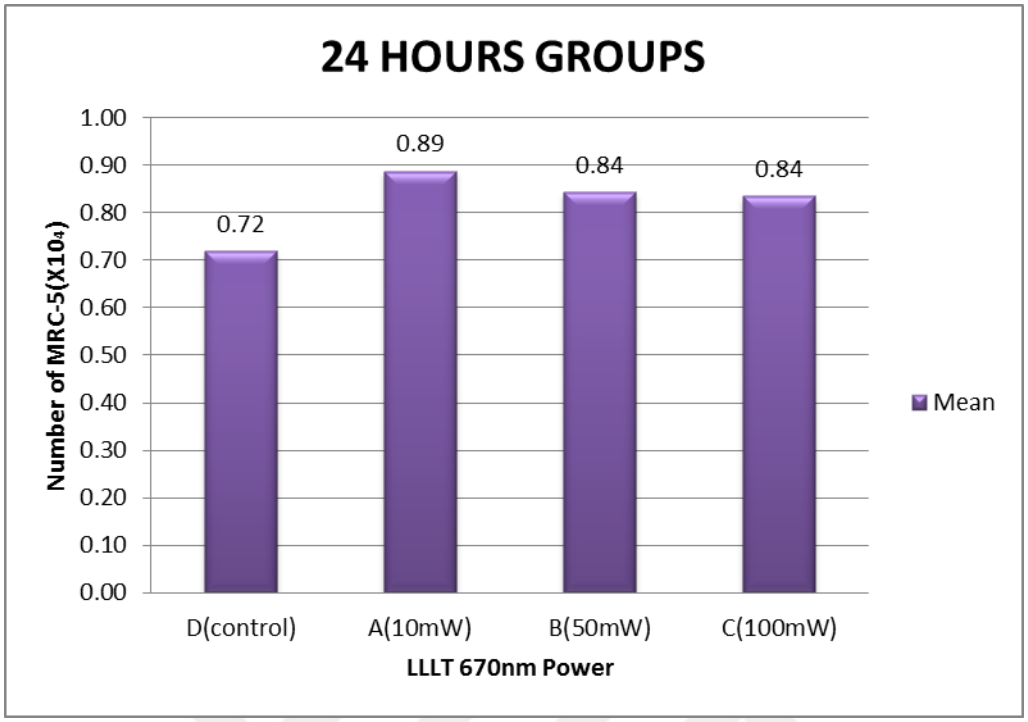


Figure 5.3 Mean proliferation of MRC-5 human fetal lung fibroblasts submitted or not submitted to laser irradiation over 24 hours incubation time.

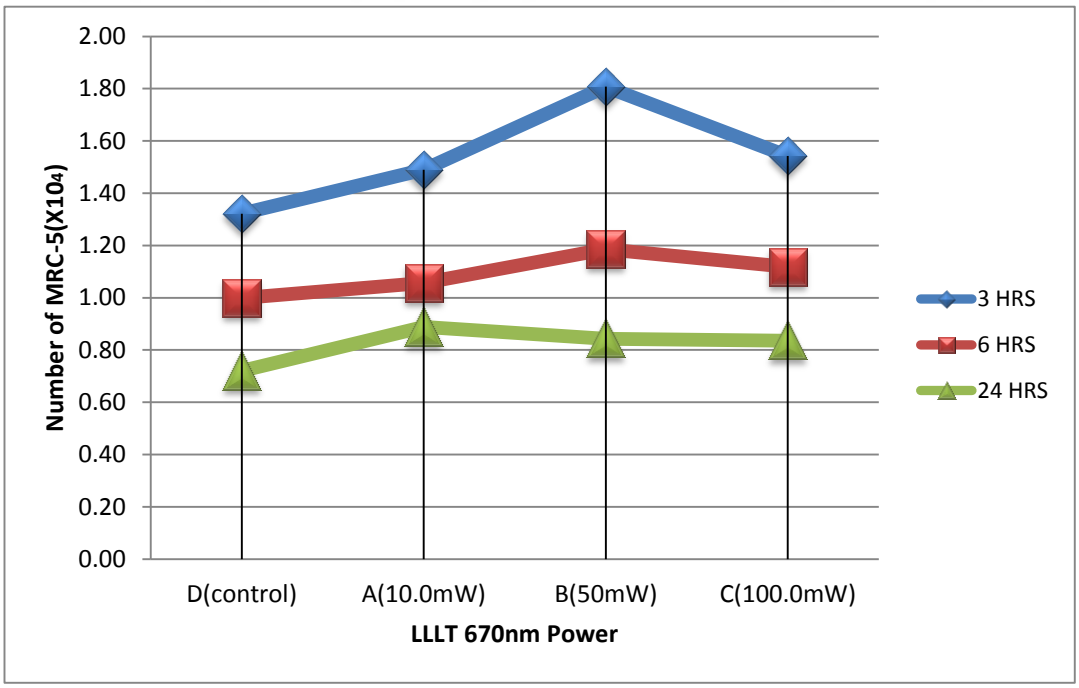


Figure 5.4 Growth curve of MRC-5 human fetal lung fibroblasts submitted or not submitted to laser irradiation over time (incubation hours).

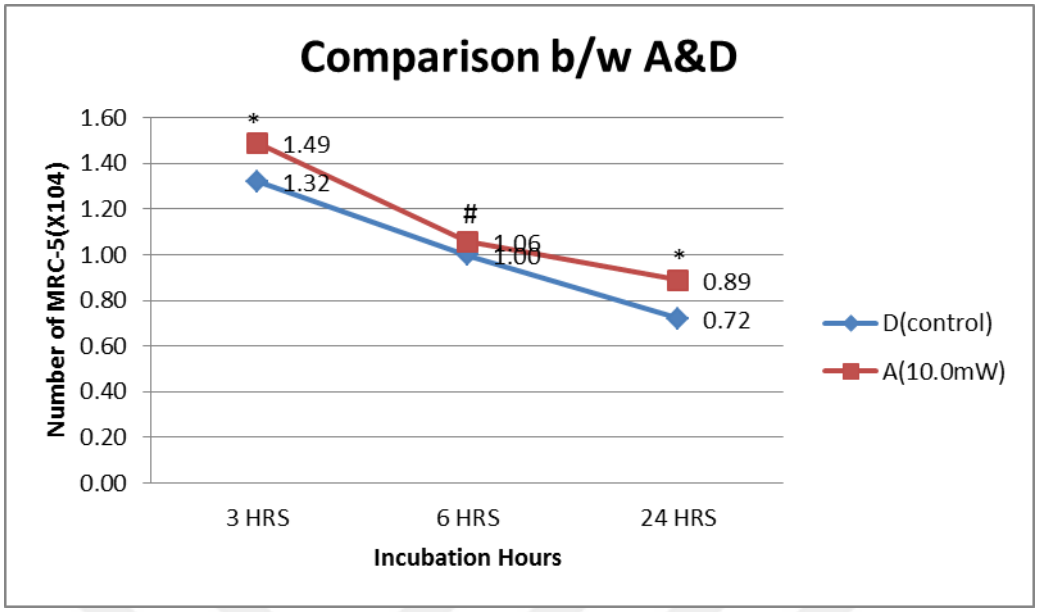


Figure 5.5 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between A (10.0 mW) and D (control) groups over times (incubation hours), * donates statistical significant difference (SSD) while # donates no SSD between A vs D.

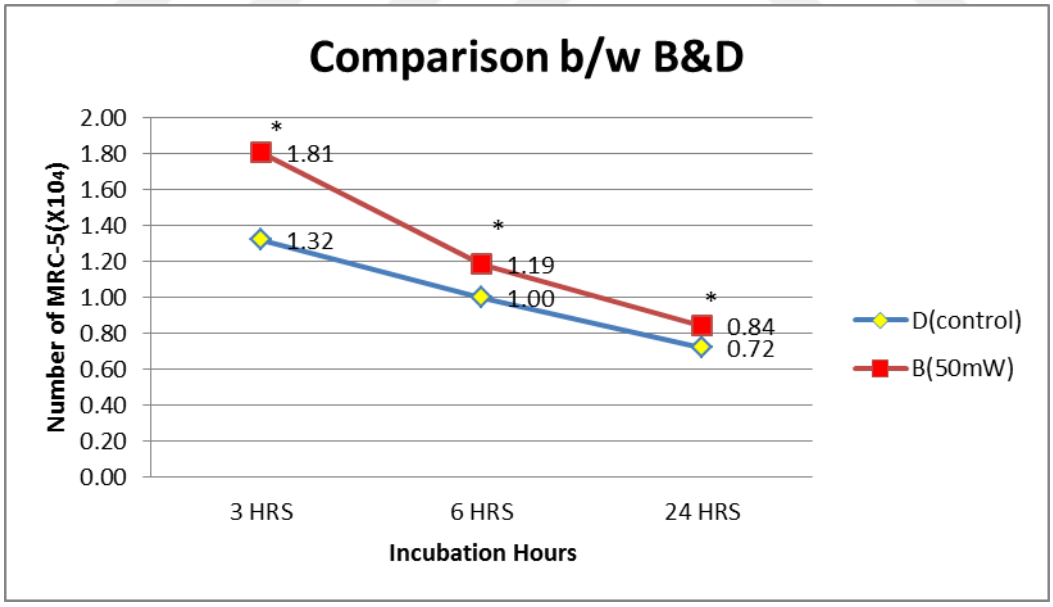


Figure 5.6 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between group B (50.0 mW) and D (control) over times (incubation hours). * donates statistical significant difference (NSS) between B vs D.

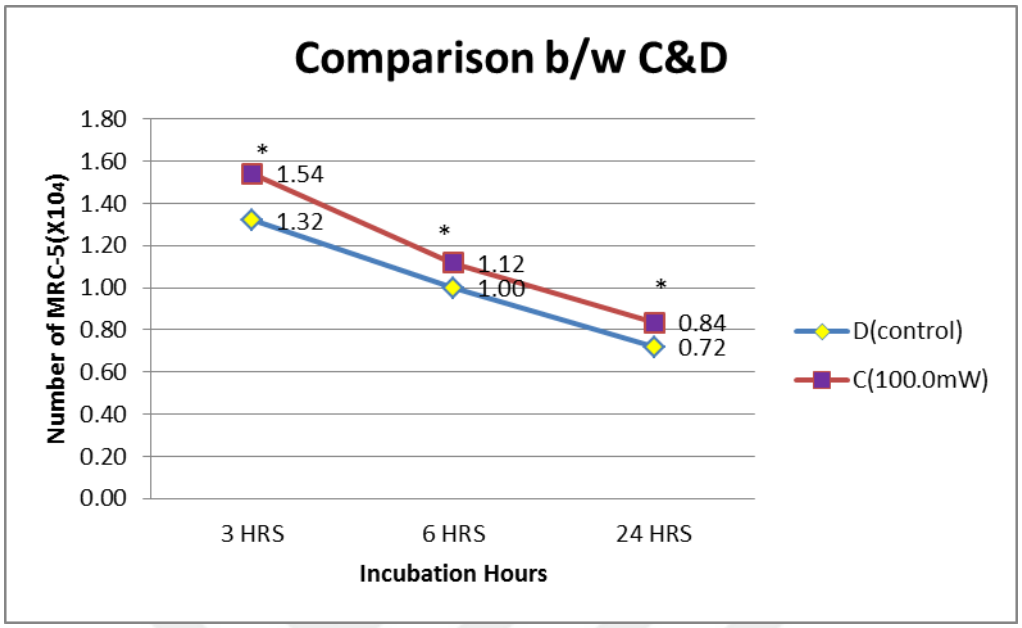


Figure 5.7 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between group C (100.0 mW) and D (control) over times (incubation hours). * donates statistical significant difference (NSS) between C vs D.

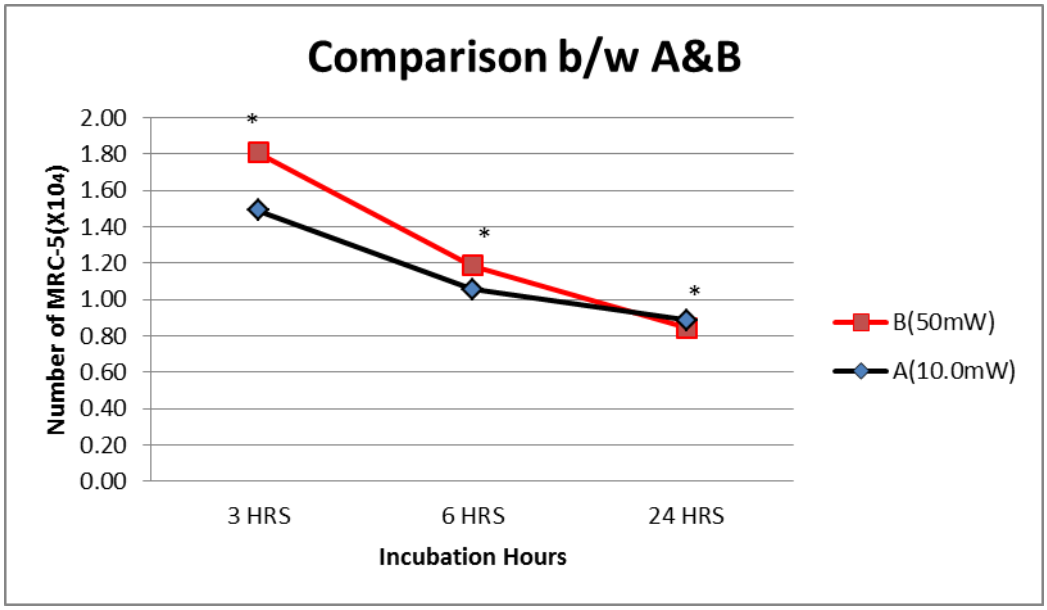


Figure 5.8 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between group A (10.0 mW) and B (50.0 mW) over times (incubation hours). * donates statistical significant difference (NSS) between A vs B.

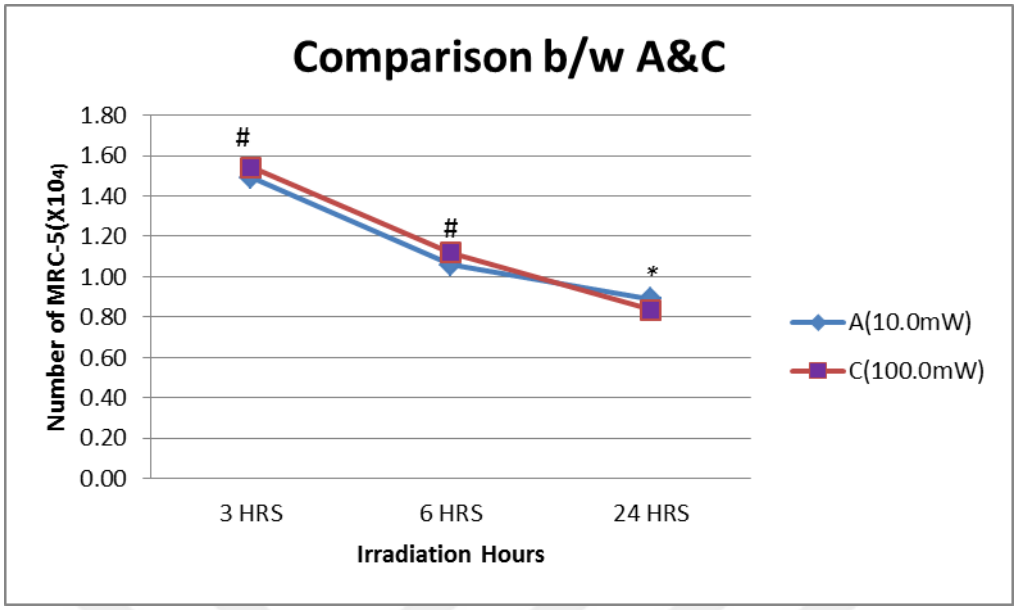


Figure 5.9 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between group A (10.0 mW) and C (100.0 mW) over times (incubation hours). * donates statistical significant difference (NSS) while # donates no SSD between A vs C.

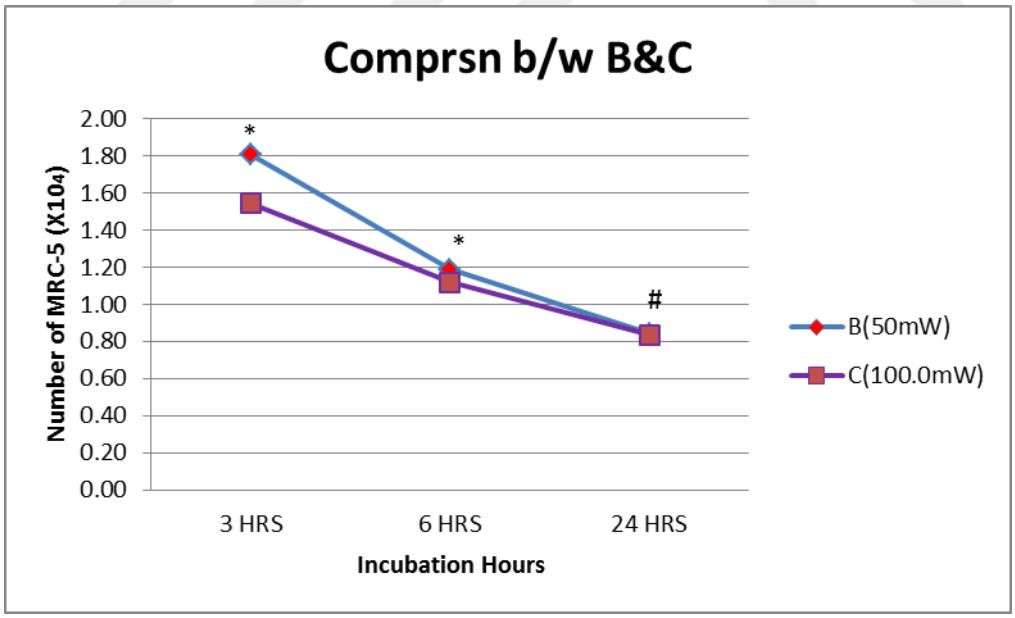


Figure 5.10 Growth curve of MRC-5 human fetal lung fibroblasts, comparison between group B (50.0 mW) and C (100.0 mW) over times (incubation hours). * donates statistical significant difference (NSS) while # donates no SSD between B vs C.

5.2 Discussion

Biostimulation and differenciation can be achieved using LLLI to different types of cells as reported by authors [146, 147, 148, 119, 149]. Sometimes, biostimulation is inhibited due to the effect of variety of factors in the process [150]. As a result, biostimulation using *in vitro* depends on laser-related parameters such as power, dose, time and also wavelength of irradiation [119, 149, 151], type of irradiated cell [105] and the physiological characteristics of the cells during irradiation [149, 151]. Thus, laser light mode of interaction with cells and tissues can lead to stimulation or inhibition of cell proliferation.

Pinheiro *et al.* observed that absorption and spreading of light are greater due to the lack of an optical barrier by applying lower doses for the irradiation to mucosa and skin wounds [152]. Many authors revieled that *in vitro* studies normally reflect the same conditions as observed in open wounds, i.e., the lack of an optical barrier, and lower doses are, thus, indicated in the case of biostimulation. On the basis of this hypothesis, doses of 1.58J/cm^2 with three different power output 10, 50 and 100 mW were used in the present study because the objective was to stimulate the proliferation of MCR-5 lung fibroblasts (ATCC® CCL171™) cell line. One research confirmed that, a dose or power increase the damages of photoreceptors, which reduces the biomodulatory effect of the laser as a result of the inhibition of metabolism and consequent cell death [153]. Another study [119] supported this assumption by revieling that laser irradiation using a dose (like 4.0J/cm^2) trigger cell proliferation but high doses caused negative effect on this biological system/process. An *in vitro* study was conducted and confirmed the enhancement of HUVEC proliferation that received LLLI at the wavelength range 670 nm and doses of 2, 4, 8 J/cm^2 [158]. Wavelengths of 600 to 700nm were used when the objective was to stimulate cell proliferation [155]. Increased cell proliferation and viability was observed in human ADSCs irradiated with diode laser (5.0J/cm^2) [147]. These results agree with the present findings that show an increased number of cells. Researchers agree that lower doses decrease the risk of cell damage and encourage the proliferation, without causing any change in the initial characteristics. Therefore LLLT showed a power output-dependent effect in the present study, as specified by the higher proliferation rate of MCR-5 lung fibroblasts (ATCC® CCL171™) cell line when

irradiated with a power output of 50 and 10 mW compared with 100mW. Another study confirmed that LLLI using a wavelength of 670nm and doses of 1.0 and 2.0J/cm^2 with output power 20 and 65 mW showed a positive biomodulatory effect on the proliferation of human osteoblasts [145]. The best results were obtained with the dose of 1.0J/cm^2, which was in agreement with the present findings showing an increased proliferation of MCR-5 lung fibroblasts (ATCC® CCL171™) cell line treated with a dose of 1.58J/cm^2.

A study investigating the effect of LLLI at different wavelengths (625, 635, 645, 655, 665, 675, and 810nm) on two types of cells (endothelial and fibroblasts) evaluating their cell proliferation rates after 24 hours of culture. Moore P *et al.* found the effective result in both cells proliferations and wavelengths except for the wavelength of 810nm [105]. The similar result was reported regarding the proliferation of fibroblasts by Evans DH *et al.* [156] using 632.8 and 830nm wavelengths in the evaluations. They concluded that effective biostimulation of fibroblast proliferation for cells treated with those wavelenths interfare with the cell response. The wavelength used in the present study (670nm) was within the range that was employed in biostimulation studies, and, as reported in the literature, we observed a positive effect on the proliferation of the cells tested.

The effect of LLLI on the proliferation of BMSCs was investigated and no significant difference was found between the groups after 7 days [148]. These results differed with the present study in which significant differences in the proliferation rate of MRC-5 were observed between the irradiated and control groups. This is due to the some factors that involved in the experimental setup which may include [148] the dose used (4.0J/cm^2) and wavelength (808nm) of the study design. As mentioned earlier, inhibitory effects on cell proliferation may occur as a result of using wavelengths outside the range of 600 to 700nm and high irradiation doses [149, 151, 155]. These results agree with the suggestion of some investigators that the effect of LLLT is directly related to the irradiation power, dose, physiological state of the cell line, and wavelength used [127, 149].

Type of cell line that is used is a factor that influences the effect of laser therapy. Because of this, a study evaluated the effect of diode laser at a dose of 5.0J/cm^2 on the

proliferation of human ADSCs and used cell line that was obtained during surgery and a commercial cell line and got different results. Commercial cell line showed no significant difference in proliferation at any of the studied time points (24, 48, and 72 hours).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

The present study showed that low-level laser irradiation promoted the proliferation of MCR-5 lung fibroblasts (ATCC® CCL171™) cell line compared to control group. The cell morphology is fibroblast-like thus present a low yield and low proliferation rate, low-level laser irradiation may be a useful tool for enhancing such cells through multiples subculturing reducing high cost and difficult to obtained. In this respect, laser therapy permits a significant increase in the initial number of MCR-5 lung fibroblasts, thus increasing the number of regenerative cells therefore enhancing healing processes.

6.2 RECOMMENDATIONS

For better understanding, it is good to ensure that correct protocols are developed for laser therapy and should include the use of appropriate power output, wavelength, dose, power density, irradiation time, as well as frequency and number of sessions as these parameters may have an influence on cell proliferation leading to achievement of good results and also avoiding controversies and empirical conclusions. However, further studies are needed to standardize the laser parameters and to test other cell types to improve the yield of cells in culture.

REFERENCES

[1] Maiman TH. Stimulated optical radiation in Ruby. Nature 1960;187:493-94.

[2] Verma SK, Maheshwari S, Singh RK, Chaudhari PK. Laser in dentistry: An innovative tool in modern dental practice. Natl J Maxillofac Surg 2012;3(2):124-32.

[3] Lin F, Josephs SF, Alexandrescu DT, Ramos F, Bogin V, Gammill V, et al. Lasers, stem cells, and COPD. J Trans Med 2010; 8:16.

[4] Mester E, Szende B, Tora JG. Effect of laser on hair growth of mice. Kiserl Orvostud 1967; 19:628-31.

[5] Huang YY, Chenet AC, Carroll JD, Hamblin RM. Biphasic dose response in low level light therapy. Dose Response 2009; 7(4):358-83.

[6] Karu TI, Afanas'eva NI. [Cytochrome c oxidase as the primary photoacceptor upon laser exposure of cultured cells to visible and near IR-range light]. DoklAkadNauk 1995; 342(5):693-5. Russian.

[7] Srinivasan S, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. Free Radic Biol Med 2012; 53(6): 1252-63.

[8] Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. Lasers Surg Med 1998; 22(4):212-8.

[9] Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O, EichlerM, et al. Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. J Biol Chem 2003; 278(42):40917-22.

[10] Zhang J, Xing D, Gao X. Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway. J Cell Physiol 2008; 217(2):518-28.

[11] Liu H, Colavitti R, Rovira II, Finkel T. Redox- dependent transcriptional regulation. Circ Res 2005; 97(10): 967-74.

[12] Gottrup F., "Optimizing wound treatment through health care structuring and professional education," *Wound Repair and Regeneration*, vol. 12, no. 2, pp. 129–133, 2004.

[13] Ueno C, Hunt TK, Hopf HW. "Using physiology to improve surgical wound outcomes". *Plast Reconstr Surg*; 117(suppl 7): 59Se71. June 2006.

[14] Trøstrup H, Bjarnsholt T, Kirketerp-Møller K, Høiby N, and Moser C: "What Is New in the Understanding of Non Healing Wounds Epidemiology, Pathophysiology, and Therapies": Review Article; Hindawi Publishing Corporation *Ulcers Volume,* 2013,

[15] Krishnan P. The scientific study of herbal wound healing therapies: current state of play. Curr Anaesth Crit Care. 17(1):21-7, 2006.

[16] Karu TI, Afanasyeva NI, Kolyakov SF, Pyatibrat LV, Welser L. Changes in absorbance of monolayer of living cells induced by laser radiation at 633, 670, and 820 nm. *IEEE Journal on Selected Topics in Quantum Electronics*. 2001;7(6):982–988.

[17] Karu TI, Pyatibrat LV, Kolyakov SF, Afanasyeva NI. Absorption measurements of cell monolayers relevant to mechanisms of laser phototherapy: reduction or oxidation of cytochrome c oxidase under laser radiation at 632.8 nm. *Photomedicine and Laser Surgery*. 2008;26(6):593–599. [PubMed]

[18] Leal-Junior EC, Vanin AA, Miranda EF, de Carvalho PD, Dal Corso S, Bjordal JM(2013) Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. Laser Med Sci Nov 19

[19] Woodruff LD, Bounkeo JM, BrannonWM, Dawes KS, Barham CD, Waddell DL, Enwemeka CS (2004) The efficacy of laser therapy in wound repair: a meta-analysis of the literature. Photomed Laser Surg 22(3):241–7

[20] Hawkins-Evans D, Abrahamse H (2008) Efficacy of three different laser wavelengths for in vitro wound healing. Photodermatol Photoimmunol Photomed 24:199–210

[21] Lodish, H., Bark, A., Zipersky, S. L., Matsudaira, P., Baltimore, D., and Darnell, J.,*Molecular Cell Biology*, 4th edition,W. H. Freeman, New York, 2000.

[22] Stryer, L., *Biochemistry*, 4th edition,W. H. Freeman, New York, 1995.

[23] Copyright © 2000-2016 Dreamstime. All rights reserved. Dreamstime is a member of PACA and CEPIC.

[24] Audesirk, T., Audesirk, G., and Byers, B. E., *Biology: Life on Earth*, 6th edition,Prentice-Hall, Upper Saddle River, NJ, 2001.

[25] Nature Reviews Molecular Cell Biology **2**; 815-826 (2001)

[26] Kumar, Abbas, Fausto and Mitchell. *Robbins Basic Pathology*; 8th Edition, Elsevier/Saunders, 2007. Chapter 6 - Neoplasia: Cell Cycle, pp 188-198.

[27] L. Huang, S. Wu, D. Xing, High fluence low-power laser irradiation induces apoptosis via inactivation of Akt/GSK3beta signaling pathway, J. Cell Physiol. 226 (3) (2011) 588–601.

[28] X. Gao, T. Chen, D. Xing, F. Wang, Y. Pei, X. Wei, Single cell analysis of PKC activation during proliferation and apoptosis induced by laser irradiation, J. Cell Physiol. 206 (2) (2006) 441–448.

[29] S. Wu, D. Xing, X. Gao, W.R. Chen, High fluence low-power laser irradiation induces mitochondrial permeability transition mediated by reactive oxygen species, J. Cell Physiol. 218 (3) (2009) 603–611.

[30] P.C. Silveira, L.A. Silva, D.B. Fraga, T.P. Freitas, E.L. Streck, R. Pinho, Evaluation of

[31] M. Monici, F. Cialdai, F. Ranaldi, P. Paoli, F. Boscaro, G. Moneti, A. Caselli, Effect of IR laser on myoblasts: a proteomic study, Mol. Biosyst. 9 (6) (2013) 1147–1161.

[32] G. Shefer, I. Barash, U. Oron, O. Halevy, Low-energy laser irradiation enhances de novo protein synthesis via its effects on translation-regulatory proteins in skeletal muscle myoblasts, Biochim. Biophys. Acta 1593 (2–3) (2003) 131–139.

[33] Wagnieres, G. A., Star, W. M., and Wilson, B. C., *In Vivo* Fluorescence Spectroscopy and Imaging for Oncological Applications, *Photochemistry and Photobiology*. **68**, 603–632 (1998).

[34] Grossweiner, L. I., and Smith, K. C., Photochemistry, in K. C. Smith, ed., *The Science of Photobiology*, 2nd edition, Plenum, New York, 1989, pp. 47–78.

[35] Kochevar, I. E., Photoimmunology, in J. Krutmann, and C. A. Elmets, eds., *Photoimmunology*, Blackwell Science, London, 1995, pp. 19–33.

[36] Karu, T.I. and Afanasyeva, N.I., "Cytochrome oxidase as primary photoacceptor for cultured cells in visible and near IR regions", *Dokl. Akad. Nauk* (*Moscow*), 342, 693, 1995.

[37] Afanasyeva NI, Karu TI, Tiflova OA. "Oxidases act as primary photoacceptors in cells Escherichia coli subjected to low-intensity visible (laser) radiation". *Doklady Acad. Nauk* (Engl. Transl.: *Doklady Biochemistry*) 345:179–181, 2005.

[38] Pastore D, Greco M, Passarella S. "Specific helium-neon laser sensitivity of the purified cytochrome c oxidase". *Int J Rad Biol*;76:863–870, 2000.

[39] Karu TI and Kolyakov SF.. "Exact action spectra for cellular responses relevant to phototherapy". *Photomed Laser Surg* 23:355-61, 2005.

[40] Wong-Riley MT, Bai C, Buchman E, Whelan HT. "Light-emitting diode treatment reverses the effect of TTX on cytochrome c oxidase in neurons". *Neuroreport.* 12:3033–3037, 2001.

[41] Wong-Riley, MT, Liang, HL, Eells, JT, Chance, B, Henery, MM, Buchmann, E, Kane, M, Whelan, HT. "Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase". *J Biol Chem*.10:1074, 2005

[42] Brown, G.C., "Nitric oxide and mitochondrial respiration", *Biochem. Biophys. Acta*, 1411, 351,1999.

[43] Sutherland JC. "Biological effects of polychromatic light". *Photochem Photobiol* 76:164-70, 2002.

[44] Karu T. "Laser biostimulation: a photobiological phenomenon". *J Photochem Photobiol* B 3:638-40, 1989

[45] Passarella S, Casamassima E, Molinari S, Pastore D, Quagliariello E, Catalano IM, and Cingolani A. "Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser". *FEBS Let*t 175:95-9, 1984.

[46] Greco M, Guida G, Perlino E, Marra E, and Quagliariello E. "Increase in RNA and protein synthesis by mitochondria irradiated with helium-neon laser" *Biochem Biophys Res Commun* 163:1428-34, 1989.

[47] Karu, Tiina, "The science of low-power laser therapy", *Gordon and Breach Science Publishers*, Amsterdam, pp. 1–40, 53–233, 1998.

[48] Karu, T.I., Kolyakov, S.F., Pyatibrat, L.V., Mikhailov, E.L., and Kompanets, O.N., "Irradiation with a diode at 820 nm induces changes in circular dichroism spectra (250–750 nm) of living cells", *IEEE J. Sel. Top. Quantum Electron.*, 7, 976, 2001a

[49] Karu, T.I., Pyatibrat, L.V., and Kalendo, G.S., "Donors of NO and pulsed radiation at λ=820 nm exert effects on cells attachment to extracellular matrices", *Toxicol. Lett.*, 121, 57, 2001b.

[50] Kolyakov, S.F., Pyatibrat, L.V., Mikhailov, E.L., Kompanets, O.N., and Karu, T.I., "Changes in the spectra of circular dichroism of suspension of living cells after low intensity laser radiation at 820 nm", *Dokl. Akad. Nauk* (*Moscow*), 377, 824, 2001.

[51] Schindi A., Schindi M., Pernerstorfer-Schon H., Kerschan K., Knobler R. and Schindi, L. "Diabetic neuropathic foot ulcer: Successful treatment by low intensity laser therapy". *Dermatology*. 198, 314-317, 1999.

[52] Stadler I., Lanzafame R., Oskoui P., Zhang R., Coleman J. and Whittaker M. "Alteration of skin temperature during low level laser irradiation at 830nm in a mouse model". *Photomedicine and Laser Surgery*. 22(3), 227-231, 2004.

[53] Dyson M. "Primary, secondary and tertiary effects of phototherapy: a review. Mechanisms of Low Level Light Therapy. Mechanisms for Low-Light Therapy" edited by Michael R. Hamblin, Ronald W. Waynant, Juanita Anders, Proceedings of SPIE Vol. 6140 (SPIE, Bellingham, WA,6140, 614005-1 — 614005-12, 2006.

[54] Theralase "Therapeutic laser treatment Laser Theory from designers and manufacturers of therapeutic medical laser systems". http://wwvv.theralase.com/technoloqv.php. Accessed on 12/04/2007.

[55] Matic M., Lazetic B., Poljacki M., Duran V. and lvkov-Simic M. "Low level laser irradiation and its effects on repair processes in the skin". *Med Pregl*. 56(3-4), 137-141, 2003.

[56] Olsen J.E., Schimmerling W. and Tobias C.A. "Laser action spectrum of reduced excitability in nerve cells". *Brain Res*. 204, 436-440, 1980.

[57] Morimoto Y., Arai T., Kikuchi M., Nakajima S. and Nakamura H. "Effect of low intensity Argon laser irradiation on mitochondria respiration". *Lasers Surg Med*. 15, 191-199, 1994.

[58] Yu W., McGown M., Ippolito K. and Lanzafame R.J. “Photostimulation of oxidative metabolism and electron chain enzymes in rate liver mitochondria”. *Photochem. Photobiol.* 66, 866-871, 1997.

[59] Giese, A.C., “Photosensitization of organisms with special reference to natural photosensitizers”, in *Lasers in Biology and Medicine*, Hillenkampf, F., Pratesi, R., and Sacchi, C., Eds., Plenum Press, New York, , p. 299, 1980.

[60] Karu, T.I., Kalendo, G.S., and Letokhov, V.S.: “Control of RNA synthesis rate in tumor cells HeLa by action of low-intensity visible light of copper laser”, *Lett. Nuov. Cim.*, 32, 55, 1981.

[61] Karu, T.I., Kalendo, G.S., Letokhov, V.S., and Lobko, V.V., “Biostimulation of HeLa cells by low intensity visible light”, *Nuov. Cim. D*, 1, 828, 1982.

[62] Brown GC.: “Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase”. *Biochim Biophys Acta* 1504:46-57, 2001.

[63] Hothersall, J.S., Cunha, F.Q., Neild, G.H., and Norohna-Dutra, A., “Induction of nitric oxide synthesis in J774 cell lowers intracellular glutathione: effect of oxide modulated glutathione redox status on nitric oxide synthase induction” *Biochem. J.*, 322, 477, 1997.

[64] Karu, T., “Mechanisms of low-power laser light action on cellular level” in *Lasers in Medicine and Dentistry*, Simunovic, Z., Ed., Vitgraf, Rijeka (Croatia), p. 97, 2000.

[65] Lane N. “Cell biology: power games”. *Nature* 443:901-3, 2006.

[66] Shiva S and Gladwin MT.. “Shining a light on tissue NO stores: near infrared release of NO from nitrite and nitrosylated hemes”. *J Mol Cell Cardiol* 46:1-3, 2009.

[67] Chopp, H., Chen, Q., Dereski, M.O., and Hetzel, F.W “Chronic metabolic measurement of normal brain tissue response to photodynamic therapy” *Photochem. Photobiol*.52, 1033, 1990

[68] Quickenden, T.R., Daniels, L.L., and Byrne, L.T., “Does low-intensity He-Ne radiation affect the intracellular pH of intact *E. coli*?” *Proc. SPIE*, 2391, 535, 1995.

[69] Storz P. “Mitochondrial ROS--radical detoxification, mediated by protein kinase D”. *Trends Cell Biol* 17:13-8, 2007.

[70] Brondon P, Stadler I, and Lanzafame RJ. “A study of the effects of phototherapy dose interval on photobiomodulation of cell cultures”. *Lasers Surg Med* 36:409-13 2005.

[71] Karu, T., Andreichuk, T., and Ryabykh, T.“Changes in oxidative metabolism of murine spleen following diode laser (660–950nm) irradiation: effect of cellular composition and radiation parameters” *Lasers Surg. Med.*, 13, 453, 1993.

[72] Forman, N.J. and Boveris, A., "Superoxide radical and hydrogen peroxide in mitochondria", in *Free Radicals in Biology*, Vol. 5, Pryor, A., Ed., Academic Press, New York, p. 65, 1982.

[73] Karu T. "Primary and secondary mechanisms of action of visible to near-IR radiation on cells". *J Photochem Photobiol* B 49:1-17, 1999.

[74] Chen AC-H, Arany PR, Huang YY, Tomkinson EM, Saleem T, Yull FE, Blackwell TS, and Hamblin MR.. "Low level laser therapy activates NF-reactive oxygen species in mouse embryonic fibroblasts". *Proc SPIE in press*, 2009b:

[75] Pal G, Dutta A, Mitra K, Grace MS, Romanczyk TB, Wu X, Chakrabarti K, Anders J, Gorman E, Waynant RW, and Tata DB. "Effect of low intensity laser interaction with human skin fibroblast cells using fiber-optic nano-probes". *J Photochem Photobiol* B 86:252-61, 2007.

[76] Zhang J, Xing D, and Gao X.: "Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway". *J Cell Physiol* 217:518-28, 2008.

[77] Karu, T.I., "Local pulsed heating of absorbing chromophores as a possible primary mechanism of low-power laser effects", in *Laser Applications in Medicine and* 46 *Surgery*, Galletti, G., Bolognani, L., and Ussia, G., Eds., Monduzzi Editore, Bologna, p. 253, 1992.

[78] Tiina I. Karu: "Low-Power Laser Therapy: Biomedical photonics Handbook" Chapter 48 pg 1-25 by CRC Press LLC., 2003.

[79] Smith K. "Light and Life: The photobiological basis of the therapeutic use of radiation from lasers. Progress in Laser Therapy": Selected papers from the October 1990 ILTA Congress. Published by Wiley and Sons, Inc. New York and Brisbane. pp 17, 1991.

[80] Tunér J. and Hode L., "Laser Therapy - Clinical Practice and Scientific Background". Prima Books AB, Grangesberg, Sweden. Chapter 1. Some basic laser physics. pp. 12, 21, 22, (ISBN: 91-631-1344-9), 2002b.

[81] Takac and Stojanovic S. "Diagnostic and biostimulating lasers". *Med Pregl*. 51(5-6), 245-249, 1998.

[82] Kiepeis V.E., Cornell-Bell A. and Trinkaus-Randall V. "Growth factors but not gap junctions play a role in injury induced Ca2 waves in epithelial cells". *J Cell Sci*. 114(Pt23), 4185-4195, 2001.

[83] Tiina I. Karu: "Low-Power Laser Therapy: Biomedical photonics Handbook" Chapter 48 pg 1-25 by CRC Press LLC., 2003.

[84] Gius, D., Boreto, A., Shah, S., and Curry, H.A., "Intracellular oxidation reduction status in the regulation of transcription factors NF-kB and AP-1", *Toxicol. Lett.*, 106, 93, 1999.

[85] Kamata, H. and Hirata, H., "Redox regulation of cellular signaling", *Cell. Signal*., 11, 1, 1999.

[86] Sun, Y. and Oberley, L.W., "Redox regulation of transcriptional activators", *Free Rad. Biol. Med.*, 21, 335, 1996.

[87] Nakamura, H., Nakamura, K., and Yodoi, J. "Redox regulation of cellular activation" *Annu. Rev. Immunol*., 15, 351, 1997.

[88] Tiphlova, O. and Karu, T., "Dependence of *Escherichia coli* growth rate on irradiation with He-Ne laser and growth substrates1, *Lasers Life Sci*., 4, 161, 1991.

[89] Vladimirovlu. A., Klebanov G.l., Borisenko G.G. and OsipovA.N. "Molecular and cellular mechanism of the low intensity laser radiation effect". *Biofizika*. 49(2), 2004.

[90] Hamblin M.R., and Deidova, T.N "Mechanisms of Low Level Light Therapy. Mechanisms for Low-Light Therapy" edited by Michael R. Hamblin, Ronald W. Waynant, Juanita Anders, Proceedings of SPIE Vol. 6140 (SPIE, Bellingham, WA, 614001-1- 614001-11, 2006.

[91] Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D, et al. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wien Klin Wochenschr. 2008;120(3-4):112-7.

[92] Huang YY, Chenet AC, Carroll JD, Hamblin RM. Biphasic dose response in low level light therapy. Dose Response 2009; 7(4):358-83.

[93] Hamblin MR. Mechanisms of low level light therapy. Proc. of SPIE 2009; 6140: 614001-1.

[94] Silva EM, Gomes SP, Ulbrich LM, Giovanini AF. "Avaliacao histologica da laserterapia de baixa intensidade na cicatrizacao de tecidos epitelial, conjuntivo e osseo: estudo experimental em ratos". *Rev Sul-Bras Odontol*.;4:29-35, 2007.

[95] Bourguignon AMF, Feitosa RCA, Beltrao GC, Pagnoncelli MR. "Utilizacao do laser de baixa intensidade no processo de cicatrizacao tecidual. Revisao de literatura. Rev" Port *Estomatol Cir Maxilofac*.; 46:37-43, 2005.

[96] Posten W, D.A. Wrone, J.S. Dover, K.A. Arndt, S. Silapunt, M. Alam, "Low-level laser therapy for wound healing: Mechanism and efficacy". *Dermatol Surg*, 31, 3, 334–40, 2005.

[97] Burd A, Zhu N, Poon VK. "A study of Q-switched Nd:YAG laser irradiation and paracrine function in human skin cells". *Photodermatol Photoimmunol Photomed*. 21(3):131–137, June 2005.

[98] Maiya GA, Kumar P, Rao L. "Effect of low intensity helium-neon (He-Ne) laser irradiation on diabetic wound healing dynamics". *Photomed Laser Surg*.;23(2):187–190, Apr 2005.

[99] Sugrue, M.E., Carola, N.J., Leen, E.J., Feeley, T.M., Moore, D.J., and Shabik, G.D.. "The use of infrared laser therapy in the treatment of venous ulceration". *Ann. Vasc. Surg*. 4, 179–181, 1990.

[100] Chromey, P.A.. "The efficacy of carbon dioxide laser surgery for adjunct ulcer therapy". *Clin. Podiatr. Med. Surg*. 9, 709–719, 1992.

[101] Nussbaum, E.L., Biemann I., and Mustard, B.: "Comparison of ultrasound/ultraviolet-c and laser for treatment of pressure ulcers in patients with spinal cord injury". *Phys. Ther*. 74, 812–823, 1994.

[102] Lubart R, Eichler M, Lavi R, Friedman H, and Shainberg A. "Low-energy laser irradiation promotes cellular redox activity". *Photomed Laser Surg* 23:3-9, 2005.

[103] Yu W, Naim JO, and Lanzafame RJ.: "The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts". *Photochem Photobiol* 59:167-70, 1994.

[104] Grossman N, Schneid N, Reuveni H, Halevy S, and Lubart R.: "780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species". *Lasers Surg Med* 22:212-8, 1998.

[105] Moore P, Ridgway TD, Higbee RG, Howard EW, and Lucroy MD. "Effect of wavelength on lowintensity laser irradiation-stimulated cell proliferation in vitro". *Lasers Surg Med* 36:8-12, 2005.

[106] Stadler I, Evans R, Kolb B, Naim JO, Narayan V, Buehner N, and Lanzafame RJ.: "In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes". *Lasers Surg Med* 27:255-61, 2000.

[107] Kreisler M, Christoffers AB, Willershausen B, and d'Hoedt B.: "Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study". *J Clin Periodontol* 30:353-8, 2003.

[108] Shefer G, Partridge TA, Heslop L, Gross JG, Oron U, and Halevy O. "Low-energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells". *J Cell Sci* 115:1461-9, 2002.

[109] Gavish L, Perez L, and Gertz SD.: "Low-level laser irradiation modulates matrix metalloproteinase activity and gene expression in porcine aortic smooth muscle cells". *Lasers Surg Med* 38:779-86, 2006.

[110] AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci 2012;27:23749.

[111] Schartinger VH, Galvan O, Riechelmann H, Dudas J. Differential responses of fibroblasts, non-neoplastic epithelial cells, and oral carcinoma cells to lowlevel laser therapy. Supp Care Cancer 2012;20:523–9.

[112] Sperandio FF, Simoes A, Correa L, Aranha AC, Giudice FS, Hamblin MR, et al. Low-level laser irradiation promotes the proliferation and maturation of keratinocytes during epithelial wound repair. J Biophoton 2014. doi: 10.1002.

[113] Sperandio FF, Giudice FS, Correa L, Pinto Jr DS, Hamblin MR, de Sousa SC. Lowlevel laser therapy can produce increased aggressiveness of dysplastic and oral cancer cell lines by modulation of Akt/mTOR signaling pathway. J Biophoton 2013;6:839–47.

[114] Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. J Biomed Sci 2009;16:4

[115] Gomes Henriques AC, Ginani F, Oliveira RM, Keesen TS, Galvao Barboza CA, Oliveira Rocha HA, et al. Low-level laser therapy promotes proliferation and invasion of oral squamous cell carcinoma cells. Lasers Med Sci 2014;29:1385–95.

[116] Al-Watban FA, Andres BL. Laser biomodulation of normal and neoplastic cells. Lasers Med Sci 2012;27:1039–43.

[117] Pellicioli AC, Martins MD, Dillenburg CS, Marques MM, Squarize CH, Castilho RM. Laser phototherapy accelerates oral keratinocyte migration through the modulation of the mammalian target of rapamycin signaling pathway. J Biomed Opt 2014;19:028002.

[118] Takeda Y: Irradiation effect of low-energy laser on rat submandibular salivary gland. *J. Oral Path. Med.*; 17(2):91–94, 1988.

[119] Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, d'Hoedt B: Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. *Lasers Surg. Med.;* 30(5):365-369, 2002.

[120] Fung DT, Ng GY, Leung MC, Tay DK: Effects of a therapeutic laser on the ultrastructural morphology of repairing medial collateral ligament in a rat model. *Lasers Surg. Med.*; 32: 286-293, 2003.

[121] Kreisler M, Christoffers AB, Willerstausen B, d'Hoedt B: Effect of low-level GaAIAS laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study. *J. Clin. Periodon.*; 30:353–358, 2003.

[122] Vinck EM, Cagnie BJ, Cornelissen MJ, Declercq HA and Cambier DC: Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation. *Lasers Med. Sci.*; 18(2):95-99, 2003.

[123] Carnevalli CM, Soares CP, Zângaro RA, Pinheiro AL, Silva NS: Laser light prevents apoptosis in Cho K-1 cell line. *J. Clin. Laser Med. Surg.*; 21(4):193-196, 2003.

[124] Matsuzaka K, Ohta K, Takahashi K, Miyake N, Hattori M, Murama T, R Sato T, Oda Y, Shimono M, Ishkawa T: Effect of dioe laser on cell proliferation, alkaline phosphatase activity, and osteopontin mRNA expression in proliferating and in differentiating osteoblastic cells. *Biomed. Res.*; 25(4):165-170, 2004.

[125] Khadra M, Lyngstadaas SP, Haanæs HR, Mustafa K: Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. *Biomaterials*; 26(17):3503–3509, 2005.

[126] Tuby H, Maltz L, Oron U: Low-level laser irradiation (LLLI) promotes proliferation of mesenchymal and cardiac stem cells in culture. *Lasers Surg. Med.*; 39(4):373-378, 2007.

[127] Renno AC, McDonnell PA, Parizotto NA, Laakso EL: The effects of laser irradiation on osteoblast and osteosarcoma cell proliferation and differentiation in vitro. *Photomed. Laser Surg.*; 25(4):275-80, 2007.

[128] Mvula B, Mathope T, Moore T, Abrahamse H: The effect of low level laser irradiation on adult human adipose derived stem cells. *Lasers Med. Sci.*; 23:277–282, 2008.

[129] Saygun I, Karacay S, Serdar M, Ural AU, Sencimen M, Kurtis B: Effects of laser irradiation on the release of basic fibroblast growth factor (bFGF), insulin like growth factor-1 (IGF-1), and receptor of IGF-1 (IGFBP3) from gingival fibroblasts. *Lasers Med. Sci.*; 23(2):211-215, 2008.

[130] Hou JF, Zhang H, Yuan X, Li J, Wei YJ, Hu SS: In vitro effects of low-level laser irradiation for bone marrow mesenchymal stem cells: proliferation, growth factors secretion and myogenic differentiation. *Lasers Surg. Med.*; 40(10):726-733, 2008.

[131] Chen CH, Hung HS, Hsu SH: Low-Energy Laser Irradiation Increases Endothelial Cell Proliferation, Migration, and eNOS Gene Expression Possibly Via PI3K Signal Pathway. *Lasers Surg Med.*; 40:46–54, 2008.

[132] Nakano J, Kataoka H, Sakamoto J, Origuchi T, Okita M, Yoshimura T: Low-level laser irradiation promotes the recovery of atrophied gastrocnemius skeletal muscle in rats. *Exp. Physiol.*; 94:1005–1015, 2009.

[133] Peplow PV, Chung TY, Baxter GD: Laser photobiomodulation of proliferation of cells in culture: a review of human and animal studies. *Photomed. Laser Surg.*; 28(1):S3-40, 2010.

[134] Frigo L, Fávero GM, Lima HJ, Maria DA, Bjordal JM, Joensen J, Iversen VV, Marcos RL, Parizzoto NA, Lopes-Martins RA: Low-level laser irradiation (InGaAlP-660 nm) increases fibroblast cell proliferation and reduces cell death in a dose-dependent manner. *Photomed. Laser Surg.*; 28(1):S151-6, 2010.

[135] Choi E, Yim J, Koo K, Seol Y, Lee Y, Ku Y, Rhyu I, Chung CP, Kim T: Biological effects of a semiconductor diode laser on human periodontal ligament fibroblasts. *J. Periodon. Implant Sci.*; 40(3):105–110, 2010.

[136] Grassi FR, Ciccolella F, D'Apolito G, Papa F, Iuso A, Salzo AE, Trentadue R, Nardi GM, Scivetti M, De Matteo M, Silvestris F, Ballini A, Inchingolo F, Dipalma G, Scacco S, Tetè S: Effect of low-level laser irradiation on osteoblast proliferation and bone formation. *J. Biol. Regul. Homeost. Agents*; 25(4):603-614, 2011.

[137] da Silva AP, Petri AD, Crippa GE, Stuani AS, Stuani AS, Rosa AL, Stuani MB: Effect of low-level laser therapy after rapid maxillary expansion on proliferation and differentiation of osteoblastic cells. *Lasers Med. Sci.*; 27(4):777-783, 2012.

[138] Yazdani SO, Golestaneh AF, Shafiee A, Hafizi M, Omrani HG, Soleimani M: Effects of low level laser therapy on proliferation and neurotrophic factor gene expression of human schwann cells in vitro. *J. Photochem. Photobiol. B: Biology*; 107:9–13, 2012.

[139] Saygun I, Nizam N, Ural AU, Serdar MA, Avcu F, Tözüm TF: Low-level laser irradiation affects the release of basic fibroblast growth factor (bFGF), insulin-

like growth factor-I (IGF-I), and receptor of IGF-I (IGFBP3) from osteoblasts. *Photomed. Laser Surg.*; 30(3):149-154, 2012.

[140] Leonida A, Paiusco A, Rossi G, Carini F, Baldoni M, Caccianiga G: Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: in vitro pilot study. *Lasers Med. Sci.*; 28(1):125-132, 2013.

[141] Bayram H, Kenar H, Taşar F, Hasırcı V: Effect of low level laser therapy and zoledronate on the viability and ALP activity of Saos-2 cells. *Int. J. Oral Maxillofac. Surg.;* 42(1):140–146, 2013.

[142] Giannelli M, Chellini F, Sassoli C, Francini F, Pini A, Squecco R, Nosi D, Bani D, Zecchi-Orlandini S, Formigli L: Photoactivation of bone marrow mesenchymal stromal cells with diode laser: effects and mechanisms of action. *J. Cell Physiol.*; 228(1):172-181, 2013.

[143] Karu T: Photobiology of low-power laser effects. *Helth. Phys.*; 56(5):691-704, 1989.

[144] Schindl A, Merwald H, Schindl L, Kaun C, Wojta J: Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation. *Br. J. Dermatol.*; 148(2):334-336, 2003.

[145] Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D, et al. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wien Klin Wochenschr. 2008;120(3-4):112-7.

[145A] Copyright © 2013 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

[146] Eduardo Fde P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, et al. Stem cell proliferation under low intensity laser irradiation: a preliminary study. Lasers Surg Med. 2008;40(6):433-8.

[147] Mvula B, Moore TJ, Abrahamse H. Effect of low-level laser irradiation and epidermal growth factor on adult human adipose-derived stem cells. Lasers Med Sci. 2010;25(1):33-9.

[148] Bouvet-Gerbettaz S, Merigo E, Rocca JP, Carle GF, Rochet N. Effects of lowlevel laser therapy on proliferation and differentiation of murine bone marrow cells into osteoblasts and osteoclasts. Lasers Surg Med. 2009;41(4):291-7.

[149] 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.

[150] Karu TI. Biomedical Photonics Handbook. Low-power laser therapy. Moscow: CRC Press LLC; 2003.

[151] Werneck CE, Pinheiro AL, Pacheco MT, Soares CP, de Castro JL. Laser light is capable of inducing proliferation of carcinoma cells in culture: a spectroscopic in vitro study. Photomed Laser Surg. 2005;23(3):300-3.

[152] Pinheiro AL, Brugnera Júnior A, Zanin FA. Aplicação do laser na odontologia. São Paulo: Santos; 2010.

[153] Karu TI. Photobiological fundamentals of low-power laser therapy. J Quantum Electron. 1987;23:1703-17.

[154] Stein A, Benayahu D, Maltz L, Oron U. Low-Level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomed Laser Surg. 2005;23(2):161-6.

[155] Wilden L, Karthein R. Import of radiation phenomena of electrons and therapeutic low-level laser in regard to mitochondrial energy transfer. J Clin Laser Med Surg. 1998;16(3):159-65.

[156] Evans DH, Abrahamse H. Efficacy of three different laser wavelengths for in vitro wound healing. Photodermatol Photoimunol Photomed. 2008;24(4):199-210.

[157] de Villiers JA, Houreld NN, Abrahamse H. Influence of low intensity laser irradiation on isolated human adipose derived stem cells over 72 hours and their differentiation potential into smooth muscle cells using retinoic acid. Stem Cell Rev. 2011;7(4):869-82.

[158] Schindl A, Merwald H, Schindl L, Kaun C, Wojta J (2003) Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation. Br J Dermatol 148:334–336

APPENDIX A

Experimental groups and Application protocol

Groups	**Control**	**Treatment**		
Subgroups	D	A	B	C
Number	6	6	6	6
Power (mW)	_	10	50	100
Power Density (W/cm^2)	_	0.01158	0.079	0.158
Energy Density (J/cm^2)	_	1.58	1.58	1.58
Laser Mode	_	CW	CW	CW
Duration (S)	_	100	20	10

Appendix B

STAT FOR MEAN ± STANDARD DEVIATION					
Control Group (D)					
	PR of D	**D-Mean**	**Y=(D-Mean)2**	**Variance ΣY/n**	**SD √ΣY/n**
3 Hours	1.321	-1.238	1.532	2.450	1.565
6 Hours	0.999	-1.560	2.433		
24 Hours	0.719	-1.840	3.385		
n	3.00				
Σ	3.039		7.350		
Mean	2.559				

STAT FOR MEAN ± STANDARD DEVIATION					
Group A					
	PR of A	**A-Mean**	**Y=(A-Mean)2**	**Variance ΣY/n**	**SD √ΣY/n**
3 Hours	1.490	0.346	0.119	0.413	0.642
6 Hours	1.056	0.088	0.007		
24 Hours	0.888	1.056	1.115		
n	3.00				
Σ	3.434		1.241		
Mean	1.144				

STAT FOR MEAN ± STANDARD DEVIATION					
Group B					
	PR of B	**B-Mean**	**Y=(B-Mean)2**	**Variance ΣY/n**	**SD √ΣY/n**
3 Hours	1.807	0.529	0.279	0.159	0.398
6 Hours	1.185	-0.093	0.008		
24 Hours	0.842	-0.436	0.190		
n	3.00				
Σ	3.834		0.477		
Mean	1.278				

STAT FOR MEAN ± STANDARD DEVIATION					
Group C					
	PR of C	**C-Mean**	**Y=(C-Mean)2**	**Variance ΣY/n**	**SD √ΣY/n**
3 Hours	1.542	0.378	0.142	0.084	0.289
6 Hours	1.117	-0.047	0.002		
24 Hours	0.835	-0.329	0.108		
n	3.00				
Σ	3.494		0.252		
Mean	1.164				

CURRICULUM VITAE

Name Surname: Nuhu Muhammad Abubakar.

Place and Date of Birth: 01/08/1986.

Address: No. 280 Link 14 Chiranci Yamma, Along Sheikh Jafar Road, Kano State of Nigeria.

E-Mail: nuuzee6@gmail.com

B.Sc.: B.Sc. Hons Biology (Kano University of Sci. and Tech. Wudil)

M.Sc. Biomedical Engineering inview

PROFESSIONAL EXPERIENCE AND REWARDS:

Certificate of Student Industrial Work Experience (SIWES) awarded by Alnoury Specialists Hospital Kano, Nigeria.

Letter of Appreciation for Dedication to National Youth Service at Government Girls Secondary School Nguru of Yobe State, Nigeria.

Letter of Recommendation for Dedication as Class Teacher to Madrasatussalafiyya Littahfiyzul Qur'an Waddarasatul Islamiyyah of Kano State, Nigeria.

LIST OF PUBLICATIONS AND PATENTS:

International Journal o f Scientific Research and Education

Incidence of Typhoid Fever Among Patients Attending Aminu Kano Teaching Hospital Laboratory, Tarauni Local Government, Kano State, Nigeria
Nuhu M. Abubakar, Hassan Muhammad Ibrahim, Dauda Danlami Ibrahim A. Sabo, Tasiu Mahmud,

PROFESSIONAL FIELD OF INTEREST:

- Medical Laser Applications (General Surgery ,Urology, ENT)
- Bio-Organic Field Effect Transistors