STUDY OF DIELECTRIC RESPONSE AND ELECTRICAL IMPEDANCE SPECTROSCOPY IN DIABETIC AND NON-DIABETIC HUMAN BLOOD INDUCED BY LOW LEVEL LASER

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Symbols/Units	**Meanings**
σ	Sigma
S/m	Siemens per metre
ε	Epsilon
F/m	Farad per metre
mW	MilliWatt
λ	Lambda
nm	Nanometre
Cm^{-1}	Reciprocal centimetre (Unit for wavenumbers in spectroscopy).
kHz	Kilo Henz
MHz	Mega Henz
J/cm^3	Joule per centimetre cube
W/cm^2	Watt per centimetre square
^0C	Degree Celsius
kg/m^3	Kilogram per volume cube
mmol/l	Millimole per litre
µL	Microlitre
Sec	Second
β	Beta
α	Alpha
ε_o	Epselon (permittivity of free space)
ε_r''	Relative dielectric loss
ε_r'	Relative dielectric constant.

Ω	Omega (uppercase)
Z	Impedance
au	Abitrary unit
ρ	Rho (density)
V^2/m^2	Volt square per metre square
mho-m^{-1}	(Reciprocal of Ohm) Ohm per metre
W/Kg	Watts per kilogram

LIST OF ABBREVIATIONS

Abbreviations	Full meanings
AC	Alternating current
ATP	Adenosine tri phosphate
BBO	Beta barium borate
Ca^{+2}	Calcium ions
CBC	Complete blood count
CCD	Charged coupled device
CCO	Cytochrome-c-oxidase
Cdl	Capacitance of the double layer
Cl^{-}	Chlorine ion
CPE	Constant phase element
CW	Continous wave
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DPSS	Diode pump solid state
DS	Dielectric spectroscopy
EDTA	Ethylenediaminetetra-acetic acid
EIS	Electric impedance spectroscopy
ESR	Erythrocyte sedimentation rate
GaAlAs.	Gallium-Aluminum-Arsenide.
HB	Human blood
Hb	Deoxyhemoglobin
HbO_2	Oxyhemoglobin

HCT	Haematocrit
He-Ne	Helium Neon
HLA	Human leukocyte antigen
ILBI	Intravascular laser blood irradiation
IR	Infrared
K^+	Potassium ion
KNB	Potassium niobate
KTP	Potassium titanyl phosphate
KTiOPO4	Potassium titanyl phosphate
LADA	Latent autoimmune diabetes in adults
LASER	Light amplification by stimulated emission of radiation.
LBO	Lithium triborate
LCR	(Inductance (L), Capacitance (C), and Resistance (R))
LCZ	(Inductance (L), Capacitance (C), and Impedance (Z))
LLLI	Low level laser irradiation
LLLT	Low level laser therapy
MCV	Mean cell volume
Mg^{2+}	Magnesium ion
Na	Sodium
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
Nd:YAG	Neodymium-doped Yttrium Aluminum Garnet
Nd:YVO4	Neodymium doped yttrium orthovanadate
TA-GVHD	Transmission-associated graft-versus-host diseases
OGTT	Oral glucose tolerance test

PDT	Photodynamic Therapy
RBC	Red blood cell
Rel	Electrolyte resistance
Rct	Charge transfer resistance
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAR	Specific absorption rate
SPSS	Statistical Package for the Social Sciences
UBI	Ultraviolet Blood Irradiation
UV-visible	Ultraviolet–visible
Zw	Warburg impedance
$\varepsilon'r - D1$	Dielectric constant of the diabetic blood as 1
$\varepsilon'r - D2$	Dielectric constant of the diabetic blood as 2
$\varepsilon'r - ND1$	Dielectric constant of non-diabetic blood as 1
$\varepsilon'r - ND2$	Dielectric constant of non-diabetic blood as 2
$\varepsilon'r - 5mRdD1$	Diabetic blood irradiated for 5 minutes
$\varepsilon'r10RmdD2$	Diabetic blood irradiated for 10 minites
$\varepsilon'r - 5mRdN1$	Non-diabetic blood irradiated for 5 minutes
$\varepsilon'r - 10mRdND2$	Non-diabetic blood irradiated for 10 minutes

LIST OF APPENDICES

STUDY OF DIELECTRIC RESPONSE AND ELECTRICAL IMPEDANCE SPECTROSCOPY IN DIABETIC AND NON-DIABETIC HUMAN BLOOD INDUCED BY LOW LEVEL LASER

ABSTRACT

The laser applications on blood helps to elucidates the interaction mechanisms of laser light with blood (biological tissues). The knowledge of blood dielectric response, biostimulation effects and specific absorption rate (SAR) through electrical impedance spectroscopy can optimise appropriate medical therapy without adverse biological alterations. The study used 208 blood samples (128 males and 80 non-pregnant females) from 104 diabetic blood and 104 non-diabetic blood samples collected in ethylenediaminetetra-acetic acid (EDTA) tubes by venepuncture. Each sample was divided into small aliquots such as control (non-irradiated group) and irradiated group samples. The irradiation was carriedout at output powers of 50, 60, 70 and 80 mW for 5, 10, 15 and 20 minutes each using diode-pumped solid-state (DPSS) laser of 532 nm wavelength. Agilent 4294A impedance analyser at frequency range of 40 Hz-30 MHz was used for impedance measurements. Results show significant increased of dielectric parameters (dielectric permittivity, dielectric loss and conductivity) in control in vitro diabetic blood compared with non-diabetic blood. The reduced haemoglobin presence in diabetic blood consequently affects oxygen affinity, reduced cell membrane resistance and induces changes in intrinsic membrane. This effected variation in dipole reactions within cell membranes and conductance transport in the extra-cellular medium to alter the dielectric reactions. Irradiated diabetic blood also showed high significant difference compared to the control counterparts. Dielectric response values; dielectric permittivity, dielectric

loss and conductivity were p = 0.003, 0.001 and 0.001 for 10 minutes exposure and p=0.000, 0.005 and 0.004 for 15 minutes exposure respectively at output power of 50 mW. At laser power output of 60 mW for exposure of 5 minutes duration, the p-values were p = 0.001, 0.000 and 0.000 respectively. Within average temperature range of $24.2 - 28.0\ ^{\circ}\mathrm{C}$ and SAR of $0.140 \leq 0.695\ W/Kg$ there is positively improved morphological structure and reduced the RBCs bloats whereas beyoud showed negative effects and inhibition due to excessive irradiation. The blood Nypuist plots based on Cole-Cole model of the irradiated diabetic blood has their electrode polarization (α-dispersion region) stretched from 40 Hz to frequency range of 11.7- 11.9 kHz similar to that of the control non-diabetic blood. Also the membrane-cytoplasm (R_1 as charge-transfer) resistance and plasma (R_2 as electrolyte) resistance ranges of 78.99 - 82.82 Ω and 113-135 Ω respectively were observed for the diabetic blood irradiated using laser power of 50 mW for 10 and 15 minutes compared to the R_1 vlaue of 73.60 Ω and R_2 of 58.06 Ω observed for the control diabetic blood. This demonstrates high resistance in the irradiated diabetic blood cytoplasm, cell membranes and blood plasma therefore low level laser irradiation effect would be of benefit in the clinical treatment of diabetic patients as it improves blood impedance and cell membrane structure.

KAJIAN TINDAK BALAS DIELEKTRIK DAN SPEKTROSKOPI GALANG ELEKTRIK DALAM KOMPONEN DARAH MANUSIA DIABETIK DAN BUKAN DIABETIK YANG DISEBABKAN OLEH LASER BERKEAMATAN RENDAH.

ABSTRAK

Aplikasi laser terhadap darah membantu untuk menjelaskan mekanisme interaksi cahaya laser dengan darah (tisu biologi). Pengetahuan mengenai tindak balas dielektrik darah, kesan biostimulasi dan kadar penyerapan spesifik (SAR) melalui spektroskopi galang elektrik dapat mengoptimumkan terapi perubatan yang sesuai tanpa perubahan biologi yang buruk. Kajian menggunakan 208 sampel darah (128 lelaki dan 80 wanita yang tidak hamil) daripada 104 sampel darah diabetes dan 104 sampel darah bukan diabetes yang dikumpulkan dalam tiub etilenediaminetetra-asetat (EDTA) oleh punktur vena. Setiap sampel dibahagikan kepada bahagian kecil seperti kawalan (kumpulan tidak disinari) dan sampel kumpulan yang disinari. Kumpulan yang disinari telah disinari dengan kuasa 50, 60, 70 dan 80 mW selama 5, 10, 15 dan 20 minit setiap satu menggunakan laser *diode-pumped solid state* (DPSS) dengan panjang gelombang 532 nm. Penganalisis galang Agilent 4294A pada julat frekuensi 40 Hz-30 MHz digunakan untuk pengukuran galang. Hasil menunjukkan peningkatan ketara parameter dielektrik seperti penyelarasan dielektrik, kehilangan dielektrik dan kekonduksian dalam darah diabetik kawalan berbanding dengan darah bukan diabetik. Ini disebabkan oleh penurunan kehadiran hemoglobin dalam darah diabetes yang akibatnya mempengaruhi afiniti oksigen, penurunan rintangan membran sel dan mendorong perubahan pada membran intrinsik. Perubahan yang dilakukan dalam tindak balas dwikutub dalam membran sel dan pengangkutan konduktans dalam medium ekstrasel untuk mengubah tindak balas dielektrik. Darah

diabetes juga menunjukkan perbezaan yang signifikan berbanding dengan yang lain. Nilai tindak balas dielektrik; permitiviti dielektrik, kehilangan dielektrik dan kekonduksian adalah $p = 0.003$, 0.001 dan 0.001 untuk 10 minit dedahan dan $p = 0.000$, 0.005 dan 0.004 untuk 15 minit dedahan masing-masing pada keluaran kuasa 50 mW. Pada output kuasa laser 60 mW didedahkan selama 5 minit, p-nilai masing-masing adalah $p = 0.001$, 0.000 dan 0.000. Di dalam julat suhu purata 24.2-28.0 ℃ dan SAR $0.140 \leq 0.695$ W / Kg, terdapat struktur morfologi sel yang diperbaiki, pembengkakan RBC kawalan yang disebabkan daripada penghasilan radikal bebas oleh pengautooksidaan glukosa, laluan poliol atau / dan glikasi protein bukan enzim. Berdasarkan Model Cole-Cole, plot Nypuist darah diabetes yang disinari mempunyai polarisasi elektrod mereka (rantau penyebaran α) yang terbentang dari 40 Hz hingga dalam julat frekuensi 11.7- 11.9 kHz serupa dengan darah bukan diabetes. Rentang sitoplasma dan rintangan plasma 78.99-82.82 Ω sebagai R1 (rintangan elektrolit) dan 64-113 Ω sebagai R2 (rintangan pemindahan caj) masing-masing diperhatikan. Ini menunjukkan rintangan yang lebih kuat dalam sitoplasma dan plasma darah jadi laser berkeamatan rendah dapat memberi faedah dalam rawatan klinikal kepada pesakit diabetes atas sebab ia boleh meningkatkan galang darah dan struktur membran sel.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Recently, continuous advances in basic and medical scientific research in the area of lasers enhanced significantly medicine performances by elucidating qualitative and quantitative measures of studying body system (blood) without and with illnesses (Zalesskaya, Sambor, & Kuchinskii, 2006). This has led to an increase in optimized market diagnostic tools for sugar levels, cholesterols, bacteria, blood aggregation test, ovulation, drugs, leukaemia cells, drugs delivery systems, etc., within different human body parts as well as the blood components (Gaur & Saran, 2016; Zalesskaya, et al., 2006).

Blood is an intricate biological system that exists throughout the human systems. It is made up of plasma ions (electrolytes) such as calcium, potassium, sodium, bicarbonate magnesium, sulphate etc (Zalesskaya, et al., 2006; Gemanam, et. al.,2020). Furthermore, it has trace amounts of other substances such as amino acid, vitamins, pigments, hormones (insulin), enzymes etc., secreted into the plasma that add up with the plasma, leucocytes, erythrocytes and the platelets to constitutes the blood. Together in certain harmony each plays its function when electric charge is passed through the blood. Much as human blood (HB) serve as complex body's main medium of transport, delivering oxygen, vitamins, nutrients, as well as metabolites to important areas of the human system; it is furthermore essential for the immune function of the blood. Accurate knowledge of its elements and properties such as the biological, physical, and chemical dynamism are crucial. In the same way information of the blood dielectric and electrical properties are very vital in nano-medicine field and appropriate diagnostic and efficient therapeutic procedures (Baer, Schulz, Notzon, Rolfes, & Musch, 2015; Wagner, Steffen, & Svetina, 2013). Mostly blood dielectric properties are fundamental for different medical purposes like separation of cells (e.g., cancerous cells from healthy blood cells), diagnose deteriorate condition of treated bloods for transfusion, and dielectric coagulometry study in the domain of artificial bloods. Furthermore, the accurate knowledge of bloods dielectric parameters is a precondition for setting threshold levels for control of electromagnetic pollutions (through conductivity and the specific absorption rate (SAR))(Gaur and Saran, 2016).

Different medical applications, such as microwave imaging, ablation and hyperthermia, also depend on the blood dielectric parameters in revealing various dynamic processes. It helps in obtaining reliable measurements and determinations of optimum and efficacy threshold limits for blood absorption of energy (Wagner, et al., 2013, Salahuddin, et al., 2017). Blood dielectric evaluations are based on quantifying its response to applied electric field. This is typically described by the blood conductivity, permittivity and specific absorption rate (SAR). Conductivity (σ), which its unit is S/m, measures the blood ability to transfer the electrical charges. Its permittivity (ε), measured in the unit is F/m, is the quantity of charge that is deposited in the blood due to the polarization of its components. The SAR measures the rate at which energy is absorbed by blood exposed to irradiation and it is measured in W/kg. The low level irradiation of the human blood characterised several dielectric response varying on the blood components and the power of the irradiating laser (Desouky, 2009, Zalesskaya, et al., 2006).

Studies of the effects of blood exposed to low level lasers are of paramount in elucidating mechanisms of its biostimulation. Blood is permanently made up of cell varieties whose membranes comprise of lipids, glucose and proteins etc. (Gaur & Saran, 2016). Although, the plasma has the most wide range of biological products among tissues, some of which are intrinsic to the organs (proteins, hormones, amino tissues, lipids, antibodies and adjusting factors), exogenous substances or component substances (clothing and defense enzymatic systems etc.)(Rauf, 2013). Various clinical treatments using low level laser irradiation have been used to different pathologic procedures, such as healing of wounds as well as repair of tissues etc (Mokmeli, 2011).

The blood and all biological tissues represent unique heterogeneous systems. It is a complex system in which different polarization mechanisms like dipole, Maxwell-Wagner's, electrochemical, etc. take place. Therefore, any change in blood physiology produced corresponding change in the blood dielectric parameters. Dielectric properties of biological tissue depend primarily on its cellular structures, therefore useful for revealing molecular and cellular properties. There are mostly considered as perfect determinant of the membrane functional state and of the cells cytoplasm (e.g., inherent membrane variations, responses of dipoles within the cellular membrane, conductivity transport in the extra-cellar medium as well as through the membrane and tissue water relaxation). The basic knowledge on tissue structure and composition, e.g. water content or presence of a tumor, may be obtained by evaluating its dielectric parameters (Sallam, et al., 2015).

It is observed that utmost studies of blood electrical properties have been restricted on erythrocyte suspension, polarization effect, and haematocrit dependency etc. With limited literature on the blood dielectric properties which is so integral to both further research and diverse medical applications, this work is has effectively closed-up the gap on dielectric parameters useful for the study of blood for patients under medical examination using optimal laser therapy of low power densities within moderate duration. in the course of this work, it is also worth mentioning that the blood dielectric response, SAR and laser optimal limit has been studied for therapeutic efficacy and biotimulation mechanisms.

1.2 Aim and Objectives

The aim of the research work is to aid in the future development of practical medicine, by providing evidence of low level laser irradiation effect on dielectric response of human blood that will yield important information on therapeutic treatment of diabetes mellitus. The research objectives are to;

1. investigate dielectric response and determine the electrical impedance spectrocopy response between diabetic and non-diabetic human blood before and after using different laser irradiation parameters as laser therapeutic treatment,
2. determine laser parameters for optimal biostimulaton and elucidates the laser biostimulation mechanisms of diabetic blood without adverse biological alterations, and
3. determine the specific absorption rate (SAR) via blood conductivity.

1.3 Problem Statement

Blood is a highly functional body fluid and a unique heterogeneous system. It is a multiplex system that different polarization mechanisms like dipole, electrochemical, etc. are observed (Frederic, et.al., 2017; Rauf, 2013). Slight variations in its morphology/physiology generate corresponding changes in its electrical and dielectric properties. There is a limited information in the literature about erythrocyte suspension, electrical impedance, polarization effect with almost no information on diabetic mellitus therapy. For example, work by Zhbanov and Yang (2018) addressed the electrochemical impedance spectroscopy of blood using the biconcave shape of erythrocytes, Liu, et. al., (2019) studied the Electrical Impedance characterization of erythrocyte response to cyclic hypoxia in sickle cell disease, Bao, et. al.,(1992) worked on the frequency domain impedance measurements of erythrocytes, Jaspard, et. al., (2003) studied the dielectric properties of blood: an investigation of haematocrit dependence, etc. According to Zhbanov and Yang (2017), dielectric properties of blood are important for basic research study and copious medical applications such as determination of the blood haematocrit, the erythrocyte sedimentation rate, the size and shape of RBC, erythrocyte aggregation and several other applications. Rauf, (2013), works on dielectric parameters using digital LCZ meter, perhaps the findings are still inadequate since readings were only evalauated at 1 kHz frequency and not for the purpose of diabetic blood therapy. Research on the effects of low level laser irradiation on both diabetic and non-diabetic blood through dielectric spectroscopy over a broad frequency range of 40 Hz to 30 MHz is crucial to understand exposure threshold therefore avoiding inhibition of human blood but optimize the use of low level laser in treatment of diabetes mellitus (DM). This will helps to elucidate the efficacy and mechanisms of interactions between low level laser and blood that is still unclear and undoubtedly a pressing issue of great concern due to the complications from the vast reactions that simultaneously occur during the low level laser irradiations (LLLI) therapy of blood. Therefore, the knowledge of dielectric properties by electrical impedance spectroscopy of human blood will give a better understanding of the low level laser irradiation mechanisms of both the diabetic and non-diabetic human blood. As diabetes mellitus (DM) affects the human blood by distorting its dieletric parameters, increasing the free radical density and density of dipoles in the diabetic blood therefore affecting the blood cells. The DM has the potential of causing changes within the plasma contents which can result to other blood constituents damage. This research work is aimed at addressing these existing gaps and providing a pathway for further research and medical applications in the DM where excessive emission which can results to inhibition and damage will be avoided. The work will further encourage evaluation of the maximum permissible specific absorption rate value for proper irradiation of the blood sample.

1.4 Significance of the Study

Low level lasers have been used for many clinical applications as a non-medicinal treatment, because of its ability to modulate blood rheology and improve biostimulation.

These humongous effects of blood laser therapy help improve not only the biostimulation but in the treatment of diabetes mellitus and indirectly in decline of certain number of transmission-associated graft-versus-host diseases (TA-GVHD) during blood transfusion. The present work elucidates the possibility of assessing the stimulating effect of low level laser therapy (LLLT) by measuring the dielectric permittivity of human blood and evaluating the blood specific absorption rate (SAR) for optimal blood low level laser therapy. The dielectric study and blood impedance spectroscopy method will help to optimise the use of radiation exposure in laser practice to avoid deterioration due to excessive thermal irradiation. Whereas also set the absorbance optimum threshold for blood specific absorption rate values and exposure parameters for efficacy to avoid inhibition of the diabetic blood during treatments.

1.5 Scope of the Research study.

This research focuses on the in-vitro biostimulation effects of different low level laser powers of 50 , 60 , 70 and 80 mW of a 532 nm diode pumped solid state (DPSS) on human blood (both diabetic and non-diabetic) at varying the laser exposure durations. The characteristics of human subjects involved in the blood sampling were of age group ranging from 22 to 54 years (average age 36 years) of both genders (male and non-pregnant female). Two groups were involved; the first constituted of those with no major medical history of serious health challenges and were not on medication. The second group were those with only diabetic mellitus as a major illness and not on serious medication. Both groups of blood samples were collected by venepuncture into ethylenediaminetetra-acetic acid (EDTA-anticoagulant) contained tubes. Some statistical analysis, graphs and certain calculations were performed using statistical package of social science (SPSS) software, origin software, microsoft excel and Z-view software for simulation and modelling of the Nyquist plots.

1.6 Outline of the Thesis.

The Thesis is broken-down into five different chapters. Chapter one presents the basic highlights of the research which are the introduction that gives a general overview of the frame research work followed by the aim and objectives of the research work the problem statement and its relevance and scope as well as the outline of the thesis. Chapter two presents the literature review where the existing research gap has been established. Chapter three describes the procedure for the human blood sampling, chemicals and few laboratory equipment that were used, experimental setup and general methodology of the data collections and analysis. Chapter four of the work presents and discusses the results using SPSS software, Z-view, origin software and UV-probe 2.31 software. Finally, chapter five summarizes the results and makes recommendations for further research work in the area considered to be novel.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Human blood as a fluid connecting tissue serves the body's main transport medium where oxygen, nutrients, information are transported to tissues and by-products and CO_2 to the organs of excretion. Blood plays different paramount roles in harmonizing individual cells into multifaceted organism systems. Research studies using laser is widely investigated because of its physiological importance. Low-level laser therapy (LLLT) has been used in various fields of medicine for a number of years as treatment principle, based on photobiomodulation effects which influence the components in the respiratory chain as primary photon acceptors. Among other things, LLLT may increase cell proliferation and have a positive impact on energy metabolism, as well as improving microcirculation and releasing growth factors. (Heu, et al. 2013). There is a significant increase in the biosynthetic activity resulted by the increase of carbohydrates, nucleic acids and proteins in the blood serum as shown by the clinical studies and intro experiments. LLLT also proven collagen content decreased in wounds subjected to laser therapy, as well as saving failing skin grafts (Vladimir, 2015; Surendranath & Arjunkumar, 2013). Also the LLLT influence brought about a lot of changes in blood peripheral; the increases in the red blood count, reduction in the sedimentation rate (ESR) of erythrocyte, couple with macrophage and lymphatic cells increased functions (Vladimir, 2015).

The molecular effects and mechanism associated with the biostimulation of blood by the use of LLLT is yet to be fully understood. However, the absorption of laser energy by the blood intracellular chromophores and converting to metabolic energy is believed to be one of the fundamental mechanisms, since after blood-He-Ne laser irradiation the cellular ATP levels are observed to increased exponentially (Kim, et. al., 2016). According to Miley, et. al.,(1997), blood glucose levels of a patient with diabetes drop from 350 – 400 to 140 – 150 after the UBI treatment is performed twice. But 3 – 4 weeks later after the UBI treatment is discontinued, the blood glucose levels are elevated back to the previous levels (Miley, et. al., 1997; Alghamdi &Kumar, 2012; Ferreira et. al., 2006). Investigation of the rabbit blood response to intravenous method of irradiation by He–Ne laser (λ= 632.8nm) through UV/visible and infra-red absorption spectra of the plasma, erythrocytes mass and whole blood constituents confirmed that hemoglobin is a major photo-absorber of this radiation (Mokmeli, 2017; Kim, et. al., 2016; Gonchokov and Lazarev, 2003). The irradiation causes a clear change in the infra-red and ultra-violet visible absorption spectra of the erythrocytes and the whole blood. These changes are connected to the fractional photodissociation of hemoglobin–ligand complexes in the course of absorption of laser irradiation. The result of photodissociation is believed as a major response of the blood exposed to low-intensity laser irradiation. (Zalesskaya and Sambor, 2005).

The experimental research of the spectral analysis on effects of low-intensity laser irradiation on blood molecule structures and its constituents were also performed by Zalesskaya, et.al., 2006. Fourier analysis of transforms IR absorption spectrum confirms changes in blood oxygen carrying characteristics when irradiated by He–Ne laser using intravenous method. The structural and conformational changes within the hemoglobin tetramer where interpreted as being initiated by the laser-induced photoreactions between the hemoglobin and oxygen. This lead to characteristic changes in the shape and intensity of the infra-red band for bond stretching vibrations, as well as amide I and amide II absorption bands. In the infra-red spectra of irradiated blood samples there is an observed increase in the absorption bands for stretching vibrations of phosphate group (945 - 1280 Cm^{-1}), which is evidence of increased nucleic acid (DNA, RNA) content. In the irradiated blood, the erythrocytes and plasma spectra there were no changes observed (Zalesskaya, et.al., 2006).

Hirsch, et. al., in 2017 study of blood -laser measured the resistance of blood,' an important factor in determining interfacial potentials of the blood systems. This accelerates research work in electrical conductive nature in different pathological conditions of the blood during clotting and clot retraction. Further research argued that the erythrocytes are the most perfect non-conductor of the electric field therefore blood resistance is directly proportional to the concentration of the erythrocytes. Subsequent investigation illustrates the electrophysical characteristics of the red cell. This indicates that only cell membranes of the erythrocytes are non-conducting that causes resistance, whereas the cytoplasm had about half plasma conductivity. Hence erythrocytes does not conduct direct current, they function as dielectric at high frequency (Hirsch, et. al., 2017).

Few researches studied the dielectric and electric properties of the blood constituents and different tissues at lower frequency as well as mechanism involves. In Rauf (2013), works on the electric parameters of plasma and whole blood revealed that the dielectric parameters at the frequency of 1 KHz were higher in plasma, low in 90% packed red blood cells and hemolysed blood. This means the significant changes in dielectric properties can be associated with cellular concentration and presence of red blood cells membranes, which separates the internal cell (haemoglobin) and outer cell (plasma). Therefore, it is cell membrane, which describes the electric parameters of the most biological tissues and the blood (Rauf, 2013).

Salahuddin, et. al., (2017) research on the dielectric properties of human blood and studied the effect of anti-coagulant agent on a wide frequency using fresh blood samples with additional and non-additional agents after extraction. The obtained results showed little changes in blood dielectric properties with and no anti-coagulant agents. These findings reflect differences in conductivity than in relative permittivity also a little difference in the dielectric properties of female and male blood samples. Based on these findings they suggest that measurements performed on blood samples with the added agents may not represent exactly the natural in-vivo and between patient variations in blood properties. In order to have an accurate in-vivo representation of the dielectric properties of blood, all factors need to be carefully considered (Salahuddin, et. al., 2017).

Basharah and Adeel, (2008) works on dielectric parameters of body tissues; liver, kidney and brain of the animal Ox at 1 kHz frequency. They attributed significant changes in these properties to the extent of hydration, molecules configuration, existence of certain trace elements, differences in structure and morphology of the cells and tissues. Hence structure constituents and molecule configuration of tissues have attributed in stimulating the dielectric parameters of the tissues. Sallam, et.al., (2015) investigates the irradiation effects of He-Ne lasers on the human blood (HB) to improve the condition of preservation for further transfusion. The results were compared with control blood and suggested the irradiation exposure dose apprpriate for increasing the duration of blood storage with little changes in parameter is 0.198 J/cm^2 and storage duration of 50 days. They further elucidates that appropriate exposure of blood with He-Ne laser could be considered a better way to enhance conservation of blood (Sallam, et. al., 2015).

In 2016, the dielectric relaxation properties of human blood were studied by Gaur and Saran, using wideband dielectric spectroscopy at various temperatures. This was carried out within the frequency range of 5MHz to determine the typical dispersion regions of blood group A^+. This investigation of dielectric relaxation was carried out on human blood of 25 years lad. They explained that variation in the intensities of dielectric relaxation behaviour with frequency and temperature had been found to be attributed to the corresponding blood structurally and morphologically variations. The dielectric permittivity, dielectric loss, relaxation time, etc, were assessed at various field and temperature conditions. The behaviour had been construed on the basis of inter facial polarization and the blood dipolar nature (Mester, 2013).

According to Zhbanov and Yang, (2015), blood conductivity slightly increased within the first minutes and thereafter decreased, when investigates the effects of cell aggregation in sedimentation of the blood and its electrical conductivity at various haematocrits. They carried out different enhancement and retardation methods of aggregation, measurements and theoretical evaluations. Finally agreed that the initial increase in blood conductivity was due to blood aggregation and the decline resulted from the deposition of erythrocytes. In 2016, Musawi, et al investigated effects of these low level laser irradiation (LLLI) dose on erythrocytes volume, by exposing it to a diode pump solid state (DPSS) ($\lambda = 405$ nm) laser. The result showed a significant decrease in RBC volume when induced with different laser doses, at 40 minutes exposure time the highest response was achieved (Musawi, et. al. 2016). Gabriel, et. al. (1996), researched on blood containing anticoagulant agents from blood banks, quoted a blood wideband spectra over various dispersion regions at $37 \circ C$ that are generally for evaluation of specific absorption rate (SAR) and other medical purposes. However, the available data is limited in scope with still many unclear aspects of the related to dielectric properties of blood. Perhaps the findings are inadequate since couldn't capture the fundamental principles that can be used for efficient therapeutic purposes (Desouky, 2009; Gabriel, Lau, & Gabriel, 1996).

Diabetes mellitus is a general health concern. These occurrence cases of diabetes mellitus diagnosis amongst Malaysia citizens of ages 18 years beyond have worsen from 11.6% in 2006 to 16.6% in 2015. Out of 19,887,000 adult populations, a total of 3,303,000 patients were discovered to be detected with Type 2 diabetes mellitus (DM) in Malaysia (Alberti, Zimmet, & Shaw, 2007; Feisul & Azmi, 2014). This consistent increase of this disease called for any urgent alternative/diverse majors in diagnosis and treatment of the DM in Malaysia and around the world.

2.2 Blood Constituents and Their Functions.

Human blood amounts 7% of weight of the body, this gives an average density of 1060kg/m^3, while that of pure water is 1000kg/m^3. An adult blood volume is estimated of roughly 5 litres, composed of plasma and different kinds of cells. Among all these components, blood plasma probably constitutes the most diverse varieties of biological constituents among tissues, some being intrinsic to the organs (lipids, hormones, amino tissues, proteins, antibodies and adjusting factors etc.), exogenous substances or component substances (clothing and defense enzymatic systems, such as compliment, etc.) (Rauf, 2013). On the average the blood composition has 55% of plasma and the formed element also known as blood Cells or Corpuscles- comprises of cells and fragments constitute the remaining 45% as in

Figure 2.1 (Zalesskaya, 2006, Anan'eva, and Dvoretskii , 2000; Lazarovits, et. al., 2015).

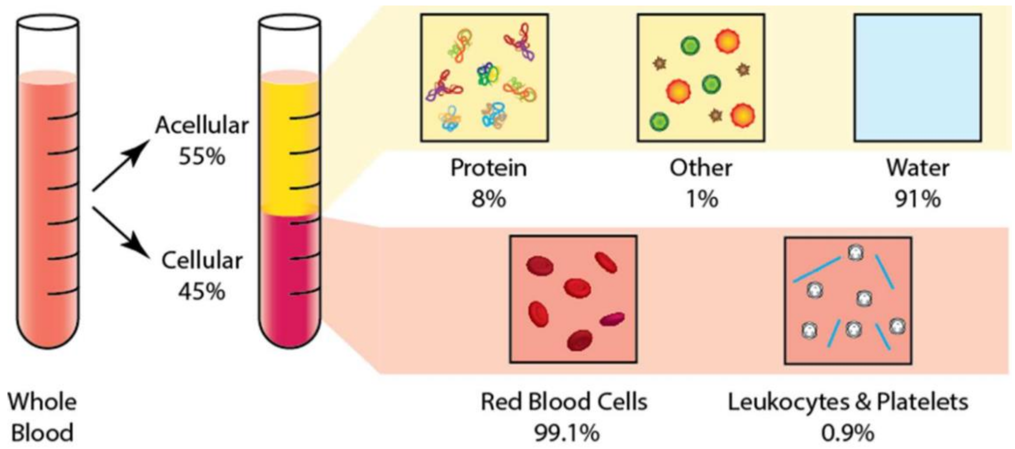

Figure 2.1 Whole blood compositions (Lazarovits, et. al 2015).

Plasma is a yellowish liquid that is 90% water, with dissolved protein, salts (electrolytes) and nutrient etc. The major proteins of the plasma are the albumin and the antibodies (also known as the globulins). Albumin keeps the fluid within the blood vessels and tissues also transport hormones and drugs in the system, example is the thrombin. Globulins which are another protein of the plasma actively serves as body defender against the virus, fungi, bacteria, clothing and cancerous cells, responsible for control bleedings. Examples are Alpha 1, Alpha 2, Beta, Gamma Factor (antihaemophilic globulin), Fibrinogen, Prothrombin and Plasma thromboplastin, etc. Plasma also serves as reservoirs capable of replenishing water insufficiency or excess water absorber from tissues therefore maintain blood pressure and thoroughly circulations (Rye, et. al. 2017; Lazarovits, et. al., 2015; Ashford, et.al., 1999).

Formed Elements; that constitute the 45% of the whole blood are also known as Blood Cells or Corpuscles, comprises of cells and fragments. The followings are the constituents; Leukocytes (leucocytes); they comprises of less than1% cells in the human blood and are primarily function as part of the immune system. These cells contain a normal nucleus and mitochondria without the haemoglobin and are comparably larger than the erythrocytes. The various types of the leukocytes are the granulocytes and agranulocytes as in Figure 2.2 (Zalesskaya, 2006, Ashford, et. al., 1999; Lazarovits, et. al., 2015).

Granulocytes are characterized by a loped nucleus and granular inclusions in the cytoplasm and typically first-reactors in time of infection or injury. Examples are; Neutrophils (or polymorphonuclear leukocytes), Eosinophils and Basophils. And the agranulocytes are the ones that lack granules in their cytoplasm. Examples are the lymphocytes including T and B cells are for adaptive immune reactors as in Figure 2.2, and monocytes grouped into macrophages and dendritic cells, react to injury and infection (Lazarovits, et. al., 2015; Rye, et. al 2017; Ashford and Comain, 2000).

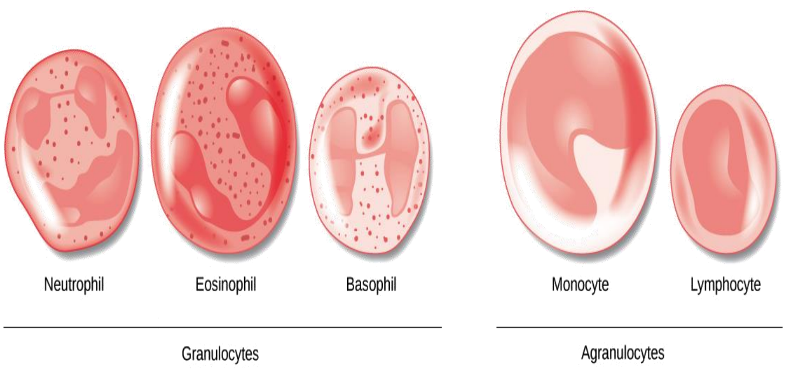

Figure 2.2 Examples of granulocytes as characterized by a lobed nucleus and granular inclusions in the cytoplasm (Rye, et. al 2017; Ashford and Comain, 2000).

The red blood cells known as the erythrocytes constitutes 99% of the formed elements, produced in the bone marrow and released into circulation, small in size to squeeze through the tiny blood vessels. The mature RBC don't nucleus which enable them to store haemoglobin, oxygen-bonding protein that enable oxygen and carbon dioxide transportation (Lazarovits, et. al., 2017). And the platelets (thrombocytes) formed less than 1% of cell fragments. They are produced by the breaking of the larger cells called megakaryocytes forming a disc-like shaped of about 2-4 μm in diameter called thrombocytes. They release signals attracting other platelets making them sticky and activating signaling cascade that converts fibrinogen (plasma water-soluble protein) into fibrin. The fibrin produced the threads that synergy the platelets clot last that preventing loss of blood (Rye, et. al., 2017; D'Alessandro, 2016).

2.3 Abnormal Erythrocytes

The diabetic mellitus affect the red blood cells (RBCs) when the glucose/sugar enters the cells increasing the blood sugar levels therefore the sugar molecules got attached to the haemoglobin capable of making the blood cells swollen, change in color and shape (Gemanam, et, al., 2020). This greatly constitutes to the formation of blood aggregates and rouleaux that result to a lot of complications. Blood aggregation is known as the blood cell clumping process that is measured either in the platelet or erythrocytes aggregation. According to Wagner, et. al., (2013), there is three major kinds of aggregation of the erythrocytes. Firstly the formation of rouleaux being resulted by the plasma macromolecules and that is presumed to be a reversible aggregation progression. Secondly, there are some symptoms that erythrocytes may become dynamically adhesive in the presence of a clotted blood. Lastly there are numerous pathological situations such as diabetes mellitus and malaria or anaemia diseases where erythrocytes are known to form considerable aggregates that hinder the easy blood flow (Wagner, et.al, 2013).

The erythrocytes are able to unite together in a form of a coin-like structure known as rouleaux. The formation of Rouleaux by the erythrocytes is a reversible occurrence that happens during low movement of blood and little shear forces in flow. Some pathological situation can modify the blood molecules constituents and the nature of erythrocytes then lead to intensified rouleaux formation, which results in oxidation of fragments and tissues (Kibria, et. al., 2014; Born and Cross, 1962). The formation of rouleaux can also be caused by resuspending the erythrocytes in physiological solutions encompassing neutral macromolecules such as dextran. Nevertheless, without any macromolecules, e.g. RBCs in a simple salt solution, no aggregation happens. The intermediated aggregation of fibrinogen RBCs upsurges consistently with increase fibrinogen concentration, while the aggregation controlled by dextran RBCs reaches the maximum at a certain dextran concentrations. The aggregation strength depends not only on the dextran concentration, but also on the molecular weight of the dextran (i.e., the radius of gyration of the dextran) (Wagner, et.al, 2013).

The forces responsible for the aggregation are feeble therefore aggregates dispersed under high shear environment in bloodstream. The variation in blood viscosity under high shear rate is recognised as the effect of blood thinning. Baskurt, et. al., 2011 observed that the formation of aggregates varies on both the surface parameters of the RBC (charge, elasticity) and suspending medium properties as shown in Figure 2.3.

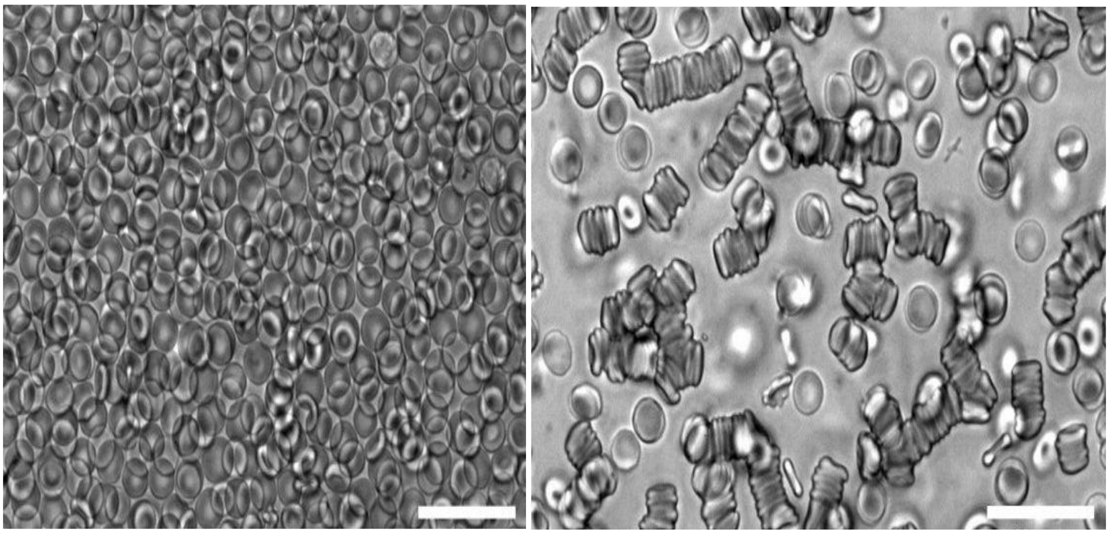

Figure 2.3 Optical images of (Left) non aggregated human RBCs in autologous plasma, (right) aggregated RBCs with 3% dextran-70 kDa PBS added to whole blood (scale bar 20 μm) (Kibria, et. al., 2014).

The rouleaux formation is influenced by the inherent parameters of erythrocytes like the elastic nature of its membrane that contribute to the erythrocytes resistance to aggregates. There is interaction between membrane elasticity and agglomerate shape. In overall, blood erythrocytes can pass through capillaries with tinny diameters than its sizes, when freezing happens, the flow may stop completely. In the incident of healing of wounds, clotting is life-saver, whereas in healthy tissue, thrombus may cause stroke and consequently death as majorly in the developed countries. Coagulation of blood is an intricate process involving several constituents and collective factors. It begins with the blood platelets activation (for example on damaged vessel walls) that discharges diversified messengers and growth factors to activate other cells to participate in formation of the blood clot. Observably, erythrocytes are main part of the thrombus, generally alleged to be only trapped in the fibrin network as a result of their predominance in the blood. Thus, the role of RBCs has always been considered to be completely passive (Wagner, et.al., 2013).

2.4 Diabetes Mellitus.

Diabetes mellitus is an intricate, dreadful ailment as well as progressively health problem that increased with incidence worldwide. By the year 2030 the World Health Organization (WHO) estimated 366 million people will be diagnosed with diabetes (5% of the world population) (Desouky, 2009). It is now available in nearly every population and epidemiological evidence shows that with no adequate prevention and control measures, occurrence will continue to upsurge globally (Alberti, et al., 2007; Lonappan, et al., 2007). This is a group of metabolic disorder characterized by hyperglycermia caused by insulin secretion deficiency, insulin action, or both. Hyperglycemia is chronic and associated with long term damages, dysfunctions, and failure of different organs, such as the eyes, vessels of the blood, kidneys etc. Diabetes mellitus is a capable cause of blindness in adults; also the risk of coronary heart and peripheral vascular diseases are higher in diabetes patients. Diabetes mellitus results in impaired lipid profile, especially increased susceptibility to lipid peroxidation, which is responsible for the increased incidence of atherosclerosis, a main complication of the diseases (Desouky, 2009).

It can usually be referred to as diabetes and known as abnormal glucose in the blood. The modality for diabetes diagnosis are symptoms of hyperglycemia and plasma glucose value of ≥11.1 mmol/l or fasting plasma glucose ≥7.0 mmol/l (total blood 6.1mmol/l) or 2-h plasma glucose ≥11.1 mmol/l during oral glucose tolerance test (OGTT)(B. Thorens., 2014). Without symptoms, diagnosis must be confirmed by the any of the above measured values (Farsaci, et. al., 2015; Kim, et. al., 2012, and Lonappan, et. al., 2007). Diabetes may be presented differently in different individuals, from severe symptoms and gross hyperglycemia to symptoms deficiencies and with blood glucose values above the diagnostic cut-off value. Where as in children, diabetes often presents with severe symptoms of very high blood glucose levels, marked ketonuria and glycosuria (Lonappan, et. al., 2007).

Hyperglycemia, which is consistent with high blood glucose levels, can cause serious oxidative stress due to the increased free radicals production. Reactive oxygen and nitrogen species (ROS and RNS) through membrane lipid peroxidation are responsible for the inducible changes in the red blood cell (RBC) structure of the cytoskeleton. Thus, the toxic effects of glucose on erythrocytes appears not only as an modification of the phospholipids' bilayer but also as an alteration of integral proteins and haemoglobin (Hb), a protein involved in oxygen transport, which is widely expressed in the RBCs (Farsaci, et al., 2015). According to Desouky (2009), high glucose values cause a toxic effect on erythrocytes, which leads to restructuring of erythrocytes membranes and electrolytes imbalance cell membrane. Dielectric spectroscopy (DS) is very sensitive to small changes.

Treatment begins as soon as the diagnosis is confirmed by blood glucose measurement. Rarely, children and adolescents are less symptomatic, and then diagnosed with a fasting blood glucose measurements and/or oral glucose tolerance tests (OGTT). This happens, for example, when a patient is found to have autoantibodies checked to identify individual at increased risk for type1 diabetes. Insufficient insulin production (produced by the pancreas and lowers blood glucose), or sensitization to insulin action cells have resulted in mechanisms that form two basics types of diabetes mellitus (Alberti, et al., 2007; Thorens., 2014).

2.4.1 Type 1 Diabetes.

This type of diabetes is also known as juvenile diabetes or insulin-dependent diabetes is a chronic condition in which the pancreas produces little or no insulin. Insulin is a hormone needed to enable glucose (sugar) to enter the cells that produce energy. It is estimated that 5-10% of the 100 diabetic people fall under this group (Alberti, et. al., 2007; Mellitus, 2005). According to Thorens , (2014), the disease is considered to be autoimmune and results from the destruction of cellular-mediated autoimmune pancreatic beta cells.

Type1 diabetes occurs mainly in children and adolescents, usually as a swiftly progressive form, and slowly in adult as latent auto-immune diabetes in adults (LADA). Few patients with Type 1 diabetes fall into the category named idiopathic diabetes (American Diabetes Association, 2010). This form of diabetes is mostly common in non-Caucasians and is strongly hereditary, has no immunological evidence for beta-cell autoimmunity, and is not human leukocyte antigen (HLA) associated.

2.4.2 Type 2 Diabetes

The Type 2 diabetes previously known as adult-onset or non-insulin-dependent diabetes can develop at any age but is commonly in adulthood (Alberti, et. al., 2007). This contributes to the majority of people with diabetes who cannot properly use insulin, which is called insulin resistance. That means this form of diabetes, which accounts for ∼90–95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, type II diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency (American Diabetes Association, 2010). When Type 2 diabetes gets worse, the pancreas makes fewer and fewer insulin, resulting to insulin deficiency.

According to American Diabetes Association (2010), there may be numerous causes of this type of diabetes, with no known etiology and no autoimmune destruction of cells. Mostly patients with this type of diabetes are obese, and obesity itself causes some degree of insulin resistance (Lonappan, et. al., 2007). Individuals with this form of diabetes often have not been diagnosed for years since their hyperglycemia is insufficient to show symptoms, but they have increased the risk of developing macrovascular and microvascular complications. Insulin sensitivity may be improved, but not completely restored, by weight loss, increased physical activity and/or pharmacological treatment of hyperglycemia (Alberti, et. al., 2007; Lonappan, et. al., 2007).

2.5 LASERS

The acronym LASER originated from Light Amplification by Stimulated Emission of Radiation. This is a device that emits monochromatic light based on the stimulated emission of electromagnetic radiation through an optical amplification process. It is considered as a landmark invention of the mid-20th century. Albert Einstein in 1916 ignited the first conceptual development plan of laser, when he proposed that photons could stimulate the release of same photons from excited atoms. Laser was firstly built by Theodore H. Maiman at Hughes Research Laboratories in 1960, while Charles Hard Townes and Arthur Leonard Schawlow laid the theoretical work (Hecht, 2010).

A laser can be distinguished from other light sources because it emits coherent, spatial and temporal light, allowing lasers to be focus to tight spaces, enabling application in all aspects of life. Spatial coherence also allows the laser beam to remain narrow over long distances (collimation), enabling applications such as laser pointer and with high temporal coordinates it can transmit light with very narrow spectrum (one color of light) (Svelto and Hanna, 2010). Having high temporal coherence properties, the laser can also produce light pulses such as in femtosecond. Lasers has wide range of applications used in optical disks drives, laser printers and barcode scanners; DNA sequencing instruments, fiber optic and free-space optical communication; laser surgery and skin treatment; cutting and welding materials; military and law enforcement tools to targets and measure distance and speed; laser exposure in entertainment (Jawad, et. al., 2011, Davarcioglu, 2010 and Svelto and Hanna, 2010).

The term "light" in a laser includes electromagnetic radiation of any frequency, not just visible light, hence the terms infrared laser, ultraviolet laser, X-ray laser, gamma-ray laser, and so on. Since the development of microwave predecessor of the laser and the maser, such devices operate on microwave and radio frequencies referred to as "masers" rather than "microwave lasers" or "radio lasers". At the Bell Telephone Laboratories, the laser was called optical maser in the early technical literature, which the term is obsolete now. Laser is known for their wavelength in a vacuum; most of the "single wavelengths" lasers actually produce radiation in several modes with slightly different frequencies (wavelengths), often not in single polarization. Temporal coherence implies monochromaticity, although there are lasers that emit a broad spectrum of light or emit different wavelengths of light simultaneously. Some are not single spatial mode and consequently have light beams that diverge more than is required by the diffraction limit. However, all these devices are classified as "lasers" based on their method of producing light that is stimulated emission. Laser is used in applications where light the required spatial or temporal coherence could not be produced using simple technology (Davarcioglu, 2010, Jawad, et. al., 2011).

2.5.1 Principles of Laser

Simulated Emission in Laser is based on classical view since electron orbiting an atomic nucleus has a larger energy for orbits further from the atomic nucleus. Though, quantum mechanical effect forces electron to take on discrete position in orbital. Therefore, electrons are found in specific energy levels of an atom as shown in Figure 2.4.

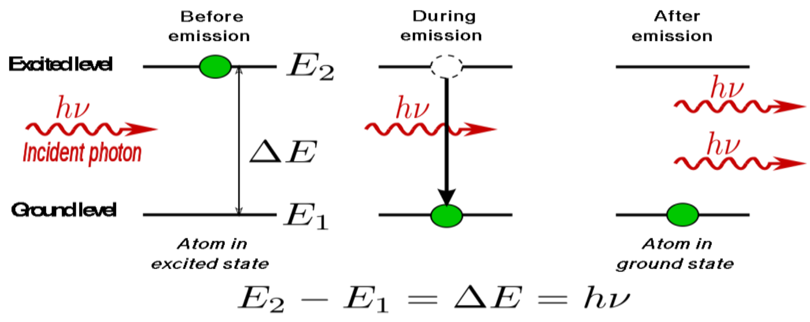

Figure 2.4 Energy transitions between discrete energy levels, when electron receives that incident quantum of energy (Svelto and Hanna, 2010).

External electromagnetic fields at transient frequency can affect the quantum mechanical state of atoms. As the electrons in the atoms make the transition between the two stationary states (which do not show dipole fields), they enter the transition state that has a dipole field, and acts as a small electric dipole, and these dipoles swing at characteristic frequency. In response to the external electric field at this frequency, the probability of an atom entering this transition state increases.

Therefore, the transition rates between the two stationary states improved as a result of spontaneous emission. Such a transition to a higher state is called absorption, and it destroys an incident photon (photon energy enters the energy of the higher state). The transition from higher to lower energy states, however, produces additional photons; this is a stimulated release process (Svelto and Hanna, 2010, Jawad, et. al, 2011)

2.5.2 Diode Pumped Solid State (DPSS) Lasers.

Diode pumped solid state (DPSS) lasers are one of the solid state lasers made by pumping a solid gain medium, with a laser diode. Lasers can be basically classified according to the nature of its medium of amplification; being gas, liquid (dye), or solid state (e.g. semiconductor, diode lasers and DPSS etc.). These classifications give an overview of the basic lasers families in terms of their uses. Among the classes, the most frequently used lasers are the solid state lasers (Svelto and, 2010, Fan and Byer, 1998, Grossi and Riccò, 2017, Hayashi, et.al., 2010 and Hecht, 2010).

According to Davarcioglu, (2010), solid state laser capture a unique place in laser progression. Solid state lasers utilize solid gains as medium, durable, easy to maintain as they efficiently of produce high power. Although they do offer some gains over gas lasers, crystals are not ideal cavities or perfect laser media. Real crystals contain variations of refractive index that interfere the wave front and the laser structure modes. High power operation promotes thermal expansion that changes the dimensions of the cavity effectively and thus adapt the mode. Laser crystals are cooled by air or force liquids, especially for high repetition rates (Davarcioglu, 2010). The most delicate aspect of solid state laser is that the output is