EVALUATION OF GINGIVAL MELANIN DEPIGMENTATION BY USING ER:YAG LASER: EX-VIVO AND CLINICAL STUDIES

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by

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بسم الله الرحمن الرحيم

"قل إن صلاتي ونسكي ومحيايومماتي لله رب العالمين"

Say: ‘Verily my prayer, my sacrifice, my life and my death are all for God, the Lord of the worlds’

Qur'an 6:162

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LIST OF ABBREVIATIONS

3Rs	Replace, reduction, and refinement
ADP	Adenosine diphosphate
DHI	5,6-Dihydroxyindole
DHICA	5,6-dihydroxyindole-2-carboxylic acid
DOPA	Dihydroxyphenylalanine
DP	De-pigmentation
Er,Cr:YSGG	Erbium, Chromium: Yttrium-Scandium-Gallium-Garnet
Er:YAG	Erbium-doped yttrium aluminum garnet
FDA	Food and Drug Administration
GLUT1	Glucose transporter 1
Gr	Group
H&E	Hematoxylin and Eosin
HIF	Hypoxia inducible factor
IR	Infrared light
KSA	Kingdom of Saudi Arabia
LASER	Light amplification by stimulated emission of radiation
LASIK	Laser-assisted in situ keratomileusis
LP	Long pulse
MSP	Micro-short pulse
Nd:YAG	Neodymium-doped yttrium aluminum garnet
PDT	Photodynamic therapy
PMN	Polymorphonuclear neutrophils

RCT	Randomized clinical trial
SP	Short pulse
TGF-β	Transforming growth factor beta
TRP-2	Tyrosinase related protein-2
TXA2	Thromboxane A2
USA	United States of America
USM	Universiti Sains Malaysia
UV	Ultraviolet light
VEGF	Vascular endothelial growth factor
VLP	Very long pulse
VS	Visible spectrum

PENILAIAN DEPIGMENTASI MELANIN GINGIVA DENGAN MENGGUNAKAN LASER ER: YAG: KAJIAN EX-VIVO DAN KLINIKAL

ABSTRAK

Hiperpigmentasi gingiva selalunya merupakan masalah bukan patologi, tetapi secara klinikal ia dianggap sebagai tidak estetik terutama dalam kes-kes persembahan gingiva yang berlebihan semasa senyuman. Banyak kaedah rawatan tersedia untuk menyelesaikan masalah seperti pengelupasan kimia, pembedahan dan ablasi laser. Laser dengan pelbagai panjang gelombangnya dianggap sebagai rawatan standard dalam penyingkiran gingiva. Er: YAG laser adalah salah satu laser yang paling banyak dikaji dalam tisu lembut dan pengurusan tisu keras. Dalam kajian ini, tetapan kuasa yang berbeza pada panjang gelombang yang sama dari laser Er: YAG telah dibandingkan antara satu sama lain dan yang paling sesuai antara mereka dipilih untuk penyingkiran gingiva. Kajian ini dijalankan dalam dua fasa bermula dengan eksperimen tisu ex-vivo diikuti oleh fasa eksperimen prospektif manusia. Dalam fasa haiwan, 24 tetapan kuasa yang berbeza telah digunakan ke atas mukosa gingiva yang berpigmen yang dipotong dari kepala biri-biri yang baru disembelih. Zon yang terubah, karbonisasi, parameter kawalan operator dan kecepatan diperiksa untuk tujuan memilih 4 tetapan kuasa terbaik untuk digunakan untuk kajian ke atas manusia. ‘Fluence’ pesakit 2J/cm^2 dan 4J/cm^2 dan tempoh denyutan SP dan VLP dipilih untuk kajian ke atas manusia. Dalam kajian manusia, seramai 40 pesakit wanita dan bukan perokok dewasa direkrut dan dibahagi secara rawak kepada empat kumpulan di mana 10 pesakit untuk setiap satu (2J/cm^{2}VLP, 2J/cm^{2}SP, 4J/cm^{2}VLP dan 4J/cm^{2}SP). Satu tetapan kuasa telah digunakan untuk setiap kumpulan. Penilaian klinikal dan biologi telah dilakukan. Kesakitan, pendarahan, kepuasan, pengkarbonan, kelajuan penyingkiran dan parameter penyiasatan histologi telah diuji. Spesimen tisu ablasi telah dihantar untuk analisa histologi. Keempat-empat tetapan kuasa membuktikan keselamatan, kepuasan pesakit dan di bawah kawalan operator, dan berkesan dalam penghapusan pigmentasi. Tidak terdapat perbezaan statistik di antara mereka mengenai histologi, kesakitan, kepuasan dan pendarahan ($P>0.05$). Terdapat perbezaan yang signifikan antara kumpulan mengenai masa ablasi ($P<0.05$). Analisa histologi menunjukkan kerosakan yang sangat sedikit di lamina propria dengan kedalaman 10 hingga 60 mikron. Sebagai kesimpulan, ‘ fluence’ $2J/cm^2$ dan $4J/cm^2$ dengan tempoh denyutan SP (0.3ms) dan VLP (1ms) untuk setiap ‘fluence’ didapati selamat, berkesan dan cekap. ‘Fluence’ $<2J/cm^2$ mungkin tidak berkesan kerana ia meningkatkan karbonisasi gingiva yang sedikit di kawasan yang dipermudahkan, dengan itu menyebabkan penglihatan pengendali kabur, dan ‘fluence’ $>5J/cm^2$ tidak dapat dikawal. Kekuatan puncak kurang kritikal daripada dos dalam aspek masa ablasi. Berdasarkan kajian haiwan dan percubaan klinikal, pemeriksaan dari aspek teknikal, klinikal dan biologikal dengan menggunakan Er: YAG laser dalam kajian ini, pengaturan kuasa optimum terakhir yang diperoleh adalah: $2J/cm^2$VLP yang optimum untuk pengamal yang bukan berpengalaman dan $4J/cm^2$SP yang optimum untuk pengamal yang berpengalaman.

EVALUATION OF GINGIVAL MELANIN DEPIGMENTATION BY USING ER: YAG LASER: EX-VIVO AND CLINICAL STUDIES

ABSTRACT

Gingiva hyperpigmentation is more often a non-pathological problem, but clinically it is considered unaesthetic especially in cases of excessive gingival display during smiling. Many treatment methods are available to solve the problem such as chemical peeling, surgery and laser ablation. The laser by its various wavelengths is considered a standard treatment in gingival depigmentation. Er: YAG laser is one of the most studied laser in soft tissues and hard tissues management. In this study, different power settings of the same wavelength of Er: YAG laser were compared between each other and the favorable of them were chosen for gingival depigmentation. The study was conducted in two phases beginning with ex-vivo animal tissue experiment followed by prospective human experiment phase. In the animal phase, 24 different power settings were applied in the hyperpigmented gingiva mucosa of a recently slaughtered sheep's head. Altered zone, carbonization, speed and operator controllability parameters were examined for the purpose of choosing 4 best power settings to be applied to the human study. 2J/cm^2 and 4J/cm^2 fluences and SP and VLP pulse durations were chosen for the human study. In the human study, a total of 40 adult female and nonsmoker patients were recruited and were divided randomly into four groups of 10 patients each (2J/cm^{2}VLP, 2J/cm^{2}SP, 4J/cm^{2}VLP and 4J/cm^{2}SP). One power setting was applied to each group. Clinical and biological evaluation was performed. Pain, bleeding, satisfaction, carbonization, speed of removal and histological investigation parameters were tested. Ablated tissue specimen was sent for histological analysis. The four power settings proved safety, patient satisfaction and dentist controllability, and efficiency in the removal of the pigmentations. There was no statistical difference between them regarding histology, pain, satisfaction and bleeding ($P>0.05$). There was significant difference between groups regarding ablation time ($P<0.05$). Histological analysis showed a very shallow damage in the lamina propria with a depth of 10 to 60 microns. In conclusion, fluence of 2J/cm^2 and 4J/cm^2 with pulse duration of SP (0.3ms) and VLP (1ms) for each fluence were found to be safe, effective and efficient. Fluence <2J/cm^2 might not be effective due to increase carbonization of the superficial gingiva of the lased areas, thus obscuring the vision of the operator, and fluence >5J/cm^2 is uncontrollable. Peak power is less critical than the dose in aspect of ablation time. Based on ex-vivo animal study and clinical trial, examining the technical, clinical and biological aspects using Er: YAG laser in this study. The final optimum power settings obtained are: 2J/cm^2 VLP which is optimum for the beginner practitioner and 4J/cm^{2}SP which is optimum for the experienced practitioner.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

1.1.1 Laser

Laser is an attractive word, which most people in the modern world are increasingly aware about it. It is going to be an important tool today and in the future, invented as a solution to today's problem (Nobelprize.org, 2014).

Laser is an abbreviation word for "light amplification by stimulated emission of radiation" which is commonly used nowadays (Gould, 1959). Since laser is a type of amplified light, understanding light behavior is a must to be able to understand laser. For decades, scientists have been trying to creat different applicable physical models to simplify the complex reality of nature with the aim of quantitative characterization and description of light.

1.1.2 Erbium laser

Erbium lasers, invented in 1975 (Zharikov *et al.*, 1975), was first used in dentistry for cavity preparation since 1989 (Hibst and Keller, 1989), because of its highly absortive nature of its wavelength (2.6 to 3μm) in water (15 times more than CO_2 laser and 20,000 times more than Nd:YAG laser) and hydroxyapatite (Walsh and Cummings, 1994). The beam it produces is a pulsed wave.

There are two Erbium laser systems that are prefered in dentistry depending on their active medium: Erbium doped Yttrium-Aluminium-Granet (Er:YAG) which produces light in the wavelength of 2.94μm, and erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) which produces light in the wavelength of 2.79μm. The pumping source is flash lamp. Both types of the lasers are used in soft and hard tissue work in the oral cavity (Gutknecht, 2007).

1.1.3 Gingival melanin pigmentation

There are several factors in which the color of gingiva depends on. These are the number and size of blood vessels, thickness of the epithelium, the level of keratinization and the amount of pigments present. The three main pigments that are responsible for the normal physiologic pigmentation of the oral cavity are melanin, carotene and oxyhemoglobin. Carotene, an intensely colored red-orange tincture is the Vitamin A that can be derived from fruits and vegetables. It helps in keeping the gingiva healthy and guarantees good healing (digest, 2016). On the other hand, oxyhemoglobin is the bright red coloring that is produced in the blood when the oxygen from the lungs and hemoglobin in blood combine. Melanin, a natural brown pigment, is produced by melanocytes that are found in the basal layer of the epithelium. Through chemical studies, melanin is found to be a high-molecular weight coloring pigment that is not soluble in water and most organic solvents. Melanin is developed exclusively in the cytoplasm of the melanin-forming cells called the melanocytes. Various factors may be responsible for the degree of pigmentation but it is greatly dependent on the activity of the melanocytes (Parwani and Parwani, 2013).

Additionally, sources of pigmented feature of gingiva may result from extrinsic and intrinsic causes. The range of color may vary from light brown to blue-black.

Usually, the coloring of gums depends on the source of pigment and how deep the pigment is in which the colors come from. Melanin is normally brown, however it can also show a blue, green, or brown color to the human vision. This different color perception can be accounted for the physical properties of light absorption and reflection, specifically explained by the light phenomenon called Tyndall effect. The Tyndall effect, also called Tyndall scattering, is the scattering of light as soon as a light beam goes through a mixture with dispersed particles that do not settle out (Fernandez-Flores and Montero, 2006).

Although melanin found on gingiva denotes a racial trait, many individuals prefer not to have them due to social reasons as they are often shown during “smiling”, particularly among those with “gummy smiles”. Therefore, many chemical and surgical methods have been advocated as standard dental procedures to remove melanin pigment from the anterior gingival part of the oral cavity. Recently, laser technology has been use for gingival melanin depigmentation but till today there is no definite Er:YAG laser power setting parameters recommended for its use.

1.2 Research question

Is there an establish standard parameter for Er:YAG laser power settings for gingiva depigmentation in the oral cavity that will give effective and reproducible clinical results? What would be the optimum power settings?

1.3 Objectives of study

1.3.1 General objective:

To investigate the optimum power setting for laser depigmentation of oral mucosal melanin pigment to achieve effective clinical, biological, esthetic results.

1.3.2 Specific objectives:

Phase I:

To assess the effect of various (24) selected power settings of laser on sheep gingival pigmentation tissue removal.

To compare between clinical outcomes and biological parameters of various selected power settings of laser on sheep gingival pigmentation tissue removal.

Phase II:

To assess the effect of various (4) selected power settings of laser on human gingival pigmentation tissue removal.

To compare between clinical outcomes and biological parameters of various selected power settings of laser on human gingival pigmentation tissue removal.

1.4 Null hypothesis

It is not possible to set the optimum power setting for laser depigmentation of oral mucosal melanin pigment to achieve favorable biological outcome and superior and stable esthetic result.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of the science of light

For a long time, scientist have been facing difficulty in explaining the nature of light. It is believed, Albert Einstein has clearly interpreted this problem. "It seems we could use sometimes the one theory and sometimes the other, and sometimes we may use either. We are faced with a new kind of difficulty of explaining the light. We have two contradictory pictures of reality; separately neither of them fully explain the phenomena of light, but together they will do" (Gould, 1959; Stadelmann *et al.*, 1998).

In ancient times, light was use to provide brightness for vision in the dark, to chase wild animals and to burn and cook hunting products for their food. Today, light has been further develop to assist humans as atechnology for curing diseases. However, understanding and explaining the science of light remain vague and controversial.

There are three models explaining the light, each of them cannot do it alone. They were developed during centuries:

i) Geometric optics

ii) Wave optics

iii) Quantum optics

The first model by timeline order is geometric optics which describe light as straight rays. It describes the basics of light propagation, reflection, and refraction. This theory includes the use of light speed, refractive index, images and imaging (images by a lens), camera obscura and its limit, reflection law, mirror resonant and effect of optical elements (Lipson *et al.*, 2010)(Fig 2.1).

Since the basic principle of geometric optics is the dealing with light as straight lines, it was the simplest theory to explain about light. Propagation, reflection and refraction may be explained by mathematical equations.

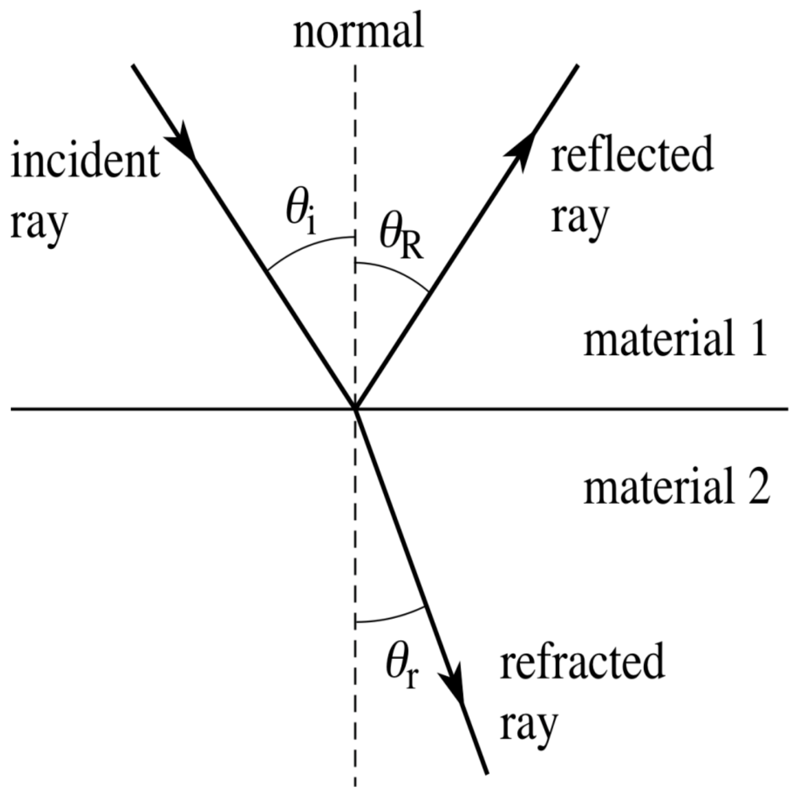

Fig 2.1: Reflection and refraction of light θR = θi (Reading, 1996)

Geometric optics theory started in 325 BC by the Greek mathematician, Euclid. There was a noticable progress in the Islamic Era which was considered significant, like the work of Al-Kindi (c. 801-873) and Ibn Sahl (c. 940-1000). Ibn al-Haythamv (known as *Alhacen* or *Alhazen* in Western Europe) (965–1040), translated the "Book of Optics" of Avicenna (980-1037) into Latin. Abū Rayhān al-Bīrūnī (973-1048) was the first to discover that speed of light is faster than the speed of sound. Abu 'Abd Allah

Muhammad ibn Ma'udh (second half of 11th century), who lived in Al-Andalus, calculated angles for morning and evening depression of the sun during twilights depending on the calculations of diffractions of sun light and found out numbers which are comparable to the modern numbers. Qutb al-Din al-Shirazi (1236–1311) and his student Kamāl al-Dīn al-Fārisī (1260–1320) gave correct explanation of rainbow phenomenon.

There is a lack of documented major advance in optics during Medieval Europe. However, there were some individuals who translated and cited Greek and Arab writings in their books and texts.

In Europe, during the Renaissance period, optics developed drastically and established the platform for modern optics. Johannes Kepler (1571–1630), a German mathematician, published in 1604, Astronomiae Pars Optica (The Optical Part of Astronomy) which is acknowledged as the ground work of modern optics (Caspar, 1959). Willebrord Snellius (1580–1626), a Dutch astronomerand mathematician, is known as one of the founders of mathematical law of refraction called “Snell’s law”. This law has also been discovered a long time before by the muslim scholar, Ibn Sahl, in 984 (Zghal *et al.*, 2007). René Descartes (1596–1650), a French mathematician, measured the angular radius of a rainbow and noted it to be 42° depending on the law of refraction (Caspar, 1959). Christiaan Huygens (1629–1695), a Dutch mathematician, proposed a wave model of light, but actually his equations were more ray depended, since his hypothesis about light waves were longitudinal waves (Zghal *et al.*, 2007) Isaac Newton (1643–1727), English physicist and mathematician had the idea that light consists of very small particles, but his mistake was that he tried to use it to explain the light diffraction theory. Then, he tried to include a wave theory to help him to do that (Dobbs, 1982).

But geometric optics is not compatible with diffraction experiment because ray equations were not right when they were applied on wavelengths larger than the objects directed on them, which would be explained when we talk about wave theory of light (Tipler and Mosca, 2007). However, geometric optics can be used widely as long as there is no wave effect. The model explaining diffraction effect (Fig 2.2) is the wave theory which was first published by James Clerck Maxwell in 1865 by his famous theory of electromagnetic waves (Maxwell, 1865).

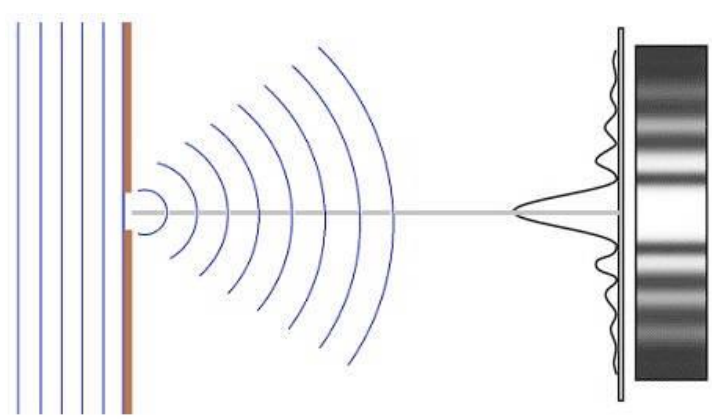

Fig 2.2: Diffraction effect. Waves spread out when they move through a slit. Image credit: modified by Helen Klus, original image by John Wetzel, an author at wikipremed.com/CC-SA

But again, this model did not explain the photo effect or "black body radiation" which was described in 1900 by Max Plank. He explained it by his term *hv*, wherin *v* is the frequency of the light and *h* is a natural constant called "Planck's constant"; based on the old physics of continuous values. It was not easy for him to quantify the energy of light. Anyway, his term was the first step of Albert Einstein's revolutionary thought, that light energy is not continuously distributed in a light wave, but it exists in discrete quantas "photons" (Tipler and Mosca, 2007).

That was the birth of quantum physics (Mehra and Rechenberg, 1982). In 1917 Einstein explained the interaction between a photon and a matter by the processes of spontaneous and stimulated emession in his paper "On the Quantum Theory of Radiation" (Tipler and Mosca, 2007). According to recent physicists, light can be explained by dual theories, wave and particles.

There is contradiction in the literature about the definition of light according to wavelength. While some references define light as a visible electromagnetic radiation within wavelengths in the range of 400–700 nanometres (nm)(CIE, 1987), there are others (especially in physics) define it aselectromagnetic radiation of any wavelength, whether visible or not (Kumar, 2003). So it includes the visible spectrum (VIS), ultraviolet light (UV) which has a wavelength shorter than 400nm up to 50nm, and infrared light (IR) which has a wavelength more than 700nm.

At the same time, visible light wavelength range differs according to the age of object that receives the light, whether it is scotopic or photopic and the laboratory conditionsavailable (Sliney *et al.*, 1976).The speed of light in vacuum is exactly 299,792,458 m/s.

2.2 History of laser

In March, 1960, Townes and Schawlow were granted US patent for the optical maser, now called a laser. In May, 1960, Theodore H. Maiman (Plate 2.1), a physicist at

Hughes Research Laboratories in Malibu, Calif., constructed the first laser using a cylinder of synthetic ruby 1 cm in diameter and 2 cm long (Bertolotti, 2005).

The first medical treatment using a laser on a human patient was performed by Dr. Charles J. Campbell of the Institute of Ophthalmology at Columbia- Presbyterian Medical Center and Charles J. Koester of the American Optical Co. at Columbia-Presbyterian Hospital in Manhattan. In 1961, An American Optical ruby laser is used to destroy a retinal tumor (Spetz, 1995).

Bell Labs reported the first yttrium aluminum garnet (YAG) laser in 1962 (Hecht, 2005) and in 1963 Herbert Kroemer of the University of California, Santa Barbara, and the team of Rudolf Kazarinov and Zhores Alferov of the A.F. Ioffe Physico-Technical Institute in St. Petersburg, Russia, independently proposed ideas to build semiconductor lasers (diode laser) from heterostructure devices. The work leads to Kroemer and Alferov won the 2000 Nobel Prize in physics.

In 1964, Nd:YAG (neodymium-doped YAG) laser was invented by Joseph E. Geusic and Richard G. Smith at Bell Labs. The laser later proves ideal for cosmetic applications, such as LASIK vision correction and skin resurfacing and the carbon-dioxide (CO_2) laser was invented by Kumar Patel at Bell Labs in the same year. The CO_2 laser became the most powerful continuously operating laser of its time and it is now used worldwide as a cutting tool in surgery and industry.

In 1975, engineers at Laser diode Labs Inc. developed the first commercial continuous-wave semiconductor laser operating at room temperature. Continuous-wave operation enables the transmission of telephone conversations (Hecht, 2005).

Plate 2.1: Theodore H. Maiman, a physicist who constructed the first laser

The development of laser technology continued and evolved from then till today in many industries and its applications in medicine and dentistry expanded to include surgery, ophthalmology and dentistry. The rules and regulations pertaining to its use in medical and dental practice was further developed involving many regulatory agencies such as the FDA.

2.3 Lasers in dentistry

The use of laser devices in dental practice is approved by FDA for soft and hard tissues (Ricki, 1995). Many different laser are used in dentistry for various applications. The wavelengths range of these lasers ranges from ultraviolet (Excimer laser) to infrared (Carbon Dioxide) (Fig 2.3).

2.3.1 Excimer laser

The first Excimer laser was invented by Basov in 1970 (Basov *et al.*, 1970). Medically, it is used in eye surgery LASIK and dermatology (Linsker *et al.*, 1984). The active medium of Excimer laser consists of unstable molecules called excimers (which is a molecule made of two species, one of them is a noble gas and the other a reactive gas).

Excimer molecules is formed by exciting the atoms. They will be stable for nanoseconds, enough for creating nanosecond pulsed laser with high peak power. Tissue ablation is done with Excimer laser based on photochemical interaction by breaking the bonds of complex molecules and turn them into volatile radicals. The ablation by this laser does not generate heat, which is an advantage for dental application. However, the disadvantages of this laser limits its uses in the oral cavity. Size and cost of the devise and the cost of the gas are expensive, the active medium has short-live, and potentially has carcinogenic effect since they may cause mutationsin the chromosomes of cell nucleus.

2.3.2 Argon ion laser

First Argon ion laser was invented by William Bridges in 1964 (Bridges, 1964). Medically, it is used in retinalphototherapy (Lexel laser). The active medium of this laser is the ions of the noble gas, Argon. It generates continious-wave beam. The uses of this laser in dentistry includes photo-polymerization because of the absorption in campherchinone. In soft-tissue surgery it causes absorption in melanin, hemoglobin, and oxyhemoglobin and also used in tooth decay prevention (Mattson *et al.*, 1998; Powell *et al.*, 1995; Westerman *et al.*, 2006). The disadvantages of this laser are that it occupy space, costly and needs strong cooling and electric supply (Beer, 2006).

2.3.3 Helium-neon laser

Helium-neon laser was the first gas laser and the first continuous laser developed in 1961. The active medium in this laser is a gas mixture of Helium and Neon. The pumping source is electrical and the beam is continuous (Javan *et al.*, 1961).In medicine and dentistry, it was the first to be used in photodynamic and low-level laser therapy (Dougherty, 1993; Saperia *et al.*, 1986).

2.3.4 Semiconductor diode laser

Diode laser was first developed in 1962. It was pulsed until 1970 when continuous-waveat room temperature was achieved. The active medium of diode laser are very small crystals of elements of groups of II and VI of the periodic table. The pumping sourse is elecric current. The long service life (more than 10,000 hours) and mass production makes it a popular used laser in medicine and other fields. In dentistry it is widely used in surgery, endodontics and other soft tisuue treatments (Gutknecht, 2007).

2.3.5 Neodymium:YAG laser

Neodymium-doped Yttrium Aluminum Garnet (Nd:YAG); Nd:$Y_3Al_5O_{12}$ was developed in 1964. It is the most commonly used solid-state laser. It emits the strongest wavelength at 1064 nm. It can produce pulsed and continuous wave (Geusic *et al.*, 1964). The active medium in this laser is the Neodymium ion which is doped in the YAG crystal. Pumping source for Nd:YAG laser can be Xenon flash lamps in pulsed mode and Krypton lamps or diode lasers in continuous mode (Yariv, 1989). In dentistry, Nd:YAG laser is used in endodontics surgery and periodontics (Gutknecht, 2007).

2.3.6 Frequency doubled Nd:YAG laser

Frequency doubled Nd:YAG laser (532 nm) is a continuous wave laser and diode laser is used as a pumping source. In dentistry, it is mainly used in the basic research (Gutknecht, 2007).

2.3.7 Erbium laser

Erbium lasers, invented in 1975 (Zharikov *et al.*, 1975), was first used in dentistry for cavity preparation since 1989 (Hibst and Keller, 1989), due to its highly absorptive property of its wavelength (2.6 to 3μm). As mentioned before, it is well absorbed in water (15 times more than CO_2 laser and 20,000 times more than Nd:YAG laser) and hydroxyapatite (Walsh and Cummings, 1994). The beam it produces is a pulsed wave.

There are two Erbium laser systems prefered in dentistry depending on their active medium: Erbium doped Yttrium-Aluminium-Granet (Er:YAG) which produces light with a wavelength of 2.94μm, and erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) which produces light with a wavelength of 2.79μm. The pumping source is flash lamp. Both of the lasers are used in soft and hard tissue work in the oral cavity (Gutknecht, 2007).

2.3.8 Carbon dioxide laser

CO_2 laser is the most powerful gas laser and was invented in 1964 (Patel, 1964). It can produce wave in pulsed and continuous modes. The active medium is a mixture of carbon dioxide (laser gas), nitrogen (exitation gas) and helium (cooling gas). The pumping source is electric current (Zhang and Killeen, 2016). It is well absorbed in water and hydroxyapatite. In dentistry it can be used effectively in both hard and soft tissue procedures (Gutknecht, 2007).

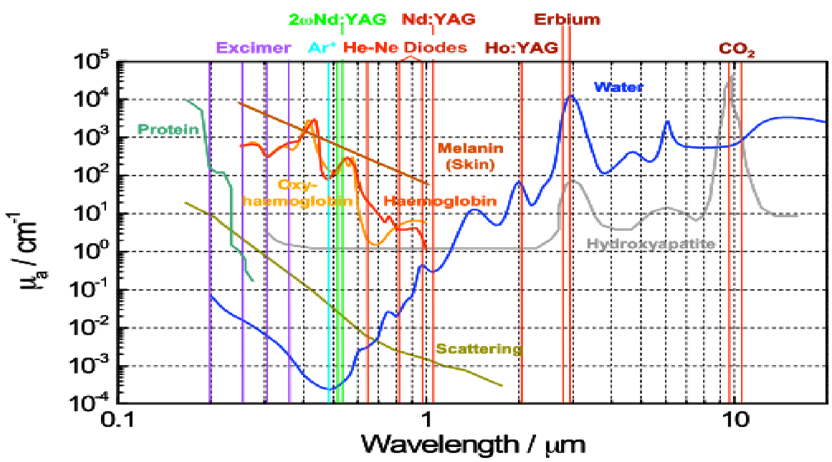

Fig 2.3: Laser absorption constant for various biological materials; by J.Meister (Gutknecht, 2007)

2.4 Laser construction

A laser system consists principally of three components (in the simplistic mode):

i) Active medium; which can be a gas, liquid, semi-conducter materials, glasses, moving charged particles or artificial gemstone is the main factor to determind the wavelength of the outcoming laser light. Usually, the name of the laser system depends on the main component of the active medium. It isresponsible for transforming the energy coming from the pumping sourse during the stimultaneous generation of light (Jeff, 1999).

ii) Pumping source; which is responsible for exciting the active medium with energy. Usually, gases are pumped by electrical charges. Liquids, glasses, and artificial gemstones are excited by flash lamp, arc lamp or lasers. Semiconductors (diodes) are excited by moving electrons in the active medium (Koechner, 2013).

iii) Optical resonator; wich is the most important part of the laser. Usually, it consists of two mirrors facing each other at the end of the active medium axis. One of the mirrors reflects 100% of the radiation, while the other reflects less than 100%; allowing emitting radiation from the active medium, amplifying and generation of laser light. At the same time, resenator can work as a filter (by reflecting specific wavelength) to get more monochromatic light (Siegman, 2000).

2.5 Laser light characteristics

The characteristics of laser light are:

a) High spectral energy or power density

b) Monochromatic light

c) High temporal and spatial coherence

d) Full amplitude stability during steady state laser operation

e) Generation of ultrashort laser pulses in the order of nano, pico and femto-seconds.

2.6 Modes of operation of laser

Laser emits light by three modes:

a) Continuous mode; wherein light produced is constant and uninterrupted.

b) Pulsed mode; wherein light is emitted in pulses, each is less than 0.25 seconds duration time. Since the pulse energy is equal to the average power divided by the repetition rate, this can sometimes be fulfilled by lowering the rate of pulses so that more energy can be built up in between pulses.

c) Chopped mode; wherein continuous beam can be interrupted (Gutknecht, 2007).

2.7 Light transmission systems of laser

Light transmission systems are selected depending on the wavelength, the pulse energy and power. It delivers the laser light from the machine to the target field. The delivery system comprise of:

a) Articulated arm with reflecting mirrors in hollow metal tubes, which is used for high energy pulses in the mid-infrared region like CO_2 and Erbium lasers.

b) Hollow waveguides with a special coated inside wall of the hollow, which is used for CO_2 and Erbium lasers as well.

c) Optical fibers, which are suitable to use for diode and Nd:YAG lasers (Katzir, 1993).

2.8 Dosimetry and definitions of laser parameters

The dose in laser application is defined as the amount of radiation energy applied during treatment in relation to the irradiated surface over a certain periode of time.

For continuous mode: $D = \Psi . t . n$ (in units of J/cm^2)

For pulsed mode: $D = \Psi_{avg} . t . n$ (in units of J/cm^2)

Whereby Ψ is the power density in W/cm^2, t is the total irrdiation time in seconds per interval, and n is the number of intervals.

2.8.1 Physical parameters for laser dosimetry

There are seven physical parameters necessary for laser dosimetry:

i) Density (fluence):

$$\Phi = \frac{E(pulse)}{A}$$

While Φ is energy density in the pulsed mode [J/CM^2], E(pulse) is the pulde energy [J], and A is its cross sectional area [cm^2].

ii) Power Density (intensity)(irradiance):

$$\Psi = \frac{P(pulse)}{A}$$

While Ψ is power density in the pulsed mode [W/cm^2], P(pulse) is the peak power of the pulse and A is its cross sectional area [cm^2]. In the continuous mode we use laser power P instead of P(pulse) in the equation.

iii) Pulse Energy:

Pulse energy can be calculated using integral equation;

$$E_{pulse} = \int_{\tau} P(t)dt$$

While τ is the pulse duration and P is the peak power of the pulse.

iv) Total Energy:

Total energy is the sum of all pulse energies.

$$E_{total} = \sum_{\text{number of pulses}} E_{pulse} = \int_{\text{total irradiation time}} P(t)dt$$

v) Average Power (Mean Power):

Average power of a pulsed laser mode is similar to the output power in the continuous laser mode.

$$P_{avg.} = E_{pulse} \times f$$

While $P_{avg.}$ is the average power in units of Watt[W], E_{pulse} is is the individual pulse energy in units of Joule [J], and f is the the pulse repetition rate in Herts.

vi) Duty Cycle:

Duty cycle is a numerical value that represent the emission duration of a pulse devided by the pulse periode (the sum of "On" time and pulse pause).

$$\text{Duty cycle} = \frac{\text{pulse duration}}{\text{pulse period}}$$

vii) Peak Power:

Peak power is one of the most important parameter in the context of laser tissue interaction. Within the same energy density, different effects can be achieved by only changing the pulse duration.

$$P_{pulse} = \frac{E_{pulse}}{\tau} \gg P_{avg.}$$

While P_{pulse} is the peak power, τ is the pulse duration (Gutknecht, 2007).

2.9 Light-tissue interaction

2.9.1 Basic interaction processes:

Three main interactions could happen when light propagates in turbid media(Hall and Girkin, 2004):

i) Reflection on the surface, which can be fresnel reflection or diffuse reflection.

ii) Interaction in the tissue, which can be absorption or scattering.

iii) Transmission through the tissues, which can be collimated or diffuse (Fig 2.4).

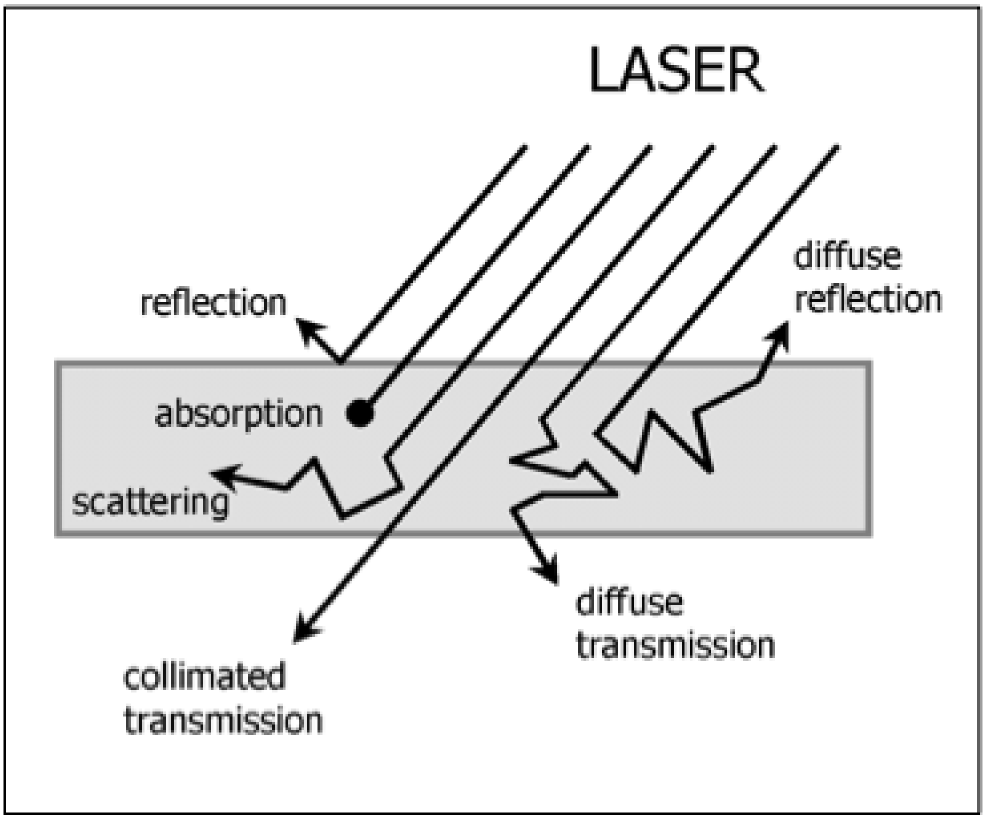

Fig 2.4: Interaction of laser light in a turbid medium, e.g. Tissue (Hall and Girkin, 2004)

2.9.2 Wavelength of the light:

There are light tissue interaction constants specific for each tissue depending on what materials the are composed of. Refractive index n, absorption constant μ_a, and scattering constant μ_s are value for materials which depend on the wavelength of light they exposed to.

The penetration depth of a light in a tissue is defined as the distance x covered in the tissue at which the incident intensity I_0 is reduced to the 1/e-th part (about 37%) (I_e). It can be described by the following formula:

$$I = I_0 \times e^{-\mu_a X}$$

And we have:

$$\frac{I_e}{I_0} = \frac{1}{e} = e^{-1}$$

That means:

$$\mu_a \times x = 1$$

In words, penetration depth is equal to the reciprocal of the absorption constant. The penetration depth of Er:YAG laser in water is 0.8 μm (Fig 2.5).

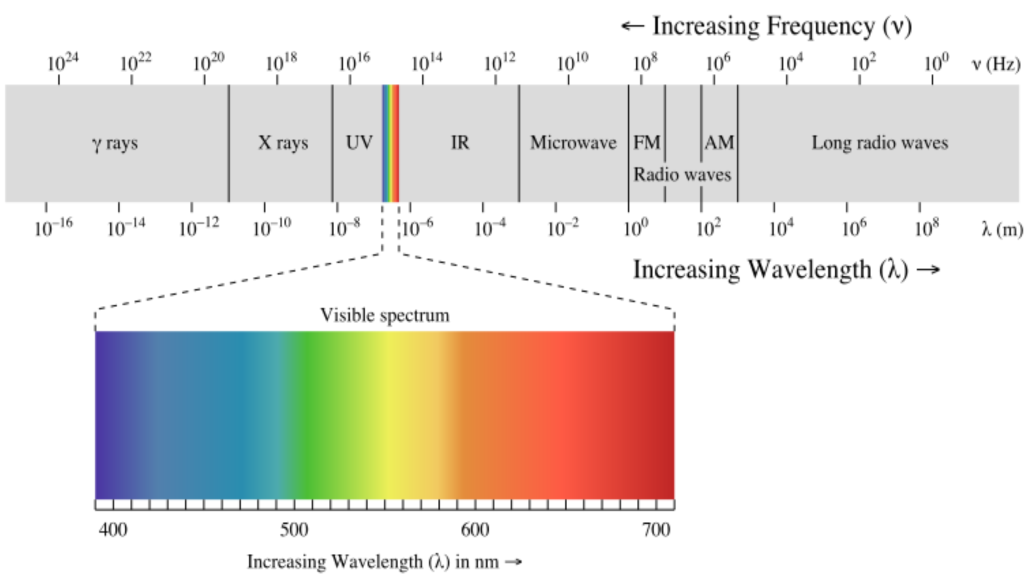

Fig 2.5: Light wavelength (wikipedia, 2007). The visible spectrum which can be seen by the human eye is shown in the diagram. Er:YAG laser, Nd:YAG laser and CO_2 laser fall under the infrared range

2.10 Laser-tissue interaction

Laser-tissue interaction occurs when light enters the tissues. It does not happen when the light is reflected. Some of interactions cannot be achieved with the normal light because it does not have the coherency and ultrashort pulsing that laser light have (Fig 2.6).

The characteristics of laser-tissue interactions can be in the form of following:

i) Photochemical interaction which cause Photodynamic Therapy (PTD), or biomodulation.

ii) Photothermal interaction which causes coagulation or vaporisation.

iii) Non-linear processes like photoablation and photodisruption (plasma forming).

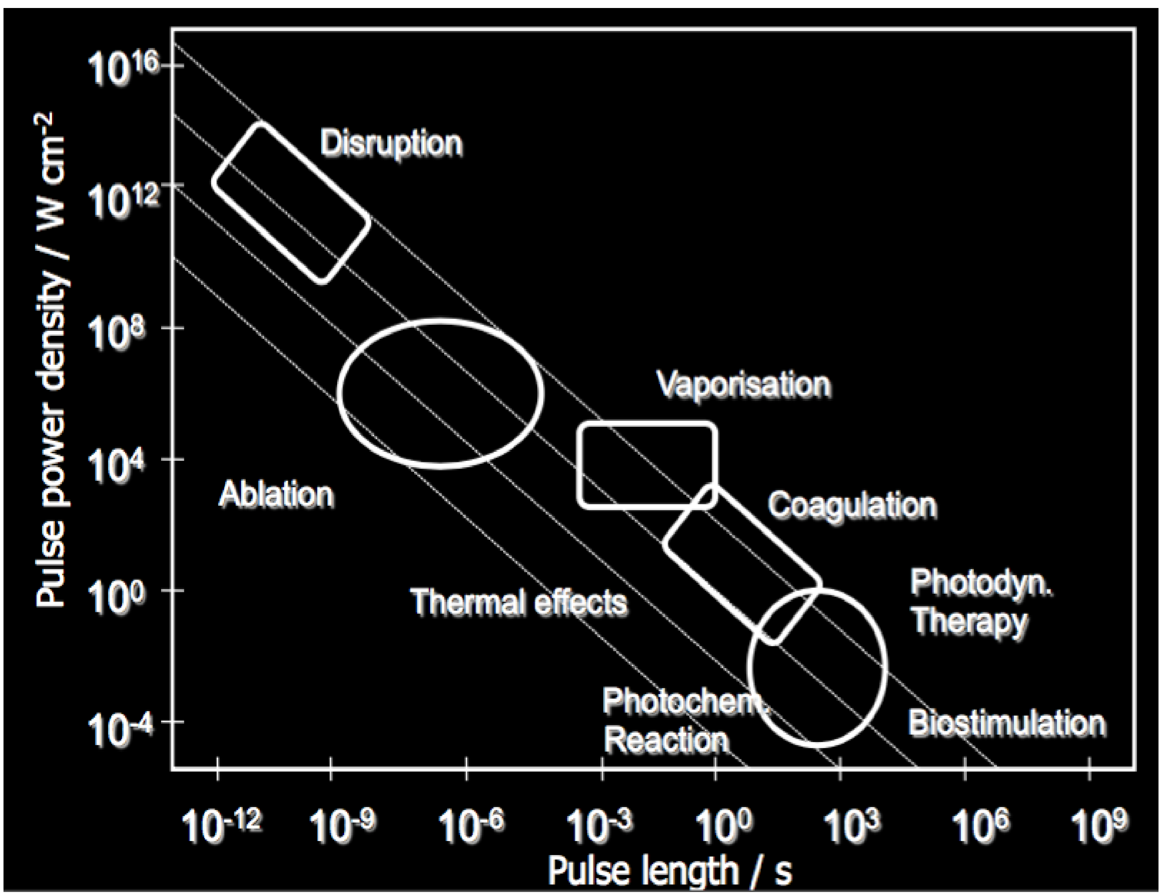

Fig 2.6: Laser tissue interaction (Gutknecht, 2007). When laser light enters the tissue, a response from the tissue occurs depending on pulse duration and pulse power density. Such response does not occur when light is reflected

2.11 Melanin

Melanin is a broad term used for a group of natural pigments found in most organisms. Melanin is a derivative of the amino acid tyrosine, however it is not itself made of amino acids and it is not a protein. The pigment is produced in a specialized group of cells known as melanocytes (Ohannesian and Streeter, 2001). Melanin is comprised of different proportions of small component molecules that give rise to three different types namely, eumelanin, pheomelanin and neuromelanin.

Eumelanin is the dominant type of melanin in humans and it is insoluble (Ahluwalia and Goyal, 2000). There are two types of eumelanin, black and brown. They exist mainly in hair and skin. They are responsible for black, brown, gray and yellow colors (Sliney *et al.*, 1976). Pheomelanin is responsible for reddish hue in skin and hair and it is alkali soluble (Susan and Arnum, 1998). Neuromelanin is composed of granules consisting of core of pheomelanin surrounded by eumelanin at the surface (Ensminger and Ensminger, 1993). It is dark and exists in brain. Its function is still unknown. However, some individuals such as patients with Parkinson disease don't have Neuromelanin (Balcheva and Balcheva, 2014).

2.11.1 Histology of epithelium-melanin unit

Melanocytes are cells located in the basal cell layer of the epithelium. Melanocytes and keratinocytes form the epithelium-melanin unit. Melanocytes are dendritic cells and their processes extend into the interstices among the surrounding epithelial cells (Fig 2.7). Although keratinocytes contain melanosomes, they do not synthesize melanin. Melanosomes are produced by melanocytes. Dendritic processes of melanocytes transfer melanosomes to the surrounding epidermal cells. The melanocytes