PHOTO DYNAMIC THERAPY (PDT) WITH BIOLOGICAL TISSUES USING ND:GLASS LASER

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ABSTRACT:

A Photo Dynamic Therapy (PDT) is a technique which is used with Laser to treat many of cancer tissues. This paper deals with the relatively new therapeutic technique (PDT) with pulsed Nd:glass Laser which was applied to human soft tissues (Ovary and Kidney tissues), and to the hard tissues (freshly extracted human teeth), with power density of 280 watt/mm^2 and exposure time 330 *u*sec. Different dyes (Blue, methylene, eosin, and orange) were applied to the area before irradiation to study the effect of the pigments on the laser interaction with biological tissues. The zone of treatment (Z-necrosis) with aid of MATLAB was determined. The relationship of zone of treatment with exposure time, accumulated damage and fraction of oxidative radicals was obtained.

الخُلاصة:

أن تقنية العلاجَ الصوّري الديناميكي تستخدم مع الليزر لعلاجَ العديد مِنْ ألانسجةِ السرطانية. يتناول هذا البحث اهمية هذه التقنية بأستعمال ليزر النيديوم الزجاجي النبضي المسلط على أنسجة بشرية لينة (أنسجة الكلية والمبيضَ)، وإلى الأنسجةِ الصلبةِ (أسنانَ بشرية منتزعة جديدا)، بقدرة كثافية 280واط/مليمتر^2 ووقت التعرّض 330ميكروثانية. صباغات مختلفة (أزرق, ميثالين، أيوسين، وبرتقالي) أُطبقتْ على مساحة الانسجة قبل التعرض للاشعاع وذلك لغرض دِراسَة تأثيرِ هذه الصبغاتِ في تفاعلات الليزرَ مع الأنسجةِ الحيويةِ. منطقة المعالجةِ وجدت ووضحت بمساعدة برنامج المحاكاة بالتعامل مع الماتلاب . أن علاقة منطقةِ المعالجةِ بوقتِ التعرّض، الاضرار المجمّعَة وتكسرَ الأكسدة الراديكاليي قد حُصِلَ عليها.

Keywords:
Photo Dynamic Therapy (PDT) technique, a pulsed Nd:glass Laser, Z-necrosis, Dyes with human tissues, Photosensitizers.

1. INTRODUCTION:

Photodynamic therapy (PDT) is the most modern and important method in the therapy of both neoplastic and non-neoplastic diseases because of its safe treatment approach for superficial human cancers and selection benign conditions. When radiant energy is absorbed by tissue, four basic types of interactions are occurring: photo chemical, photo thermal, photo mechanical, photo electrical. (PDT) involves light activation chemotherapy in the presence of molecular oxygen, of certain dyes (photosensitizers) that are taken up by the target tissue.

A photon is absorbed by a photosensitive drug which moves the drug into an excited state. The excited drug can then pass its energy to oxygen to create a chemical radical called "singlet oxygen". Singlet oxygen attacks cellular structures by oxidation. Such oxidative damage might be oxidation of cell membranes or proteins. When the accumulation of oxidative damage exceeds a threshold level, the cell begins to die, as shown in **Fig.1.1**.

The PDT treatment window is shown in the **Fig.1.2**. There must be enough photosensitizing DRUG and activating LIGHT to achieve effective PDT treatment. A threshold dose is defined as:

LIGHT x DRUG = threshold dose

Too much drug may lead to "dark toxicity." Too much light can lead to photo destruction or "photo bleaching" of the drug (Patterson MS, 1990).

In the clinic, one doctor must be sure that there is sufficient DRUG in the tissue and sufficient light penetrating the tissue to the desired depth so as to enter the PDT treatment window (**Fig.1.2**).

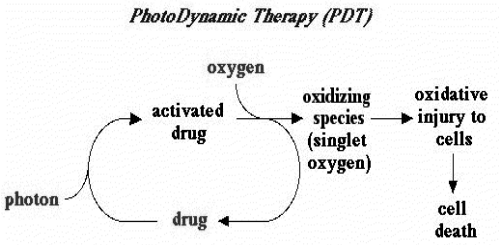


Fig.1.1 *The PhotoDynamic Therapy [PDT]* (Patterson MS, 1990).

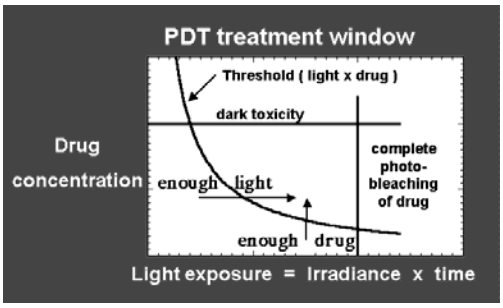


Fig.1.2 *PDT treatment* window (Patterson MS, 1990).

1.1. The Mathematics of PDT Dosimetry for Cancer Treatment:

This section provides a working description of PDT dosimetry using definitions summarizing PDT with a flow diagram as shown in **Fig.1.3**.

As illustrated in this figure, PDT depends on the amount of light delivered (**L**), the amount of photosensitizing drug (**D**) in the tissue, and the amount of oxygen ($\mathbf{O^2}$) in the tissue. Absorption of light converts **D** into an activated drug (**D***). Reaction of **D*** with oxygen yields oxidizing radicals (**R***, primarily singlet oxygen). A fraction (**f**) of these radicals attacks critical sites within the cell causing an accumulated oxidative damage (**A**). When the accumulated damage exceeds a threshold, ($\mathbf{A > A_{th}}$), then cell death occurs (Foster TH, 1991).

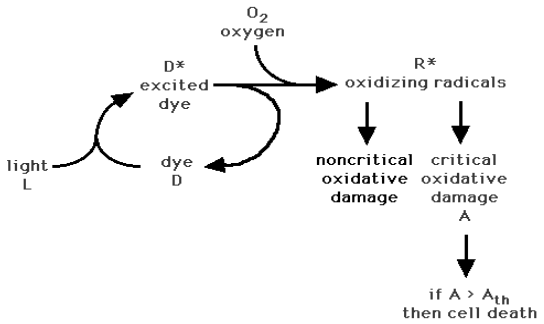


Fig.1.3 *Schematic diagram for PDT* (Allison RR, 2004).

The light provided by a delivered fluence rate (ϕ) can be expressed in units of photon concentration:

$$L = \frac{\phi}{c}\frac{\lambda}{hc}\frac{1000}{6x10^{23}} \quad , [\text{moles/liter}] \qquad \textbf{(1.1)}$$

where:

ϕ **:** is the fluence rate of light [W/cm^2] or [J/ (cm^2.s)].

λ**/(hc) :** is number of photons per J of energy [ph/J].

λ **:** is the photon wavelength in [cm].

c **:** is the speed of light, 3.0x10^{10} [cm/s].

h **:** is Planck's constant, 6.6x10^{-34} [J s].

The rate constant ($\mathbf{k_1}$) for drug activation (**D >D***) is:

$$\text{rate constant for drug activation } k_1 = c\varepsilon \quad , [s^{-1}\,(\text{moles/liter})^{-1}] \qquad \textbf{(1.2)}$$

where:

ε **:** is the extinction coefficient of the photosensitizing drug [(cm^{-1}) / (moles/liter)].

The rate of production of activated drug is:

$$\text{rate of drug activation} = k_1 LD \quad , [(\text{moles/liter})\,s^{-1}] \qquad \textbf{(1.3)}$$

The total amount of activated drug (**D***) produced per unit volume of tissue is:

Where:

T **:** is the time of light exposure in second.

The probability that an activated drug (**D***) will transfer its excited state energy to oxygen to yield an oxidative radical (**R***) is specified by the quantum yield (Φ) which depends on the oxygen concentration in the tissue. The total amount of **R*** produced is:

$$\text{total amount of oxidative radicals } R^* = \Phi D^* \quad , [\text{moles/liter}] \qquad \textbf{(1.5)}$$

A fraction (**f**) of **R*** succeeds in oxidative damaging critical sites in the cell which contributes to cell death. The remaining fraction (**1 - f**) will attack relatively inert or no critical sites. The accumulation (**A**) of critical oxidative damage is:

$$\text{accumulation of critical oxidative damage} = A = f\Phi\varepsilon\phi DT\frac{\lambda}{hc}\frac{1000}{6x10^{23}} \quad , [\text{moles/liter}] \qquad \textbf{(1.6)}$$

If the accumulated damage exceeds a threshold, $\mathbf{A > A_{th}}$, then cell death occurs.

$$\text{if } A > A_{th} \text{ then cell death} \qquad \textbf{(1.7)}$$

1.2. Photodynamic Dose (D*):

Currently, few laboratories concerned with rigorous PDT dosimetry routinely document the light reaching a tissue site (Φ), the amount of photosensitizing drug that accumulates in that tissue site (**D**), and the light exposure time (**T**), then calculate the total amount of drug activated during the light exposure period (Tromberg BJ, 1990).

This factor is quantifiable and therefore a practical dosimetric parameter which has been called the **"photodynamic dose"**. We have used the symbol **D*** and the units of [moles/liter] to describe the "photodynamic dose", Which have described as the number of photons absorbed by photosensitizing drug per gram of tissue [ph/g].

$$\text{"photodynamic dose" } D^* = \varepsilon D\phi T\frac{\lambda}{hc\,\rho} \quad , [\text{ph/g}] \qquad \textbf{(1.8)}$$

where:
ρ **:** is the density of tissue [g/cm^3].

The "photodynamic dose" (**D***) does not consider the quantum yield (Φ) of oxidative radicals, the effect of oxygen on (Φ), or the fraction (**f**) of radicals that oxidize critical sites. However, "photodynamic dose" is the dosimetric parameter most commonly documented. There is logic in this choice since light (**L**), drug (**D**), and exposure time (**T**) are parameters under experimental or clinical control. Experimental determination of the margins of necrosis induced by a well-defined **D*** can specify the threshold dose (**D*$_{th}$**). The criteria for necrosis are then:

$$\text{if } D^* > D^*_{th} \text{ then cell death} \qquad \textbf{(1.9)}$$

1.3. Treatment Zone:
Consider a treatment using topical irradiation of a tissue surface with a broad beam of light a couple (cm) in diameter (Jacques, 1992). The light penetration into the tissue can be described by the one-dimensional expression:

$$\phi = E\, k_s \exp(-z/\delta) \qquad \textbf{(1.10)}$$

where:
E **:** irradiance at tissue surface [W/cm^2].
ks **:**the backscattering factor which accounts for how reflected light from the tissue augments delivered light [dimensionless]
z **:**depth into the tissue [cm]
δ **:**the optical penetration depth [cm], the path length which causes the concentration of light to drop to 1/e or 37% of its initial concentration.
Assume that the depth of necrosis from such a topical PDT treatment is located at **z_necrosis** which corresponds to the depth at which the threshold accumulation of oxidative damage, **A$_{th}$**, occurs. Then combining equations (1.9) and (1.10) and inserting **z_necrosis** and **A$_{th}$** yields:

$$A_{th} = f\Phi\varepsilon DT \frac{\lambda}{hc} \frac{1000}{6x10^{23}} Ek_s \exp(-z_{necrosis}/\delta) \ , [\text{moles/liter}] \qquad \textbf{(1.11)}$$

Finally, rearrange equation (1.11) to solve for **z_necrosis**:

$$z_{necrosis} = \delta \ln\left[\frac{f\Phi\varepsilon\phi DTEk_s}{A_{th}} \frac{\lambda}{hc} \frac{1000}{6x10^{23}}\right] \ , [\text{cm}] \qquad \textbf{(1.12)}$$

Eq. (1.12) shows how the depth of necrosis depends on all the various parameters that affect PDT. Notice that **z_necrosis** is linearly related to the optical penetration depth δ but logarithmically related to all other parameters.
Again consider the practical dosimetry based on **D***, the "photodynamic dose". If the irradiance at the tissue surface yields a **D*$_{surface}$** at the surface, then the depth of necrosis can be expressed:

$$z_{necrosis} = \delta \ln\left[\frac{D^*_{surface}}{D^*_{th}}\right] \ , [\text{cm}] \qquad \textbf{(1.13)}$$

$$\text{where } D^*_{surface} = \varepsilon DTEk_s \frac{\lambda}{hc} \frac{1}{\rho}$$

2. EXPERIMENTAL WORK:
2.1 Method:
The Nd:glass laser system which was used in this experiment (in the Institute of Laser for Postgraduate Studies / Baghdad University) is a homemade laser (**Fig.2.1**). It operates in the pulsed mode giving a single pulse for each shot. This system gives a beam of (1064 nm) wavelength. The active medium of the system is a glass rod Silicate type ED-2 doped with ($2.83x10^{20}$ cm^{-3}) concentration of Nd^{+3} ions. The rod length is 20 cm with 1.25 cm in diameter. The Nd:glass rod is optically pumped with a xenon filled flash lamp to pump the laser rod. The flash lamp supported with voltage pulse by using pulsing power supply in which connected with a charge capacitor to give out a voltage pulse within about (4–6 KV) to the flash lamp. The out-put laser energy increases with

increasing the charging of the capacitor "flash lamp power". The output laser energy was measured by using a joulemeter which is a thermal detector (Orazio Svelto, 1998), (W.T. Silfvast, 2004).

The joulmeter was connected to an oscilloscope to measure the voltage generated by the joulmeter as a result of the incidence of the laser beam on its surface .The calibration of the output voltages displayed in the oscilloscope were divided by the detector factor which is about 10.3 Volt/joule, to obtain the amount of incidence energy "in joule" of the laser beam on the detector. The output laser power is obtained by dividing the output laser energy to the pulse width "330 microseconds". In order to calculate the power density, the spot diameter is controlled partly by the focal length of the lens of 10 cm to focus the beam (**Table.2.1**).

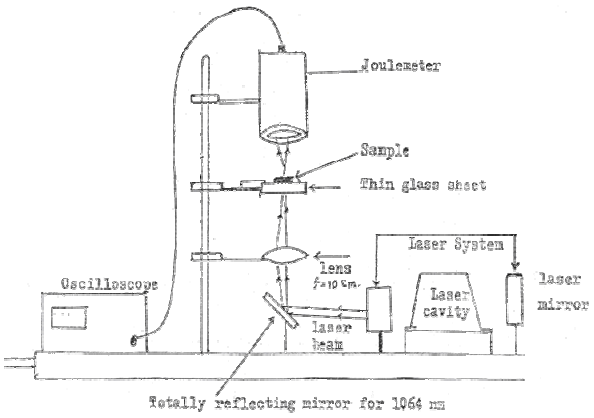


Fig.2.1 *Schematic diagram of Nd:glass laser.*

Table.2.1 *The physical properties of the glass-rod.*

Glass type	Silicate
Nd-ion concentration	2.83 x 1020
Density	2.547 gm/cm^3
Refractive index	1.555
Wavelength	**1064 nm**
Photon energy "for single photon"	1.88 x 10^{-19} J
Thermal conductivity	**1.35 x 10^{-2} w.cm^{-1}.k^{-1}**
Pulse width	330 micro-second

2.1.1 Experiment (1):

Soft tissues were obtained, from human kidney and ovary, and served as homogeneous biological test media. The samples were taken from Pathology Department / College of Medicine / Baghdad University. All samples used were fixed with 10% formalin. Each sample was supported along its length by two razor blades, each mounted and cut into a few millimeters in thickness, 3-5 mm. The soft tissue is almost perfectly colorless in its intrinsic state, and served as control. The other samples were stained with different dyes, methylene-blue, eosin and orange dye.

Dyes and Stains are frequently used in biology and medicine to highlight structures in biological tissues for viewing. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells. In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound (Horobin RW, 2002).

The samples were irradiated with pulses of Nd:glass laser at 1064 wavelength, emission energy 0.0184 joule, and pulse duration 330 μsec.

2.1.2 Experiment (2):

Freshly extracted human teeth were obtained from Surgery Department/College of Dentistry/Baghdad University. These samples were chosen because of their inhomogeneous appearance; represent bones as hard tissues and with inclusions of caries. All extracted teeth were washed with fresh water immediately and fixed with 10% formalin. The tooth without staining "carries existing" served as a control. While the other teeth stained with methylene blue, orange and eosin dye, were irradiated with pulses of Nd:glass laser, emission energy 0.0184J, beam diameter 0.5 mm , and pulse duration 330 μsec.

2.2 Readings:

The used laser parameters:

- Incident energy "E_o" = 0.0184 joule.
- Spot diameter = 0.5 mm
- Spot radius = 0.25 mm = 0.025 cm
- Spot size in "cm^2"= $3.14x(0.025)^2 = 2.0x10^{-3}$ cm^2

2.2.1 Fluence:

Table.2.2 shows the measurements of the transmitted joulemeter in term of "Joule", of the control and stained samples with different dyes. Where **Table.2.3** shows the calculations of the transmitted fluence "Energy Density (J/cm2)" of the control and stained samples with different dyes.

- Energy density or Fluence = $\frac{0.0184 \quad J}{2 \times 10^{-3}} = 9.2\ Joul\ /\ cm^2$
- Pulse duration = 330 μsec.

Table.2.2 *Measurements of the Joulemeter in term of "Joule".*

Sample	*Control "J"*	*Orange "J"*	*Eosin" J"*	*Blue "J"*
Ovary	0.00854	0.00504	0.00103	0.0066
Kidney	0.01456	0.01514	0.01456	0.01553
Intact tooth	0.00543	0.00737	0.00348	0.00691
Caries-tooth	0.00407	0.00427	0.00485	0.00582

Table.2.3 *Calculations of the Transmitted Fluence "Energy Density (J/cm^2)".*

Sample	*Control "J/cm^2"*	*Orange" J/cm^2"*	*Eosin" J/cm^2"*	*Blue"J /cm^2"*
Ovary	4.27	2.52	0.515	3.3
Kidney	7.28	7.57	7.28	7.77
Intact tooth	2.72	3.69	1.74	3.1
Caries-tooth	2.04	2.14	2.43	2.91

2.2.2 Power:

Table.2.4 shows the calculations of the power densities of the control and stained samples with different dyes.

- $power = \frac{\text{Energy} \quad \text{(Joule)}}{\text{Time} \quad \text{(sec)}}$
- Incident power = $\frac{0.0184 \quad J}{330 \times 10^{-6}} = 55.8 Watt$
- Incident power density = $\frac{\text{Power}}{\text{Area}} = \frac{Watt}{cm^2} = \frac{55.8}{2 \times 10^{-3}} = 28 \times 10^3\ Watt\ /\ cm^2$ $= 280 Watt\ /\ mm^2$

Table.2.4 *Calculations of the Power Densities.*

Sample	*Control "W/mm2"*	*Orange "W/mm2"*	*Eosin "W/mm^2"*	*Blue "W/mm2"*
Ovary	129	76	16	100
Kidney	221	230	221	235
Intact-tooth	112	112	53	91
Caries tooth	62	65	74	88

2.2.3 Transmission Coefficient:

Transmission coefficient is defined as the percentage ratio of the transmitted energy density (J/cm2) to the incident energy density (J/cm2). **Table.2.5** shows the transmission coefficient calculations of the control and stained samples with different dyes.

Table.2.5 *Calculations of the Transmission Coefficient.*

Sample	*Control %*	*Orange %*	*Eosin %*	*Blue %*
Ovary	46	27	6	36
Kidney	79	82	79	85
Intact-tooth	30	40	19	34
Caries-tooth	22	23	26	32

2.2.4 Optical Density:

Table.2.6 shows the calculations of the optical densities "OD" of the control and samples coated with different dyes. The depth of laser light transmission is governed by the wavelength dependent optical density "OD" of the tissue which is defined as:

$$OpticalDensity = \log \frac{\text{Incident Fluence}(J/cm^{2})}{\text{Transmitted fluence}(J/cm^{2})}$$

Table.2.6 *Calculations of the Optical Densities "OD".*

Sample	*Control*	*Orange*	*Eosin*	*Blue*
Ovary	0.333	0.562	1.252	0.445
Kidney	0.102	0.084	0.102	0.073
Intact-tooth	0.529	0.396	0.723	0.472
Caries-tooth	0.654	0.633	0.578	0.499

The optical density is directly proportional to the concentration of light absorbing chromophores and the tissue thickness or light path length [8].

3. SOFTWARE AND RESULTS:

In this work, the zone of treatment (z_necrosis) is determined by using a simulation program which was dealt with MATLAB. The relationship of zone of treatment with exposure time, accumulated damage and fraction of oxidative radicals is obtained. As well as the calculations of the penetration depth, by using the eq.s (1.10), (1.11) and (1.13). **Fig.3.1** shows the flow chart of the program for PDT technique.

In this work, the values of parameters which used to calculate the Penetration depth (Z-necrosis) are taken from **Table.3.1**.

The curves which obtained from execution this program with the readings from the above experiments are shown in figures below.

Fig.3.2 shows that the zone of treatment (Z-necrosis) will change with exposure time .When the exposure time increased Z-necrosis will be increased quickly until reached to 12cm after this point the Z-necrosis is increased slowly with increasing exposure time. Then from this curve Z-necrosis is linear proportional with exposure time.

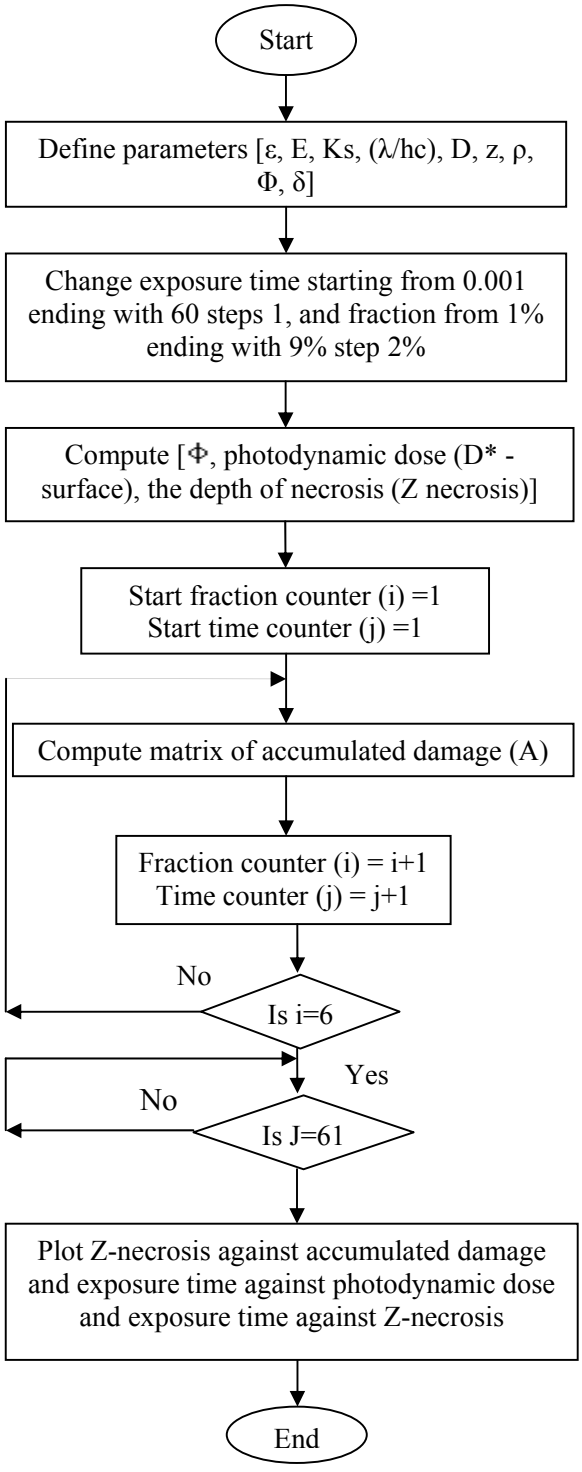
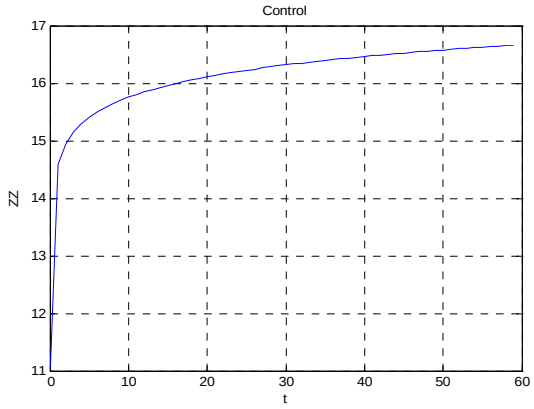
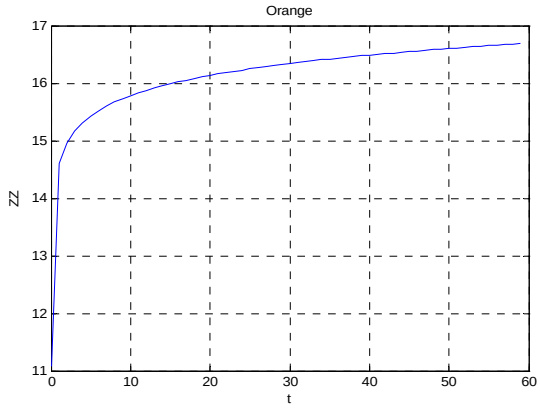


Fig.3.1 *Flow chart of the program of determining penetration depth.*



(a)



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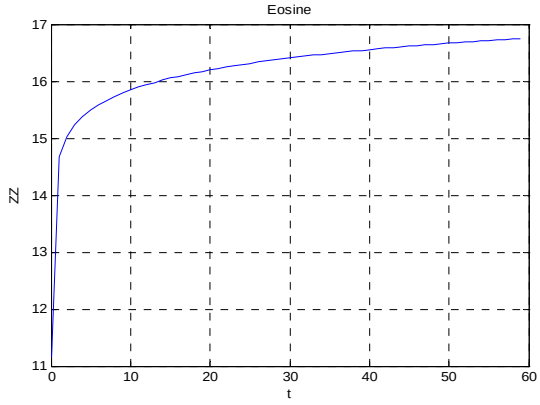

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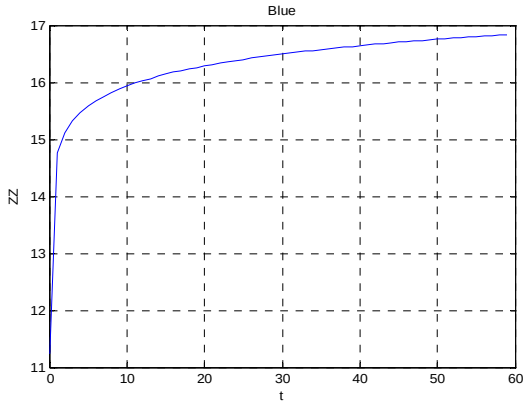


(d)

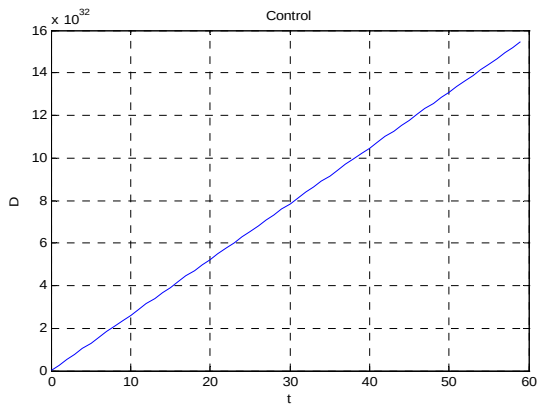
Fig. 3.2 *The relationship between Z-necrosis and exposure time of the* ***(a)*** *Control,* ***(b)*** *Orange,* ***(c)*** *Eosin and* ***(d)*** *Blue.*

Table.3.1 *The parameters that used in Z-necrosis.*

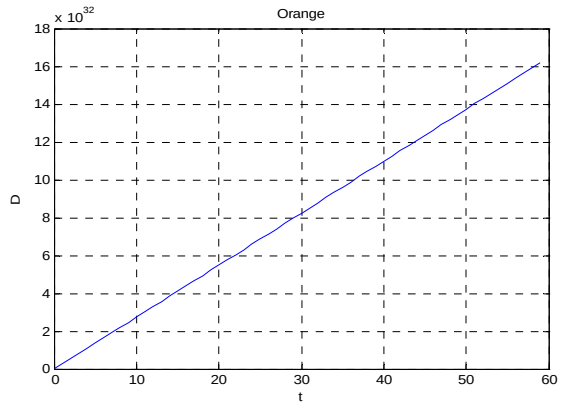
photon		
Wavelength	λ	1064 nm = 1.064 *u*m
Irradiance	E	From Table. 2.2 & 2.3
Exposure time	T	10 min = 600 s
Optical penetration depth	δ	0.51 cm
Optical backscatter factor	ks	4.4 [dimensionless]
Conversion constant	λ/hc	3.2x10^18 photons/J
Photosensitive drug		
Administered drug dose	-	5 mg/kg body weight = 5 ug/g.tissue

Molecular weight of drug	MW	600 g/mole
Tissue density	ρ	1 g.tissue/cm^3
Tissue concentration of drug	D	3 ug/g.tissue = (3x10^-6 g/g.tissue)(1 mole/600 g)(1 g.tissue/cm^3)(1000 cm^3/liter) = 5x10^-6 moles/liter
Extinction coefficient of drug	ε	10^4 (cm^-1)/(mole/liter)
Quantum efficiency of activating radicals	Φ	0.1 [dimensionless]
"Photodynamic dose" at surface	D*_surface	8.4x10^19 ph/g = 143 mmoles/liter
Tissue treatment zone		
Threshold toxic product	D*th	10^19 ph/g = 17 mmoles/liter
Zone of treatment	z_necrosis	1.1 cm

Fig. 3.3 explains that the relationship between the photodynamic dose and exposure time was linear.



(a)



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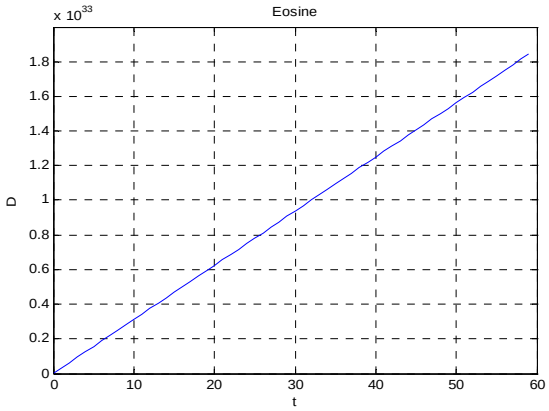

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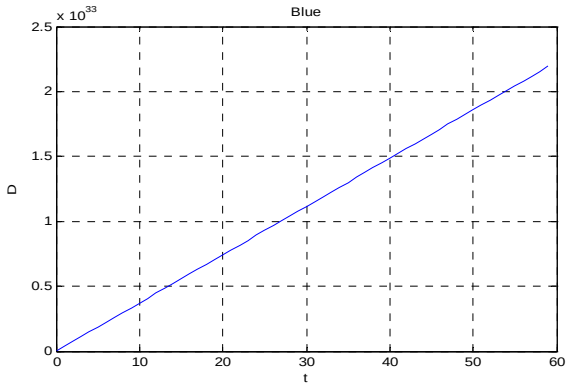
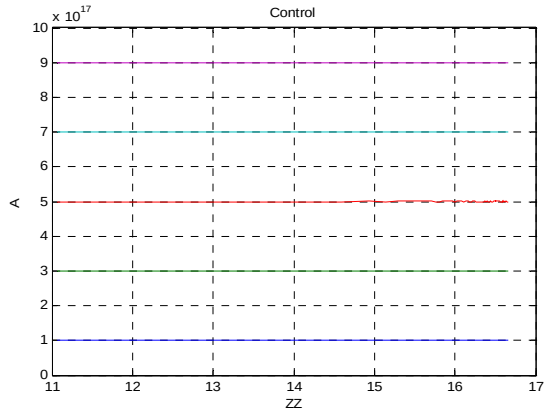


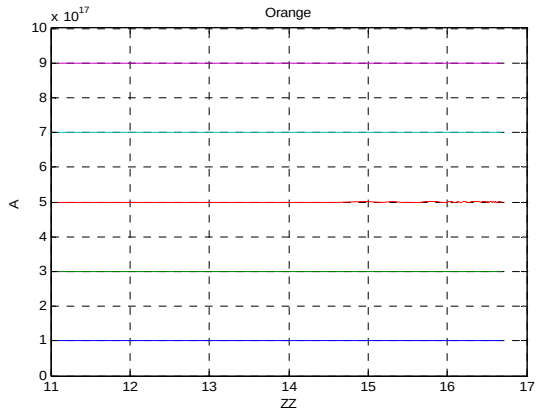
(d)

Fig. 3.3 *The relationship between D*_Surface and exposure time of the* ***(a)*** *Control,* ***(b)*** *Orange,* ***(c)*** *Eosin and* ***(d)*** *Blue.*

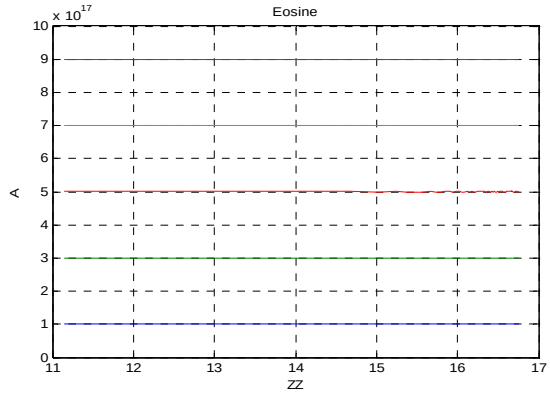
Fig. 3.4 shows that the accumulated damage remained constant when Z-necrosis increased, but when change the fraction of oxidative radicals the accumulated damage increased and remained constant with Z-necrosis.



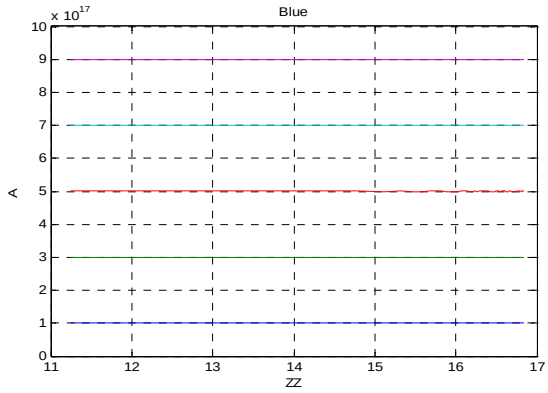
(a)



(b)



(c)



(d)

Fig. 3.4 *The relationship between accumulated damage and different fractions with Z-necrosis of the* ***(a)*** *Control,* ***(b)*** *Orange,* ***(c)*** *Eosin and* ***(d)*** *Blue.*

All the above results showed that the samples coated with blue stain increased the power density and reduced the scattering effect, whereas the effect of eosin dye was to increase the scattering effect.

4. DISCUSSION:

Laser light Incident on tissue can be reflected, transmitted or absorbed. The distribution of light in tissue is governed by wavelength dependent, optical properties of the target, primarily absorption and scattering, as well as by physical parameters of the incident laser beam "e.g., energy, power density, exposure time and spot size ".

The optical properties of tissue may be altered by exposure to laser light, such that the distribution of light during or after an Initial exposure may be completely different.

4.1 The Soft Tissues:

In **Table.3**, the transmitted fluences of the ovary tissues stained with different dyes were less than the control samples. The significant reduction of the output light was in eosin-ovary tissue as well as with orange dye. These results might indicate that the orange and eosin dyes were increase the

volume of the target in which interact with Incident laser, and reduced the result of energy density. The effects of the dyes were noticed in **Table.5**, where the transmittance of the stained samples with eosin, orange, and blue dyes were 6.0%, 27%, and 36% respectively. All of these coefficients were less than the control.
Since the optical properties of tissue may be altered by exposure to laser light, **Table.6**, clearly indicated that the optical densities of the stained-ovary samples were more than the control that might be due to the concentration of light-absorbing chromospheres and the depth of the light within the targets during the exposure. Mean while, there were a little change of optical density in stained kidney samples, that might be reflected to the amount of chromospheres present in tissue targets (Chromospheres: any substance that absorbs light).

Scattering Effect:
Scattering has the effect of increasing the volume of tissue in which the photons of the incident laser beam are distributed and may eventually be absorbed (Jon H, 2004). This effect actually increases the spot size of the laser beam within the target tissue and thus decreases the concentration of photons per unit volume tissue; (i.e, decreases power density). Scattering, particularly backscattering, along with absorption contributes to the exponential decrease in light intensity with increasing depth in the target tissue. The amount of scattered light reflected from or transmitted through tissue depends on internal reflectance and on the absorption properties of the tissue.
Table.4 showed a significant reduction in transmitted power density of the eosin-ovary sample than the other dyes and control sample; that's, because of the strong scattering of the light within the target. While the effect of the blue dye was less scattering, this might be was due to the selective absorption of pulses Nd-laser. The power density of the blue-kidney samples was more than the other samples with orange, eosin and control samples, that; indicated the blue dye was increased the absorption of the light by reducing the amount of scattering effect .

4.2 The Hard Tissues:
In **Table.3**, there was reduction in the amount of the transmitted fluence in the intact tooth tissues stained with eosin dye, whereas, the effect of the other dyes were to improve the amount of light to the tissue targets. The transmission coefficients of eosin and blue dyes were less than the control, where there was a significant enhancement of transmission of the light in the orange dye. These results explain the physical and biological interaction of these dyes with the targets due to the ability of these dyes or biological stains to react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas (Horobin RW, 2002). **Table.6**, showed these effects, where the optical density of the eosin sample was more than control sample. The power density of the eosin sample was less than the control, that's due to the scattering effect, while there was significant power density in the orange-dye samples, that's due to the selective absorption effect.
Determination of the reflection and absorption of laser light by tooth tissues showed that the absorption of Nd-laser radiation in unstained sections of teeth varies and depending on the present or absence of caries.

5. CONCLUSIONS:
Photodynamic therapy (PDT) technique has been successfully applied in various biomedical and clinical applications; like in the treatments of head and neck, lung and skin cancers. In this work when the program of determined the penetration depth is written and execution, there are three curves are obtained which are explained the relationship between exposure time, accumulated damage, fraction of oxidative radicals and Z-necrosis.
The relationship between Z-necrosis and exposure time is linear as well as the relationship between photodynamic doze and exposure time is linear.

When the fraction of oxidative radicals is constant, accumulated damage is remained constant when Z-necrosis is changed. While when fraction is changed then the accumulated damage is changed too but remained constant with changing in Z-necrosis.
This work also showed that the tooth tissues with caries and for all dyes have effect of increase the amount of laser to the target tissues, whereas the power density of the blue dye sample was more than the control and other dyes samples.
The results showed that from all dyes that was used; blue dye gave the greatest potentiating of the effect.

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