STUDY THE DAMAGE TO BLOOD CELLS WHEN EXPOSED TO LASER RADIATION AND HOW TO AVOID IT

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ABSTRACT

This research studied the damage caused by lasers on red blood cells (R.B.C.) and white blood cells (W.R.C.) in humans when common medical treatments , such as: sterilization of blood samples, treatment of tumors and removal of the birthmark, then a method for avoiding these damages.

The study was divided into two axes: The first axis: the study of the laser effect in terms of (its power, power density, exposure period, and wavelength) using three powers whose spectrum is located within the visible area of the electromagnetic spectrum : - Diode laser with power (5 mW) and wavelength (632.8 nm), Diode lasers have power (4.2 mW) and wavelength (632.8 nm), and (Nd: YVO4) laser with power (10 mW) and wavelength (532 nm). The second axis: studying the effect of the laser when placing the sample under the temperature (20 & 37^{0}C), for three irradiation periods (4,6,8 min). Fresh blood samples were irradiated from a healthy donor (his group B-).

The results showed a clear damage in (R.B.C.) counts when irradiating with a Diode laser, as the damage gradually increased to reach the highest value (4x10^7 cell / ml) at the power density (2.48 mW \ cm^2) for the period (8 min). The damage was also observed in the number of (W.R.C.) to increase with the increase in

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power and period to reach the highest damage (5.2 x 10^6 cell / ml) in the number of (W.R.C.) at the same period and the same power density.

However, less damage was observed in the numbers of red and white blood cells when the samples were irradiated at (20^{o}C) for all densities and irradiation periods if compared with the results of (37^{o}C) for any laser in the study. Also, no damage was observed in red and white blood cells (damage 0.0%) when using a diode laser (4.2 mW) with a power density of (0.791 mW / cm^2) during the period (4 min).

The study concluded that it recommends to use the diode laser (4.2 mW), which it deems to be successful, appropriate and safe in medical uses without harming red blood cells and white blood cells when used with a power density (0.791 mW / cm^2) , period (4 min) and temperature (20^{o}C) , especially when adopted in sterilization of blood bags against bacteria and viruses in blood banks, or when treating tumors in surgical operations.

دراسة الاضرار في كريات الدم عند تعرضها لأشعة الليزر وكيفية تلافيها

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الخلاصة

درس هذا البحث الاضرار التي يسببها الليزر في كريات الدم الحمر والبيض في الانسان عند المعالجات الطبية الشائعة مثل : تعقيم عينات الدم وعلاج الاورام وازالة الوحمة ، ثم طريقة تلافي هذه الاضرار.

قسمت الدراسة على محورين : المحور الاول : دراسة تأثير الليزر من حيث (قدرته وكثافة القدرة و فترة التعريض والطول الموجي) باستعمال ثلاث قدرات يقع طيفها ضمن المنطقة المرئية من الطيف الكهرومغناطسي :- ليزر الدايود Diode ذو القدرة (5mW) وطول موجي (632.8 nm) . وليزر الدايود Diode ذو القدرة (4.2 mW) وطول موجي (632.8 nm) . و ليزر (Nd:YVO4) ذو القدرة (10 mW) وطول موجي (532 nm). المحور الثاني : دراسة تأثير الليزر عند وضع العينة تحت درجتي

الحرارة (20 & 37^oC) ، لثلاث فترات تشعيع (8,6,4 min) . وقد تم تشعيع عينات دم جديدة (fresh) من متبرع سليم فصيلته (B-).

اظهرت النتائج ضررا واضحا في اعداد كريات الدم الحمر عند التشعيع بليزر (Diode)، حيث ازداد الضرر تدريجيا ليبلغ اعلى قيمة ($4x10^7$ cell/ml) عند كثافة القدرة (2.48 $mW\backslash cm^2$) للفترة (8 min) . ولوحظ الضرر كذلك في اعداد كريات الدم البيض ليزداد مع زيادة القدرة والفترة ليبلغ اعلى ضرر في اعداد الكريات البيض (5.2 $x10^6$ cell\ml) عند الفترة نفسها وكثافة قدرة نفسها.

ولكن لوحظ حصول ضرر اقل في اعداد كريات الدم الحمر والبيض عند تشعيع العينات في (20^oC) لكل الكثافات وفترات التشعيع اذا ما قورنت مع نتائج (37^oC) لأي ليزر في الدراسة. كما لوحظ انعدام الضرر في كريات الدم الحمر والبيض (الضرر 0.0 %) عند استعمال ليزر الدايود (4.2 mW) بكثافة قدرة (0.791 $mW\backslash cm^2$) في الفترة (4 min).

توصلت الدراسة الى ان توصي باستعمال ليزر الدايود (4.2 mW) الذي تعده ناجحا ومناسبا ومأمونا في الاستعمالات الطبية دون الاضرار بكريات الدم الحمر والبيض عند استعماله بكثافة قدرة (0.791 $mW\backslash cm^2$) وفترة (4 min) ودرجة حرارة (20^oC)، لاسيما عند اعتماده في تعقيم أكياس الدم من البكتريا والفيروسات في مصارف الدم ، أو عند معالجة الاورام في العمليات الجراحية.

INTRODUCTION

The laser was discovered in 1960, and in 1962, lasers has been used in micromanipulation [1]. Laser applications provided distinguished research in medical fields, such as using it as an optical tweezer in cell rotation. In the last four decades, laser beams found to be very useful in a variety of applications in the fields of medicine, surgery, ophthalmology, surgery, ophthalmology, industry, automatic control, communications, chemistry and other fields.

Low Level Laser therapy (LLLT) is the application of light to a biologic system to promote tissue regeneration, reduce inflammation and relieve pain. Unlike other medical laser procedures, LLLT does not have an ablative or thermal mechanism, but rather a photochemical effect which means the light is absorbed and cause a chemical change [2].

Laser can also be used in sterilization of blood in blood bank which purifies blood from bacteria and viruses [3]

Burduli and Pilieva, studied the time course of changes in the activity of the protein C system and other hemostatic parameters under intravascular laser irradiation of blood (ILIB) in patients with community-acquired pneumonia [4] .

Nabihah , et al., studied the effect of Low Level Laser Irradiation of ATP content on anaemic human blood cells. This work measured the optical density of RBCs to study the effect of ATP level after irradiated by the laser. They showed that the ATP level is increased for anaemic RBCs after the irradiation for (20 mW), (40 mW) and (60 mW), each at (15 min), (30 min) and (45 min) of exposure time. The cell viability for the anaemic also shows an increase compared to the un-irradiated one [5].

Mohsen HK, mentioned the effect of the laser (532nm) studied on blood viscosity using Laser (532nm). He found the decreases of viscosity in blood with effect of laser where the decrement was (2%) after (15 min) and (8%) after (20 min). of laser irradiation [6].

The laser has become a tool for treating cancer, through photodynamic-therapy , where a light-sensitive substance is injected into the patient's body, and then we use a laser of a wavelength of (630 nm) for the purpose of conducting a chemical reaction with the substance to produce a toxic substance that works to destroy tumor cells. Lasers have also been used to sterilize the blood in the blood banks, as it works to cleanse the blood from existing bacteria and viruses.

In 1985, Nakagawa performed selective damage to the skin by exposing it to a wavelength of (532 nm). It was found that the appearance of deformation of the blood cells increases with the increase in the power density of the laser. He noticed that the deformation of the globules changed their shape from concave to amoebic shape. Other blood cells were completely destroyed [7] .

In 2006, a group of scientists studied the mechanical deformation of a red blood cell during its heat transfer between room temperature and (42°C), when heating is done by using a laser, it was found that the deformation of the cells increases with increasing temperature [8] . In the same year, researchers found that exposure of T- cells to laser radiation of (632.8 nm) length notes an increase in the activity of immune cells through their response to the production of activating proteins for immune cells. However, as the laser power and exposure time increased, their immune response decreased [9].

Photobiology is the study of the interaction of non-ionizing electromagnetic radiation with biological cells and their biological responses. Photo-medical changes are concerned with wavelengths from (200-800 nm) and photochemical changes that affect the viability or function of living materials. Therefore, Photo-Medical is considered to be an application of photo-biology principles to diagnose, understand and treat diseases. The photon energy in the 200-400 nm ultraviolet spectrum and in the (400-700 nm) visible spectrum is sufficient to cause electron hybridizations of specific dye molecules that lead to specific chemical reactions. And the use of (visible - ultraviolet) rays leads to the possibility of choosing specific pigment molecules from among many different numbers and causing chemo-photoelectric reactions, so the longer and shorter wavelengths of that may be less important than those biological reactions.

Laser Damage on Biology system : Scientific experiments have confirmed that electromagnetic waves that include visible light, ultraviolet and infrared rays, have harmful biological effects when they are absorbed by human tissues or by all other living tissues. The tissues of the human skin and eye come in the first place in terms of their effect by laser beams. Scientific experiments have confirmed that the laser has severe biological effects that are very harmful in these two organs when the absorbed doses exceed certain limits. The damage to the human eye or the skin occurs when laser beams fall on them as a result of specific physical processes during energy transferred from the beam of rays to the exposed organ. The transfer of the energy of the laser beam to the exposed organ can lead to any of the following three processes: arranged according to the degree of its greater impact:

1- Raising the temperature of the cells that absorb the beam energy to an extent that leads to the death of the cells and damage of the organ.

2- The effect of chemical reactions by the action of the absorbed light in some components of the cells.

3- The possibility of ionizing some cells exposed to the laser beam - high energy - whose spectral lines fall within the ultraviolet rays.

In general, damage of the human tissue or organ by these three processes depends on the wavelength of the laser beam, and the type of the exposed tissue or organ.

Laser Damage in Blood : The advanced technologies of the laser have led to its use in the biological sciences, medicine and surgery. Therefore, it is important to understand the mechanics of the effects of any high-energy incident radiation that can cause damage to the cells as a result of (photochemical) processes [10] . Erythrocyte cells are very important due to their simplicity , large numbers and its medical physical importance [11].

The deformation of the blood corpuscles plays a fundamental role in the blood circulation, especially when they pass through the capillaries, which have a small diameter compared to the size of the corpuscles. The deformation is responsible for the decrease in the viscosity of the large arteries. Although blood is mostly composed of cells half its volume, simple deformation of blood cells can reduce the rate of entry into capillaries, and neglecting any decrease in the deformation of these cells will lead to a series of diseases such as diabetes, high blood pressure, and anemia. Blood, the wide variety of diseases can be diagnosed by less deformation of blood cells [12]. Cell deformation requires the transformation of structures by cellular reactions to the protein. When the temperature increases, the entropy (energy measure) of the internal components of the protein system decreases, causing deformation in the cells. At high temperature deformations, the elasticity of the cells disappears [13] .

Reasons for interest in studying the deformation mechanism of red blood cells:

1- Red blood cells are distinguished by their simple structure, and the fact that they do not contain a nucleus made them a model system in the study of single living cells.
2- The simplicity, symmetry and concave shape are relatively more suitable for detailed theoretical study, as well as for a computational model compared to other cells of great engineering complexity.
3- Its disc shape facilitates the mechanical deformation experiments such as optical forceps.
4- The deformation of red blood cells has important effects on the blood flow, which can be determined by mechanical and engineering factors such as the surface area, elasticity, viscosity of the membranes and their size.
5- The deformation is studied for its close relationship between some inherited diseases and the mechanical deformation characteristics of the red blood cell, where in the case of cell disease with

anemia resulting from a deficiency in hemoglobin composition is known as (Sickle blood anemia) because the shape of the globule resembles a sickle.

6- The mechanical deformation of red blood cells can arise from the maturation of cellular parasites that can lead to a succession of fatal diseases as in malaria, it is the most widespread parasitic disease. [14,15,16].

PROCEDURE

Preparation of Blood

Taking blood from a vein (3ml) for a healthy person, who does not suffer from blood diseases, by tying the vein by the (Tourniquet) band around the arm, the area was sterilized with alcohol (70%) then the needle was inserted to draw the blood. the drawn blood was put in tubes containing (EDTA), an anti-coagulant, sealed well. The blood samples were placed in a magnetic-stirrer for the duration of successive examinations, at a small rotational speed, at room temperature.

Examination of Blood

The blood samples were examined before the irradiation process at 37°C & 20°C, this reading represents the control model. Counting process was carried out using the counting chamber method (Tahoma's chamber) for both red and white blood cells. The centrifuge was used to determine the hemoglobin percentage. The samples were irradiated with the lasers and the tests are performed again after each irradiation.

The blood sample was placed in a water bath for raising the blood temperature to 37°C or to 20°C. The blood samples were treated through periods (4 , 5 and 8 min.) with many run of different lasers as following:

1. Diode laser (5 mW) irradiation of blood at 37°C.

2. Irradiation with a diode laser (5 mW) at (20°C).

3. Irradiation with a diode laser (4.2mW at 37°C)).

4. Irradiation with a diode laser (4.2mW) at (20°C).

5. Irradiation with a laser (Nd: YVO4) at 37°C).

6. Irradiation with a laser (Nd: YVO4) at (20°C).

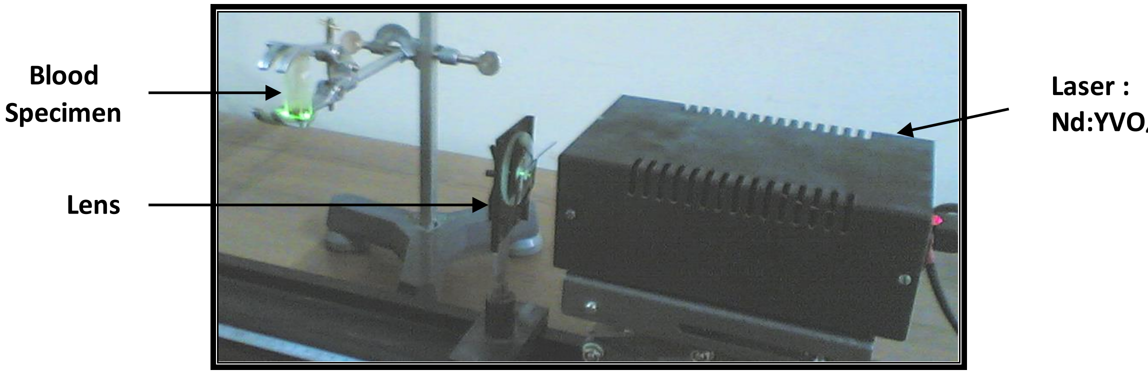
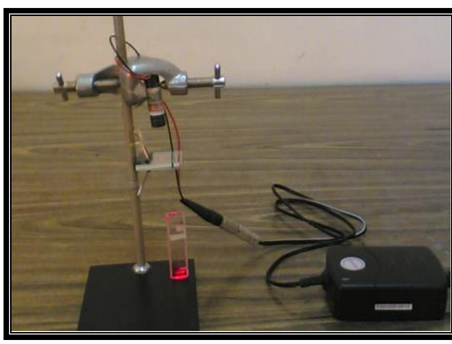


Figure 1 : Laser Nd:YVO4



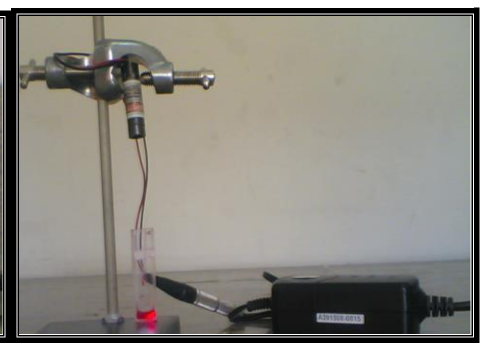


Figure 2 : Diode Laser (4.2 mW) **Figure 3 : Diode Laser (5 mW)**

RESULTS

1. **Effect of temperature on damage of red and white blood cells using a 5mW diode laser.**

The effect of temperature on the damage of red blood cells was noted from (Figure 4). The figure indicates a high percentage of red blood cells damage, which is gradually from the lowest density of capacity up to the highest, to reach the highest percentage of damage about (20%) when the power density (2.48 mW / cm^2)

and the irradiation period (8 min), and the lowest damage percentage is at (1.31mW / cm^2) and the irradiation period of (4%) is about (1%).

It is noticed at (20°C) that there is a percentage of damage to the pellets, but it remains small if it is compared with the damage rate at (37°C), where the highest percentage of damage was about (3%) at (2.48 mW / cm^2) for the period of 8min, and the highest percentage of damage was about (0%) at (1.31 mW / cm^2) for all irradiation periods. The damaged cells showed deforming as in (figure 9-1).

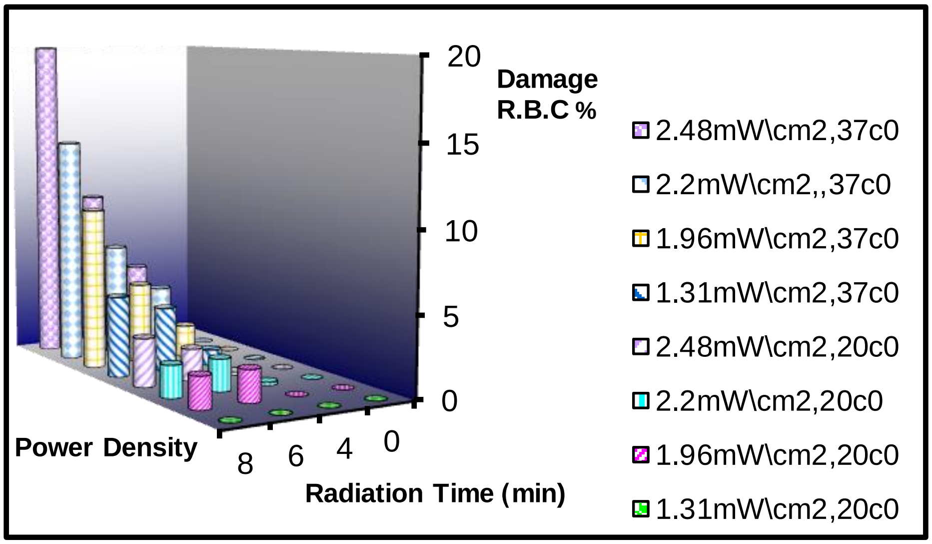


Figure 4 : The effect of a (5 mW) diode laser on the red blood cell damage ratio at (37 & 20 °C) in different irradiation periods and different power densities.

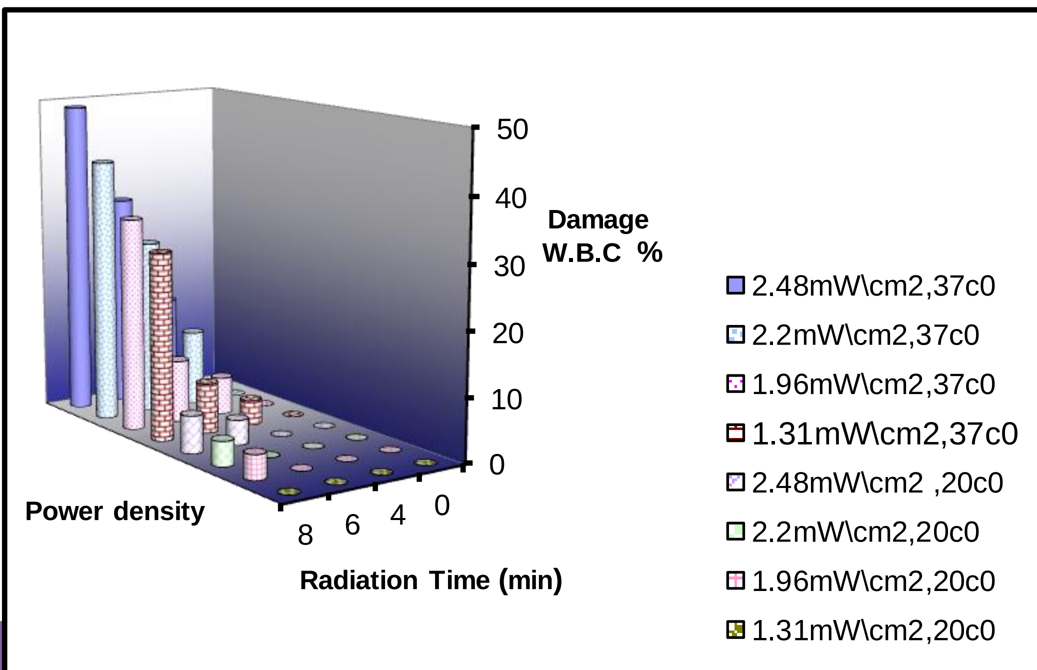


Figure 5: The effect of a (5 mW) diode laser on the white blood cell damage ratio at (37 & 20 ^{0}C) in different irradiation periods and different power densities.

(Figure 5) shows an increase in the damage of white blood cells to reach 50% at the highest intensity and the highest irradiation period. This damage is due to the thermal effect of the laser in addition to the temperature increasing to (37°C).

While the difference is easily noticed when the samples are cooled to (20°C) to reach the damage ratio to (0%) at the lowest power density and the lowest irradiation period. It is noticed from the figure that the high percentage of white blood cells damage over the red blood cells damage percentage in both (20&37°C) by diode 5 mW. It is noticed from the above figure that the highest percentage of red blood cell damage was (10%) at a power density of (1.65 mW / cm^2), and the highest irradiation period was at (37°C), then the lowest percentage was (1%) at the power density (0.791mW / cm^2) for all irradiation periods.

2. Effect of temperature on the number of red and white blood cells using a 4.2mW diode laser

(Figure 6) shows the low percentages at (20°C) to reach the lowest percentage (0%) at (0.791 mW / cm^2). In the period (4 min), it can be distinguished that the RBC damage rate for the two degrees is less than that of the diode laser (5mW).

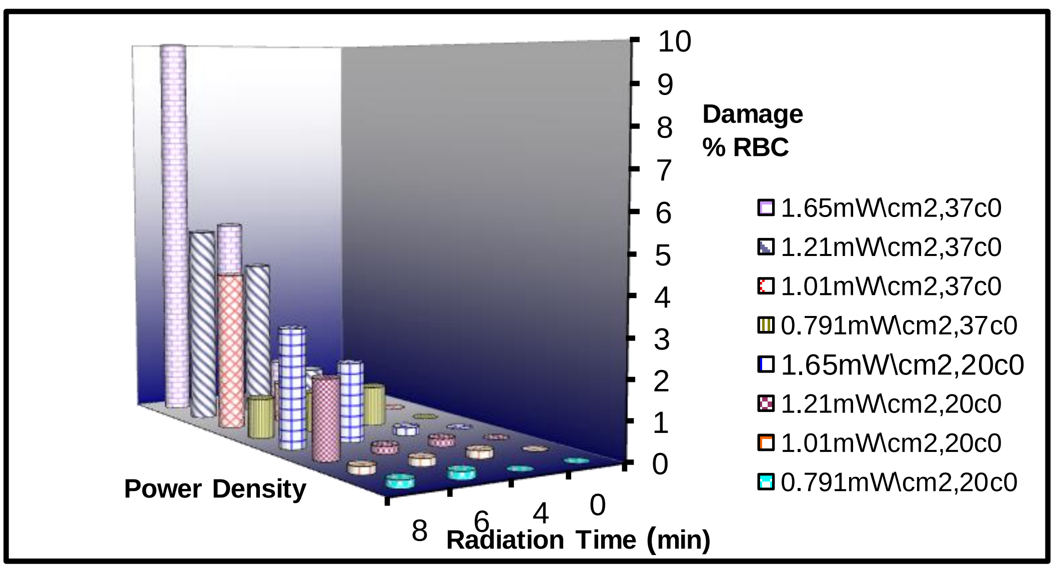


Figure 6: The effect of (4.2mW) diode laser on red blood cell damage ratio at (37 & 20°C) in Different irradiation periods and different power densities.

(Figure 7) indicates an increase in the percentage of white blood cells damage to reach 30% at the highest power density and the highest irradiation period, then the gradual decrease begins with the decrease in the power density and the irradiation period, and it is also noted that the rates of damage to white blood cells on the red blood cells are high, but it is noted reducing damage rate when cooling to 20%, so that the lowest damage percentage is 0% at low power density and low irradiation period.

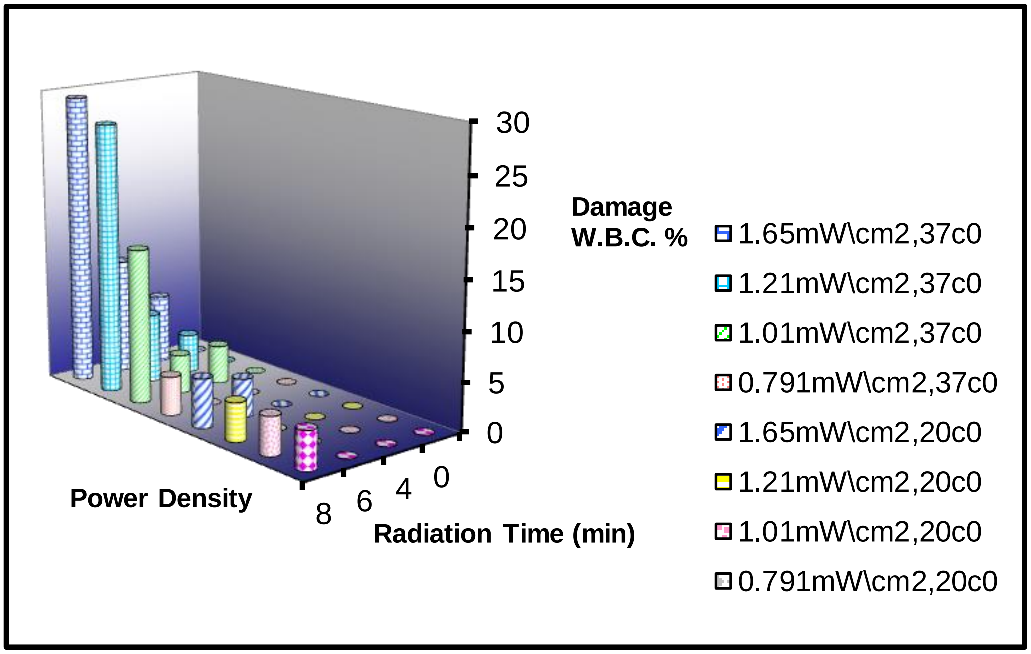


Figure 7: Effect of (4.2mW) diode laser on white blood cell damage ratio at (37 & 20°C) with different irradiation periods and different power densities.

3. Effect of temperature on the number of red and white blood cells using an (Nd: YVO4) laser with a power of (10mW).

It is noticed from (Figure 8) the correlation of the percentage of damage to the red blood cells with the temperature, as the percentage of damage increases to reach (20%) at (2.866mW / cm^2) and the highest irradiation period is at (37°C), then the lowest damage percentage is about (0.2%) not (0%) at the lowest power density for the period (8min). Also can be noticed in the same figure notes the convergence between the results of both (5&10 mW) lasers despite the difference in power and the difference in the two wavelengths, but it is noted that there is a percentage of damage even at low power densities and for low irradiation periods compared to a (5 mW) laser.

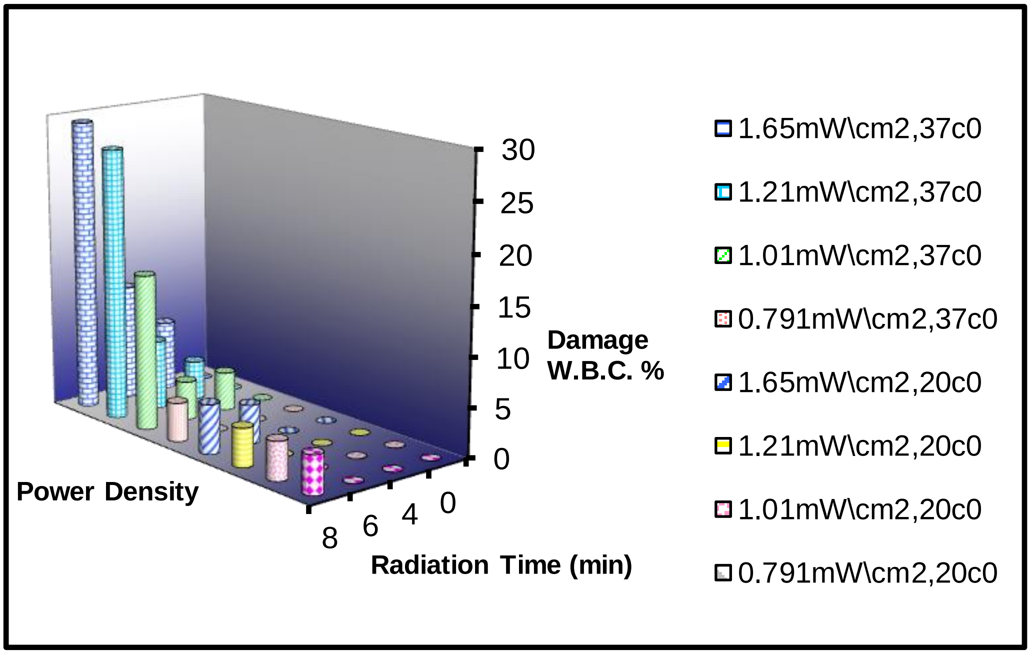


Figure 8: The effect of the Nd: YVO4 laser on the red blood cell damage ratio at (37 & 20) ^{0} C at different irradiation periods and different power densities.

It is noticed from (Figure 9) that the percentage of damage to white blood cells is approximately (50%), which is similar to the percentage of damage to the cells in the diode laser (5 mW), and this may be due to the same power density and the same irradiation periods, but the percentage of damage to the cells remains for the rest of the densities and irradiation periods higher than pellet damage ratio of 5mW laser. When cooling, the lowest percentage of damage (0%) for density is (0.716 mW / cm^2) at (4 min), but it is (5%) for the same density but at 8min. It is evident from the above that the damage caused by the laser in blood components can be controlled and treated by lowering the temperature of treated organ (treated by laser) from the normal body temperature of (37℃) to a degree of (20℃) in order for the laser treatment to be done without all these damages occurring in the red and white blood cells . Increase in the pressurized red cells and the flow of hemoglobin from them to the plasma loses its chemical role and the required vital function. Thus, laser technology becomes safe in human treatment and other health uses.

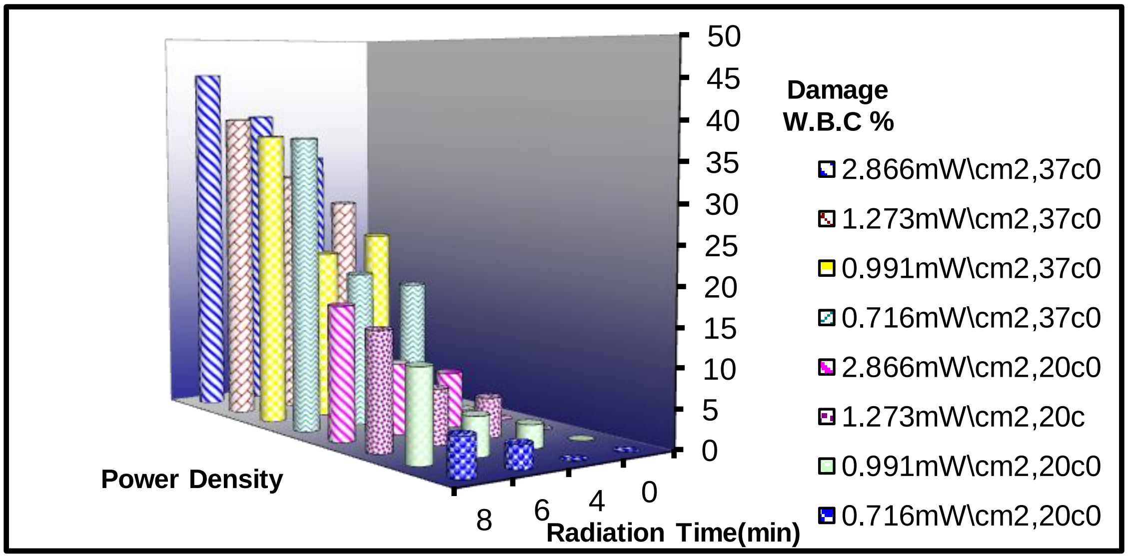


Figure 9: The effect of the (Nd: YVO4) laser on the white blood cell damage ratio at (37 & 20^0C) at different irradiation periods and different power densities

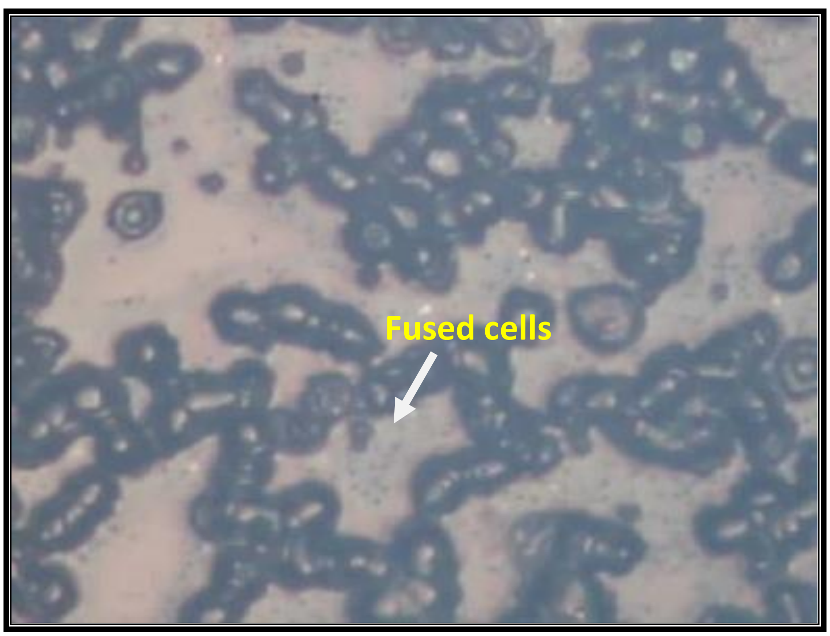


Figure 10 : Effect of laser (5mW) on Blood cells to become Fused.

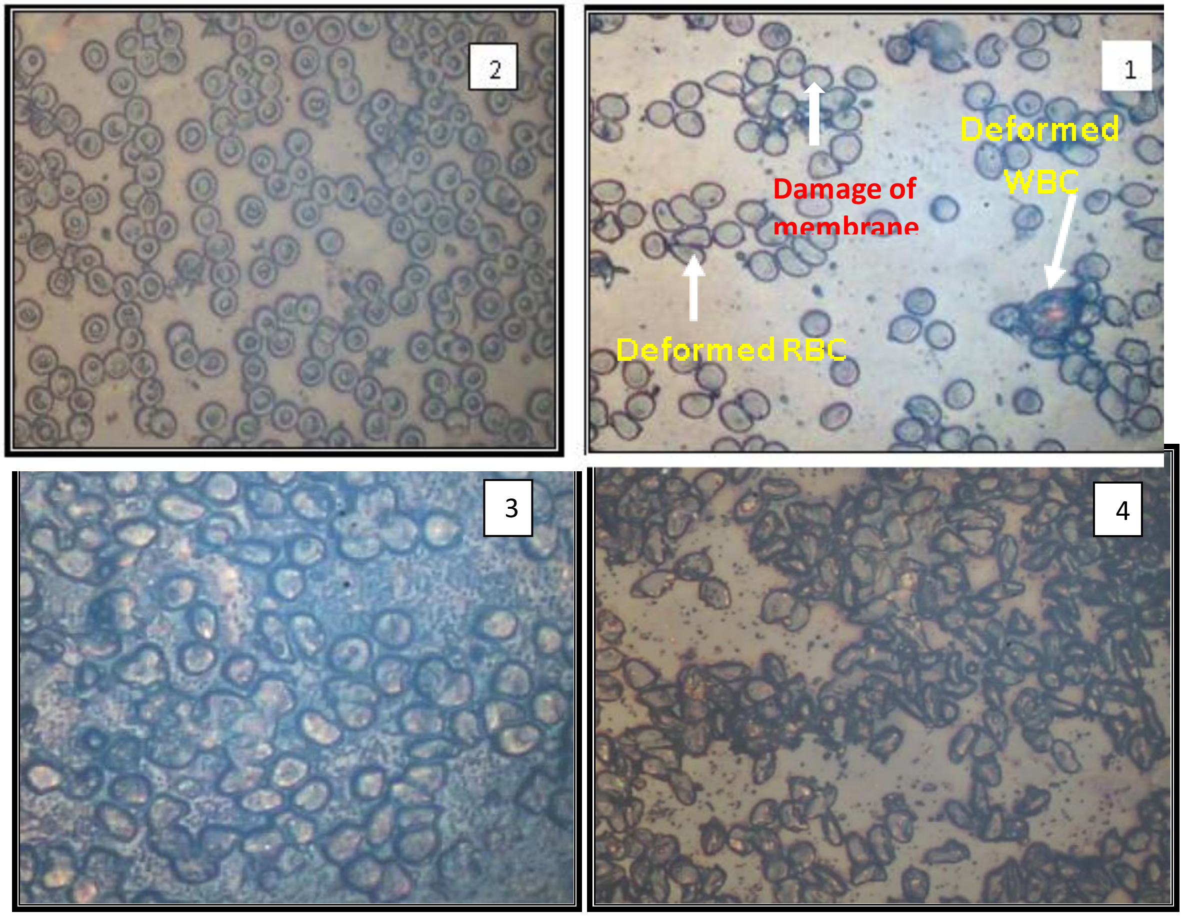


Figure 11 : Effect of laser (5mW) on Blood cells to become Deformed.

Image 1 : represents blood exposure to a (5 mW) diode laser for a 4 min irradiation period and a power density of (1.96 mW / cm^2) at (37^0 C).
Image 2 : the effect of a (5 mW) laser cooling at (20^{0}C) (2.20 mW / cm^2) at (8 min).
Image 3 : represents a green (10 mW) laser effect in pellets red and white blood and at (6 min) and at a power density of (2.886 mW / cm^2) at (37^{0}C).
Image 4 : represents a laser effect (10 mW) at (4 min) irradiation period and a power density of (2.866 mW / cm^2) at (37^{0}C).

DISCUSSION

The study showed that red and white blood cells maintained their shape and effectiveness at low laser power and short exposure periods, while in high laser power the blood cells did not maintain their normal shape, so in addition to their thermal effect, the high power radiation leads to the accumulation of the effect that causes chemical changes, also high laser doses lead to stresses that reduce the capabilities of cells and their activities, and then prevent their vitality [17].

The laser effect appears clearly at high power densities and irradiation periods (1.65 mW / cm^2 , 8 min). This is consistent with published research, it has shown that the change in the shape of blood cells occurs during cooling to (20^oC) with higher power density and the use of the same wavelength [18].

The increase in red blood cell damage - at high power lasers - is attributed to the thermal effect of the laser on the cells. When the cells are exposed to laser radiation, it causes changes in the structure and functions of cells and affects permeability of the cell membranes [19].

The percentage of damage to red blood cells in the published research (20%) after exposure to the laser, while the percentage of damage in this research reached (15%) [20].

This damage is consistent with published research, as when the laser beam falls on a red blood cell, there is part of the energy absorbed - not negligible - (transformed into heat), which can increase the transient heat that leads to a change in the structures and cell membranes as a result of the occurrence of reactions (Chemical - biological) in the membrane [21].

In white blood cells the damage is due to the effect of the laser beams themselves. However, it is noticed that the damage is high in the numbers of these cells when compared with the damage in the numbers of red blood cells, and this is due to the nature of their composition, as the red blood cells are soft membranes whose reflectivity to the incident light is more than that of the white blood cells that contain colored chromatin granules with a tendency to absorption of light falling on it, while red blood cells are simple in structure and do not contain such granules. In addition, the life span of white blood cells does not exceed (3-4 days), while red blood cells reach (120 days) [22].

The damage caused to the white blood cells is evidence of their susceptibility to low lasers, and it was easily observed when compared with the control samples. It should be noted that the effect increases with increasing power with the period of exposure [23].

The damage was caused by the use of a (5 mW) diode laser with a low power application like in published research [24].

This is also in agreement with the published research which showed damage to white blood cells at low power due to the micronucleus of the cell, but the greatest damage is observed at high laser power that lead to a cell-killing effect [25].

Research has shown that the use of wavelength (523 nm) causes an increase in deformation compared to the wavelength (632.8 nm). This is due to the effect of wavelength (532 nm) as it has a high absorbency and this is reflected to make hemoglobin the target of the first reaction under the influence of the laser [26] .

The effect of laser on red and white blood cells: Through this study and observing the shapes of blood cells under microscopes, it became clear that the lasers used affected these cells in different patterns, and they are as follows: -

1- Deformation of the shape of the globule: Here, laser rays can affect the molecular structure of the blood cells, as the molecules with long structures are in nature subject to bending and folding when affecting the bonds that preserve the natural shape of the folds and thus have random folds that lead to deforming the final shape of the globule (as in figure 11) and giving it a shape abnormal (deformed) which affects its biochemical properties.

2- Cohesion of the globules together: The protein structures with long chains contained in the globules, if damaged more by laser radiation, then develop from bending or folding to becoming fused with each other because their many folds lead to many twists in their outer membranes, which leads to twists in the adjacent globules and thus the adhesion or fusion of their winding membranes with each other as showed in (figure 10). Thus, the cells lose their vital function and may lead to the occurrence of clumps that obstruct the bloodstream. [22]

3 - The breakdown of cell membranes: The red and white blood cells are characterized by their containment of thin membranes, so exposure of the cells to laser radiation can lead to the rupture of the outer membranes and the exit of its internal contents, thus separating the membrane from the cell, which leads to the exit of cell components into the plasma, leading to a lack of hemoglobin inside the red globule due to its leakage out of it and becomes ineffective in the biological transport of respiratory gases.

The occurrence of rupture of membranes and the breakdown of the protein chains forming them into smaller chains is in itself a criterion for the physico-chemical damage of radiation to proteins that leads to disturbance in the secondary structures and the breakdown of the amino acid chains and peptide chains. [22]

4. Cell breakage or rupture: Polymerized molecules consisting of identical repeating units will be split into a number of smaller molecules due to the laser radiation. This leads to the breakdown of the bonds of the

organic compounds, and may even extend to the re-joining of the fragmented parts to form clusters that express strange distorted formations that flow into the bloodstream without any benefit [22].

The effect of different types of laser on damaging blood cells: The changes that occurred in the red and white blood cells were not caused by all the studied lasers. Rather, some damages were caused by a specific laser with a certain power density and for a certain period, so the study continued these effects according to the type of laser and its operating conditions to find out which ones have less effect on damage in order to recommend that it be the best in the required deal.

CONCLUSIONS

1.The emergence of a clear effect of the lasers used to damage red and white blood cells and changing their shape.

2. Not equal to the damage of a 5mW diode laser of (632.8 nm) wavelength with a (Nd laser: YV04) , (10 mW) of wavelength (532 nm), on blood components.

3. The lack of effects resulting from all types of lasers used at low irradiation periods and low power densities, and the effect increases with their increases.

4. Low blood cell damage for all types of lasers at (20°C) compared to (37^{o}C).

5. Recommending the adoption of the diode laser (4.2 mW) as the best laser in sterilizing blood bags from bacteria and viruses in blood banks, or when treating tumors in surgical operations with the application of a power density (0.791 mW / cm2), a period (4 min) and a temperature (20^{o}C), especially when it is used in the treatment of organs with less damage to blood components in the human body.

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