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The Tensile Test of Irradiated Skin

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Abstract

Irradiation can complicate surgical skin treatment, yet little is known of the importance of the time between surgery and irradiation on this process. This study investigated the impact of postoperative irradiation on tensile strength in a rat skin model. Irradiation on the same day as irradiated skin. In contrast, postoperative irradiation delivered at 1, and 3 days transiently enhanced irradiated skin tensile strength with different lasers, as measured 3 but not 4 or 5 weeks later. This effect was independent of the inclusion (hemibody) or exclusion (skin alone) of the hematopoietic system in the field of irradiation. Radiation-enhanced tensile strength was greater and occurred earlier after higher radiation doses with both Er:yag and Co2 lasers. Even though the effect of irradiation skin in enhancing tensile strength is transitory, it could be important in assisting early ski treatment.

Keywords: laser , tensile test, CO2 laser , Er:YAG laser

Introduction

The laser is a device that generates a high intensity beam of single wavelength electromagnetic radiation. The basic principle of operation the laser can describe in terms of light /matter interaction where the electron in atoms exist has sates and each atom possess electrons that are characteristic to specific element .The transition of this atom from one state to another is called the quantum transition which may occur in different interactions. This commonly occurs when atoms interact with optical radiation .In general, the light from a laser has a much higher intensity than normal light, including the sun, and the light generated is at a fixed wavelength corresponding to photon energy release on atom de-excitation.The following describes the basic physics of the two lasers that were used for the experimental work in this thesis; the Erbium YAG laser and the CO_2 (Carbon Dioxide) laser. The electrical attraction between the positive and negative charges holds the entire complex together. If the electron absorbs the energy in the form of photons it will transform into an excited state. The atom can stay in an excited state for a short period of time and then the energy is released by exited the electron in other electron state and back to resting state in the form of photon. This emission of photons is random and appears in all directions which refer to spontaneous emission as shown in Figure 1. The laser light in the visible portion of the electromagnetic spectrum creates a visible beam in different colors depending on the wavelength. For example, lasers within the infrared section of the electromagnetic spectrum, such as YAG and CO2, generate an invisible beam of laser light [1].

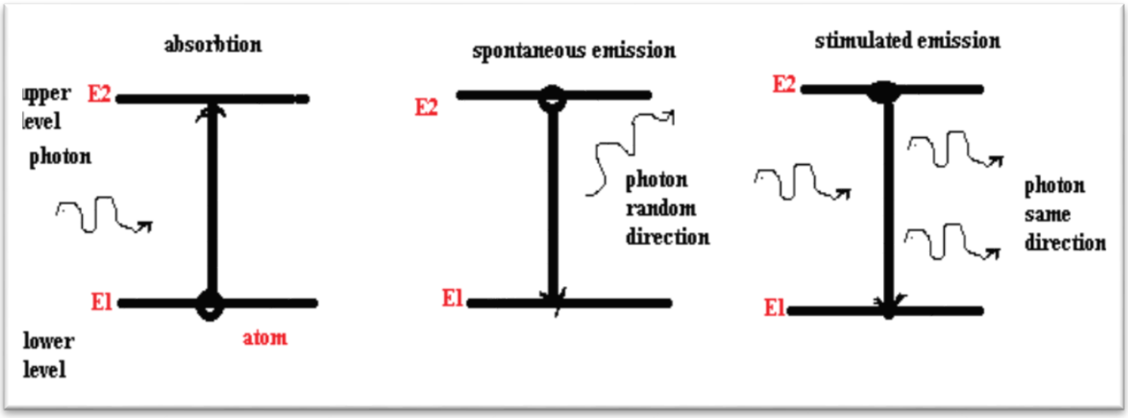

Fig.1: Mechanism of the interaction between an atom and a photon (The photon has an energy hν equal to the difference between the two atomic energy levels).

The skin covers the whole body and therefore is the large organ of the body. At its most basic role, skin provides an efficient barrier to the outside world. The layers of skin consists of water, as well as boarder keratinocytes which results in varying epidermis thickness around the whole of the human body to provide different levels of protection depending on the body's needs [2, 3]. The epidermis consists primarily of epithelial keratinocytes, but also contains Langerhans and melanocyte cells. The dermis is 300-400 μm thick and is positioned to provide skin with strength, shape, and overall structural completeness. The dermis consists of resident fibroblasts that attach to an extracellular matrix (ECM). When skin is loaded with tension it exhibits a nonlinear concaved stress-strain characteristic. The ramification of this stress-strain curve is that under normal conditions skin is elastic, but becomes stiff when loaded with tension.

In the work of a laser skin interaction in, several aspects are considered to limit the scope of the study. These include the selection of laser wavelength, laser energy, laser influences and skin target. In this study, white rats were chosen as the in-vivo skin tissue model. This selection is based on its physical skin properties and its viability. Although, it has limitation because of its little different between rat skin structure and human skin structure. The irradiation sources of the laser treatments were done by using, CO_2 lasers , Er:YAG laser and dual beam of CO2/Er:Yag. In order to observe the laser effect on skin, a video Camera was used to capture the pictures. Beam profiler was used to measure the beam quality, and Power meter used to measure the output power and tensile test machine.

Apparatus

In this project we using instruments relevant to our study, including a Power meter (Wizard™ 250), AM microscope (Olympics light microscope), warmer slide, microtome machine, embedding machine, water bath and tensile machine, jar glass, beam meter, diode 808-nm power meter, Slid boxes.

Animal studies

Preparation of Animals Studies

This study involved 120 rats 12 week old male Sprague Dawley rats weighing 200-250g.These rats were obtained from the animal house of Universiti Teknologi Malaysia under license number shown in appendix A as shown in Figure (2). They were fed standardized food and kept in a constant temperature and humidity environment with a 12-hour light/dark cycle.

Sample Preparation for Laser Irradiation

Surgical procedure

The rats were anesthetized with xylazine hydrochloride (10 mg/kg body weight) and ketamine hydrochloride (75 mg/kg body weight) intramuscularly. All injection procedures were performed the same way. After an adequate level of anesthesia was obtained, the animals were fixed in the prone position on a holder. Each rat was weighed before and after laser irradiation. The rats were anesthetized by intramuscular injection of 10% ketamine hydrochloride (0.1 ml/ 100 g/kg) at the same dose (Martins *et al.* 2006)[4] to anaesthetize them for the experimental work. Next, the 2x2 mm marked area on the rat‘s back and the hair at the back skin surface was shaved gently using a razor blade and shaving machine, before being cleaned with 70% alcohol to avoid infection. The target skin tissue was marked for laser radiation, and the laser was focused directly on marked target of rat back as show in Figure (3). The full-thickness skin from the dorsal back was excited and prepared for histological examination.

Laser Irradiation

The left grid of marked area was for laser irradiation, leaving the right side as the control. Four different laser pulses were applied for all rats with one, three, six and ten pulses: the laser was fired repeatedly without moving the target until the desired number of pulses had been achieved. Each exposure was done on the back of the rat in order to get duplicate samples for histology studies. Control samples were obtained from the unexposed skin and processed under the same condition as the exposed one.

Laser Procurer

To make an effect on the surface of the skin, we focused the laser beam and orientated the sample to be perpendicular to the incident direction of the beam, before irradiating with the desired number of pulses. Some parameters were varied in the study, including the laser wavelength, laser fluences and the number of laser pulses.

The ERBIUM: Yttrium-Aluminum-Grant Laser (Er:YAG) system setup

we used an Erbium (Er:YAG) laser with a fundamental wavelength of 2940 nm. The output operates in external trigger mode with a single pulse, the duration of which is 432 ns. The beam spot size is 3 mm and was employed as a source of irradiation and manufacture from San Diageo California USA. The laser is operated at a variable voltage in the range of 750-1350 V. An Er3+-doped YAG crystal was used as an active medium in this laser system in terms of decreasing the effect of thermal lensing. Both end-faces of the laser rod were concave, with a radius of curvature of 5m. Both sides were AR-coated. The laser rod was pumped by a single xenon

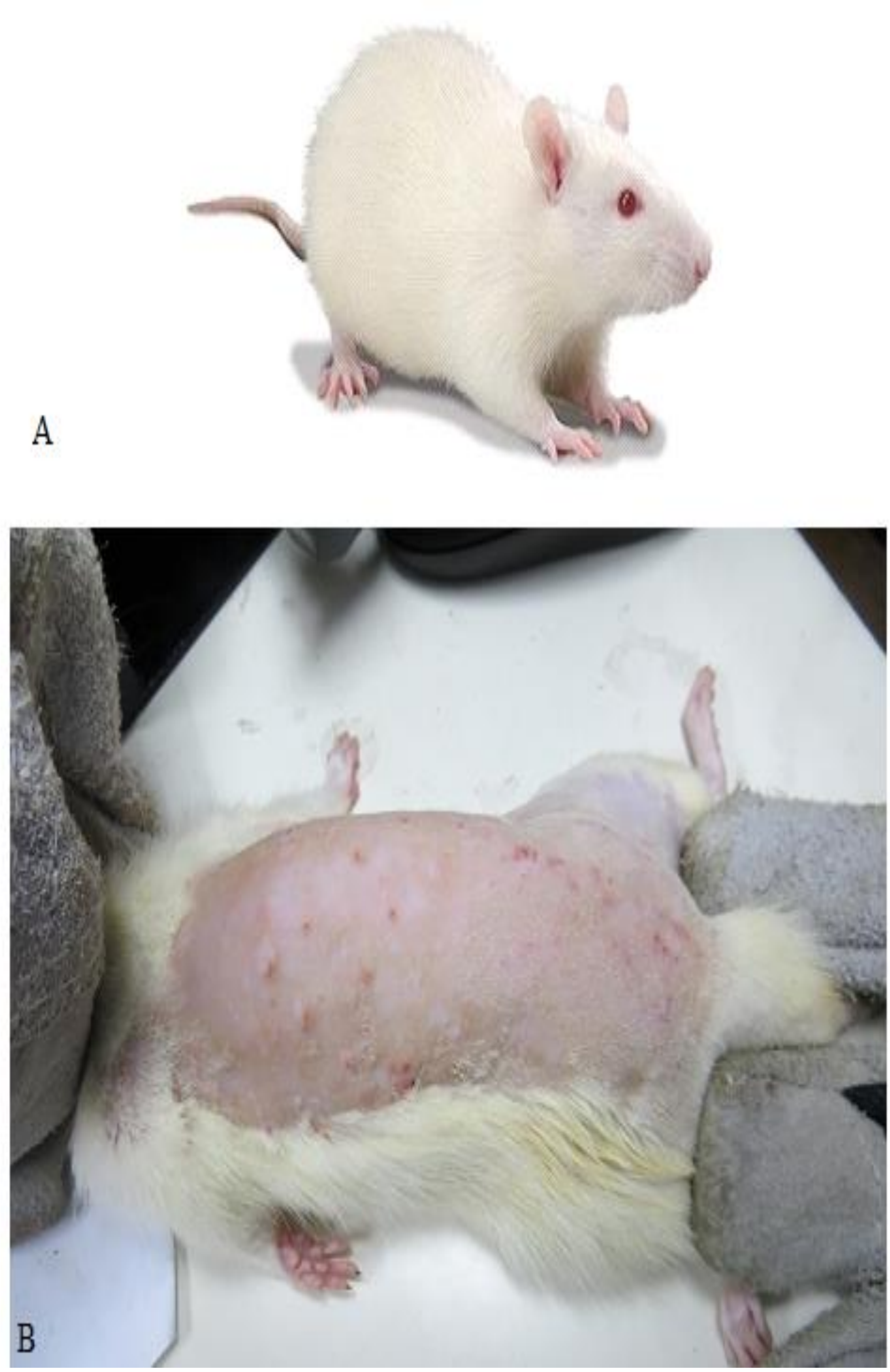

Figure 2-(A) shows the Sprague Dawley rat, (B) shaving rat

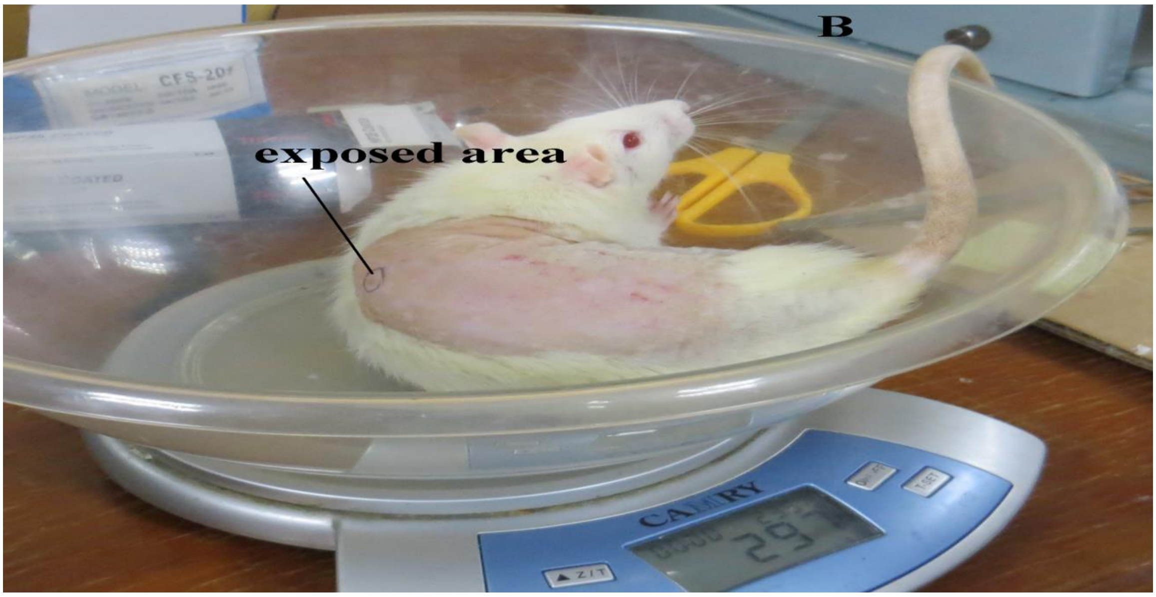

Fig. 3 -the weight process after laser irradiation.

flashlamp, with typical pump energy of up to 100 J and a repetition rate of 1-12 Hz, housed in a single diffuse cavity LMI 1610. The 39.5 cm-long optical cavity was formed by two flat dielectric mirrors M1, M2 with reflectivity's of 85% and 100%, respectively.

3.5.2 The Carbon Dioxide (CO_2) Laser.

The CO_2 laser, manufactured by COLE Technology Co. Ltd from China, was used. The laser was modulated into a pulsed continued laser as shown in Figure 5. The power supply is designed for 80 Watt Laser products with wavelength from 800-10600 nm. It's suitable for the laser apply un engraving, cutting or medication equipment. Power is controllable from TTL (20 K Hz or an analog signal 0-5 V).output is controllable in the of continually or in pulse, operating in a single pulse with duration of 1 ms per pulse. The beam spot size was 3.5 mm.

Measurement Equipments and Other Components

In this study, there is some equipment required for measurement process. The measurement equipment used this study were power/ energy meter, beam profiler and

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oscilloscope. The specifications of measurement equipment will be describe in the following sections

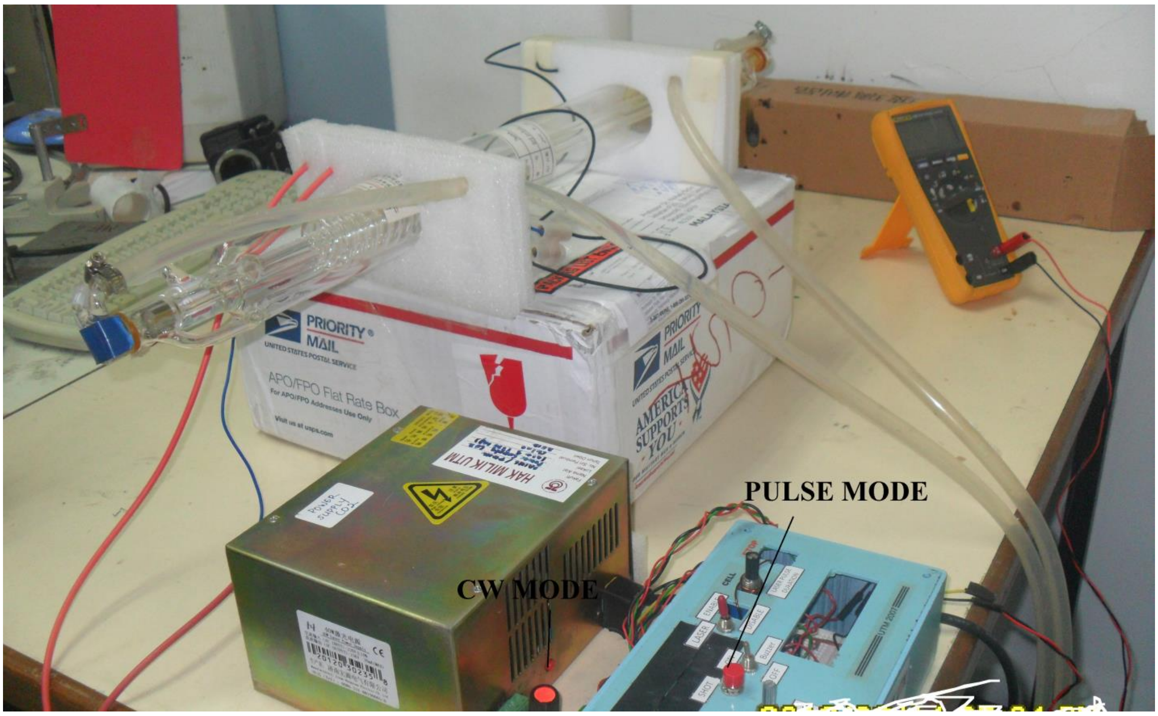

Fig. 4- shows the CO_2 laser setup

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The CO_2 Laser Power Meter

One of the most important parameter measurements for a laser is the output power and energy. In this present work, the CO2 laser energy is measured by a Power Wizard™ 250 which has a high sensitivity to ensure maximum laser performance power. This device can measure the output power of a CO_2 laser from 1-250 W, which it displays on an LCD screen. The CO_2 laser is set to pulse laser setting and fired on to a power meter, which displays the output power on-screen.

Beam Profiler

Figure 3.9 shows OphirBeamstar CCD beam profiler which was used to capture laser beam cross sectional intensity distributions. The spectral range this beam profiler is 350 nm to 1100 nm due to silicon based CCD sensor used. The beam profiler capable of measuring both continuous and pulsed laser output beam profile. Maximum beam size

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limit of the beam profiler is 4.7mm×3.6mm (width × height) in dimension. By optimizing included filter, built in gain and shutter speed, one can capture beam profile of beam with microwatt to watts power range. The CCD sensor recessed from front surface of the beam profiler by 4.5 mm. The beam profile connected to computer through PCI card and captured beam profile will be displayed in Beamstar software.

Tensile Test

. The rats were then killed three hours after irradiation according to international rules of animal care. The samples were cut out with a specific mould which has the same geometric dimension of 7mm in length, including the 1 cm clamp allowance at both ends to be exactly 5 cm between the clamps in length and 2mm in width. To get similar dimensions for all harvested specimens from the rat skins, we flattened them on a surface with 0.4N deed weights. The dimensions were perpendicular to the direction of the preload. The thickness of all samples were then measured with a digital caliper and taken at three different locations before getting the mean value. There was a slight but nevertheless significant effect in thickness with increasing laser pulses. The rats used were about the same age of 8 weeks to ensure the thickness of

Fig. 5: Ophir beamstar CCD beam profiler

their skin were similar. The skin strip was placed between the two clamps of the tensiometer, which was a universal Testing Unite (USA) model and the clamps were secured to avoid any slippage of the sample. Subcutaneous tissue was scarified near both clamps. These samples were used directly for the tensile test at a loading rate of 30mm/min and sampling until they broke.

Result and Discussion

As shown in figure7 , we can clearly observe that, the skin tissues which were irradiated with both Er:YAG and CO2 lasers more elasticity than skin tissues that were irradiated with either the Er:YAG laser and CO2 laser. The samples that were irradiated with ten pulses of the Er:YAG laser followed by one pulse of CO2 laser has stretch factor of 13.065 MPa, which is considered as a high value of stress stretch. The lowest value obtained for the samples that was irradiated by the dual laser system

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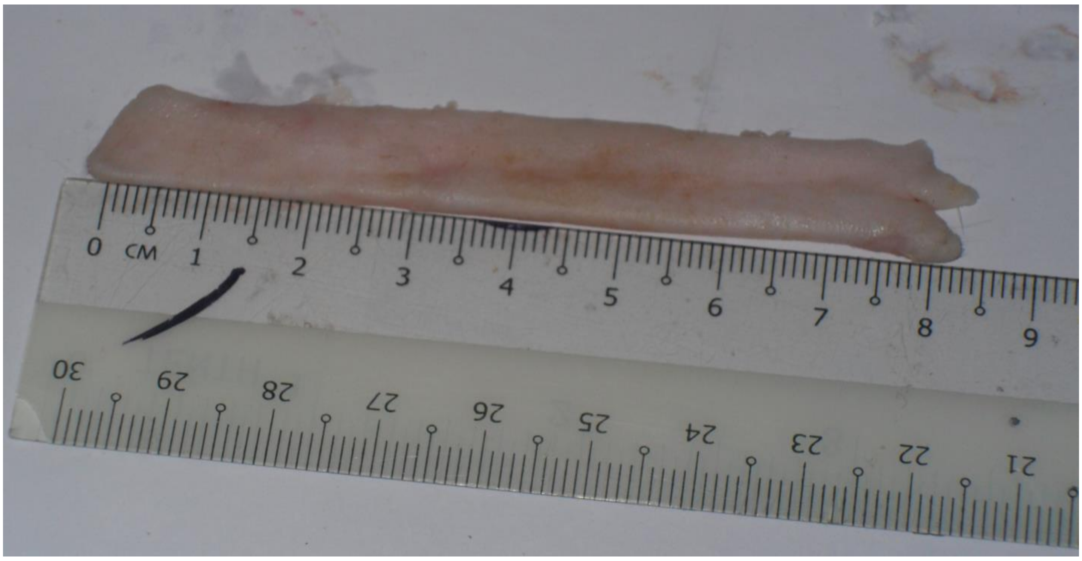
was 7.227 MPa, which is higher than the values that were obtained from the samples irradiated by Er:YAD laser and the CO2 laser alone , which had stretch factors of 1.569 MPa and 1.003 MPa respectively.



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The laser light interacts with tissue in four different ways, transmission, reflection, absorption and scattering. Transmission refers to the passing of the laser light through tissue without any changes to tissue and light properties itself. When the light is exposed to the tissue it is either scattered or absorbed. Scattering is the process involve the light change its direction through the tissue without change its wavelength due to the heterogeneous structure of the tissue. The new direction of the beam depends on the size and shape of particles in the tissue as well as the wavelength of the laser. Scattering distributes the light within the tissue, resulting in radiation of a larger area of tissue than expected, which leads to limited penetration because the light can move forward and backward. The amount of scattered light depends on the wavelength which is considered as an important factor to determine how the light interacts with tissue [5].

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Reflection is when light returns off the surface with no penetration or interaction with the tissue at all. Reflection is in common an unrequited effect in laser-tissue interactions, but in some cases is useful such as when the Erbium laser is reflected off titanium that to safety trimming of gingiva around implants abutments. In general, approximately 4% to 7% of laser light is reflected off the skin. The reflection of light increases as the angle of accidence increases and decreases when the laser light is directed perpendicular to the tissue [6]. If there is a lot of reflected light then damage to unwanted targets can be occurred, for example, the CO_2 laser with a wavelength of 10,600 nm can cause damage to the cornea because of its water contents, whereas a reflected 595 nm pulsed dye laser can cause damage to the retina because of its pigment contents. When the laser is exposed to the tissue, some of the light, is reflected (R) and this value of reflected light is given by the nature of the exposed tissue. This reflected value is affected by the light wavelength, λ, for transparent media.Reflection of the laser at the surface of the skin varies with pigmentation and important only at visible and near infrared spectrum [7]. Absorption is an important mechanism, if the light isn’t absorbed by tissue, there is no energy transfer through tissue, and thus there will be no effect on the tissue. The absorption of photons may change the electron structure of molecules so that the electrons in the outer levels will jump to a higher energy level. The light absorbed by different components of tissue is called chromospheres. When dual laser applied which lead to High laser light with short pulse duration has enough energy to separate the electrons from atoms and

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Fig. 6 shows the sample held in the tensile test machine.

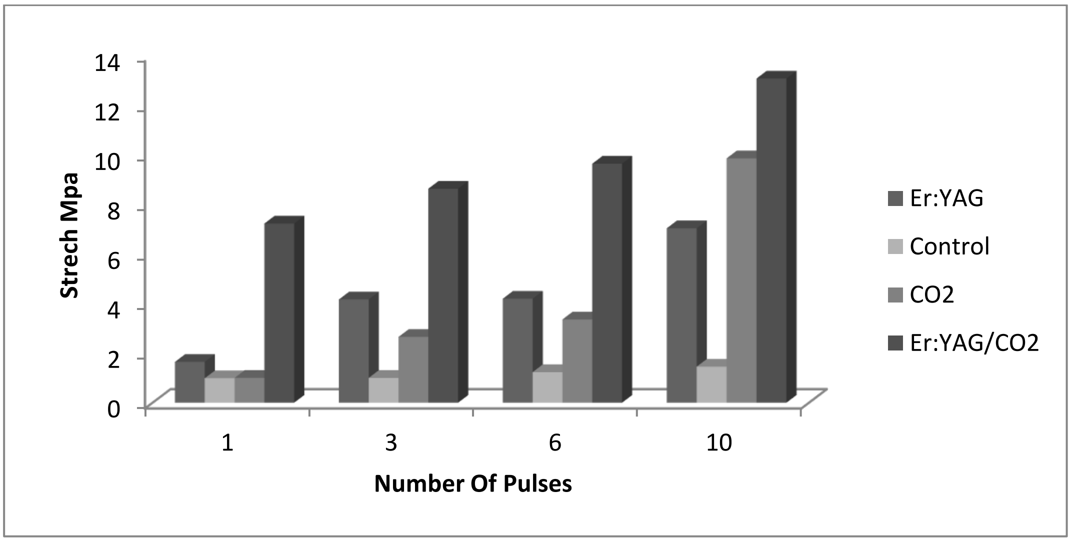

Fig.7: the tensile test of rat skin taken after three sessions of laser treatment

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molecules, ionizing them to induce secondary mechanical effect. Near infrared lasers with a short pulse can disrupt the tissue by using a small spot size and extremely short duration. High laser irradiation leads to ionization of the matter in a small volume of space, generating plasma. Once the plasma forms, the light absorbs or scatters, thereby shielding underlying tissue from photons arriving later in the light pulse. Secondly, faster expansion generates an acoustic wave that tears tissue adjacent to the target. Other disruption appears because of the stress presented in the target when the slot laser is applied which lead to the starch of irradiated skin will be more than control skin.

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