

## Tendon Repair by Suturing Vs Carbon Dioxide Laser Welding: An in-Vivo Study in Rabbits

TAREK F. EL-WAKIL, M.D.\*; EL-KHARBOTLY A., M.D.\*\* and HOSSAM HUSSEIN, M.D.\*\*\*

*The Departments of General Surgery\*, Otolaryngology\*\*, National Institute of Laser Enhanced Sciences and Pathology Department\*\*\*, Faculty of Medicine, Cairo University.*

### ABSTRACT

Tendon injury is a clinical problem, which requires a repair followed by an early postoperative mobilization. Optimum tendon repair requires re-establishment of a normal smoothly gliding tendon. Laser could be one of the new technique used for tendon repair, which theoretically initiates a thermal bonding to induce a strong repair. The aim of the present study is to evaluate carbon dioxide (CO<sub>2</sub>) laser tendon welding in relation to the suturing repair from the histopathological and functional points of view. Thirty Achilles tendons of thirty mature adults Raex rabbits were randomly divided into two equal treatment groups. Group (A), was the sutured tendons and group (B) was the CO<sub>2</sub> laser welded tendons. Three weeks latter, the repairs were evaluated by light microscopic, scanning electron microscopic and biomechanical evaluating parameters. Ten opposite Achilles tendons of the normal uninjured side were served as a control group for biomechanical evaluations. It was found that suturing technique had superior results when it is compared to CO<sub>2</sub> laser welding for tendon repair at all the evaluating parameters. However, the presence of dense fibroproliferative changes with suturing repair may be the cause of the following dense adhesions. With further refinement of the technology and technique of the laser welding, it may become a useful tool in different reconstructive surgeries.

### INTRODUCTION

The photothermal effect of laser is now being applied for many clinical purposes. It is used to coagulate, cut and to ablate tissues with different reported success rates [1]. There are many factors that influence the effect of laser energy on the target tissues. These include the type and wavelength of laser used, how that particular wavelength of energy can affect the tissues upon which it strikes and the laser power to which the target tissue is exposed [2,3].

As the experience with the applications of

lasers in medicine widened, many researchers began to explore whether laser energy especially at low powers could be sufficiently controlled to eliminate the areas of coagulation and vaporization for precise and accurate tissue effect with the least possible side effects. An early work [4], reported the successful sealing effect of laser welding by the use of Neodymium: Yttrium Aluminum Garnette (Nd:YAG) laser (wavelength of 1064 nm) for the closure of arteriotomies and venotomies. Subsequent investigators [5] have shown the same beneficial effects to make fast and watertight vascular anastomoses by the use of CO<sub>2</sub> laser (wavelength 10600 nm) and argon laser (wavelength 514 nm). There were many ongoing studies [6-10], which reported the improving results of laser welding using different lasers at different wavelengths on various target tissues like in urology, gynaecology, gut, nerves and tendons especially with the introduction of a adjuvant protein solder.

Various types of protein solders have been used. These include albumin of different species at different concentrations, fibrin glue, fibrinogen suspension, red blood corpuscles and egg white [11-13]. The amount and concentration of protein solder were found to affect the strength of the formed bond. Excess protein solder blocks the regenerating tissues at the site of the welding and thick protein solder coagulates superficially with weak deeper portions of the bond [6]. Recently, introduction of dyes enhanced the protein solder effectiveness. Many investigators [14,15] have shown further improvements by using dye-enriched protein solder. Dye acts as a chromophore for the laser tissue welding to minimize

the area of tissue damage by minimizing the collateral thermal damage and concentrating the heat generation of laser energy at the solder-tissue interface. Addition of the dye, as well as, the selection of which dye to be used depend on the wavelength of the laser used for welding rather than the type of the target tissue. Moreover, the depth of energy absorption and heat generation and hence the tensile strength of the mould were predominantly determined by the dye concentration. These findings suggest the importance of the bond strength for successful laser welding, however, a new hypothesis has been recently elicited suggesting that laser welding reflects the photothermal coagulation of the cytoplasmic peptides and nucleic acids liberated at the coaptation interface rather than formation of intermolecular collagen bonds [16].

Tendons have a unique pattern as regard the structure and function. Tendons are composed of densely packed highly well organized collagen bundles able to withstand significant tensile strength loads while gliding within their sheaths. Tendon injuries are a common problem requiring a repair followed by early postoperative mobilization. Treatment of the ruptured and lacerated tendons has undergone much advancement especially with the advent of surgical techniques especially with the aid of the surgical microscopy. These techniques could apply precise placement of sutures with lesser tissue damage and subsequent minimal inflammation that were caused by unnecessary manipulation [10]. For better results of tendon repairs, active rather than passive movements are allowed. However, active movements increase the incidence of dehiscence. The golden aim at tendon reconstruction is to establish a permanent repair of the injured tendon, which could withstand significant tensile strength loads and to glide smoothly through the surrounding tissue without any interference against movements [17]. These targets stimulated the prompted investigations of the thermal bonding as a strategy for strengthening tendon repairs, which triggered the investigators to start evaluating the role of laser welding to achieve these goals.

The aim of the present study is to evaluate the effectiveness of tendon repair by CO<sub>2</sub> laser welding in relation to the suturing technique from the histopathological and functional points of view.

## MATERIAL AND METHODS

### *Animal model:*

The present study was carried out at a National Institute of Laser Enhanced Sciences (N.I.L.E.S.), Cairo University. Thirty adult mature Raex rabbits (3.5-4 Kg body weight and age of 6-7 months) were anaesthetized by a single intramuscular injection of Ketamine hydrochloride (35 mg/Kg body weight) and Xylazine hydrochloride (5 mg/Kg body weight). After shaving and scrubbing of the skin, Achilles tendon were exposed unilaterally (n = 30), through a 3 cm incision over the tendon and were isolated from the surrounding fascia (Fig. 1). The tendons were then sharply transected with a scalpel in their midsubstance, about 1 cm from its calcaneal insertion (Fig. 2). Then the tendons were divided randomly into two equal treatment groups, 15 Achilles tendons for each group.

### *Group A: Suturing technique for tendon repair:*

Achilles tendons were immediately repaired using modified Kessler suture technique as described by Kleinert et al. [18]. The sharply cut tendon ends were approximated centrally and repaired by using 4/0 prolene (Ethicon Inc., N.J.) (Fig. 3). The wounds were then irrigated with isotonic saline and finally the skin was closed by 3/0 catgut suture.

### *Group B: CO<sub>2</sub> laser welding technique for tendon repair:*

Achilles tendons were immediately repaired by CO<sub>2</sub> laser (wave length 10600 nm), DEKKA, Italy, mounted on an operating microscope (ZEISS OPMIFC, Germany) using a joy-stick micro-manipulator (Cooper Laser Sonic LS-11) under X 40 magnification. Both tendon ends were welded by a delivered laser beam of 5 pulses, 1.0 second pulse duration, 200 milliwatt power and a spot size of 320 micrometer with addition of 20% human albumin dropwisely as a solder and coagulated by the laser energy. This technique was previously suggested [12] to reach the optimum strength for the liquid protein solder repairs. The cut tendon ends were approximated and held in place with a fine temporary pre-stay suture, which was removed by the end of the welding procedure. The laser was applied until a visual change in colour and consistency of the protein solder, indicating coagulation was achieved (Fig. 4). The helium-neon target spot

of the laser was centered over the tendon edges and CO<sub>2</sub> laser beam was swept over the field. The tendon ends were welded in a circumferential manner. The surface temperature, at which welding was achieved, was measured by Thermocouple (Fluke 52 K/J thermometer). The wounds were then irrigated with isotonic saline and skin was closed by 3/0 catgut suture.

For both treated groups, after wound closure, the operated unilateral hindlimbs were immobilized in polyurethane casts for five days. The rabbits were returned back to their cages and were fed ad libitum with addition of a prophylactic antibiotic to their drinking water. After the 5th postoperative day, the polyurethane casts were removed and free movement within the cages was permitted.

Three weeks later, all animals were anaesthetized again by the same procedure. The operated unilateral Achilles tendons were transected with preservation of the musculotendinous junctions and calcaneal attachments to observe the histo-pathological and biomechanical changes after which the rabbits were sacrificed. At each group, the specimens were randomly assigned for light microscopic, scanning electron microscopic and tensile strength evaluations (n = 5 for each evaluating parameter). On the opposite hind-limbs, ten contralateral normal Achilles tendons were randomly assigned for tensile strength evaluation to serve as a control group to evaluate the biomechanical readings of both treated groups on the 21st postoperative day of the repair.

#### *Evaluating parameters:*

##### *I- Histopathological evaluations:*

###### *A- Light microscopy evaluation:*

Specimens were trimmed and immediately fixed in 4% formaldehyde for 2 days, then were washed by distilled water and left in 70% ethylalcohol overnight at room temperature. Dehydration of the specimens was completed by 96% followed by absolute ethylalcohol for an hour. Subsequently, the specimens were emerged in 1% celloidin methyl benzoate overnight at room temperature then were embedded in paraffin. From each paraffin block, 5 sections of 5 µm thickness were obtained at the longitudinal plane. Sections were subjected to Haematoxylin and Eosin (Hx & E) and examined in a blind manner by using an objective lens of X100 magnification.

By light microscopy, specimens were evaluated as regard the degree of inflammatory reactive changes, as well as, the amount and organization of the fibroproliferative response.

###### *B- Scanning electron microscopy (S.E.M.) evaluation:*

Specimens were cut with a scalpel to expose the solder/tissue interface. They were immediately fixed in 4% gluteraldehyde solution buffered with cacodylate. The specimens were dehydrated in a graded acetone series by the critical point drying process. The specimens were mounted on aluminum stubs with colloidal silver paint, sputtered with gold-palladium and examined in a Jeol JSM 840 A scanning electron microscope (Jeol Datum, Tokyo, Japan) at different magnifications.

By S.E.M., specimens of both treated groups were evaluated for separation of tendon ends or gaps at the site of the repair, organization of the fibroproliferative repair for better alignment of the neo-tendon. Moreover, specimens of the tendon repaired by CO<sub>2</sub> laser welding were evaluated as regard the shape and uniformity of the coagulum, solder attachment at the solder / tissue interface, as well as, the presence of any air vacuole which denotes weak bonding.

##### *II- Biomechanical evaluations:*

The biomechanical evaluations were done by using an Instron machine (model 1011, Instron Corp., Canton, M.A.). Both ends of each specimen were grasped in serrated Instron grips. The specimens were kept moist during this procedure, to avoid tensile strength changes associated with drying. Each tendon was loaded to failure (i.e. till tendon rupture) at a constant Instron crosshead speed of 50 mm/min. All the repaired tendons disrupted at the sites of the repair. Load cell output versus displacement was calculated by a series IX Automated Materials Testing System 6.02. The mechanical properties of each tendon were measured from the force/deformation curve. The evaluating biomechanical parameters were the ultimate tensile strength (Max. Force by Newton, N.), load at break (Newton, N.), extension at break (mm.) and the energy to failure (defined as the area under the curve, Nmm.). The force/deformation curves were digitalized and the energy to failure was calculated by using Sigma Scan (Jandel Scientific, San Rafael, C.A.).

## RESULTS

### I- Histopathological evaluations:

They were done in a blinded manner.

#### A- Light microscopic evaluation:

In group A, those tendons repaired by the suturing technique, Fig. (5) shows a marked dense inflammatory tissue reaction with the presence of foreign body giant cell granulomatous reaction around the suture material across the site of the repair, with very prominent capillary-type vascular proliferation. The fibroproliferative response was great with active plumbly fibroblastic cellular proliferation in an edematous matrix and with evidenced prominent vascular proliferation. The organization was more obvious and of well-organized pattern from active plumbly fibroblastic zones to more mature, less cellular and more collagenous areas. It showed broad bands of collagen from less to more mature fibrous areas with thin well-arrayed bands of collagen and with diminished vascular pattern.

In group B, those tendons repaired by CO<sub>2</sub> laser welding, Fig. (6) shows less marked dense inflammatory reaction with less prominent capillary-type vascular proliferation and with no foreign body giant cell granulomatous reaction. The fibro-proliferative response was minimal with mild plumbly fibroblastic cellular proliferation in a less edematous matrix and with less vascular proliferation. The organization pattern was minimal. The fibroproliferative organization showed less mature fibrous areas with poorly arrayed bands of collagen.

#### B- Scanning electron microscopic (S.E.M.) evaluation:

In group A, those tendons repaired by the suturing technique, Fig. (7) shows well coapted both ends of the repaired tendon with apparently no gap. However, on higher magnification at the site of repair, a gap was reported as shown in Fig. (8). The fibroproliferative regeneration of the neo-tendon showed well-organized pattern of better alignment as shown in Fig. (9).

In group B, those tendons repaired by CO<sub>2</sub> laser welding, Fig. (10) shows a good coagulum regarding its shape and uniformity without any reported air vacuoles. In spite of, protein solder in the liquid form makes the control of its thickness virtually impossible, the coagulated protein solder showed well adherence at the solder/tissue

interface. Creeping regenerating neo-tendon was observed across the area of coagulated protein solder at the site of repair as shown in Fig. (11). The fibroproliferative regenerating neo-tendon showed a less organization pattern with less favorable alignment as shown in Fig. (12). At only one specimen, a detachment of the protein solder from the underlying tissue substance was found, with evidence of thermal damage of the underlying tissue as shown in Fig. (13).

### II- Biomechanical evaluation:

Regarding the ultimate tensile strength or the maximum force by Newton (N), on the 21st postoperative day of the repair, both modes of repair resulted into tensile failure at consistently lower levels than those required for the opposite normal uninjured tendons. The mean ultimate tensile strength or the max. force of the control group was 312.57 N with a standard deviation of 18.06 N. However, the ultimate tensile strength for the sutured and CO<sub>2</sub> laser welded tendons were 235.56±24.21 N and 192.62±6.39 N respectively. These represent 75.36% and 61.63% respectively of the control group values.

Regarding load at break, on the 21st postoperative day of the repair, the mean value of the control group was 67.28 N with a standard deviation of 7.56 N. All the experimentally treated tendons of both groups displayed lower values as compared to the normal control group. The load at break for the sutured and CO<sub>2</sub> laser welded tendons were 37.69±6.01 N and 29.45±0.46 N respectively. These represent 56.02% and 43.77% respectively of the control group values.

Regarding the extension at break, on the 21st postoperative day of the repair, the control group had a mean of 16.74 mm with a standard deviation of 4.39 mm. The extensions at break for the sutured and CO<sub>2</sub> laser welded tendons were 16.22±3.41 mm and 11.86±1.73 mm respectively. These represent 96.89% and 70.85% respectively of control group values.

Regarding the energy to failure, on the 21st postoperative day of the repair, the control group had a mean of 2881.1 Nmm with a standard deviation of 481.61 Nmm with lower energies to failure values for the experimentally treated tendons. The energies to failure of the sutured and CO<sub>2</sub> laser welded tendons were 2368.2±227.79 Nmm and 1186±254.65 Nmm respective-

ly. These represent 82.2% and 41.17% respectively of the control group values. The overall biomechanical readings are summarized in Table (1). Figs. (14,15 & 16) show the force / de-

mation curves of the biomechanical evaluation of specimens of the normal contralateral tendon, sutured and CO<sub>2</sub> laser welded tendon repairs respectively.



Fig. (1): Dissected Achilles tendon.

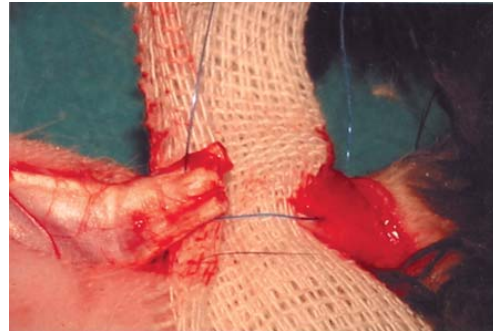


Fig. (2): Achilles tendon was sharply transected at its midsubstance and a fine temporary pre-stay suture approximated both ends.



Fig. (3): At the end of the suturing technique. Note: arrowheads mark the site of the repair.

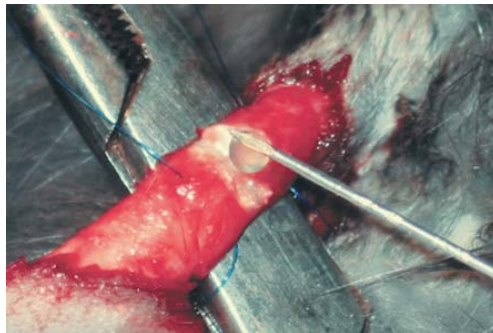


Fig. (4): CO<sub>2</sub> laser welding technique almost at its end. Note: The albumin solder was applied dropwisely at the site of repair and coagulated by the laser energy as was denoted by its whitish discolouration. The fine temporary pre-stay suture is still present to approximate both ends of the cut tendon till the end of CO<sub>2</sub> laser welding procedure when it is removed.

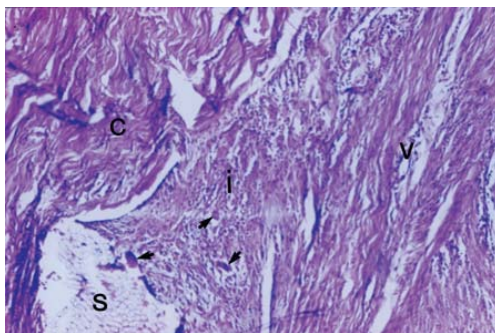


Fig. (5): Light microscopic examination of a specimen of group A, those tendons repaired by the suturing technique, (H & E, Mag. X 100). It shows a remnant suture material (s), with multiple giant cell reaction (arrowheads) and dense inflammatory reaction (i). Moreover, prominent vascular proliferation (v) and well arrayed bands of collagen (c) were elicited.

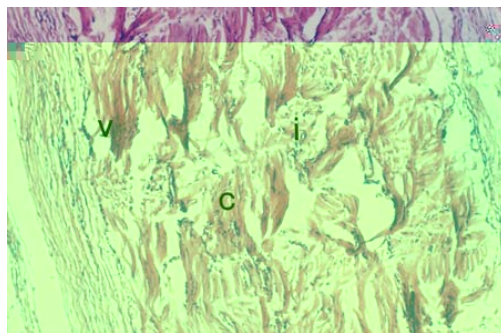


Fig. (6): Light microscopic examination of specimen of group B, those tendons repaired by CO<sub>2</sub> laser welding technique (H & E, Mag. X 100). It shows less inflammatory reaction (i) with less prominent vascular proliferation (v) and poorly arrayed bands of collagen (c).



Fig. (7): S.E.M. examination of specimen of group A, those tendons repaired by the suturing technique (Mag. X 270). It shows well coapted both ends of the cut tendon at the site of repair as was marked by arrowheads.

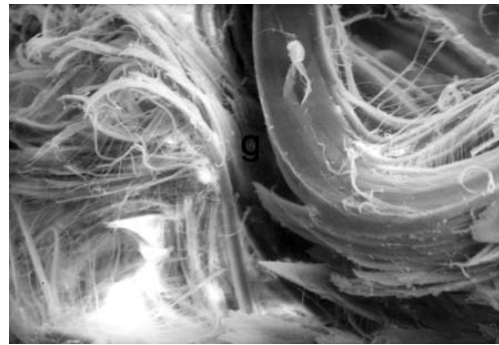


Fig. (8): S.E.M. examination of specimen of group A, those tendons repaired by the suturing technique (Mag. X 480). It shows the site of the repair at a higher magnification with an obvious gap (g).



Fig. (9): S.E.M. examination of specimen of group A, those tendons repaired by the suturing technique (Mag. X 380). It shows well-organized pattern and good alignment of the regenerating neo-tendon.

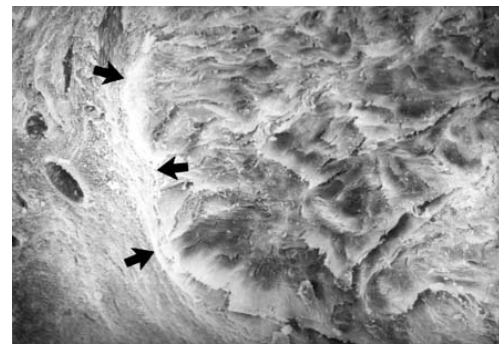


Fig. (10): S.E.M. examination of specimen of group B, those tendons repaired by CO<sub>2</sub> laser welding technique (Mag. X 130). It shows adherent good coagulum as regard its shape and uniformity. Note: arrowheads mark the solder/tissue interface.

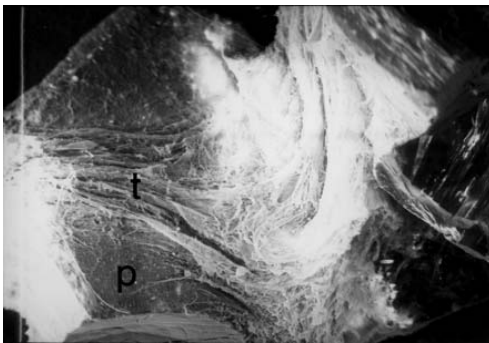


Fig. (11): S.E.M. examination of specimen of group B, those tendons repaired by CO<sub>2</sub> laser welding technique (Mag. X 85). It shows creeping regenerating neo-tendon (t) from both ends of the cut tendon across the area of coagulated protein solder (p).

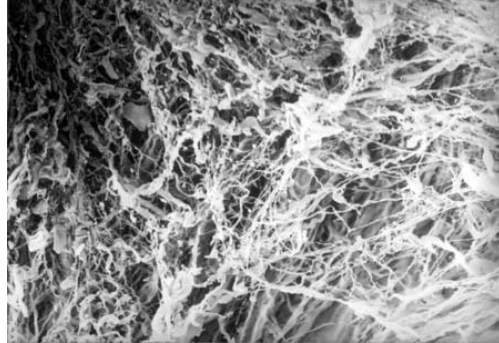


Fig. (12): S.E.M. examination of specimen of group B, those tendons repaired by CO<sub>2</sub> laser welding technique (Mag. X 380). It shows less organized pattern of the regenerating neo-tendon.

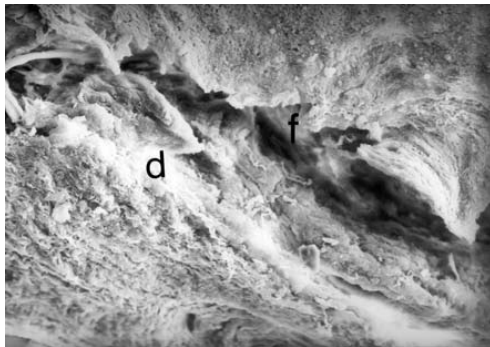


Fig. (13): S.E.M. examination of specimen of group B, those tendons repaired by CO<sub>2</sub> laser welding technique (Mag. X 160). It shows a detachment of the protein solder at the solder/tissue interface (f) with evidenced thermal damage (d) of the underlying tissue.

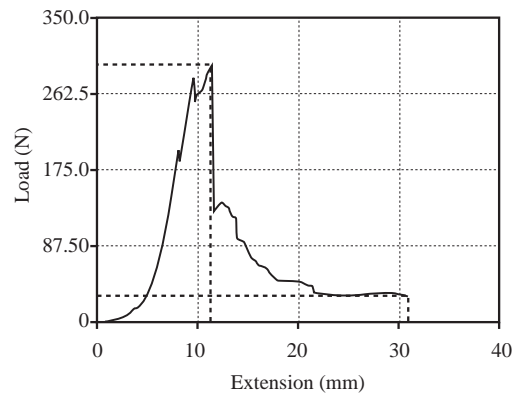


Fig. (14): Force/deformation curve of biomechanical study of a specimen of normal contralateral uninjured Achilles tendon.

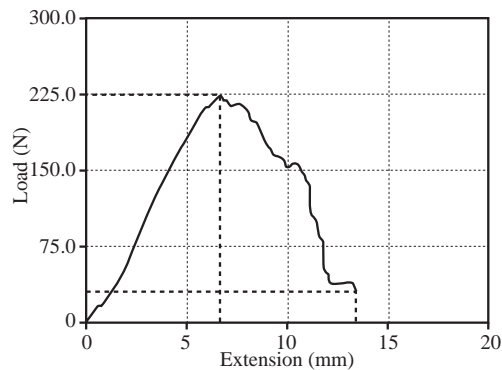


Fig. (15): Force/deformation curve of biomechanical study of a specimen of group A, tendon repair by suturing technique.

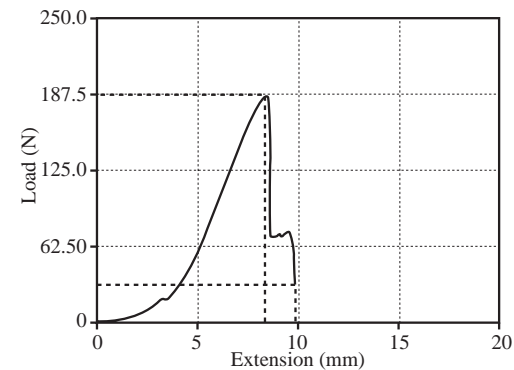


Fig. (16): Force/deformation curve of biomechanical study of a specimen of group B, tendon repair by CO<sub>2</sub> laser welding technique.

Table (1): The biomechanical results. All the data were calculated into means ± standard deviations.

Treatment groups	Ultimate tensile strength (N)	Load at break (N)	Extension at break (mm)	Energy to failure (Nmm)
Normal control	312.57±18.06	67.28±7.56	16.74±4.39	2881.1±481.61
Sutured	235.56±24.21	37.69±6.01	16.22±3.41	2368.2±227.79
CO <sub>2</sub> laser welding	192.62±6.39	29.45±0.46	11.86±1.73	1186±254.65

### DISCUSSION

Lasers have been applied for welding of a variety of tissues with different reported success rates. Laser welding has theoretically many advantages over the suturing repair. Laser welding produces less degrees of both acute and chronic inflammatory response [5]. However, suture materials produce a marked foreign body reaction, which may interfere against the natural healing process and promote for the following dense adhesions.

The exact mechanism by which CO<sub>2</sub> laser energy can influence tissue welding process is not clear. There is growing evidence that supports the theory of thermal remodeling of tissue proteins by laser energy [2,3]. Recently, the influence of CO<sub>2</sub> laser energy upon external protein solder has been established [16]. Albumin as protein solder for laser welding was found to coagulate completely at 70°C [13,19].

Regarding the histopathological evaluation in the present study, the light microscopic anal-

ysis showed a greater inflammatory response among specimens of group A, those tendons repaired by the suturing technique, than those repaired by CO<sub>2</sub> laser welding in group B. This observation could be attributed to the suture material itself. This, in addition to the observed marked fibroproliferative response among group A, may be unfortunately the cause of the following dense adhesions among this group. These findings in the present study differ from those previously cited [10] that CO<sub>2</sub> laser welding of tendons expressed a more marked inflammatory reaction. They explained this observation by the thermal injury caused by laser itself. This is not the case in the present study, particularly due to the monitoring of the surface temperature at which welding was achieved by thermocouple. Moreover, the crush injury away from the site of repair, which was previously reported [5,10], could be overcome in the present study. This was achieved by the application of a fine temporary pre-stay suture rather than vascular clamps to establish withstand close approximation of both ends of the injured tendon during laser welding repair. This pre-stay suture was then removed at the end of the CO<sub>2</sub> laser welding procedure.

Regarding S.E.M. evaluation, superior favorable results of suture technique for tendon repair among group A were observed at many aspects. These include better coaptation, fibroproliferative organization, as well as, better alignment of the regenerating neo-tendon. These favorable results with the suturing technique over CO<sub>2</sub> laser welding repairs were in spite of formation of a good coagulum of protein solder as regard its shape and uniformity among group B of CO<sub>2</sub> laser welding technique. However, the liquid nature of the protein solder makes the control of its thickness virtually impossible as was previously reported [12,15]. For this reason and to increase the strength of the liquid protein solder bond, it is recommended to apply the liquid protein solder in the form of small drops and are fully coagulated dropwisely. This technique was previously shown to reach the optimum strength of the protein solder bond [12].

Regarding the biomechanical evaluation, those tendons repaired by the suturing technique at group A, showed better biomechanical properties than those repaired by CO<sub>2</sub> laser welding technique. These findings were previously re-

ported [10] but in different values in relation to the normal control group. These biomechanical findings in the present study could be explained on a histopathological basis. The better biomechanical findings among group A, those tendons repaired by suturing technique, could be due to the better organization of the fibroproliferative response, better alignment of the neo-tendon, as well as, by the marked fibroproliferative response collectively. However, the relatively acceptable ultimate tensile strength and load at break readings of specimens of group B (81.77% and 78.14% of those readings in group A respectively), could make CO<sub>2</sub> laser welding a promising technique for tendon repair especially with the observed minimal inflammatory response with laser welding.

Globally, the ultrastructural and functional observations with CO<sub>2</sub> laser welding technique for tendon repair in the present study suggest the simple thermal denaturation theory, which agree with what are previously cited [10,16,17]. The results of the present study suggest that when successful tissue welding has been observed, the mechanism is unlikely to be due to formation of intermolecular collagen bonds only as was hypothesized. This could be explained by the reported successful welding of cell-rich tissues such as bowel, vessels and nerves and at the same time could explain the observed relatively less success rate of laser welding in collagen-rich tissues of relatively hypocellular structure like tendons and cartilages.

#### *Conclusion:*

Tendon repair by CO<sub>2</sub> laser welding technique is possible. However, the favorable results of the suturing technique for tendon repair at both histo-pathological and biomechanical evaluations make the suturing technique is still superior for tendon repair. However, we feel that with further refinement of the technology and technique of laser welding, it may become a useful adjunct in many reconstructive surgeries.

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