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An in-Vivo Experimental Evaluation of He-Ne Laser Photostimulation in Healing Achilles Tendons

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ABSTRACT

Tendons must glide smoothly and withstand significant tensile strength loads. No method of treatment for tendon injuries has proved to accelerate the rate, or improve the healing quality. Non-invasive laser has gained a considerable attention for enhancing tissue repair. However, there is still a controversy regarding the effectiveness of laser photostimulation for improvement of healing process of surgically repaired tendons. Accordingly, the present study was conducted to evaluate the role of He-Ne laser photostimulation on the process of tendon healing. Thirty unilateral Achilles tendons of 30 Raex rabbits were transected and immediately repaired. They were divided into two equal groups: Group (A), was subjected to He-Ne laser photostimulation, while group (B), served as a control group. Two weeks later, the repaired Achilles tendons were histopathologically and biomechanically evaluated. The results of the present study indicate the favorable beneficial effects of He-Ne laser photostimulation during the healing process after tendon repair for better functional outcome. However, the favorable results of the qualitative evaluation of the newly synthesized collagen of the regenerating neotendon at the present study makes this subject a suitable topic for further quantitative evaluation.

INTRODUCTION

Tendons have unique structure and function able to withstand significant tensile strength loads. Tendon injury is a problem that requires a repair followed by an early mobilization. However, it actually heals slowly and frequently leaves scar tissue **[1,2]**. The golden aim at tendon repair is to establish a permanent repair that could withstand significant tensile strength loads and to glide smoothly without any interference against movement. However, no method of treatment has proved to accelerate the rate, or improve the healing quality **[3]**.

Non-invasive laser has gained a considerable attention for enhancing tissue repair at a wide spectrum of applications. These included the use of low level laser photostimulation for wound healing, musculoskeletal complications, and pain **[4-7]**.

Although the beneficial effects of laser photostimulation are now generally accepted, the mechanisms by which laser photostimulation can facilitate tissue healing remain poorly understood **[8,9]**. Previous studies suggested that laser photostimulation increases adenosine tri-phosphate (ATP) synthesis, promotes nucleic acid production and augments cell division **[4,7,10]**. In addition, it has been shown that Helium-Neon (He-Ne) laser (632.8nm) is capable of stimulating matrix collagen production by skin fibroblasts. In-vivo studies on wound healing indicated that laser photostimulation enhances collagen synthesis in wound area resulting into an increased tensile strength. Additionally, elevated procollagen messenger ribonucleic acid (mRNA) levels have been reported in cutaneous wounds after treatment with He-Ne laser **[11,12]**.

However, there is still a controversy regarding the effectiveness of He-Ne laser photostimulation for improvement of healing of surgically repaired tendons **[3,9]**. The mechanism by which laser photostimulation facilitates collagen production in regenerating tendons is not clear. This may be attributed to alteration in gene regulation or modulation of enzymes that are involved in collagen metabolism **[13,14]**. A theory proposed that mitochondria are the photoreceptors for the laser energy. The absorption of laser energy by respiratory chain may cause an oxidation of dihydronicotinamide adenine dinucleotide (NADH), producing changes in rodox status in both mitochondria and cytoplasm. Activation of electron transport chain results in an increase in the electrical potential across mitochondrial membrane, an increase in ATP pool, and activation of nucleic acid synthesis **[9]**.

On the basis of in-vitro studies proposed that laser energy at certain frequencies can modulate cell proliferation and release of growth factors from fibroblasts. Therefore, the positive effects of laser biostimulation on enhancing tendon healing may involve the enhancement of growth factor release, which in turn, promotes extracellular matrix production and degradation [15,16].

Apart from the mechanism by which laser photostimulation can affect tendon healing, it was found of interest to study in the present work the role of He-Ne laser photostimulation on healing of surgically repaired Achilles tendons through histopathological and biomechanical evaluations.

MATERIAL AND METHODS

Animal Model:

Thirty Raex rabbits (body weight, 3.5-4.0kg and age 6-7 months) were anaesthetized by a single intramuscular injection of Ketamine hydrochloride (35mg/kg body weight) and Xylazine hydrochloride (5mg/kg body weight). Of each rabbit, unilateral Achilles tendon was exposed and isolated, after shaving and scrubbing of the skin, through about 3cm incision at hindlimb (Fig. 1). The tendon was then sharply transected with a scalpel at its mid-substance, about 1cm from its calcaneal insertion (Fig. 2). The ends of the severed Achilles tendon were approximated and immediately repaired by 4/0 prolene (Ethicon Inc., N.J.) using modified Kessler suture technique (Fig. 3) as described by Kleinert et al. [17]. The wound was then irrigated with isotonic saline and finally the skin was closed by 3/0 simple interrupted catgut sutures. Afterward, the operated unilateral hindlimbs were immobilized using custom designed premolded polyurethane casts taking into consideration to split the casts over the wounds making windows for application of laser photostimulation at the proposed experimental group. Achilles tendons were randomly assigned into two equal groups, 15 repaired Achilles tendons for each group; He-Ne laser photostimulated tendons group (A) and control group (B). All rabbits were returned back to cages and were fed ad libitum with addition of a prophylactic antibiotic to their drinking water. On the 5th postoperative day, all immobilization casts were removed and free movements of the animals within their cages were permitted.

He-Ne Laser-Photostimulation:

Experimental Group (A) was subjected to laser photostimulation using He-Ne laser (632.8nm, level laser systems, M300), CW mode, at 1 Joule/cm^2. Laser photostimulation was carried out on a daily basis, transcutaneously by a computerized scanning software through the preformed windows at the polyurethane casts starting from the 1st-5th postoperative day then continuing after removal of the casts for up to the 14th postoperative day (Fig. 4).

Two weeks after tendon repair, each rabbit was anaesthetized again as was previously described. Following wound's re-exploration, the operated unilateral Achilles tendon was transected below its musculotendinous junction and above its calcaneal attachment after which the animal was sacrificed. At each group, the excised neotendons (n=15) were randomly assigned for light microscopic, scanning electron microscopic, and biomechanical evaluations (n=5 Achilles tendons for each evaluating parameter). In addition, 10 random contralateral normal Achilles tendons were assigned for biomechanical evaluations to serve as normal reference.

Evaluating Parameters:

I- *Histopathological Evaluations:*

A- *Light Microscopy:*

Specimens were trimmed and immediately fixed in 10% formaldehyde for 2 days, then washed by distilled water and left in 70% ethylalcohol overnight at room temperature. Dehydration of specimens was started by 96% ethylalcohol followed by complete dehydration by the use of absolute ethylalcohol for an hour. Subsequently, specimens were immersed in 1% celloidin methyl benzoate overnight at room temperature then embedded in paraffin. From each paraffin block, 5 sections of 5μm thickness were obtained at the longitudinal plane. They were subjected to Haematoxylin and Eosin (Hx & E) and examined by an objective lens of X 80 magnification.

B- *Scanning Electron Microscopy (S.E.M.):*

Specimens were immediately fixed in 3% glutaraldehyde solution buffered with cacodylate, then dehydrated in graded acetone series. The specimens were mounted on aluminum stubs with colloidal silver paint, sputtered with gold-palladium and examined in a Jeol JSM 840A scanning electron microscope (Jeol Datum, Tokyo, Japan) at different magnifications.

II- *Biomechanical Evaluations:*

Biomechanical evaluations were done using an Instron machine (Model 1011, Instron Corp., Canton, M.A.). Both ends of each specimen were grasped in serrated Instron grips. The specimen was kept moist during the procedure in order 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 50mm/min. Load cell output versus displacement was calculated by a series IX Automated Materials Testing System 6.02. The mechanical properties for each tendon were measured from the force/deformation curve. The evaluated biomechanical parameters included 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, N.mm.). The force/deformation curves were digitalized and the energy to failure was calculated using Sigma Scan (Jandel Scientific, San Rafael, C.A.).

RESULTS

I- *Histopathological Results:*

A- *Light Microscopy:*

Regarding He-Ne photostimulated tendons, group (A), Fig. (5) shows well-organized fibroproliferative changes with a properly aligned pattern of collagen bands. Moreover, less marked inflammatory tissue reaction could be elicited without prominent signs of inflammatory reaction.

However, regarding control group (B), Fig. (6) shows less organized fibro-proliferative changes with poorly aligned collagen bands. A marked inflammatory tissue reaction with an obvious foreign body granulomatous reaction could be elicited.

B- *Scanning Electron Microscopy (S.E.M.):*

Regarding He-Ne photostimulated tendons, group (A), Fig. (7) shows a more favorable organization pattern of the regenerating neotendons with an obvious well-aligned pattern of collagen bands.

However, regarding control group (B), Fig. (8) shows a bizarre-arrangement of the regenerating neotendons that appear at a less favorable pattern of organization with a prominent poorly-aligned pattern of collagen bands and fibrin threads.

II- *Biomechanical Results:*

Firstly, the site of tendon disruption was constantly found at the site of the repair in both groups. Both groups (A and B) expressed lower values for all biomechanical parameters when compared to those of the normal contralateral uninjured tendons.

Figs. (9-11) show the Force/Deformation Curves of the normal contralateral uninjured tendons, He-Ne photostimulated tendons group (A), and the control group (B) respectively. Moreover, Table (1) summarizes collectively the results of the biomechanical parameters of both experiment groups (A and B), as well as, of the normal contralateral tendons.

DISCUSSION

Tendon injury is a problem that requires a repair. Tendons must withstand significant tensile strength loads and glide smoothly without any interference against movement. However, no method of treatment for tendon injuries has proved to accelerate the rate, or improve the healing quality [1-3]. Non-invasive laser has gained a considerable attention for enhancing tissue repair at a wide spectrum of applications. However, there is still a controversy regarding the effectiveness of He-Ne laser photostimulation for improvement of healing of surgically repaired tendons [3,9].

From the results obtained from the present study, it can be seen that treatment with a He-Ne laser after surgically repaired Achilles tendons had favorable beneficial effects as regard the collagen production and its quality at the regenerating neotendon.

Table (1): The biomechanical evaluations for the experiment groups (A and B) and for the normal contralateral tendons. Readings are presented in the form of means ± standard deviations.

	Normal contralateral tendons	He-Ne laser photostimulation group (A)	Control group (B)
Ultimate tensile strength (N)	367.21±23.17	323.88±27.55	263.03±14.82
Load at break (N)	74.03±6.95	63.61±6.12	49.16±4.33
Extension at break (mm)	17.11±5.66	16.23±2.76	14.21±3.27
Energy to failure (N.mm)	3041.02±411.07	2711.68±209.49	2314.82±194.37

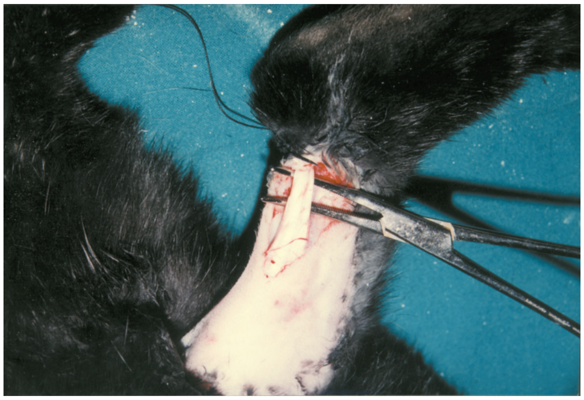

Fig. (1): Achilles tendon is exposed and isolated.

Fig. (2): Achilles tendon is sharply transected at its midsubstance.

Fig. (3): Achilles tendon ends are approximated and immediately repaired.

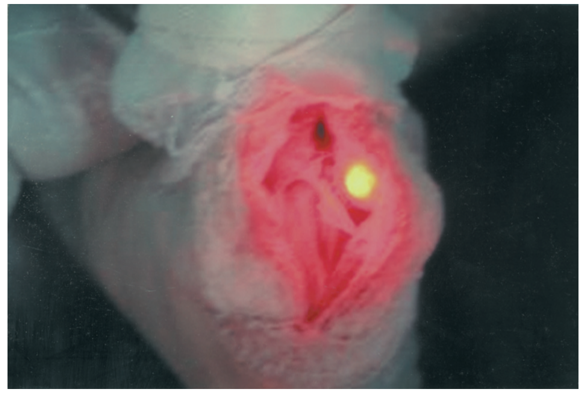

Fig. (4): He-Ne laser-photostimulation.

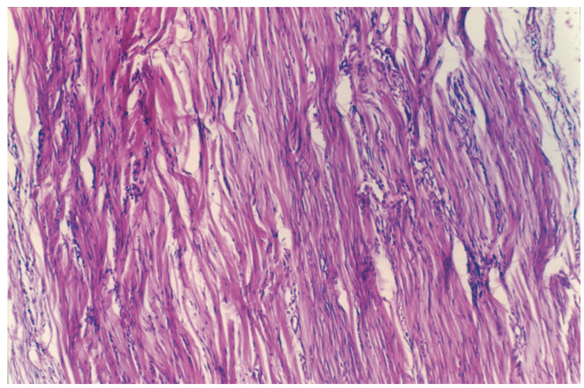

Fig. (5): Light microscopic findings of He-Ne laser-photostimulated tendons group (A). It shows well-organized fibroproliferative changes with a properly aligned pattern of collagen bands. Mild inflammatory tissue reaction could be elicited without prominent foreign body granulomatous reaction.

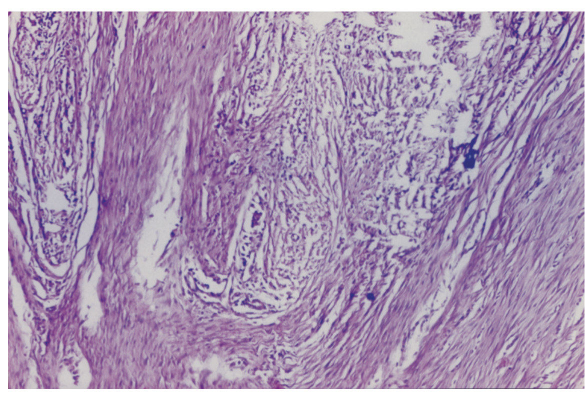

Fig. (6): Light microscopic findings of control group (B). It shows less-organized fibroproliferative changes with poorly aligned collagen bands. Marked inflammatory tissue reaction with an obvious foreign body granulomatous reaction could be elicited.

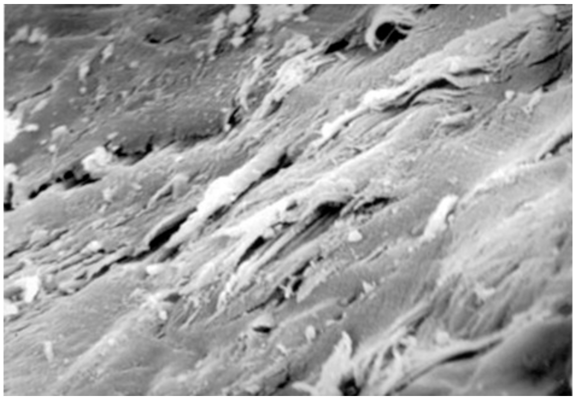

Fig. (7): S.E.M. findings of He-Ne laser-photostimulated tendons group (A). It shows more favorable organization pattern of the regenerating neotendons with an obvious well-aligned pattern of collagen bands.

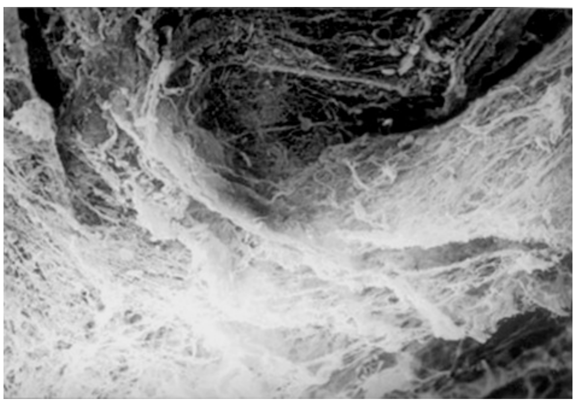

Fig. (8): S.E.M. findings of control group (B). It shows less favorable pattern of organization of the regenerating neotendons with prominent poorly-aligned pattern of collagen bands and fibrin threads.

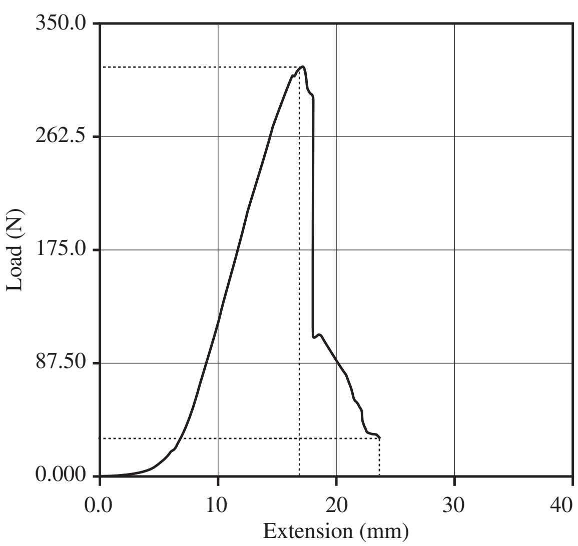

Fig. (9): Force/deformation curve of normal contralateral uninjured tendon.

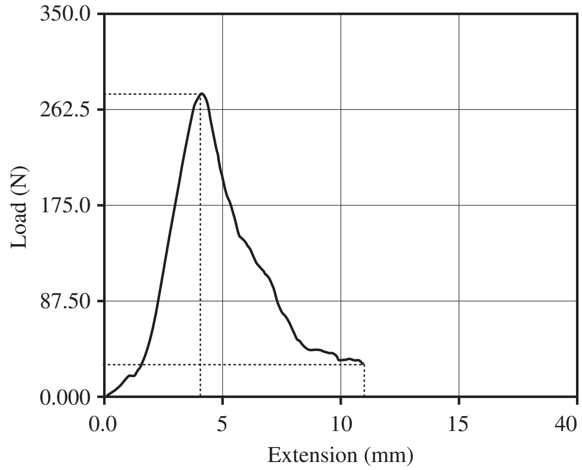

Fig. (10): Force/deformation curve of He-Ne photostimulated tendons group (A).

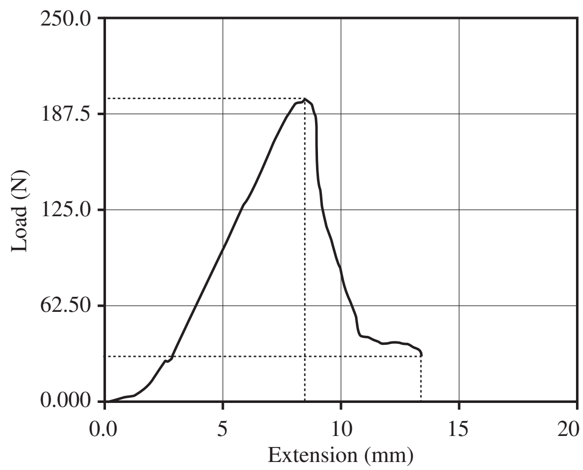

Fig. (11): Force/deformation curve of control group (B).

Regarding the histopathological findings; well organization and proper alignment of the newly synthesized collagen at the regenerating neotendon could explain the better results of the He-Ne laser photostimulation after tendon repair of surgically repaired Achilles tendons among group (A) rather than control group (B) in the present study. These histopathological findings could also explain the favorable biomechanical results among group (A) rather than group (B). Moreover, the observed less prominent inflammatory reactions among group (A) supports the anti-inflammatory role of laser photostimulation that may have a role for facilitation of the free movements of the regenerating neotendon with minimal adhesions for a better functional performance. These findings may support previous studies that proposed the same favorable results of laser treated tendons during tendon healing **[13-16]**.

Regarding the biomechanical evaluation; the ultimate tensile strength (N) among groups (A and B) represented 88.20% and 71.63% respectively when compared to those readings of the normal contralateral uninjured tendons and 85.93% and 66.41% respectively when compared to those readings of the normal contralateral uninjured tendons as regard load at break (N). Moreover, Regarding the extension at break (mm), groups (A and B) represented 94.86% and 83.05% respectively when compared to the normal contralateral uninjured tendons readings and 89.17% and 76.12% respectively when compared to the normal contralateral uninjured tendons readings as regard the energy to failure (N.mm). These better biomechanical results of He-Ne laser photostimulated tendons at group (A) are not surprising. There is an interacting relationship between the histopathological and the biomechanical results in the present study. The former histopathological findings could explain the later biomechanical results that in turn, could ensure these histopathological findings. Once there is well-organized, properly-aligned newly synthesized collagen at the regenerating neotendon, there must be better functional results for the haling process of the repaired tendon **[3]**. These findings could explain the desirable beneficial effects of laser photostimulation for tendon healing from the functional point of view.

Collectively, the present findings may support the previously proposed anti-inflammatory effect of laser photostimulation **[4,5,7]** that may have a useful role during tendon healing for better smooth functional results. Moreover, the results of the present study could support the favorable effects of He-Ne laser photostimulation for tendon healing against the previously reported longstanding debate **[3,9,13,15]**. These results are due to the better qualitative collagen production in the regenerating neotendon that may support multiple previous studies frequently reported the same effect during cutaneous wound healing **[4,5,7,12]**. However, the favorable results of the qualitative evaluation of the newly synthesized collagen of the regenerating neotendon at the present study makes this subject a suitable topic for further quantitative evaluation.

Conclusion:

The results of the present study indicate the favorable beneficial effects of He-Ne laser photostimulation for the healing process after tendon repair for better functional results. Accordingly, this form of treatment, laser photostimulation, may be of value after surgical repair of ruptured and injured human tendons.

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