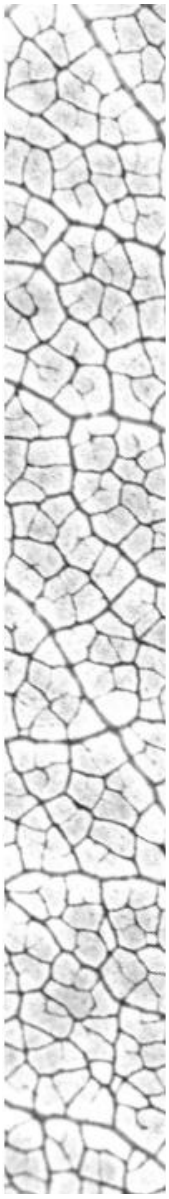

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# The effects of low-energy laser light in increasing the number of fibroblasts during the healing process of tendon achilles rupture in white rats (*Rattus Norvegicus*)

**Erwin Ramawan [1], Putut Sugiantoro [1], Pramono Ari Wibowo [1], Kukuh Dwiputra Hernugrahanto [1], Dwikora Novembri Utomo [1*]**

[1] Department of Orthopaedi and Traumatology, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo Regional Public Hospital, Surabaya 60131, INDONESIA
*Corresponding author: dwikora-novembri-u@fk.unair.ac.id

**Abstract**

**Background:** The recovery process of tendon injury is slower and results in weaker scar tissue. The increase in metabolism and the formation of new blood vessels in the tendon occurs in the tendon healing mechanism. Laser energy that hits the tissue increases the blood flow and lymph flow in the tissue. **Purpose:** This study aims to determine the effects of low energy laser therapy on the tendon healing process. **Method:** A total of 21 white rats were divided into three groups, i.e., G1 as the control group without additional therapy, G2 with the therapy in the inflammatory phase (day 3), and G3 with the therapy in the proliferative phase (day 8). G2 and G3 received the therapy for five days at a dose of 1 Joule. The experiment animals were terminated on the 21st day for evaluation, and the obtained and collected data at the end of the study were compared and analyzed using ANOVA statistical tests. **Results:** The groups that received the treatment using a low energy laser experienced an increase in the average number of fibroblasts by 78.99% per visual field in G2, and 79.23% in G3 in comparison to the control group. **Conclusion:** The administration of low energy laser therapy to the Achilles tendon healing of white rats brings positive results with an increase in the average number of fibroblasts.

**Keywords:** low energy laser, tendon, fibroblast

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## INTRODUCTION

Tendons are structures formed by fibroblasts and connect muscles to bones, which are very vital musculoskeletal components in the motion system. Anatomically, tendon tissue has a relatively low blood supply and a small number of cells. Therefore, its recovery or healing is slower and less effective. Generally, there are three phases of tendon recovery, i.e., the inflammation in the first seven days, the proliferation from day 7 to 21, and the remodeling and maturation on day 21 to one year. These phases are not fragmented, but they overlap with various ranges depending on the location of tendon damage (Rhatomy, et al. 2019. Lin, Cardenas, & Soslowsky, 2004. Gomez, 1995).

In the process of tendon formation, fibroblast cells proliferate in areas with many blood vessels. Fibroblasts synthesize procollagens in cells, which then are released as collagen. Those fibroblast cells are also contained in the endotenon of loose connective tissue that encloses fascicles where there are blood vessels, nerves, and lymphatics. Fibroblasts (tenoblasts) in this endotenon repair damaged collagen fibers. The information about fibroblasts becomes a reference in several studies of the construction or reconstruction process that occurs in connective tissue (Juliastuti, Budi, & Maula, 2016. Ernawati, & Puspa, 2018. Nirwana, Rachmadi, & Rianti, 2017; Shahabi, et al, 2016).

Photobiological reactions occur when the tissue absorbs the light of a certain wavelength through a light receptor contained in the cell, which is the photo acceptor molecule. The light absorbed by this photoreceptor causes a change in the electron balance at the molecular level in the form of a reduction-oxidation reaction and an acceleration of electron transfer in the respiration chain reaction (Vo-Dinh, 2003). The cells in abnormal conditions, especially the pathological cells, respond more to low energy lasers compared to normal cells where pathological cell condition is more sensitive

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to an increase in energy transfer and metabolic activity (Nascimento, & Callera, 2006).

Low energy laser therapy is one of the therapeutic modalities developed today. The use of laser therapy began to be widely used for connective tissue or soft tissue injuries, such as tendons, ligaments, muscles, and even bones. Laser energy hits the tissue functions by increasing blood flow and lymph flow into the tissue and reducing prostaglandin production which causes inflammatory processes and the onset of pain. In the process of tendon healing, there is an increase in the formation of new blood vessels. Thus, an increase in blood flow to the traumatic tendon greatly improves the tendon recovery process. The determination of the right dose is very important to achieve optimal treatment. The therapeutic window of the laser is approximately 0.1 – 3 J/cm$^2$, with power density 5 – 21 mW/cm$^2$, with the administration duration of 3-5 times per week (Bjordal, Couppe, & Ljunggren, 2001).

Accordingly, it is necessary to analyze the impact of administering low energy laser as a therapeutic instrument during the tendon healing process, where the parameter used is the number of fibroblasts after laser therapy.

## METHOD

In this study, white rats were used and cut sharply on their right Achilles tendons in the middle part. Then tendon repair was conducted with one horizontal mattress suture. The experimental animals were divided into three groups, i.e., the first group without additional therapy as the control group, the second group received adjuvant therapy in the form of low energy laser light in the inflammatory phase (day 3), and the third group received low energy laser light therapy in the proliferative phase (day 8). Each group of low-energy laser therapy received the therapy for five days at a dose of 1 Joule. The experiment animals were terminated on the 21$^{st}$ day to evaluate the quality of tendon healing by assessing the histopathological examination of the tendon by counting the number of fibroblasts as a parameter. By conducting the microscope observations, the average number of fibroblasts was obtained with 400 times magnification by five visual fields.

The rats' Achilles tendons were cut after they were disinfected using 10% povidone iodine and drapping by making a vertical skin incision in the posterior part of the right cruris, then by identifying the paratenon which was vertically incised, following the skin incision. Next, the incision was performed using sharp knife in the middle part. After the tendon was cut, the process was continued by the primary suture using non-absorbable nylon thread (monofilament) with a single horizontal mattress suture. The paratenon was restored and the skin was sutured using one-on-one interrupted suture. The sutures were then treated and covered using sterile gauze. The extremity immobilization was not performed in the surgical procedure.

**Table 1.** The Calculation Results of the Number of Fibroblasts in Experimental Animals Group 1

| G1 | Day | | | | | Total | Average |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | |
| 1 | 60 | 105 | 130 | 81 | 101 | 477 | 95.4 |
| 2 | 101 | 131 | 125 | 147 | 121 | 625 | 125 |
| 3 | 82 | 71 | 77 | 148 | 136 | 514 | 102.8 |
| 4 | 118 | 88 | 119 | 128 | 109 | 562 | 112.4 |
| 5 | 132 | 151 | 85 | 90 | 139 | 597 | 119.4 |
| 6 | 102 | 96 | 86 | 112 | 124 | 520 | 104 |
| 7 | 79 | 86 | 106 | 118 | 96 | 485 | 97 |

The treatment and care of the experimental animals were carried out by placing all experimental animals in cages with a size of 30 x 40 x 50 cm, where each cage was filled with a maximum of five animals that are provided with standard food and drink. The sample population in this study utilized white male rats with the following criteria, i.e., Rattus Norvegicus of Wistar strain, male, 2-3 months old, 190-210 grams of weight, and healthy as indicated by their active movements. The number of samples in this study was seven rats for each control group and the treatment groups.

The independent variable in this study was low energy laser light, whereas the dependent variable was the number of fibroblast cells. The control variables in this study included the type of low energy laser, i.e., He-Ne, the wavelength of the low energy laser light, i.e., 632.8 nm, the dosage of the laser light, type of experimental animal, the cutting of the experimental animals' tendon, tendon suturing, and experimental animal care.

The data obtained and collected at the end of the study from the treatment group were compared and analyzed using the ANOVA statistical test to determine whether there was a difference between G1 and G2; G1 and G3; as well as G2 and G3.

## RESULTS

After the 21$^{st}$ day, the average number of the fibroblasts per five visual fields was obtained in the joining area of each experimental animal group. Next, the results compared the number of fibroblasts in the group which received low-energy laser therapy (treatment) on the 3$^{rd}$ day, 8$^{th}$ day, and the group which did not receive laser therapy (control). Analysis of Variance (ANOVA) was employed as a statistical test in this study where the data of the three groups must be normally distributed and the three variants of the data groups must be identical.

Based on the results stated in **Table 4**, the three data groups had a normal distribution with a p-value of more than 0.05. Furthermore, to conduct the ANOVA test, the variant homogeneity test was performed in which the variance value must be the same. **Table 5** indicates the results of the homogeneity test (Levene's test), where the number of fibroblasts obtained had a significance or

**Table 2.** The Calculation Results of the Number of Fibroblasts in Experimental Animals Group 2

| G1 | Day | | | | | Total | Average |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | |
| 1 | 154 | 188 | 161 | 189 | 202 | 894 | 178.8 |
| 2 | 323 | 200 | 185 | 147 | 183 | 1038 | 207.6 |
| 3 | 184 | 209 | 184 | 166 | 213 | 956 | 191.2 |
| 4 | 173 | 280 | 217 | 181 | 179 | 1030 | 206 |
| 5 | 215 | 179 | 139 | 195 | 157 | 885 | 177 |
| 6 | 185 | 204 | 186 | 196 | 187 | 958 | 191.6 |
| 7 | 204 | 190 | 207 | 198 | 206 | 1005 | 201 |

**Table 3.** The Calculation Results of the Number of Fibroblasts in Experimental Animals Group 2

| G1 | Day | | | | | Total | Average |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | |
| 1 | 198 | 176 | 201 | 159 | 233 | 967 | 193.4 |
| 2 | 188 | 182 | 228 | 210 | 165 | 973 | 194.6 |
| 3 | 169 | 161 | 202 | 191 | 238 | 961 | 192.2 |
| 4 | 219 | 239 | 212 | 186 | 275 | 1131 | 226.2 |
| 5 | 201 | 188 | 173 | 213 | 125 | 900 | 180 |
| 6 | 185 | 203 | 170 | 168 | 206 | 932 | 186.4 |
| 7 | 150 | 178 | 203 | 195 | 185 | 911 | 182.2 |

**Table 4.** The Normality Test for Transformation Data on Fibroblasts

| | Group | Kolmogorov-Smirnov | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | 0f | Sig. | Statistic | Df | Sig. |
| Fibroblast | Control | .198 | 7 | .200 | .935 | 7 | .590 |
| | Day 3 of laser | .169 | 7 | .200 | .903 | 7 | .351 |
| | Day 8 of laser | .316 | 7 | .033 | .809 | 7 | .051 |

**Table 5.** The Homogeneity Test Results for the Number of Fibroblasts

| Levene Statistics | df1 | df2 | Sig. |
|---|---|---|---|
| 1,493 | 2 | 18 | .251 |

**Table 6.** The One Way ANOVA Test Results on the Fibroblast Numbers Data

| | Sum of Square | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .302 | 2 | .151 | 116,937 | .000 |
| Within Groups | .023 | 18 | .001 | | |
| Total | .325 | 20 | | | |

**Table 7.** The Test Results of Multiple Comparisons with Post-Hoc on Fibroblast Numbers Data

| Treatment | Treatment | Sig. |
|---|---|---|
| Control | Day 3 of laser | .000 |
| | Day 8 of laser | .000 |
| Day 3 of laser | Control | .000 |
| | Day 8 of laser | .991 |
| Day 8 of laser | Control | .000 |
| | Day 3 of laser | .991 |

probability value of 0.251. Hence, the data obtained had the same population variant. Furthermore, the statistical tests were performed using ANOVA to determine the differences among the three groups that received radiation treatment.

The calculation result employing the one-way ANOVA test obtained a p-value of 0.000 ($p < 0.05$). The results suggested that there were at least significant differences between the two sample groups compared to the multiple comparison tests with post hoc test, which was used to determine the differences in each sample group. Based on the results of the post-hoc test in **Table 7**, it was observed that there was a significant difference between the number of fibroblasts between the control group and the groups receiving laser therapy on day 3 ($p = 0.000$) and day 8 ($p = 0.000$). Meanwhile, there was no significant difference between the number of fibroblasts on day 3 and day 8 of the laser therapy ($p = 0.991$).

## DISCUSSION

This study indicated that the group received treatment using low energy lasers experienced an increase in the average number of fibroblasts per visual field in G2 and G3 compared to the control group. The results of data analysis, performed through the one-way ANOVA statistical test on the number of fibroblasts variable in each treatment group and compared to the control group, had a significant effect.

The mean value of fibroblasts per visual field reached 108.00 cells in the G1 control group, while the mean value in the treatment group amounted to 193.31 cells at G2 and 193.57 cells at G3. The fibroblast increase in this study was greater than in previous studies on the pigs' Achilles tendon which amounted to 13% at the same dose (Chen, et al. 2009).). The different results could be influenced by the types of experimental animals used due to the different biological responses in each species. Anatomically, the thickness of different tendons affects different penetration power on the same dose

The study results suggested that the increase in the average number of fibroblasts in the G2 treatment group received radiation on day 3 (inflammatory phase) compared to the G3 group received radiation starting on day 8 (proliferative phase) did not imply a significant difference ($p>0.05$). The mean value of fibroblasts, amounting to 193.31 cells in G2 and 193.57 cells in G3, indicated no significant increase in the average number of fibroblasts which received a low-energy laser radiation treatment.

## CONCLUSION

The administration of low-energy laser therapy to the Achilles tendon recovery of white rats indicates positive results with an increase in the average number of fibroblasts in experimental rats. An increase of fibroblasts is evident in the treatment group per visual field, rather than in the experimental rats that do not receive low energy laser radiation therapy (the control group). The treatment between G2 and G3 at the beginning of low-energy laser radiation does not indicate a significant difference in the number of fibroblasts per visual field in the white rats' Achilles tendon.

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