

Aging Effect Towards Crimp Pattern, Collagen Type I, and III Composition in Achilles Tendon Rupture (Study on Sprague-Dawley Rats)

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Abstract— Achilles tendon rupture happens more frequently in the elderly. The aim of this study is to analyze the utilization of exercise rehabilitation program in the achilles tendon injury on the elderly. This is quasi-experimental study which were conducted by mix method approach. There were six Sprague-Dawley (SD) old male rats which were used as sample in this research. The experimental group was involved in the exercise rehabilitation program. The collagen type I, collagen type III, and crimp pattern were observed as independent variables. The result, experimental group shows better recovery by the higher percentage of collagen type I and lower collagen type III. In conclusion, the finding of this study could give more evidences about the benefit of doing exercise rehabilitation on achilles tendon rupture in elderly every age. Further study could be conducted by adding more research sample and involving other research variable.

Keywords— Achilles tendon, collagen type I, collagen type III, crimp pattern, exercise rehabilitation program

I. INTRODUCTION

Physical activity is very important to improve human body capability. Furthermore, performing physical activity regularly in elderly could maintain the health status. However, the injury may appears more frequently in the elderly. Biomechanical and physiological changing in elderly could be related to decreasing of physical ability. One of the injury which mostly happened in the middle-age and older person is Achilles tendon ruptures [1]. Achilles tendon is the part in lower musculoskeletal system, it is connected gastrocnemius and soleus muscles to the calcaneus tubercle[2]. It is a soft connective tissues which have multi-unit hierarchical structure from the molecular scale up to the whole tendon itself[3]. Collagen fibers are the smallest hierarchical tendon unit[4]. However, even though collagen fiber is the smallest unit, it is constructed by the other smaller unit which is called collagen fibril. A collagen fibril diameter is 10 – 500 nm[5]. Then Collagen fibers group together in to a bundle of collagen fibers, some bundles of collagen fibers are gathering together and wrapped in to an endotenon, and some endotenon are unite in to an epitenon.

Age is a nature factor that could not be avoided by everybody, because the ability and function are determined by the mechanical properties of tendon. Maturity causes the tendon loses its elasticity and resistance gradually. The decreasing of muscle mass, fiber cross sectional area (CSA)

and collagen content in tendon cause the significantly reduction of Achilles tendon strain in middle aged and older individuals compared to younger [6]. On other hand, exercise regularly increases the size and capacity of Achilles tendon become more resistant to stretching [7]. Regular physical activity leads muscle stronger and correspondingly stronger tendon. Exercise does not only have a good benefit to maintain the function of the tendon, but also was proved in the former studies about the Achilles tendon healing management, there are many evidences that exercise rehabilitation was able to help restore the tendon’s ability better than the sedentary. Early exercise rehabilitation encourages the improvement of tendon properties and increasing tensile strength [8]. However, there are only few studies which investigate the effect of age and exercise rehabilitation treatment of Achilles tendon healing process especially at remodeling phase. The aim of this study is to find out the fact about age and exercise rehabilitation in remodeling phase of Achilles tendon injury.

II. METHODS

A. Research Design

This is a quasi-experimental study which we conducted mixed-methods approach. There were six Sprague-Dawley (SD) old male rats which were used as sample in this research. All of them are 58 weeks old or more. Three rats were in the control group and the other are in the experimental group. The treatment in this study is called Achilles tendon rupture exercise rehabilitation program. It used walking and running with the gradual speed increasement (Table 1) using TMR/M Treadmill Machine from Chsin M & I CO. LTD.

TABLE I. RESEARCH DESIGN

Time	Research Design		
	Experimental Group	Control Group	Information
-2nd day	Surgical	Surgical	-
5th day	4 meters/minute	-	-
14rd day	12 meters/minute	-	-
21th day	20 meters/minute	-	-
35th day	Collecting the tissue	Collecting the tissue	-
36 th day	Data Analysis	Data Analysis	-

B. Research Procedure (Surgical)

The rats were anesthetized by the injection of a mixture of 0.1 mL/100g of Zelotil (Zolazepam + tiletamine) and 0.03 mL/100 g of Rompun (Xylazine) before make the incision in Achilles tendon[9]. The incision was made in the Achilles tendon which is located in the right hind legs area of the rats and nearby calcaneal tubercle. The incision cut the entire Achilles tendon, so it caused rapture completely. After thirty days treatment or in the 35th day after surgical, the right hind legs rats' Achilles tendon was extracted from the musculotendinous junction and the attachment site of calcaneus. There were six tissues samples from each group that were analyzed and observed by physiological procedure (Figure 1).

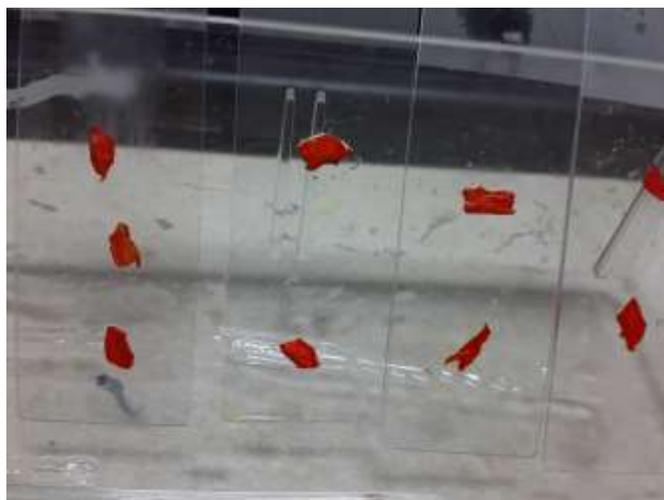


FIGURE 1. ACHILLES TENDON TISSUES

There four steps before the tissue can be observed in the microscope. The first one is cutting process. In this phase, the tendon must be cut in to a slice and put in to the microscope glass slide. The second phase is colorings the tendon by anti-collagen I and III. The third phase is observing the tissue in to electron microscope. The last phase is determining the collagen type I and III proportion or percentage by JAVA software which is called 胶原蛋白變別軟體.jar.

C. Research Instrument

Electron microscope was applied to observe the microscopy object such like Collagen fiber and fibrils. It was used to figure out the data about the crimp pattern and collagen type in the Achilles tendon tissue. Furthermore, Achilles tendon sample components of Sprague–Dawley rats were observed and compared by Quantitative reverse transcription and PCR analysis (qRT-PCR) to find out collagen I, III, and V mRNA expression[6].

III. RESULT AND DISCUSSION

Overall, almost all research subjects have been using social media as their tools in marketing. However, there is only a few of them who utilize the internet to develop their business by establishing collaboration with other institutions and foreign companies.

TABLE 2. RESEARCH RESULT

	Result		
	Collagen Type I	Collagen Type III	Crimp Pattern
Experimental	76.61 %	16.26 %	Obvious and unorderly
Control	68.06 %	19.18 %	Not clear and unorderly

A. Experimental group

The collagen composition in the experimental old group shows that the average of collagen I is 76.61 % and collagen III is 16.26 % (Figure 2). The maximum collagen type I proportion of this group is 89.79 % and the lowest is 60.70 %. For collagen type III, the maximum and minimum are 32.25 % and 9.15 %.

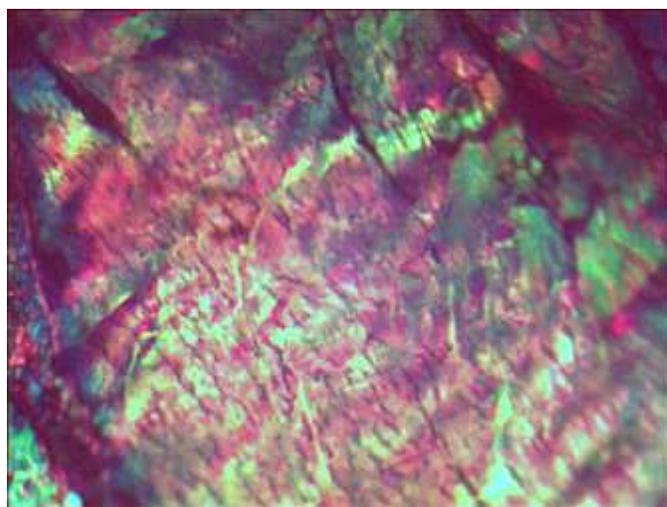


FIGURE 2 COLLAGEN AND CRIMP PATTERN IN EXPERIMENTAL GROUP

The color of the tissue is dominated by red color. It is indicating the appearance of the collagen I. However, there are other variance colors in experimental old tissue sample. Those many kinds of color are including green. The apparent of green color on this tissue sample is indicating the existence of collagen type III. Furthermore, the crimp pattern could be observed clearly in thi tissue. The crimp pattern in the experimental old Achilles tendon tissue makes the texture looks rough and wavy but not really orderly.

B. Control group

The collagen composition in the control old group shows that the average of collagen I is 68.06 % and collagen III is 19.18 % (Figure 3). The maximum collagen type I proportion of this group is 89.58 % and the lowest is 64.71 %. For collagen type III, the maximum and minimum are 44.39 % and 9.31 %.



FIGURE 3 COLLAGEN AND CRIMP PATTERN IN CONTROL GROUP

The color of the tissue is also dominated by red color. It is indicating the appearance of the collagen I. However, there are many variances of green colors in control old tissue sample. Those many kinds of color are including green color on its tissue sample. The appearance of green and blue color on this tissue sample is indicating the existence of collagen type III. The crimp pattern could be observed, but it is not really clearly (figure 4.12). The crimp pattern in the control old Achilles tendon tissue sample is not really smooth and orderly. Furthermore, the wave is not appeared clearly.

IV. CONCLUSION

Experimental group shows better recovery by the higher percentage of collagen type I and lower collagen type III. In conclusion, the finding of this study could give more evidences about the benefit of doing exercise rehabilitation on achilles tendon rupture in elderly every age. Further study could be conducted with more research sample and involving other research variable.

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