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# Experimental tendon rehabilitation model, histologic stages of healing associated with tensile strength restoration

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***Keywords*— Collagen, histologic stages, Cartilage formation**

***Abstract*— *The tendon repair process is the focus of a lot of current study. It is common to use histological and biomechanical tests to evaluate various gene therapy and tissue engineering procedures. There is some overlap between the two methods, but they have historically been viewed as two distinct things. To compare data from different research or even within the same study, there is no approved scoring scale for histological examination. Biomechanical testing can produce reliable results, but it comes at a higher price and requires more time and effort. An objective, verified scoring system for histological results and biomechanics was anticipated to be the best way to compare histolog ical findings. Histological grading scale for assessing tendon repair has been devised in Achilles tendon model. Angiogenesis, collagen orientation, and cartilage induction are graded by the system. Histology scores were plotted against biomechanical testing findings of healing tendons, and the results showed a strong linear association between histological qualities and biomechanical characteristics. Concurrently, this study presents a practical and financially possible way to evaluate repair while accounting for both the histology and biomechanical features observed in surgically repaired, regenerating tendon.*

## I. INTRODUCTION

According to Yoon, et al., (2020), there are four stages of tendon recovery. A hemorrhagic phase occurs in the first few hours after a tendon is ruptured. The cytokines released by a blood clot attract polymorphonuclear leukocytes and lymphocytes, which fill in the void. The arrival of macrophages at 24–48 h marks the beginning of an inflammatory phase. Neovascularization and granulation tissue production are induced by the phagocytosis of necrotic tissue by these cells, which produce growth factors. During the third phase, fibroblasts enter and begin making collagen and matrix proteins. This occurs within the first week. Remodeling and maturation take place in the final stages of development. Healed tissue loses its cellularity with time, while the matrix thickens and becomes more longitudinally orientated. Type I collagen predominates, as does collagen turnover, water content, and the collagen I/III ratio, all of which return to normal levels at this point in the process. A recovery to normal collagen concentration occurs during 12 to 14 weeks after maturation (Bulut, et al., 2020).

One of the most important areas of research in tendon restoration is the use of biological mechanisms to improve surgical repair of tendon injuries and influence the reported histological phases of healing. Validated outcome metrics are the only way to confirm the utility of these biological approaches. Direct readings from tensile testing devices now validate mainly biomechanical effects (Loder, et al., 2018). Pineda, the International Cartilage Repair Society, the o'Dris-col scale, and the Oswestry Arthroscopy Score are only a few of the objective grading systems for evaluating cartilage repair. However, there are no comprehensive, unbiased methods for determining whether or not a tendon repair was a success. To better understand tendinosis, Soslowsky and his colleagues devised a subjective scale to measure the severity of the condition (Shekha, et al., 2013). However, biomechanical testing has never been used to verify the efficacy of this strategy (Han, et al., 2020).

We could greatly benefit from the creation of a validated histological scoring system Another reference point would be used to remark on the results of investigations and to compare different repair methods. Animal research is also very pricey. An alternative to expensive biomechanical testing could be justified if histological scoring systems were found to correlate well with biomechanical outcomes. Our goal was to create a histological scoring system that could detect even the smallest variations in tendon composition and use that information to determine how the tendons' biomechanical qualities had changed (Jiang, et al., 2020).

A new histological grading system for evaluating the tendon–tendon healing process is described in this article. Since the results of biomechanical testing were expected to significantly influence our scoring system, we developed a new score called the Grande Histological Biomechanical Correlation Score (GHBCS), which we believe will accurately reflect the biomechanical strength of the nonruptured tendon. This score, we believe, will give researchers an additional tool for evaluating and comparing the results of tendon healing investigations (Liu, et al., 2018).

## II. MATERIALS AND METHODS

Aseptic conditions were maintained during the surgical process. Scalpel transection was used to execute the midsubstance tenotomy. Recombinant Human Growth and Differentiation Factor 5 (rhGDF-5) sutures were utilized to heal Achilles tendons utilizing the Mason–Allen stitch technique. Each repair included an average of 2.9 centimeters of suture. Biomechanical testing was carried out on the tendons that were not surgically removed. The involvement of rhGDF-5 in the production and healing of tendons has been well described in previous research [5–8, 17]. [7, 8] We used the biomechanical and histological data from two of these earlier investigations to establish a grading scheme for this project.

The 96 rats were divided into two groups: a 3-week and a 6-week time period. Randomly assigned to one of four rhGDF-5-coated suture groups (n=12 in each group), the rats in each time protocol were assigned to one of the four growth-factor-coated suture groups (n=12 in each group). Each group had four rats tested for histology and eight rats for biomechanics. These allocations were kept a secret from the researchers involved in this investigation.

Following surgery, the animals were euthanized 3 or 6 weeks later. Both the right and left hindlimbs of each of the four rats sacrificed for histology were used to acquire histological sections. Hematoxylin and Eosin were used to stain ten slides per sample, which contained three specimens per slide (H&E). Collagen grade, angiogenesis degree, and cartilage induction were assessed using our new histological scoring system, which evaluates these three factors. Angiogenesis was assessed on a scale of 0–2, whereas collagen orientation and cartilage induction were graded on a scale of 0–3. The lower the score, the better the histology results.

There were three impartial, blinded observers (JD, DG and LW) who assessed each slide. Students were graded on the basis of their ability to see clearly. A minimum of ten sections were used to grade each specimen. Grading was done in the area between the suture placements, which was close to the site of the transection. For observation and grading, an area of 3 mm2 was usual. Using sections stained with H&E and Masson trichrome, grading was performed at 100 and 200. Sections stained with Sirius red and viewed under polarized light were also used to examine collagen orientation. The overall histology score for each rat was calculated by averaging the grades given to each specimen. Its ultimate tensile strength ranged from 0 to 8, with lower numbers indicating the best results. The test results were then compared among the therapy groups.

The tensile grips of an EnduraTec ELF 2100 were used to assess the mechanical behavior of eight rats in each treatment group (Rashid, 2017). As a power study demonstrated, eight samples were needed for the four groups to obtain an accuracy level of 0.05. An 80 percent power to detect hypothesis differences at the 0.05 level was found utilizing a two-tailed test with a sample size of eight subjects per group. Cold-frozen dissected leg parts from rats have been used in the study. Samples were thawed to 4°C and drawn in uniaxial strain to failure at each time point (Delgado Caceres, et al., 2021). The load was documented to 0.005 N with an accuracy of 2Hz by using an open-loop control with a 0.025 mm/s displacement rate (Rashid & Basusta, 2021). Data on load and displacement were used to build time-independent load-displacement curves. Each sample's ultimate tensile strength (the maximum force supported by the sample normalized to the cross-sectional area) was determined.. Measurements of the length of the major and minor axes of the tendon were used to calculate the cross-sectional area. In order to determine the efficacy of treatment, two-way ANOVA tests were conducted.

A scatter plot analysis in Microsoft® Excel was used to calculate the coefficients of determination (R2). The correlation coefficient was squared to get this number. The contralateral, unoperated tendon was used as a control in the comparison of biomechanical data between the experimental and control groups. An appropriate UTL (UTL) % was calculated for the experimental and surgically repaired groups by using the control tendon's tensile load biomechanics. Using the UTL function, histology scores were plotted against the coefficient of determination (R2) for each treatment procedure. An optimal percentage of optimal UTL was determined by plotting each UTL value as the y-coordinate on the graph. UTL data was also correlated with each specific histological parameter. On the basis of their close association, we were able to determine the histological intervals at which different biomechanical scores became apparent. The Grande Histological Biomechanical Correlation Score (GHBCS) was determined as a result of this. In this way, the ultimate tensile strength of a non-ruptured tendon can be calculated as a percentage of its non-ruptured biomechanical score.

## III. RESULTS

It was found that postoperative week 3 collagen orientation was improved in sutures treated with GDF-5 at low dose (24 ng/cm) and high dose (556 ng/cm), compared to controls. Results at 6 weeks were similar regardless of therapy [8]. Week 3 of suture treatment revealed isolated hyaline cartilage in tendons, regardless of the procedure used. De novo cartilage creation was more prevalent six weeks after surgery. The majority of this chondrogenesis took place in the areas where sutures were placed. Compared to untreated tendons, treated tendons showed more neovascularization (Table 1). The restored tendons were shown to be weaker than the contralateral, unoperated tendons in terms of ultimate tensile load (P .001). However, the UTL of the growth factor-treated sutures differed significantly from the control group (P .01). The experimental tendons resembled the unoperated tendons more, but they still differed significantly (P .02) from each other. Three-week tendons have half the UTL biomechanics of six-week tendons.

*Table 1: Descriptive Test*

| | UTL | Histology | Caetilage | Angiogensis | Collagen |
|---|---|---|---|---|---|
| 0 | 0.29±0.075 | 1.898 | 0.061±0.69 | 0.311±0.046 | 0.0576±0.062 |
| 24 | 0.61±0.38 | 1.354 | 0.211±0.56 | 0.454±0.44 | 0.84±0.28 |
| 55 | 0.49±0.33 | 2.112 | 0.76±0.049 | 0.69±0.067 | 0.61±0.056 |

**Histological Grade**

Reparative histology (a low histology score) and the experimental groups' ultimate tensile strength ratios were shown to be directly related in each suture strategy. Histological scores greater than 2.2 are associated with ultimate tensile stresses that are less than 30% of those of an unoperated control specimen. Test results less than 1.6 show that tensile loads will be more than twice that of an unoperated control, indicating that repair conditions must be improved (Table 2). Sections of disordered collagen, little to no angiogenesis, and moderate to extensive cartilage production are seen at histological scores greater than 2.2 Combined, these characteristics are associated with a lower than 30 percent rate of UTL biomechanics. There is just a slight infiltration of new blood vessels and no chondrogenesis seen in tissue with a histology score of less than 1.6. Tendons with such features have a higher biomechanical strength. More than half the strength of unoperated controls can be found in one tendon. Correlations between the healed tendon's histology qualities and its biomechanical traits were found to be 0.91. There was also a correlation coefficient of 0.89 between ultimate tensile strength and the orientation of collagen, angiogenesis, and cartilage production .

*Table 2: Correlation Scores*

| UTL | Histologic |
|---|---|
| >1.9 | <20 |
| 2.0-2.4 | 20-29.5 |
| 1.8-1.9 | 30.5-32 |
| 1.6-1.7 | 32-33.5 |
| 1.5-1.6 | 33.5-35 |
| <1.5 | >40 |

## IV. DISCUSSION

An Achilles tendon model from a rat was used as a basis for developing a method for assessing postoperative biomechanical strength. The Grande Histological Biomechanical Correlation Score was used to accomplish this. There was no discernible difference between the experimental and control groups in the six-week study, despite enhanced tendon healing observed in the 3-week study. Data from all rats, regardless of the changes between the three-week and six-week investigations. When we looked at 6-week experimental and control groups in our previous study, we found that both groups had similar histological properties and similar biomechanical properties (Linderman, et al., 2018). These results corroborated the findings of the three-week examination. In other words, histology and biomechanics share a significant link. As more biological investigations are carried out in animal models, this approach will become even more valuable in the future. There is still some subjectiveness in the histological grading scale, which was developed in part to reduce the subjectiveness that existed in previous grading systems. However, we were able to demonstrate a link between histology and biomechanics by combining the findings of three different experts. All of these people had a background in histology, therefore the scale could be used correctly and objectively by all of them. Histology and biomechanics had the best correlation using this approach. Since histology and biomechanics are linked, researchers can get a good idea of biomechanical performance without having to undertake the tests themselves.

Each parameter's average histology score and ultimate tensile strength were calculated. The more closely the two factors correlate, the more expensive the undertaking is. As a result, we recommend that the histological sections be graded by three independent observers. We used the coefficient of determination to look at the connection between histology and UTL (R2). An R2 score of 1.0 indicates that the regression line fully fits the data, and it is a statistical measure of how well it approximates real data points. A great deal of care must be taken when using the coefficient of determination in correlation studies. Despite its name, correlation does not prove the existence of a causal link. There is no guarantee that when one of these two variables changes, the other will likewise change, despite the excellent correlation found in this study between UTL and histological grade (R2=0.91). However, it means that it is quite probable. To reiterate, this applies to any attempts to demonstrate a connection between two variables and is not limited to the findings of this study. These tendon healing processes are part of the body's normal response to injury and take place even in the absence of elevated levels of growth factor. Some may argue that a histology score of 2.376 (control suture group) is not indicative of a bad biomechanical rating, given that a score of 8 is the worst conceivable grade. To get scores considerably higher than 2.376, however, it appears implausible given the body's inherent propensity to mend and the data from the 24 controls.

Each histology property was associated with a specific correlation coefficient for the ultimate tensile load. Collagen histology grading alone was not sufficient to determine UTL, as evidenced by the coefficient of determination of 0.59 when comparing collagen with UTL. Meaning that there was no clear link between the reported collagen properties and any significant differences in UTL levels. Poor biomechanics and a poorly repaired tendon are both associated with disorganized collagen fibrils, according to research (Ruan, et al., 2021). Further research has shown that the parallel arrangement of collagen fibers is a significant contributor to collagen's tensile strength (Bulut & Rashid, 2020). This is not something we contest because it correctly highlights the role collagen plays in tendon recovery. While collagen properties are important, we do not feel that they are the only thing that must be taken into account in order to accurately determine tendon healing using UTL. As a result, our histological investigation includes both angiogenesis and chondrogenesis.

The second criteria was vascular infiltration, because a better blood supply is necessary for tendon recovery when it is wounded. The tenosynovium's cells can be disrupted by tendon injury, which should lead to angiogenesis (Abdou, et al., 2019). Repair is hampered by a lack of nutrients and growth factors delivered to the healing region by the bloodstream. In fact, blood-derived mesenchymal cells and fibroblasts are required for the initial repair of a tendon (Lu, et al., 2019). Despite this, we found no link between UTL and angiogenesis. Because they have an effect on tendon healing as well, histological qualities must also be considered while evaluating this criterion.

In damaged tendons, cartilage-inducing genes have been demonstrated to be upregulated. Tendons from rats with tendinopathy caused by overuse regimens have been discovered to have a cartilage phenotype (Rashid, et al., 2018). Achilles tendon cartilage formation in rats has been documented in other research (Kokubu, et al., 2020). Using cartilage as an indicator of an inferior repair process and worse biomechanics, we created our model. Our research showed that UTL and subsequent tendon repair are harmed by the presence of cartilage. (R2 = 0.89) between chondrogenesis histological grades and UTL (Misir, et al., 2020); the lower the level of cartilage production, the higher UTL. For specific histological markers, only cartilage development was found to have a significant link with the

UTL. Angiogenesis and collagen creation must also be taken into consideration when looking at the association between tendon injury and chondrogenesis, even if these results confirm prior studies.

## V. CONCLUSION

Using the total histological grade, which integrates scores from each of the three factors, the strongest coefficient of correlation may be found. Because no single measure correlates with biomechanics as strongly as the whole histological score, this provides more evidence for its usefulness. Whereas grading tendon restoration, prior studies have focused on collagen alone, when in fact, two additional factors (as stated above) can provide a more accurate assessment of the repairing tendon's status. When measuring tendon restoration, a scoring system that allows for a quantitative evaluation of biomechanical strength as a function of histological features is essential. A unique histological scoring scale and the biomechanical features of tendon restoration in a rat model were shown to have a good association in this model.

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