Differentially expressed proteins on postoperative 3 days healing in rabbit Achilles tendon rupture model after early kinesitherapy

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【Abstract】Objectives: Surgical repair of Achilles tendon (AT) rupture should immediately be followed by active tendon mobilization. The optimal time as to when the mobilization should begin is important yet controversial. Early kinesitherapy leads to reduced rehabilitation period. However, an insight into the detailed mechanism of this process has not been gained. Proteomic technique can be used to separate and purify the proteins by differential expression profile which is related to the function of different proteins, but research in the area of proteomic analysis of AT 3 days after repair has not been studied so far.

Methods: Forty-seven New Zealand white rabbits were randomized into 3 groups. Group A (immobilization group, \(n=16\)) received postoperative cast immobilization; Group B (early motion group, \(n=16\)) received early active motion treatments immediately following the repair of AT rupture from tenotomy. Another 15 rabbits served as control group (Group C). The AT samples were prepared 3 days following the microsurgery. The proteins were separated employing two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). PDQuest software version 8.0 was used to identify differentially expressed proteins, followed by peptide mass fingerprint (PMF) and tandem mass spectrum analysis, using the National Center for Biotechnology Information (NCBI) protein database retrieval and then for bioinformatics analysis.

Results: A mean of 446.33, 436.33 and 462.67 protein spots on Achilles tendon samples of 13 rabbits in Group A, 14 rabbits in Group B and 13 rabbits in Group C were successfully detected in the 2D-PAGE. There were 40, 36 and 79 unique proteins in Groups A, B and C respectively. Some differentially expressed proteins were enzyme with the gel, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). We successfully identified 9 and 11 different proteins in Groups A and B, such as GAPDH, phosphoglycerate kinase 1, pro-alpha-1 type 1 collagen, peroxiredoxin 1, alpha-1-antiproteinase E a-1 and MAD2L1 binding protein, etc. And some with the molecular chaperone, oxidative stress, energy metabolism, signal transduction, coupled with the tendon cell expression and protein synthesis, proliferate, differentiate and are closely related to the AT healing. The GAPDH protein was further validated through Western blotting. It was indicated that some differentially expressed proteins were involved in various metabolism pathways and may play an important role in initial healing of AT rupture.

Conclusion: Differentially expressed proteins in rabbit healing AT model may contribute to 3 days healing of AT rupture through a new mechanobiological mechanism due to the application of postoperative early kinesitherapy.

Key words: Achilles tendon; Rupture; GAPDH protein; Polyacrylamide gels; Mechanotransduction, cellular; Databases, protein; Muscle stretching exercises
Aftet open surgical repair of Achilles tendon (AT) rupture, cast immobilization, orthosis or the synthetic anterior below-the-knee slab is not necessary indeed. Marti et al reported firstly in the 1970s that early functional exercise is necessary. Since 1980s, many studies demonstrated that postoperative early motion facilitates the healing of AT rupture and the early functional exercises reduce the re-rupture rate. Recently, Yotsumoto et al treated 20 patients with improved operation methods and obtained satisfactory outcome. These patients started early functional exercise on the first day after operation. After one week, they started partial weight bearing exercise. After four weeks they started complete weight bearing exercise. One patient was able to exercise and perform standing on tiptoe 41 days after operation. All patients had no complications after operation and the outcome was certainly satisfactory.

A detailed comparison of postoperative early motion and postoperative cast immobilization to repair AT rupture is available in the studies performed by Schizas et al and Tang et al. Tang et al compared the rabbits that received postoperative cast immobilization and the rabbits that received early motion treatment immediately following tenotomy of the tendon. On the 21st day following the microsurgery, through matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique, they found two polypeptides from serum, whose molecular weight was 958 and 1 434 respectively to express more highly in mobilization group. Furthermore, Schizas et al concluded that the immobilization at 2 weeks significantly reduced maximum force, energy uptake, stiffness, tendon length, transverse area, stress, organized collagen diameter and collagen III-LI occurrence by 80%, 75%, 77%, 22%, 47%, 65%, 49%, and 83% respectively compared with free mobilization in rats AT repair model.

The time to start tendon mobilization after surgery is an important factor, which has been collaborated by other studies, but a deep insight into the mechanobiology mechanism involved in the tendon healing process 3 days following microsurgery employing differential proteomic techniques has not been gained yet. Therefore, this study aimed to investigate the significance of postoperative early motion treatment during the AT healing process through a proteomic analysis of the differential expression of proteins in the AT of rabbit model within postoperative 3 days.

**METHODS**

**Experimental protocol**

Forty-seven New Zealand white rabbits (healthy; mean age: six months; male; weight: 2.5 kg±0.2 kg) were provided by the Animal Centre of First Teaching Hospital of Xinjiang Medical University, Urumqi, China. They were divided into 3 groups according to a predetermined prospective animal experiment based on random number table. Sixteen rabbits were included in Group A (immobilization group) and Group B (early motion group) respectively, while 15 rabbits served as control in Group C. The experimental protocol was previously approved by the local research ethical committee (No: A-20080114008).

The rabbits in Group A and Group B received a tenotomy and subsequent AT microsurgery. The tenotomy was performed at a site of 1.6 cm above the tendon's attachment point with the calcaneus of unilateral AT of the rabbits following sterilization and hypnotic induction. Local anaesthesia was induced using lidocaine hydrochloride injection. The AT microsurgery was carried out using a novel ‘Pa’-bone suture method designed by our group. The rabbits in Group A were treated by postoperative cast immobilization, with the knee joint in flexion at 75° and the ankle joint in plantar flexion at 90° (Figure 1A). The rabbits in Group B received a simulated postoperative early motion treatment through an induction for food and water. The rabbits received the movement of standing up and squatting down, which was considered to have stretching exercises or eccentric training on AT, or was considered as kinesitherapy (Figure 1B). The frequency of the movement was about (150±15) times per day.

The rabbits in each group were excluded from this study in the following situations: (1) death of the rabbits; (2) loosening of the plaster cast; (3) infection of the ruptured site of the AT; and (4) gap at the ruptured site of the AT larger than 1.0 mm. Consequently, a total of 40 rabbits were accepted for further experiment, with 13, 14 and 13 rabbits remaining in Groups A, B and C, respectively.
Preparation of samples

Three days (72 hours) following AT microsurgery, all rabbits were sacrificed and a piece of 0.5 cm × 0.5 cm × 0.5 cm healing tendon tissue was cut off from the site of the ruptured AT. The epitenon tissues attached circumference was removed with caution, and the samples were rinsed thoroughly three times using 0.9% saline solution at 4 °C, and then frozen in liquid nitrogen. All these procedures were performed quickly in order to ensure that the difference of rehabilitation period for the AT of the rabbits was controlled within an hour.

The AT tissues were thawed, cut into small pieces with a pair of scissors, and further rinsed using phosphate buffered saline to remove any impurity involved. Then the AT tissues (weight: 50.0 mg) were crushed with a mortar, and the protein lysates were extracted with a Cocktail solution (4% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (chaps), 50 mmol/L dithiothreitol, 0.1% phenylmethanesulfonyl fluoride (PMSF), 7 mol/L urea, 2 mol/L thiourea) associated with a centrifuge tube at a speed of 16 000 r/min for 45 minutes at 4 °C. The protein concentration was quantified based on Bradford method using ReadyPre 2-D Cleanup Kit (Bio-Rad Laboratories, Inc, USA).

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

The proteomic experiments were performed for 3 times. The proteins were first separated through isoelectric focusing (IEF) according to their isoelectric point. Then 400 µg of protein sample was loaded per 18 cm immobilized pH gradient (IPG) strip with a non-linear pH range of 3-10 by in-gel rehydration. It was noted that each strip was overlaid with 2-3 ml mineral oil to prevent evaporation during the rehydration process. The IPG strip was then placed on the tray, and IEF was performed at 20 °C using IPG-Phor isoelectric system (Amersham Pharmacia Biotech Inc, Sweden). The initial voltage was set at 250 V and raised step-wise until 4 000 V to remove salt. The proteins were focused for 8 hours at 8 000 V.

After focusing, the IPG strip was removed and the mineral oil was drained off the IPG strip using wet filter paper. Proteins immobilized on the IPG strip were then placed on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel (thickness: 1 mm) and separated based on their molecule weight using Protean II electrophoresis system (Bio-Rad Laboratories, Inc, USA) at 15 mA/gel. Subsequently, the two-dimensional gel was silver-stained according to a modified method which enabled a direct mass spectrometry characterization. The gel was then scanned using a GS-800 molecular imaging system (Bio-Rad Laboratories, Inc, USA) and spot detection, spot matching, and quantitative intensity analysis were performed using PDQuest software version 8.0. The gel images were normalized according to the total quantity in the analysis set, and differentially expressed proteins were defined by measuring the density values of the protein spots. A difference in the abundance of the protein spots of Group B were 5-fold abundance than Group A, then it was nominated as the threshold. This was analyzed employing Student’s t test based on the six density values obtained for one protein spot, and a probability value <0.05 was considered statistically significant.

Mass spectrometry analysis

Differentially expressed spots were excised from the gel using a spot cutter (Amersham Pharmacia Biotech Inc, Sweden), and proteins were in-gel digested with trypsin and extracted as peptides based on the method described by Gharahdaghi et al. Each sample was suspended in 0.8 µl of 0.5 g/l matrix solution (a-cyano-4-hydroxycinnamic acid in acetonitrile/water (1:1, v/v) acidified with 0.1% (v/v) trifluoroacetic acid). Then the mixture was immediately spotted onto the stainless steel MALDI-TOF MS target plate, and allowed to dry and crystallize under room temperature. The mass spectrometry was performed using a 4700 Proteomics Analyzer (TOF/TOF™, Ap-
plied Biosystems, USA) equipped with a 337 nm Nd: YAG laser.

The proteins were identified by peptide mass fingerprinting (PMF) and tandem mass spectrometry using the program MASCOT version 1.9 (Matrix Science, London, UK) against SWISS-PROT database with GPS explorer software (Applied Biosystems, USA).

**Validation of differentially expressed spots by Western blotting**

Western blotting was performed for three times to verify the differentially expressed proteins in 3 groups at postoperative 3 days. Selected proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. The blots were probed by anti-GAPDH antibody (Santa cruz, California, USA), using ECL Western blotting detection system (Pierce, Rockford, USA). BCA Protein Assay Kit was obtained from Bio Technology Co, Ltd (Beijing, China), and the results were visualized by the Pierce ECL detection system. The digital image was obtained by scanning the film and gray value analysis. The mean optical density (OD) values of GAPDH were compared among three groups.

**RESULTS**

**Comparison in 2D-PAGE**

The two-dimensional electrophoresis maps of the proteins indicated that most of the protein spots were concentrated in the pH 3.5-9.0 region. A total of 446.33±7.00, 436.33±4.51 and 462.67±11.59 protein spots were detected in the gels of rabbit AT model samples in Groups A, B and C (Table 1). Protein spots were detected from the 2D-PAGE in Group A and Group B (Tables 2 and 3). There were 9 protein spots in Group A with expression abundance five times higher than Group B, 11 protein spots in Group B five times higher than Group A.

**Identification of differentially expressed proteins and bioinformatics**

The proteins with the high expression abundance more than 5 times in the AT samples of two groups were considered to be differentially expressed proteins (P<0.05). According to this criterion, many differentially expressed proteins were identified in Group A and Group B (Tables 2, 3). In Group A, 7 proteins was identified as the unique protein, and 3 proteins had expression abundance 5 times higher than that of Group B. In Group B, 4 proteins was identified as the unique proteins, and 7 proteins had expression abundance 5 times higher than that of Group A. In Group B, the unique proteins identified were gi|1334227 protein, which is an unnamed protein, phosphoglycerate kinase 1 protein, osteoglycin protein and pro-alpha-1 type 1 collagen protein. The high abundance proteins identified were glyceraldehyde-3-phosphate dehydrogenase (GAPDH), peroxiredoxin 1, chain A, rabbit serum transferrin at 2.6 A resolution, MAD2L1 binding protein, gi|74181566 to NCBI-Access.-Nr, Carbonic anhydrase I and alpha-1-anti-proteinase E a-1 proteins. Those unidentified spots were considered as protein mixtures and would be further separated and identified. The relative protein expression in Group A and Group B indicated that there was significant difference in the expression abundance.

**Western blotting**

One of the differentially expressed proteins at 3 days after treatment, GAPDH (Table 2), was selected to be validated through Western blotting. Seven samples were randomly selected in each group. Figure 2 shows the results of Western blotting, displaying a comparison of GAPDH among three groups. The mean OD value of GAPDH were 0.40, 2.25 and 0.80 in Groups A, B and C, respectively. The OD value of Group B was higher than that of Group A (P<0.01).

![Figure 2](image-url)
It was indicated that there was significant difference between Group A and Group B with regard to expression abundance of GAPDH. However, significant difference was observed on expression abundance of GAPDH between Group B and Group C. These data agreed with the results in the two-dimensional electrophoresis.

Table 2. Identification of differentially expressed proteins for the AT samples of the rabbits in Group A

<table>
<thead>
<tr>
<th>No.</th>
<th>Spot No.</th>
<th>NCBI-Access.-Nr</th>
<th>Protein name</th>
<th>Molecular weight</th>
<th>pI</th>
<th>Protein score</th>
<th>Functional association</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3110*</td>
<td>gi</td>
<td>73986005</td>
<td>Tetranectin precursorCLECT super-family</td>
<td>26515.4</td>
<td>8.53</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>0319*</td>
<td>gi</td>
<td>19851928</td>
<td>CLL-associated antigen KW-4 splice variant 2</td>
<td>89459.4</td>
<td>9.17</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>5125*</td>
<td>gi</td>
<td>55732136</td>
<td>Hypothetical protein</td>
<td>34684.3</td>
<td>5.29</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>3428*</td>
<td>gi</td>
<td>27806891</td>
<td>Keratocan</td>
<td>40407.9</td>
<td>6.76</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>1133*</td>
<td>gi</td>
<td>178067</td>
<td>Actin prepeptide</td>
<td>36783.2</td>
<td>5.19</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>7133*</td>
<td>gi</td>
<td>73947204</td>
<td>Similar to asporin precursor</td>
<td>42447.1</td>
<td>8.93</td>
<td>112</td>
</tr>
<tr>
<td>7</td>
<td>5234△</td>
<td>gi</td>
<td>126723746</td>
<td>Serum albumin precursor</td>
<td>68865.3</td>
<td>5.85</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>2120△</td>
<td>gi</td>
<td>73999700</td>
<td>Actin</td>
<td>16540.2</td>
<td>6.11</td>
<td>184</td>
</tr>
<tr>
<td>9</td>
<td>7307*</td>
<td>gi</td>
<td>38649027</td>
<td>ASPN protein</td>
<td>43863.4</td>
<td>6.03</td>
<td>89</td>
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</table>

*The unique proteins identified for the AT samples in Group A. △ The proteins with the expression abundance in Group A 5 times higher than that of Group B.

Table 3. Identification of differentially expressed proteins for the AT samples of the rabbits in Group B

<table>
<thead>
<tr>
<th>No.</th>
<th>Spot No.</th>
<th>NCBI-Access.-Nr</th>
<th>Protein name</th>
<th>Molecular weight</th>
<th>pI</th>
<th>Protein score</th>
<th>Functional association</th>
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<tr>
<td>1</td>
<td>0105*</td>
<td>gi</td>
<td>1334227</td>
<td>Unnamed protein product</td>
<td>6693.3</td>
<td>4.61</td>
<td>66</td>
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<tr>
<td>2</td>
<td>9306*</td>
<td>gi</td>
<td>52783777</td>
<td>Phosphoglycerate kinase 1</td>
<td>44574.1</td>
<td>8.64</td>
<td>103</td>
</tr>
<tr>
<td>3</td>
<td>2310*</td>
<td>gi</td>
<td>126723013</td>
<td>Osteoglycin</td>
<td>33866.8</td>
<td>5.93</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>6225*</td>
<td>gi</td>
<td>5732934</td>
<td>Pro-alpha-1 type 1 collagen</td>
<td>20413.1</td>
<td>9.26</td>
<td>105</td>
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<tr>
<td>5</td>
<td>8214△</td>
<td>gi</td>
<td>77404273</td>
<td>GAPDH</td>
<td>35845.3</td>
<td>8.50</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>8112*</td>
<td>gi</td>
<td>56599887</td>
<td>Peroxiredoxin 1</td>
<td>18963.7</td>
<td>6.41</td>
<td>233</td>
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<tr>
<td>7</td>
<td>6628*</td>
<td>gi</td>
<td>15825992</td>
<td>Rabbit serum transferrin at 2.6 A resolution</td>
<td>74741.7</td>
<td>6.35</td>
<td>277</td>
</tr>
<tr>
<td>8</td>
<td>4721△</td>
<td>gi</td>
<td>13543201</td>
<td>MAD2L1 binding protein</td>
<td>31091.6</td>
<td>5.44</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>5210*</td>
<td>gi</td>
<td>74181566</td>
<td>Unnamed protein product</td>
<td>12621.7</td>
<td>5.11</td>
<td>69</td>
</tr>
<tr>
<td>10</td>
<td>7130△</td>
<td>gi</td>
<td>164840</td>
<td>Carbonic anhydrase I</td>
<td>25677.1</td>
<td>8.00</td>
<td>63</td>
</tr>
<tr>
<td>11</td>
<td>2515△</td>
<td>gi</td>
<td>126722876</td>
<td>Alpha-1-antiproteinase E a-1</td>
<td>45655.7</td>
<td>5.72</td>
<td>58</td>
</tr>
</tbody>
</table>

*The unique proteins identified for the AT samples in Group B. △ The proteins with the expression abundance in Group B 5 times higher than that of Group A.

DISCUSSION

The newly developed proteomic analysis is a potentially useful tool to elucidate the rationale behind the success of postoperative early eccentric training via a mechanobiological mechanism and from the viewpoint of system biology, but literature survey has shown that research in the proteomic analysis of AT is few. In this study, the proteomic analysis of differential protein expression in rabbit model of AT rupture treated by postoperative cast immobilization and postoperative early eccentric training was performed based on 2D-PAGE and mass spectrometry. Additionally, the differentially expressed proteins were validated through Western blotting. The analysis of identified proteins in Groups A and B indicated that some proteins may contribute to healing of AT rupture, including GAPDH protein, NCBI-Access.-Nr gi|1334227, phosphoglycerate kinase 1, osteoglycin, etc, which were detected in the early eccentric training group.
GAPDH protein is considered as a housekeeping protein expressed constantly, used for the internal standard of protein qualification.\textsuperscript{18-20} It is considered that classical glycolytic protein is involved in cell-based energy production. However, recent evidence suggests that it is a multifunctional protein that displays novel activities which are significantly different from the traditional metabolic function of this kind of protein. These new roles of GAPDH may depend on its subcellular localization, oligomeric state or cell proliferation state.\textsuperscript{24}

In Group B, gi|1334227 protein is an unnamed protein, associated with Ca\textsuperscript{2+}-binding protein. Phosphoglycerate kinase 1 protein is associated with carbohydrate degradation and glycolysis. Osteoglycin is related with osteoinductive factor. Pro-alpha-1 type 1 collagen is associated with tissue regulation. Peroxiredoxin 1 protein is related with thiol-specific antioxidant (TSA), which can inhibit the destruction to DNA and albumen in cells of oxide. Transferrin at 2.6 A resolution protein is connected with transferring. MAD2L1 binding protein is associated with protein of unknown function (DUF1135) and pfam06581. gi|13543201 protein, also an unnamed protein, can regulate rDNA transcription. Carbonic anhydrase I protein functions as carbonic anhydrases (CAs). Alpha-1-antiproteinase E a-1 protein functions as serine protease inhibitors.

GAPDH protein, comprised of four subunits with molecular weight of 30-40, is a key enzyme in classical glycolysis process. In addition to participating glycolysis in the cytoplasm, in mammalian cells, GAPDH is involved in membrane fusion, microtubule bundling, phosphotransferase activation, export of nuclear RNA, DNA replication, DNA repair and other cellular biochemical processes.

In the present study, GAPDH was considered as force-generating protein, kinesin, involved in microtubule-based motility. GAPDH in different physiological and pathological states still has large fluctuations in shape change.\textsuperscript{22} According to Vale’s theory, GAPDH may be one of the important force-generating proteases. From mechanobiological perspective, in eccentric training period, GAPDH starts to differentially emerge in the early stage and demonstrates high metabolic changes while the AT healing has not yet demonstrated significant histological and biomechanical changes. This may suggest a foundation of vigorous tissue growth in eccentric training. It is also considered that tendons via centrifugal stress are able to respond to mechanical forces by altering AT structure, composition, and mechanical properties at late stage.

Previous research has shown that postoperative early kinesitherapy may promote an overall reconstruction of the ruptured AT through a mechanobiological mechanism.\textsuperscript{23} The timing to start tendon mobilization after surgery is an important factor. In a study that examined canine flexor tendons after surgical repair, immediate mobilization is superior to delayed mobilization in recovering range of motion (a 50% increase) and dramatically better than immobilized tendons (more than a five-fold improvement in angular rotation).\textsuperscript{24} The biomechanical experiment on the injury model of canine flexor tendon shows that the exercises increase maximum intensity of tendon more than the non-motion group (61.6 N and 41.0 N respectively).\textsuperscript{30} Clinically, it is commonly seen that re-rupture occurred soon after removing cast immobilization and the reported rate is 5.6%-11.4%.\textsuperscript{25} It was indicated in the present study that both of the quality and quantity of different protein expressions may play a key role in this healing process. By the contrary, postoperative cast or anterior below-the-knee slab immobilization treatment for many weeks resulted in a situation where the tendon was in a tension-free or disused state and healing of AT rupture was restrained to a certain degree.

Although proteomic analysis to reveal the nature of changes in a biological way is available in many studies,\textsuperscript{16,20,26-29} the research in treatment of AT rupture based on the analysis of differentially expressed proteins has been reported rarely. The eccentric training promotes the healing process of AT on the postoperative 7 days and 12 days\textsuperscript{30} to have the biomechanical changes, or have histological changes at postoperative 3 weeks.\textsuperscript{12,23} These changes are not likely to happen in one night, and it is a gradually developed material accumulation process. During the early phase of AT healing (72 hours), AT has no histological or biomechanical changes, but kinesitherapy induces metabolic changes in AT, which may lead to biomechanical changes of AT at the postoperative 7 days, histological changes in postoperative 3 weeks. This is the reason why we used the newly-developed proteomic technique and chose the third day after operation to analyze early-micro-difference changes between immobilization group and early

\textsuperscript{15,23}
motion group during the healing process. We firstly conducted proteomic analysis in treatment of AT rupture on postoperative 3 days and provided a deep insight into the contribution of postoperative early kinesitherapy to the healing of AT rupture, however, some differentially expressed proteins were not successfully identified in this study. Therefore, further research is required to thoroughly investigate this issue using RT-PCR, Western blot, etc, to validate these proteins and their function, signal conduction network during the process of AT healing.

The self repairing and protecting instinct of humans has always been ignored in the classical postoperative treatment of AT rupture using various immobilization methods. With new operative technique, the experimental outcome has shown that the repaired tendon can resist the load from traction in eccentric training of AT. The new surgical suture method proposed the excellent experimental outcome resulted from the postoperative early kinesitherapy. Differentially expressed proteins were detected in the AT samples of the rabbits treated by early kinesitherapy postoperatively, and mechanobiological mechanism was considered to be essential to promote healing of AT rupture. This research, as an initial study in proteomic analysis of AT rupture at postoperative 3 days, provided a deep insight into the mechanobiological mechanism or mechanism of mechanotransduction on postoperative early kinesitherapy and the foundation of overall reconstruction of AT from system biological point of view.

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