Model preparation

The model generated has been shown in Figure 1 below. The epineurium was cut longitudinally and the nerve tissues were separated. The nerves were cut and then a 5 mm nerve defect area was created while preserving the epineurium. The incision of the epineurium was 3 mm to prevent the nerve tissue from retraction after being cut. **Figure 1A** shows a schematic diagram depicting the model generated. **Figure 1B** and **C** depict the pictures of the model group, and **Figure 1D** shows the picture of the treatment group.

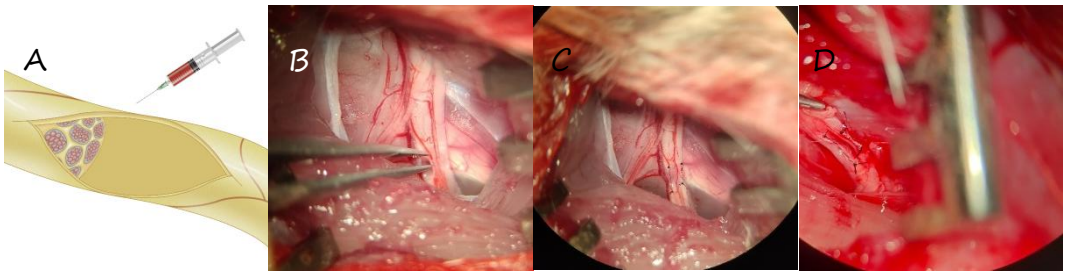

Figure 1 A schematic diagram depicting the model generated.

(A) Schematic diagram of model preparation. (B, C) Model group. (D) Treatment group that has injected PRP.

Auricular artery approached for blood collection

After the rabbit was anesthetized, the central ear artery on the back of the ear was identified and the skin was wiped with alcohol to fully dilate the blood vessels. Thereafter, 0.9 mm needle was connected to a 20 mL syringe to collect the blood. Normally, 20-30 mL of blood can be collected from a rabbit. During the blood collection, the movement should be gentle and the stimulation of the artery should be reduced to prevent vasospasm. **Figure 2** included below shows the process of blood collection.

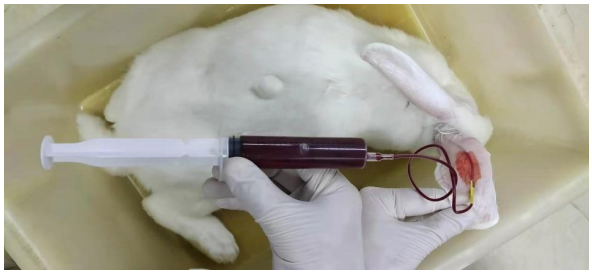

Figure 2 Blood collection from auricular artery.

Characterization of PRP

In the low-concentration group, the platelet in PRP was $866.67 \pm 91.54 \times 10^9$/L and the

enrichment index was 3.05 ± 0.38-fold. In MODERATE CONCENTRATION, the platelet concentration was 1654.29 ± 212.34 × 10^9/L and the enrichment index was 5.44 ± 0.62-fold, while in high concentration, the platelet concentration was 2548.33 ± 198.01 × 10^9/L and the enrichment index was 8.15 ± 0.48-fold. There was a statistically significant difference among the three groups ($P < 0.05$).

Result of GO and KEGG

Result of GO and KEGG was shown in **Table 1**.

Table 1 GO and KEGG pathway analysis on protein expression changes after PRP treatment on sciatic nerve injury

Category	Term	Count	Gene symbol	P-value	Fold enrichment
Gene Oncology					
GOTERM_BP	GO:0006886~intracellular protein transport	4	*CSE1L*, *SNX9*, *COPG1*, *TNPO1*	0.03	5.47
GOTERM_CC	GO:0070062~extracellular exosome	28	*A1BG*, *THY1*, *ALAD*, *STK10*, *CA1*, *PRDX4*, *ITGB8*, *AOX1*, *SNX9*, *TNPO1*, *MAMODERATE CONCENTRATIONK1*, *ACE*, *IDH1*, *ANXA5*, *HPCAL1*, *PLXDC2*, *ALDH3A2*, *GNPDA1*, *FABP5*, *CRISPLD2*, *BLMH*, *UBA1*, *GALK1*, *ARF6*	0.00	2.67
GOTERM_MF	GO:0005509~calcium ion binding	8	*GSN*, *MYL2*, *ANXA5*, *HPCAL1*, *PLS3*, *LTBMODERATE CONCENTRATION*, *ITGB1BMODERATE CONCENTRATION*, *MATN3*	0.01	3.29
KEGG(kyoto encyclopedia of genes and genomes) pathway					
KEGG_PATH	ocu04510:Focal adhesion	6	*MAMODERATE CONCENTRATIONK1*, *ROCK1*, *MYL2*, *COL5A2*, *ITGB8*, *ILK*	0.00	6.51

WAY					
KEGG_PATHWAY	ocu04810:Regulation of actin cytoskeleton	5	*MAMODERATE CONCENTRATIONK1*, *GSN*, *ROCK1*, *MYL2*, *ITGB8*	0.01	5.35
KEGG_PATHWAY	ocu01130:Biosynthesis of antibiotics	5	*ALDH3A2*, *HK3*, *IDH1*, *ACADM*, *PAPSS2*	0.02	4.82
KEGG_PATHWAY	ocu00520:Amino sugar and nucleotide sugar metabolism	3	*HK3*, *GNPDA1*, *GALK1*	0.02	14.20
KEGG_PATHWAY	ocu00280:Valine, leucine and isoleucine degradation	3	*ALDH3A2*, *AOX1*, *ACADM*	0.03	11.51
KEGG_PATHWAY	ocu03320:PPAR signaling pathway	3	*FABP5*, *ILK*, *ACADM*	0.04	9.27
KEGG_PATHWAY	ocu01100:Metabolic pathways	11	*CKMT2*, *ALAD*, *ALDH3A2*, *HK3*, *GNPDA1*, *IDH1*, *AOX1*, *ACADM*, *PAPSS2*, *PAFAH1B2*, *GALK1*	0.04	1.90
WAY					

Liquid chromatography-mass spectrometry analysis (the method of sample process)

Preparation of samples:

1. The tissue samples were transferred to 1.5 mL centrifuge tubes in 1× phosphate-buffered saline (pH 7.5) and pre-chilled at 4°C. The tissue was then ground with a grinding rod, centrifuged at 2392 × *g* for 2 minutes, and the phosphate-buffered saline was removed. This was repeated three times until the tissue was colorless.
2. The sample was then transferred to a 2 mL centrifuge tube, cut up, and protein lysate (7 M urea, 2% sodium dodecyl sulfate, 1× protease inhibitor cocktail) was added.
3. The tissue was homogenized with a handheld homogenizer (Jingxin Technology, Shanghai, China) for 15 seconds, then placed on ice for 30 seconds. This step was repeated three times. If tissue pellets were present in the suspension, the steps were repeated.
4. The samples were then sonicated using an ultrasonic cell disruptor (Scientz-IID, Xinzhi, Ningbo, China) for 2 seconds bursts with 5 seconds intervals on ice for a total of 1 minute, and lysed on ice for 2 hours.
5. The lysate was centrifuged at 16,170 × *g* for 20 minutes at 4 °C, and the supernatant was transferred to a 1.5 mL centrifuge tube. The supernatant was then centrifuged at 16,170 × g for 20 minutes at 4°C and the middle layer was aspirated into a new 1.5 mL centrifuge tube.
6. Six volumes of 100% acetone were added, and the sample was precipitated overnight at -20°C.
7. After one day, the sample was re-centrifuged, and the precipitate was washed twice with 500 μL of pre-cooled solution (ethanol: acetone: acetic acid = 50:50:0.1) and centrifuged at 16,170 × *g* for 15 minutes at 4°C.
8. The precipitate was then re-dissolved in 6 M guanidine hydrochloride, 300 mM triethylammonium bicarbonate solution and the concentration was measured again. The samples were stored in a refrigerator at 4°C and partially diluted to determine the concentration by the bicinchoninic acid assay method (Olson and Markwell, 2007).

Filter aided proteome preparation enzymolysis

1. One-hundred microgram aliquots were taken from each sample and made up to 100 μL with 25 mM ammonium bicarbonate.
2. Dithiothreitol (2 μL, 1 M dithiothreitol/100 μL protein, final concentration 20 mM) was added to the protein solution for reductive alkylation, then the sample was mixed and incubated at 57°C for 1 hour. Ten microliters of 1 M iodoacetamide/100 μL solution (final concentration of 90 mM iodoacetamide dissolved in 25 mM ammonium bicarbonate immediately before use) was added, mixed, and kept away from light for 40 minutes at room temperature.
3. The reduced and alkylated protein was placed in a 10 K ultrafiltration tube and centrifuged

at 13,778 × *g*, after which the solution at the bottom of the collection tube was discarded.

4. Ammonium bicarbonate (dissolution buffer) was added to the ultrafiltration tube and the material was washed four times.

5. Trypsin prepared in the dissolution buffer was added, and the sample was digested at 37°C overnight.

6. The next day, the digested peptides were collected by centrifugation and concentrated and dried by centrifugation. After centrifugation and drying, the peptides were desalted using a Monospin desalting column, dried and prepared for mass spectrometry analysis.

Multiple reaction mode target protein verification

After filter-aided proteome preparation enzymatic digestion and desalting, the target proteins were screened for quantitative signature peptides with the following conditions: 1. It must be a unique peptide; 2. The length must be 6-25 amino acids; 3. At least three fragments with the highest response intensity must be selected, which included b and y ions; 4. Information of the 2+, 3+ and 4+ parent ions were retained and the ions with higher overall signal intensities were preferentially selected.

The MRM mode was used to detect the target peptides in the peptide complex using the following screening conditions: 1) Liquid phase conditions: Phase A: 2% ACN, 0.1% FA; Phase B: 98% ACN, 0.1% FA; Flow rate: 5 μL/min; Gradient conditions: 0-5 minutes, 95% A, 5% B; 5-105 minutes, 70% A, 30% B; 105-115 minutes, 20% A, 80% B; 115-120 minutes, 98% A, 2% B. 2). The mass spectrometry conditions: ion source of electrospray ion source; positive ion mode detection; scanning mode of multiple reaction monitoring (MRM); injection voltage of 5500 eV; temperature of 150°C; gas curtain gas of (CUR, N2) 30 psi; collision gas pressure of (CAD, N2) high mode, auxiliary gas GAS1 pressure of 20 psi; auxiliary gas GAS2 pressure of 15 psi; scan time of 5 ms. The decluster voltage and collision energy used scheduled MRM methods, with a total scan time of 1.7S and an MRM detection window of 300S. The proteins with fold change ≥ 1.2 or c ≤ 1/1.2 and $P < 0.05$ were considered as differentially expressed proteins between groups.

Statistical method

The statistical method was summarized in **Figure 3.**

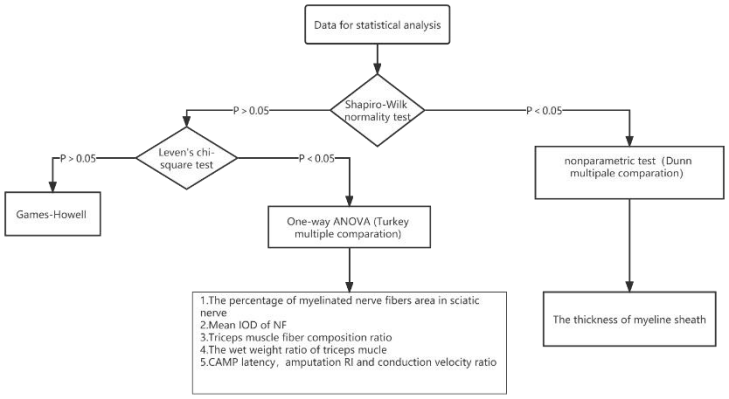

Figure 3 Statistical method.

Reference

Olson BJ, Markwell J (2007) Assays for determination of protein concentration. Curr Protoc Protein Sci Chapter 3:Unit 3 4.