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Original Article

Experimental Study of Autologous Platelet-rich Plasma Combined With Sodium Hyaluronate on Tendon-bone Healing After Rotator Cuff Injury Repair in Rabbits

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Abstract

Objective: To investigate the therapeutic effects of autologous platelet-rich plasma (PRP) combined with sodium hyaluronate on tendon healing following rotator cuff injury repair in rabbits. **Methods**: New Zealand white rabbits were randomly assigned to five groups: sham operation group, control group, PRP group, sodium hyaluronate group, and combined group, each comprising 12 rabbits. A rotator cuff injury model was established in all groups except the sham operation group. At 8 weeks post-surgery, 12 lateral rotator cuff specimens were taken from each group. Four specimens were randomly selected from each group for biomechanical testing, and analyses were conducted on the expression of vascular endothelial growth factor (VEGF), the fiber area ratio of COL-I and COL-III, and tissue morphology. **Results**: The combined group exhibited the highest biomechanical strength in the cuff tissue of white rabbits ($P < 0.05$). There was no significant difference in VEGF levels among the five groups ($F = 0.814$, $P = 0.523$). However, a significant difference was observed in the ratio of fiber area between COL-I and COL-III groups ($F = 11.600$, $P < 0.001$), with the combined group scoring the highest (3.82 ± 0.47 minutes). The inflammatory infiltration in tendon-bone tissue was minimal, and histological morphology was optimal. **Conclusion**: The combination of PRP and sodium hyaluronate effectively promotes the repair of rotator cuff injuries and accelerates tendon-bone healing.

Keywords: Platelet-rich Plasma, Rotator Cuff Injury, Sodium hyaluronate

Introduction

Rotator cuff injury is a prevalent cause of shoulder pain and dyskinesia, particularly among middle-aged and elderly individuals. The incidence rate among those over 60 years old is approximately 40%[1]. Rotator cuff tears, whether resulting from acute injury or substantial damage, often necessitate surgical intervention. Regardless of the chosen treatment approach—be it conservative or surgical—swift recovery plays a pivotal role in determining the patient's prognosis. Establishing a suitable animal model is crucial in identifying rapid and effective recovery methods.

Platelet-rich plasma (PRP) is obtained by centrifuging whole blood, resulting in a plasma rich in platelets. PRP, a concentration of autologous platelets, contains abundant growth factors and cytokines that effectively promote tissue healing[2,3]. PRP harnesses the body's innate repair capacity, making it applicable for various tissue repairs and wound healing[4]. Its autologous origin ensures safety. Sodium hyaluronate, commonly known as hyaluronic acid, is a non-species-specific glucuronic acid polysaccharide inherent to the human body. Widely distributed in the cytoplasm and extracellular matrix of cells and tissues such as placenta, amniotic fluid, lens, articular cartilage, and dermis, it provides lubrication and nourishment to contained cells and organelles. Research indicates its efficacy in treating conditions like ankle osteoarthritis[5]. This study aims to investigate the therapeutic effects of combining PRP and sodium hyaluronate for tendon-bone healing after rotator cuff injury in rabbits. Through the establishment of a rabbit shoulder cuff injury model, the study aims to provide a favorable basis for related research.

The authors have no conflict of interest.

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Materials and Methods

Laboratory animals

Sixty healthy adult male New Zealand white rabbits, with weights ranging from 2.5 to 3.0 kg and aged between 6 and 12 months, were sourced from the Animal Experiment Center of Weifang Medical University. The rabbits were accommodated in a clean animal room at Weifang Medical University, and the experiment commenced after a period of adaptive feeding lasting 7 days. Considering the anticipated effect and statistical efficiency (80%), we calculated the sample size, leading to the utilization of 12 white rabbits in each group.

Preparation of medicine

Preparation of Heparinization Instrument: A 10 mL syringe was used to draw 9.6 mL of normal saline. The pre-filled low molecular weight heparin in the syringe was then mixed with the saline through gentle pumping, resulting in a diluted solution of 500 IU/ml low molecular weight heparin. After a 3 to 5-second interval, the mixture was injected into a centrifuge tube, yielding a heparinized syringe and a heparinized centrifuge tube.

Autologous PRP Preparation: Twenty-four rabbits were randomly selected, anesthetized, and immobilized with sodium pentobarbital (40 mg·kg-1, 1% concentration). Using a heparin syringe, 9 mL of blood was collected from the rabbit auricular central artery, and a 0.1 mL sample was used for whole blood platelet count (0.41 × 1012/L). The remaining venous blood was centrifuged at 400g for 10 minutes, removing the bottom erythrocytes, followed by a second centrifugation at 1200g for 15 minutes. After settling, the pale-yellow gel was extracted as PRP and placed in a sterile tube. By adding 80u/mL thrombin (10% CaCl2 solution + bovine thrombin), a PRP gel (approximately 1 mL) was obtained, with 0.1 mL taken for PRP platelet count (2.47 × 1012/L).

Animal grouping modeling, drug administration and specimen collection

Twenty-four rabbits, prepared with autologous PRP, were randomly divided into the PRP group and the combined group, with 12 rabbits in each group. Another 36 rabbits were randomly divided into the sham operation group, control group, and sodium hyaluronate group, with 12 rabbits in each. All rabbit rotator cuff injury models were established except in the sham operation group[6].

Establishment and Repair of the Rabbit Rotator Cuff Injury Model: Procedure involved administering the operation on the left shoulder joint of all rabbits after an 8-hour fasting period. A preventive measure against infection was taken by intramuscular injection of 0.096 g penicillin (equivalent to 160,000 units). White rabbits were anesthetized with pentobarbital sodium (40 mg·kg-1, concentration 1%) and securely fixed on the operating table. Following skin preparation and disinfection, a 2 cm longitudinal incision was made along the long axis of the attachment point of the greater tubercle of the humerus. Subcutaneous tissue was separated, exposing the supraspinatus tendon at the attachment point of the greater tubercle of the humerus. The supraspinatus tendon was then cut at the endpoint of the greater tubercle of the humerus, and it was peeled off along the greater tubercle of the humerus towards the abdomen of the supraspinatus using a blade, creating a gap of approximately 0.5 cm × 0.5 cm. After establishing the rabbit model of full-thickness rotator cuff injury, the tendon was sutured using a bone-penetrating suture technique. In the control group, the incisions were sutured layer by layer without any drug treatment. In the PRP group, PRP gel was evenly applied to the injured area, and the fascia was sutured to wrap and fix it to prevent loss. The sodium hyaluronate group was injected with 0.2 mL sodium hyaluronate solution into the injured area, and the damaged fascia was completely wrapped. In the combined group, PRP gel was uniformly applied to the injured area, and 0.2 mL sodium hyaluronate solution was injected into the injured area simultaneously. In the sham operation group, only the supraspinatus of rabbits was exposed without any treatment. After the operation, the affected limbs were not fixed, and the rabbits in each group moved freely, having free access to drinking water and food. Gentamicin (3 mg/kg) was injected intramuscularly into the hind legs to prevent infection, twice a day for 3 days. Throughout the experiment, all rabbits successfully survived.

Rabbits in each group were anesthetized with pentobarbital sodium (40 mg/kg, 1% concentration) 8 weeks after surgery and then sacrificed by neck dislocation. The supraspinatus tendon and proximal humerus were taken from the center of the supraspinatus, and the proximal scapula, distal humerus, and other soft tissues were removed. They were then divided into two parts: one part was wrapped in wet gauze soaked with normal saline and stored at a low temperature for biomechanical testing. The other part of the specimens was placed in formalin solution (10%) and stored at 4°C.

Biomechanical test of rotator cuff tissue of white rabbits in each group

The biomechanical properties of the specimens were tested using a tensile test with an electronic universal testing machine. The specimens underwent pretreatment with a 5N tensile force for 1 minute, followed by a tensile fracture test (load rate: 5mm/min), and measurements were taken for the maximum load and stiffness.

Expression of Vascular endothelial growth factor (VEGF) in lateral tendon and bone of rabbits in each group

Four specimens were randomly selected from each group to assess Vascular Endothelial Growth Factor (VEGF) expression. The specimens, fixed in formaldehyde solution, underwent decalcification with EDTA, dehydration with gradient ethanol, transparency with xylene, and embedding in paraffin. Subsequently, they were sectioned along

Table 1. Comparison of biomechanical test results of rotator cuff tissue of white rabbits in each group [$\bar{x} \pm s$, n=12].

Group	Maximum load (N)	Stiffness (N/mm)
Sham operation group	78.54±3.90	59.84±3.77
Control group	70.87±2.69[a]	48.64±4.57[a]
PRP Group	75.14±4.13[ab]	50.29±3.63[ab]
Sodium hyaluronate group	72.23±4.28[abc]	50.45±4.08[ab]
Joint group	78.15±4.52[bcd]	60.39±3.20[bcd]
F	9.048	25.940
P	<0.001	<0.001

Note: Compared with sham operation group, [a]P < 0.05; Compared with the control group, [b]P < 0.05; Compared with PRP group, [c]P < 0.05; Compared with sodium hyaluronate group, [d]P < 0.05.

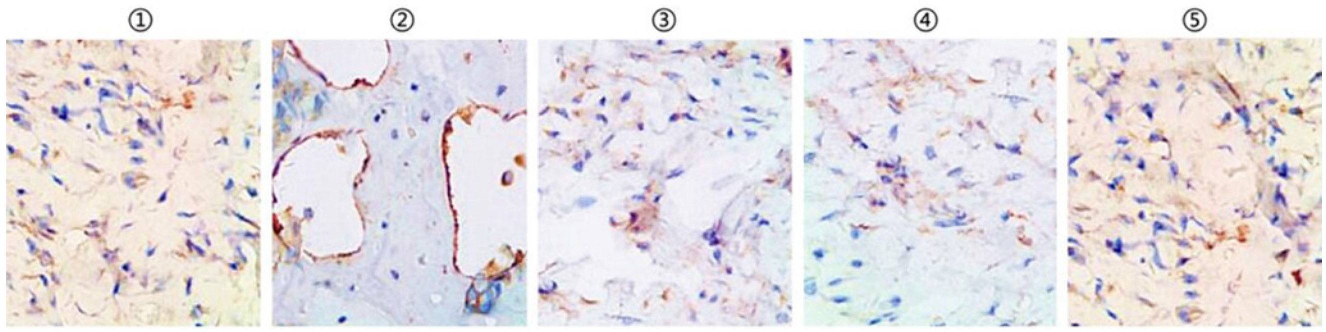

Figure 1. Immunohistochemical staining of VEGF in rabbits of each group (200 ×). Note: ① Sham operation group; ② Control group; ③ PRP Group; ④ Sodium hyaluronate group; ⑤ Combined group.

Table 2. Comparison of VEGF scores of white rabbits in each group [$\bar{x} \pm s$, n=12, Score].

Group	Immunohistochemical score
Sham operation group	3.71±0.28
Control group	3.50±0.46
PRP Group	3.71±0.49
Sodium hyaluronate group	3.73±0.52
Joint group	3.82±0.47
F	0.814
P	0.523

the longitudinal direction of the tendon-bone interface (thickness: 4 μm). The distribution of VEGF was observed, and its expression was evaluated through immunohistochemical staining.

The immunohistochemical scoring criteria were as follows[7]: Under an optical microscope (×400), five visual fields were randomly selected from each section. Scores were assigned based on the proportion of positive cells and staining intensity. Positive cell ratio: No cell staining-0 points, positive cell ratio < 10%-1 point, positive cell ratio 10%-50%-2 points, positive cell ratio 51%-80%-3 points, positive cell ratio > 80%-4 points; Staining intensity: colorless-0 points, light yellow-1 point, yellow-2 points, brown/brown-3 points. The product of these scores resulted in the immunohistochemical score (total score 0 ~ 12 points). A higher score indicates a higher expression of VEGF.

Rabbit rotator cuff tendon-bone recovery in each group

Four specimens from each group underwent staining with Sirius Red picrate, and the distribution of Collagen type I (COL-I) and Collagen type III (COL-III) fibers was observed using a polarized light microscope. Under polarized light, COL-I exhibited a yellow-red color, while COL-III appeared green. The Image-ProPlus software was employed to calculate the fiber area ratio of COL-I and COL-III, aiding in the evaluation of rotator cuff tendon-bone recovery.

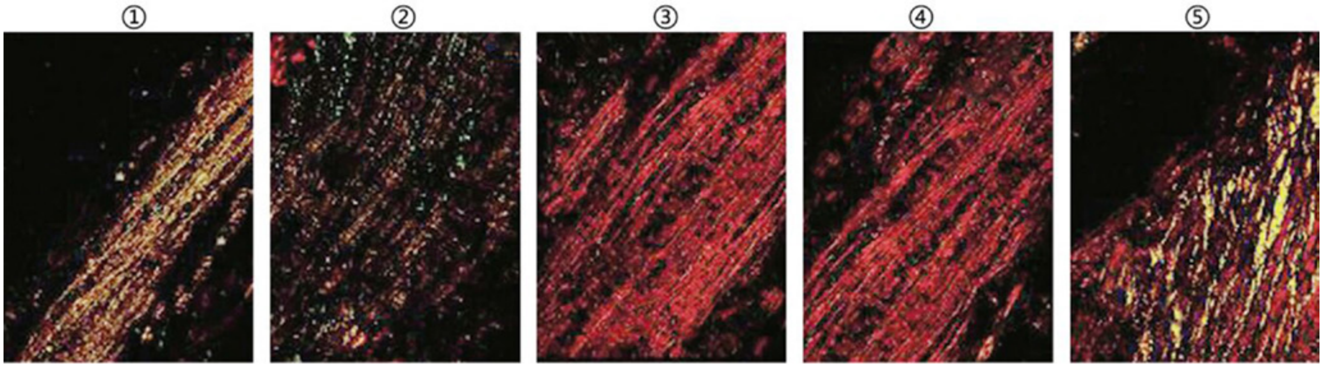

Figure 2. Rotator cuff tendon-bone picric acid Sirius red staining (200 ×). Note: ① Sham operation group; ② Control group; ③ PRP Group; ④ Sodium hyaluronate group; ⑤ Combined group.

Histomorphological observation of lateral tendon-bone interface in white rabbits of each group

Four specimens were randomly selected from each group and subjected to toluidine blue staining and Masson staining. The specimens, fixed in formaldehyde solution, underwent decalcification using EDTA, followed by dehydration with gradient ethanol, transparency with xylene, and embedding in paraffin. Subsequently, sections were prepared along the longitudinal direction of the tendon-bone interface (thickness: 4 μm). The results of toluidine blue staining and Masson staining were then observed.

Statistical analysis

GraphPad Prism 8 software was used for statistical analysis. The data in each group followed a normal distribution. Mean ± standard deviation ($\bar{x} \pm s$) was used to describe the measurement data. One-way ANOVA was employed for group comparisons, and Least Significant Deference test (LSD-t) method for pairwise comparisons. The significance level was set at $P < 0.05$

Results

Biomechanical test of rotator cuff tissue of white rabbits in each group

The maximum load in the combined group represented the optimal force, excluding the sham operation group, which measured (78.15 ± 4.52) N. The stiffness reached the highest value among the five groups, measuring (60.39 ± 3.20) N/mm. Significant differences were observed between the groups ($F_{maxload}$ = 9.048, $P_{maxload}$ < 0.001; $F_{maxstiffness}$ = 25.940, $P_{maxstiffness}$ < 0.001). Compared to the sham operation group, both the maximum load and stiffness in the control group, PRP group, and sodium hyaluronate group were significantly reduced ($P < 0.05$), but there was no significant difference in the combined group ($P > 0.05$). In comparison to the control group, both the maximum load and stiffness in the PRP group, sodium hyaluronate group, and combined group were significantly increased ($P < 0.05$). While the maximum load of the sodium hyaluronate group decreased significantly compared to the PRP group ($P < 0.05$), there was no significant difference in stiffness ($P > 0.05$). The combined group showed a significant increase in both maximum load and stiffness, significantly surpassing the values of the sodium hyaluronate group ($P < 0.05$), as shown in Table 1.

Table 3. Comparison of fiber area ratio of COL-I and COL-III in white rabbits of each group [$\bar{x} \pm s$, n=12].

Group	Ratio
Sham operation group	2.34±0.15
Control group	2.03±0.21^{a}
PRP Group	2.35±0.17^{b}
Sodium hyaluronate group	2.34±0.14^{b}
Joint group	2.53±0.23^{abcd}
F	11.600
P	<0.001

Note: Compared with sham operation group, $^{a}P < 0.05$; Compared with the control group, $^{b}P < 0.05$; Compared with PRP group, $^{c}P < 0.05$; Compared with sodium hyaluronate group, $^{d}P < 0.05$.

Expression of VEGF in lateral tendon-bone of white rabbits in each group

There was no significant difference in VEGF scores among all groups (F = 0.814, P = 0.523). However, the VEGF scores of the PRP group, sodium hyaluronate group, and combined group were significantly better than those of the control

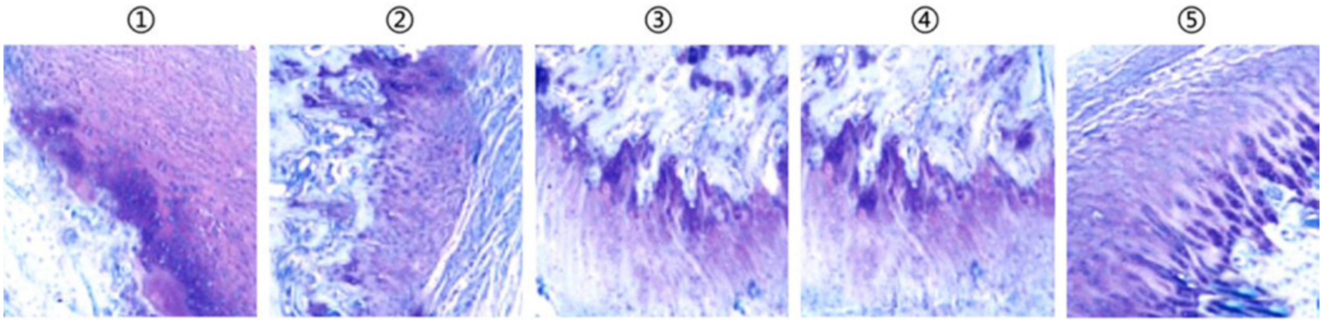

Figure 3. Toluidine blue staining of lateral tendon-bone tissue of white rabbits in each group (200 ×). Note: ① Sham operation group; ② Control group; ③ PRP Group; ④ Sodium hyaluronate group; ⑤ Combined group.

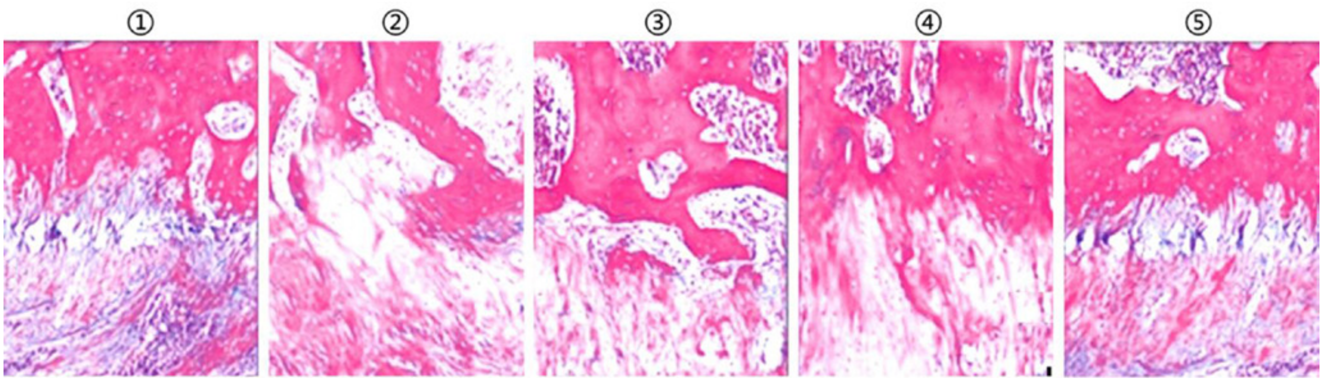

Figure 4. Masson staining of lateral tendon-bone tissue of white rabbits in each group (200 ×). Note: ① Sham operation group; ② Control group; ③ PRP Group; ④ Sodium hyaluronate group; ⑤ Combined group.

group, with the combined group exhibiting the best results. The results of immunohistochemical staining for VEGF in rabbits were observed. When compared with the control group, the expression of VEGF-positive cells in the sham operation group, PRP group, sodium hyaluronate group, and combined group was significantly higher. Moreover, the expression difference of positive cells among the sham operation group, PRP group, sodium hyaluronate group, and combined group was smaller, as shown in Table 2 and Figure 1.

Recovery of rotator cuff tendon-bone in white rabbits in each group

The ratio of COL-I and COL-III fiber area in each group of rabbits was statistically significant ($F = 11.600$, $P < 0.001$), and the combined group had the highest ratio of COL-I and COL-III fiber area, measuring (2.53 ± 0.23). Compared with the sham operation group, the ratio of COL-I and COL-III fiber area in the control group was significantly lower, but significantly higher in the PRP group, sodium hyaluronate group, and combined group ($P < 0.05$). Additionally, compared with the control group, the ratio of the PRP group, sodium hyaluronate group, and combined group was significantly higher than that of the PRP group and sodium hyaluronate group ($P < 0.05$). There was no significant difference between the PRP group and sodium hyaluronate group ($P > 0.05$). The staining results showed a small amount of COL-III in the control group, and the distribution of COL-III was less in the other groups, with the least observed in the combined group (Table 3 and Figure 2).

Toluidine blue staining of lateral tendon-bone tissue of white rabbits in each group

Observe the results of toluidine blue staining in rabbits from each group. The sham operation group exhibited a substantial presence of fibroblasts and chondrocytes, with normal tissue morphology. In the control group, a few fibroblasts were identified, new chondrocytes were scarce, and tissue connections were loose. Both the PRP group and sodium hyaluronate group displayed an increased number of fibroblasts and chondrocytes, with the lateral tendon-bone connection becoming closer. In the combined group, the morphology essentially returned to normal. No significant difference was observed between the PRP group and sodium hyaluronate group (Figure 3).

Masson staining of lateral tendon-bone tissue of white rabbits in each group

Observe the results of Masson staining in white rabbits from each group: Collagen fibers were clearly and orderly arranged in the lateral tendon-bone tissue of white rabbits in the sham operation group. Occasionally, a small amount of inflammatory infiltration was observed between tissues. In the control group, collagen fibers exhibited loose binding, and a substantial amount of inflammatory infiltration was observed between tissues. The PRP group, sodium hyaluronate group, and combined group showed reduced inflammatory infiltration, and the morphology of collagen fibers was essentially normal. The tissue morphology of the combined group was the most favorable, as depicted in Figure 4.

Discussion

In this study, the combined intervention of autologous PRP and sodium hyaluronate led to a higher degree of tendon-bone recovery and normalized tissue morphology in rabbits with rotator cuff injuries. Vascular endothelial growth factor (VEGF) serves as a potent, specific angiogenic factor, playing a pivotal role in angiogenesis and biomechanical activity. 'Load' encompasses external forces acting on the rotator cuff, such as gravity, inertia, and friction, while 'stiffness' denotes the tissue's resistance to deformation under load. Studies have indicated a close relationship between stiffness and load, signifying that higher stiffness results in less deformation under external load. However, patients with rotator cuff injuries exhibit diminished rotator cuff function, tissue damage, reduced VEGF expression, and decreased maximum load and stiffness. These manifestations are clinically observed as shoulder joint movement disorders and shoulder weakness[8]. PRP is a highly concentrated platelet-rich growth factor (GF) known for its beneficial effects on cell proliferation and differentiation. Its capacity to mobilize the self-healing functions of cells and tissues makes it a common choice for repairing bone defects, addressing meniscus joint cartilage damage, and treating tendon and ligament injuries[9]. Autologous PRP contains numerous growth factors, such as endothelial growth factor and transforming growth factor, synergistically enhancing their effectiveness at the site of injury. This makes PRP a valuable option for treating injuries to cartilage, ligaments, and tendons[10]. Additionally, PRP can concentrate in damaged joint and soft tissue areas, promoting the secretion of VEGF. This, in turn, enhances the natural recovery ability of aging and injured tissues, ultimately improving biomechanical indices.

Sodium hyaluronate creates a conducive microenvironment for cell metabolism while ensuring lubrication between cells and organs. It aids in clearing oxygen free radicals, improving circulatory metabolism, and fostering the rapid healing of damaged areas[11]. Sodium hyaluronate possesses an adhesive effect that enables it to aggregate VEGF in tissues and cells at the injured site, expediting the repair process of rotator cuff tissue. When directly injected into joints, sodium hyaluronate not only accelerates wound healing but also moisturizes the joints. Previous studies have demonstrated the effective role of sodium hyaluronate in promoting the repair of the scapular tendon in stroke patients with rotator cuff injuries, thereby reducing the incidence and severity of congestion[12].

Huang et al.[13] showed that PRP effectively reduces the pain levels in patients with rotator cuff injuries. They confirmed that the various growth factors and bone morphogenetic proteins abundant in PRP play a crucial role in guiding neovascularization, promoting tendon cell proliferation, and enhancing collagen synthesis. In a study by Louis et al.[14], where VEGF combined with sodium hyaluronate was injected into rabbits with allogeneic bone-patellar tendon-bone reconstruction, it was found that the sustained release of sodium hyaluronate kept VEGF at a closer proximity to physiological concentrations for an extended period, thereby promoting continuous blood vessel regeneration. Cai Yu et al.[15] used a subacromial injection of PRP combined with sodium hyaluronate to treat partial rotator cuff tears and observed that the treatment effect was significantly superior to using PRP alone. This finding aligns closely with the results obtained in our study. The combination of PRP and sodium hyaluronate effectively enhances the expression of VEGF in rabbits with rotator cuff injuries, thereby improving their biomechanical indices. Collagen, a vital component of the extracellular matrix (ECM), plays a crucial role in tissue repair. As VEGF accumulates in substantial amounts to facilitate the repair of damaged rotator cuff tissue, a significant quantity of ECM is accumulated outside cells. Among these, COL-I and COL-III are closely related and work synergistically to maintain the coherence and stability of boundary cells' structure[16]. COL-I is associated with promoting keratinocyte production. Some studies have revealed that incorporating keratinocytes into the collagen matrix can effectively reduce inflammatory reactions and stimulate VEGF synthesis. During the tissue repair process, the ratio of COL-I to COL-III increases, signifying that COL-III is gradually replaced by COL-I due to the remodeling of the cytoplasmic matrix during tissue repair and vascular reconstruction. This ratio change is indicative of the gradual recovery of tendons[17]. Karayannopoulou et al.[18], in their study injecting PRP into skin photoaging rats, observed a significant increase in COL-I and COL-III in the rats' skin. Activated PRP demonstrated better promotion of COL-III expression compared to unactivated PRP. Stryja et al.[19], in a study using sodium hyaluronate injection and subchondral drilling to repair cartilage injuries in rabbits, noted a significantly higher expression of cartilage-specific genes, COL-I and Collagen type X (COL-X), in the sodium hyaluronate group, resulting in better repair.

The COL-I to COL-III ratio was lowest in the control group, but in the combined group, it was better than in other groups. This suggests that PRP combined with sodium hyaluronate can effectively reduce inflammatory reactions in tissues and promote the rapid repair of damaged rotator cuff tissues. Masson staining was employed to show the fibrous structure and the degree of inflammatory infiltration. Toluidine blue staining was used to evaluate the injury in the rabbit rotator cuff tissue. Xu et al.[20] discovered that PRP effectively reduces inflammatory mediators in tissues of Achilles tendon disease model rabbits, leading to a significant decrease in inflammatory infiltration in Masson staining results. Kartika et al.[21] intervened with patients suffering from knee osteoarthritis using PRP combined with sodium hyaluronate, resulting in a significant improvement in knee joint function and a notable reduction in inflammatory factors in the serum.

There are some limitations in this study. The optimal dosage of PRP combined with sodium hyaluronate remains unclear, necessitating further experimental study. The modeling method employed in this article may lean more towards rotator cuff degradation or tearing, and future research should explore more precise modeling methods to enhance the reliability of research results. The strength of this study lies in the selection of modeling animals and the design of experimental plans. The bone canal formed by the rabbit coracoid process and subpelvic nodules is remarkably similar to the human structure, and the rabbit tendon is substantial. The article takes into account the role and mechanism of autologous PRP and sodium hyaluronate in tendon-bone healing, providing a more reasonable treatment plan for the repair of rotator cuff injuries.

In conclusion, PRP combined with sodium hyaluronate effectively promotes the repair of rabbit rotator cuff injuries, enhances the expression of VEGF, improves its biomechanical indexes, enhances the recovery degree of the rabbit rotator cuff tendon-bone interface, and improves the morphology of the rotator cuff tissue. This renders it of high research value.

Ethics approval

The animal experiment procedures were approved by the Experimental Animal Ethics Committee of Weifang Medical College (Approval No.: 2021SDL392). The care and experimentation of animals adhered to the Guiding Opinions on Treating Experimental Animals issued by the Ministry of Science and Technology of China. The methods employed followed the 3R principles.

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