Scholar Publishing Group
International Journal of Educational Innovation and Science
https://doi.org/10.38007/IJEIS.2022.030406
ISSN 2789-7184 Vol. 3, Issue 4: 69-81

Platelet Rich Plasma Combined with Collagen Matrix Promotes Chondrocyte Regeneration in Arthritis and the Effect of Nanoparticles on Oxygen Free Radicals

Qiumin Li*

Gansu Normal University for Nationalities, Hezuo, China

**corresponding author*

Keywords: Platelet Rich Plasma, Arthritis Cartilage, Cartilage Repair, Collagen Matrix

Abstract: With the development of economy and the improvement of medical level, the treatment technology of osteoarthritis has also made a great breakthrough. At present, surgical treatment is the mainstream way for patients with severe disease. Although surgical treatment can solve the problem of cartilage damage, it is still not enough to fundamentally treat the disease. It is the key to achieve the goal of cartilage repair. In view of the lack of research in this field, this paper proposed platelet rich plasma combined with collagen matrix to promote the regeneration of articular chondrocytes and the effect of nanoparticles on oxygen free radicals. The research of this paper is mainly divided into three parts. The first part is the research of theoretical basis and core concepts. Through this part of the study, we believe that PRP and collagen matrix can strengthen the self-healing ability of tissue, and achieve ideal effect in theory. The second part is animal experimental model. Based on the above theoretical basis, the experimental model of white rabbit is established. In order to compare the experimental effect, 18 rabbits were randomly divided into two groups, 9 in each group. In the third chapter, the experimental principle and design scheme are given in detail. The third part is a comparative experiment. In order to verify the actual effect of this model, a number of experimental analysis including histological observation of new cartilage, biomechanical properties of new cartilage and expression of type II collagen were carried out. Through the analysis of experimental data, PRP combined with collagen matrix accelerated the regeneration of articular chondrocytes, and had a close relationship with the effect of nanoparticles on oxygen free radicals.

1. Introduction

With the development of society and the progress of science and technology, more and more attention has been paid to articular cartilage injury. The common causes of articular cartilage injury include fracture caused by trauma, osteoarthritis caused by long-term wear of articular cartilage, acromegaly, hemophilia and other physical diseases. Traditional methods of treatment of cartilage injury conservative treatment and surgical treatment, conservative treatment does not take oral

non-steroidal anti-inflammatory drugs or intra-articular injection of sodium hyaluronate. This method is only to slow down the further development of articular cartilage injury, relieve pain, and does not fundamentally solve the problem of articular cartilage injury. Surgical treatment, including local micro fracture technique, cartilage transplantation and joint replacement, is how to solve the problem of articular cartilage injury, but it also has a corresponding indication and shortcomings. The new tissue of microfracture fibrocartilage, which alleviate the short-term clinical symptoms of patients, especially for young patients with femoral cartilage damage effect is significant, but the long-term effect is poor, and the effect is not significant for patients with injury greater than 2.5cm2. Cartilage transplantation includes allogeneic cartilage transplantation and autologous cartilage transplantation. Allogeneic cartilage transplantation source has the potential risk of disease transmission. Autogenous cartilage transplantation is usually used in patients under 46 years old with an area of 2.5-3.5 square centimeters. Due to the more weight of autologous cartilage, the source of cartilage is limited, and the curative effect of large area of cartilage injury patients is poor. Due to the limitation of the service life of the prosthesis, joint replacement is suitable for the elderly patients with lower requirements for physical activity. Due to the above reasons, the current treatment of articular cartilage injury is not satisfactory. In recent decades, scholars have been looking for a method to repair articular cartilage by itself.

Platelet rich plasma (PRP) is a blood product obtained by concentrating and separating autologous blood. Its characteristic is that platelet concentration is 5-6 times higher than that of whole blood. Platelet activation can release a large number of high concentration growth factors, stimulate cell proliferation and differentiation, and promote soft tissue repair. Recent studies have shown that PRP can promote the regeneration and repair of bone, cartilage and soft tissue. High levels of growth factors in PRP repair damaged cartilage more effectively than hyaluronic acid injection. The biomechanical characteristics of tibiofemoral joint after cartilage injury is one of the causes of knee joint degeneration. Degradation and synthesis imbalance of chondrocytes, extracellular matrix and subchondral bone under mechanical and biological factors can cause knee joint degeneration. After cartilage injury, it is necessary not only to restore the morphology of cartilage tissue, but also to restore the biomechanical properties close to normal cartilage.

The research on the biomechanical characteristics of articular cartilage and the change of knee joint movement after cartilage injury repair is not comprehensive. The research and repair of cartilage injury can not only help to clarify the role of biomechanical factors, but also the pathogenesis of osteoarthritis and joint injury. Moreover, it can provide new research ideas and technical methods related to mechanical factors in cartilage repair, and provide theoretical basis for the final treatment of cartilage injury. In this paper, platelet rich plasma combined with collagen matrix promotes the regeneration of articular chondrocytes and the effect of nanoparticles on oxygen free radicals.

First of all, this paper makes an in-depth study on the basic theory of osteoarthritis and PRP. Through the study, this paper believes that arthritis, as a common disease in daily life, brings serious health hazards to people. For the treatment of the disease, the serious is mainly treated with surgery. The effect of surgical treatment has been improved in recent years, but there is still a lack of methods in repairing cartilage. PRP is the key point to solve this problem. It is of great significance to study the relationship between PRP and articular cartilage repair. Then, the relevant animal experimental models were established. 18 male New Zealand white rabbits were taken as samples, and the knee joint cartilage was damaged by surgery to form arthritis. Then through random grouping, 9 rats in the experimental group and 9 rats in the control group. The experimental group was treated with PRP combined with collagen matrix, while the control group was treated with natural recovery. In the third chapter, the experimental principle and specific operation steps are given in detail. Finally, in order to further verify the actual effect of this model, a number of

experimental analysis including histological observation of new cartilage, biomechanical properties of new cartilage and expression of type II collagen were carried out. Through the analysis of the experimental data, we can see that the experimental group using PRP combined with collagen matrix repair method within 4W, 8W and 16W after surgery has obvious cartilage repair compared with the control group, the effect is significant, with obvious statistical significance. Therefore, PRP combined with collagen matrix has a positive effect on articular cartilage repair, which can be used in the actual treatment of patients [1-3].

2. Basic Theory and Core Concepts of This Paper

2.1. Definition of Osteoarthritis

The term osteoarthritis (OA) itself is a misnomer because it does involve the inflammatory process. The term was originally coined by John spender in 1888 as an alias for rheumatoid arthritis, which is not the concept we understand now. "Osteoarthritis" has been used for several generations in English speaking countries. Pathologically, it is ready to replace "degenerative arthritis" or "osteoarthritis". However, there is no consensus on the definition of osteoarthritis so far. According to the possible mechanical or biological causes, OA disease is generally defined as the result of the interaction of mechanical and biological factors and the normal imbalance of the synthesis and degradation of articular chondrocytes, extracellular matrix and subchondral bone. However, OA can be caused by a variety of factors, including genetic, developmental, metabolic and traumatic factors. OA disease can affect all tissues of active joints. The final manifestations of oa disease are: cartilage softening, fibrosis, ulcer and reduction, subchondral bone sclerosis and ivory shape, osteophyte formation and subchondral cyst due to morphological, biochemical, molecular biological and biomechanical changes of chondrocytes and matrix. When the clinical symptoms are obvious, OA disease is characterized by joint pain, tenderness, limited movement, torsion, occasionally associated joint exudation, varying degrees of joint inflammation, and no systemic reaction [4-5].

2.2. Development History and Epidemiological Study

Osteoarthritis and rheumatoid arthritis were initially confused as genetic susceptibility, but there was no clear definition of OA. Since the word OA was proposed for more than a century, its definition has not been unified. First, because of the limitation of science and technology and the lack of emphasis on OA, OA's research progress is slow. In recent years, with the rapid progress of science and technology and the acceleration of global aging, OA incidence rate has increased significantly. In 1987, the American Society of Rheumatology defined OA as a joint syndrome caused by the damage of articular cartilage integrity and the corresponding pathological changes of articular margin and subchondral bone. The International Symposium on osteoarthritis in 1994 proposed that osteoarthritis is the result of the imbalance of degradation and synthesis of chondrocytes, extracellular matrix and subchondral bone under the joint action of mechanical and biological factors. However, the current general definition of OA is that osteoarthritis is a synovial joint, which is a joint disease caused by joint marginal bone hyperplasia and articular cartilage loss. Only when cartilage lesions and bone hyperplasia occur simultaneously can it be called osteoarthritis.

The incidence rate of osteoarthritis increased with age. According to the X-ray survey, 12% of the 15-24-year-old group had X-ray manifestations of osteoarthritis, 83% of osteoarthritis of the age group over 50 had X-ray findings, and 1/9 had symptoms and movement disorders. According to preliminary investigation abroad, the total prevalence of osteoarthritis is about 16%, and the incidence rate of osteoarthritis is about 6% under 45 years old. The incidence rate of patients aged

60-75 years is over 55%, and the incidence rate is even higher than 75 years old. The incidence rate is even 85%. In China, the prevalence of osteoarthritis is 12-15% among people over 16 years old. The prevalence of osteoarthritis is 50 in people over 50 years old. The prevalence of osteoarthritis in the population over 79 is 55-75%, and the incidence rate of OA in China is on the rise. Since 2000, the World Health Organization (who) has designated October 12 as "world osteoarthritis day" and the first decade of the 21st century as "osteoarthritis decade". Osteoarthritis is becoming a common concern of doctors and patients. China has also designated 2002-2012 as China's "decade of bone and joint health", that is, the "decade of bone and joint health" [6-8].

2.3. Pathogenesis of Knee Osteoarthritis

Modern medicine believes that knee osteoarthritis is a kind of aseptic chronic progressive disease characterized by cartilage degeneration, involving bone, synovium, joint capsule and other joint structures. At present, there are two kinds of conditions that can lead to knee osteoarthritis: one is the abnormal change of cartilage, but the stress is normal, the cartilage cannot bear the normal stress, degeneration, leading to osteoarthritis. In the other case, the cartilage itself is normal, but it is under abnormal pressure, the cartilage cannot withstand excessive abnormal pressure, it degenerates, leading to knee osteoarthritis. Its etiology is mainly attributed to mechanical and biological factors, mainly summarized as follows.

When the cartilage bears constant load for a long time, the fluid in the cartilage is squeezed out, and the synovial fluid is squeezed out from the articular cartilage surface and directly contacts the joint surface. At this time, the articular activity will cause obvious wear on the cartilage surface. The wear of cartilage surface and the relaxation and fracture of collagen fiber network can cause the leakage of proteoglycan components in cartilage. And then affect the stability of collagen fiber network, forming a vicious cycle, leading to the continuous destruction of cartilage matrix. In addition, with the destruction of cartilage, osteoarthritis is always accompanied by the process of cartilage repair. In the process of matrix degradation, TGF - β, IGF - 1, FGF, growth factor release and proliferation can induce chondrocyte proliferation. To promote the production of various macromolecular matrices, especially cartilage, deep polymerization and increased concentrations of modified elements, these reactions partially offset the decomposition of matrix metalloproteinases. However, the damage response of chondrocytes always exceeds or equals to the repair response. When the damage response exceeds the repair response, the cartilage is gradually destroyed. When the two are equal, the cartilage is still intact. This provides theoretical guidance for the treatment of knee osteoarthritis. It is now believed that in the natural process of knee osteoarthritis, the repair response is unlikely to exceed the injury response, so progressive cartilage damage will occur, and the progress of knee osteoarthritis is also progressive.

2.4. Definition and Characteristics of Platelet Rich Plasma

PRP is a plasma component rich in platelets. Because PRP is a native platelet concentrate, it contains a variety of growth factors and cytokines that promote the healing of soft and hard tissues.

(1) Contents

The growth factors in platelet rich plasma have the effect of treating various injuries. The concentration of these growth factors is also the theoretical basis for the application of platelet rich plasma in tissue repair and regeneration. They can be activated by platelet growth factor A or platelet rich granules from plasma. It mainly includes platelet growth factor, transforming growth factor, fibroblast growth factor, insulin growth factor 1, insulin growth factor 2, vascular endothelial growth factor, epidermal growth factor, interleukin 8, keratinocyte growth factor and connective tissue growth factor. Each factor has its own function and characteristics, so that platelet rich

plasma has a strong repair function and application prospects.

(2) Preparation

At present, there are two main methods to produce platelet rich plasma, which have been approved by FDA. PRP was obtained centrifugation twice, but whole blood (citrate anticoagulant) was obtained before centrifugation, and then red blood cells and PPP were separated by centrifugation twice. For humans, whole blood platelet content is about 300000 / μ L, and platelet concentration in therapeutic PRP more than 6 times that of whole blood platelet concentration. However, the platelet concentration varies with the equipment and extraction method.

(3) Clinical application

Platelet rich plasma is widely used in clinic, such as nerve injury, membranous inflammation, osteoarthritis, myocardial injury, bone tissue repair and regeneration, plastic surgery such as breast reconstruction, breast augmentation, fat filling, wrinkle removal, dilution staining, oral and neck surgery, etc. Platelet rich plasma has been widely concerned by the media because it can be used to treat sports injuries of professional athletes.

However, the clinical application of platelet rich plasma is still in the early stage, and the basic research and preclinical research have not been confirmed by large-scale clinical trials. For example, PRP has been proved to be effective in the clinical treatment of nerve injury and sports medicine, but it cannot be followed for a long time and is still in its infancy. For the potential therapeutic effect of platelet rich plasma on joint, muscle binding, cartilage, ligament and muscle injury, few controlled clinical trials can fully prove its safety and effectiveness, but there is no effective evidence.

However, some people think that the disadvantage of platelet rich plasma therapy is the limited availability of platelet kits, and the number of platelet rich plasma varies with human factors and sources. Different platelet concentration devices may change the characteristics of platelet degranulation, thus affecting its clinical therapeutic effect [9-11].

2.5. Properties and Repair Properties of Collagen

Collagen is one of the main components of extracellular matrix (ECM). No matter which animal it comes from, its biological and chemical properties are very similar. The main properties of collagen are as follows: (1) good cell compatibility. (2) The immunogenicity is low and non-toxic. (3) It can interact with platelets and play the role of coagulation and hemostasis. (4) It can be degraded and absorbed in vivo. (5) The degradation rate can be adjusted by crosslinking modification.

Once the collagen is in contact with blood, due to its structural characteristics, it will quickly adsorb blood, gather, concentrate and adsorb platelets in the blood, thus promoting the release of coagulation factors and triggering coagulation phenomenon. Since the discovery of the coagulation properties of collagen, collagen has been processed into various forms, such as fibrin, collagen and felt, and used for hemostasis in various procedures, such as oral surgery, visceral surgery, laparoscopic surgery and general surgery. Compared with the traditional bone hemostatic materials, the collagen complex has obvious advantages such as good biocompatibility, wide use temperature, good biodegradability, good control of coagulation factors, and good tissue repair. Collagen can quickly absorb blood and combine with wound tissue. The hemostatic time is shorter than other hemostatic materials. Collagen also plays an important role in wound healing. Collagen, as a tissue sponge, can be used as a transport medium for cells in dermal wound repair and growth factors for bone repair. Collagen fiber is one of the best biological sponges, which is conducive to cell proliferation and migration [12-13].

3. Animal Models and Experimental Methods

3.1. Experimental Materials and Grouping

There were 18 male New Zealand white rabbits, aged 2-4 months, weighing 1.9-2.8kg, with an average of 2.4kg. According to the principle of consistent balance between groups, they were randomly divided into two groups with 9 people in each group. Anterior cruciate ligament resection (ACLT) was used to make osteoarthritis model. The experimental group underwent bilateral anterior cruciate ligament resection, while the control group only underwent bilateral knee arthrotomy, without anterior cruciate ligament resection.

3.2. Experimental Materials and Grouping

Experimental group: after intramuscular injection of copper matte, New Zealand rabbits were given ketamine of 0.9 ml / kg, skin preparation of both knees, lying on the experimental table for fixation, towel disinfection. The skin and subcutaneous fascial layer is cut open, the joint cavity is opened, the bone is out of order, and the ACL is exposed to extreme bending of the knee. The ACL is cut directly from the eye with a sharp knife, and the surrounding tissue is not injured. The joint cavity was washed with normal saline, the joint cavity and skin were sutured with silk thread, and fixed with sterile dressing. After operation, each animal was injected with penicillin 450000 U / D for 9 consecutive days.

3.3. Main Reagents

In this experiment, 16 kinds of reagents including trypsin were used in this experiment. See Table 1 for details

Table 1. List of main reagents

name	manufacturer
Trypsin	Hyclone
Collagenase type II	Sigma
fetal bovine serum	Hyclone
DMEM-H	Hyclone
CCK-8	PhD Bioengineering
DMSO	Sigma
DEPC	Gibco
chloroform	Chengdu Kelong Chemical Reagent Factory
Isopropanol	Chongqing Maoye
Loading buffer	TAKARA
Green view nucleic acid dye	BioRule
agarose	Sigma
Anhydrous ethanol	Biochemistry of Anwei Anta
2xTaq PCR Master Mix	TIANGEN
DNA marker	TIANGEN
RNAiso Plus	TaKaRa

3.4. Animals Were Killed and Materials Were Collected

At 16 weeks after operation, the animals were killed by air embolism. The femur and tibia were cut off at 4.5cm above and below the right knee joint with pendulum saw, the surrounding muscle tissue was removed, the medial and lateral meniscus were cut off, and the medial and lateral

collateral ligament were cut OFF. The left knee was used as a control, all labels and soaked in 15% neutral formalin solution were used.

3.5. Preparation of Platelet Rich Plasma

A 60 ml sterile syringe (containing 10 ml sodium fluoride) was used to draw 50 ml venous blood from femoral vein, and the syringe was gently shaken to make the blood fully mixed with anticoagulant. After centrifugation for 15 minutes, venous blood was divided into three layers: erythrocyte membrane, white membrane and plasma layer. Plasma layer was taken and 2300g was centrifuged for 15 minutes to obtain platelet rich plasma and lower platelet mass. The platelet rich plasma was transferred to another new centrifuge tube to regulate platelet rich plasma concentration. Platelet rich plasma was prepared by suspension of low platelet weight in 15 ml of dilute platelet plasma. Platelets were counted at 150 h [14].

3.6. Experimental Methods

All specimens were thawed at room temperature one day before the experiment. The conditions of thawing day were controlled at room temperature of 23 ℃ and humidity of 45%. The rabbit knee joint specimens were fixed on the fixture in the straight position (knee joint bending 10 degrees), and the femur and tibia were firmly fixed to avoid sliding phenomenon affecting the experimental results. First of all, the preloading test was carried out to measure the general range of the knee joint. The pressure-sensitive paper was cut into the size and shape suitable for the knee joint, and then wrapped with preservative film. Because of the small medial space, the medial collateral ligament was cut off to fully expose the knee joint. The pressure-sensitive film sealed with plastic film was put into the knee joint of rabbit. The angle and position of pressure-sensitive film were adjusted to ensure that the pressure-sensitive film was located between the tibiofemoral joint. The biomechanical position was adjusted to avoid the movement of the pressure-sensitive film.

The maximum pressure was 60N, 6 mm / min. Keep the pressure at 60N for 3 minutes to stabilize the staining of pressure sensitive film. After 3 minutes, take off the pressure sensitive film, fix the color surface of the pressure sensitive film on A4 printing paper, mark and record one by one. A total of 36 knee joint operations were performed on 18 rabbits. After recording all the experimental results, the balance was placed next to the pressure-sensitive film to take photos, and the results were sent to the computer. AutoCAD software was used to measure the area, multi-line was used to measure the irregular area, and the pressure of the femoral and tibial joint was measured and recorded. FPD-306E and FPD-305E pressure sensitive films produced by Fuji company in Japan were used to measure the medial and lateral pressure of tibial femoral joint, and the results were recorded and marked clearly.

3.7. Determination of Cell Growth Curve

After counting, the cells in good condition were inoculated into 24 well plate with 2 × 104 / ml. The cells of 4 wells were counted every other day and the average value was calculated. The cells were cultured for 3-5 days. Taking the culture time as the horizontal axis and the number of cells as the vertical axis, the counting points were connected into a curve.

3.8. Proliferation of Chondrocytes Was Measured by MTT

The well-growing cells were digested with 0.33% trypsin and cultured in monolayer. These cells were mixed with 15% IMDM to form a single cell suspension. After counting, the cells were seeded

in 100 well plate, 0.2ml/well, cells adhered to the wall, and the medium was discarded. Before the experiment, 120 μ L cell cultures with different drug concentrations (6.56 μ g / ml, 13.9 μ g / ml, 30 μ g / ml) were prepared. The plates were moved to a CO2 incubator and cultured at 36 ℃, 5.5% CO2 and saturated humidity for 4 days. 15 μ l MTT solution (5mg / ml) was added into each well and incubated at 36 ℃ for 5 hours. The supernatant was carefully cultured in the absorption and discarding wells. 80 μ l DMSO was added to each well, and the crystal was fully dissolved by shaking for 15 min.

3.9. Biomechanical Test

The biomechanical test was performed by BOSB 3500 biomechanical testing machine. Proper amount of self-setting defined tooth powder and denture were melted into paste, and the tibia was placed in paste mixture vertically, waiting for heating to solidify slowly. The circular groove mold was fixed firmly on the platform under the biomechanical testing machine, and the femoral end of knee joint was fixed on the upper part of the biomechanical testing machine with clamps. Adjust the relative position of medial femoral condyle cartilage defect area and tibial plateau to make it fully stressed, generally 175 ° straight position. After adjusting the position, incise the anterior and posterior cruciate ligament, raise the upper part of the biomechanical testing machine, and fully expose the knee joint. Cut the pressure-sensitive paper with a size of 3 * 3cm, put the ground surface on the tibial plateau, and adjust the angle and position of the pressure-sensitive film. The maximum pressure was 0.05mm/s, 60N. When the maximum pressure is reached, the machine will stop automatically and keep the pressure for 3 minutes so that the pressure sensitive film can be dyed stably. All 36 right knees and 10 left knees were treated with pressure sensitive film. The area of indentation is measured by AutoCAD software, and the area of irregular figure is measured by multi line.

3.10. Statistical Methods

All measured data are expressed as mean ± standard deviation ($\bar{x} \pm s$). SPSS17.0 was used for data processing. One-way ANOVA was used for pairwise comparison between groups. Homogeneity test of variance was used for mean comparison of multi group samples. Dunnett's T3 method was used for variance heterogeneity comparison. LSD method was used for data satisfying normality and homogeneity of variance. When $p < 0.05$, the difference was statistically significant.

4. Experimental Results and Analysis

4.1. Gross Morphological Observation and Scoring of Cartilage

According to the results of gross morphological evaluation in Figure 1, there was no significant difference in postoperative repair effect between the two groups at 4W. In the 8W control group, the cartilage defect was obviously depressed, and the repair effect was poor. A small amount of fibrous tissue was filled and the defect boundary was clear. In the experimental group, there was no obvious depression in the cartilage defect, and the repair effect was good. There was a large amount of hyaluronic acid cartilage in the base of the experimental group. The surface of the new cartilage was relatively flat, and the boundary between the defect and the normal cartilage was almost invisible.

In the 16W control group, the cartilage defects were depressed, but still more obvious, no cartilage like tissue formation was observed, some fiber tissue filled the defect, and the repair of the central defect area was not obvious. In the experimental group, the pitting of cartilage defect

disappeared completely, and the surface of new cartilage was very flat, which could not be distinguished from the boundary of normal cartilage.

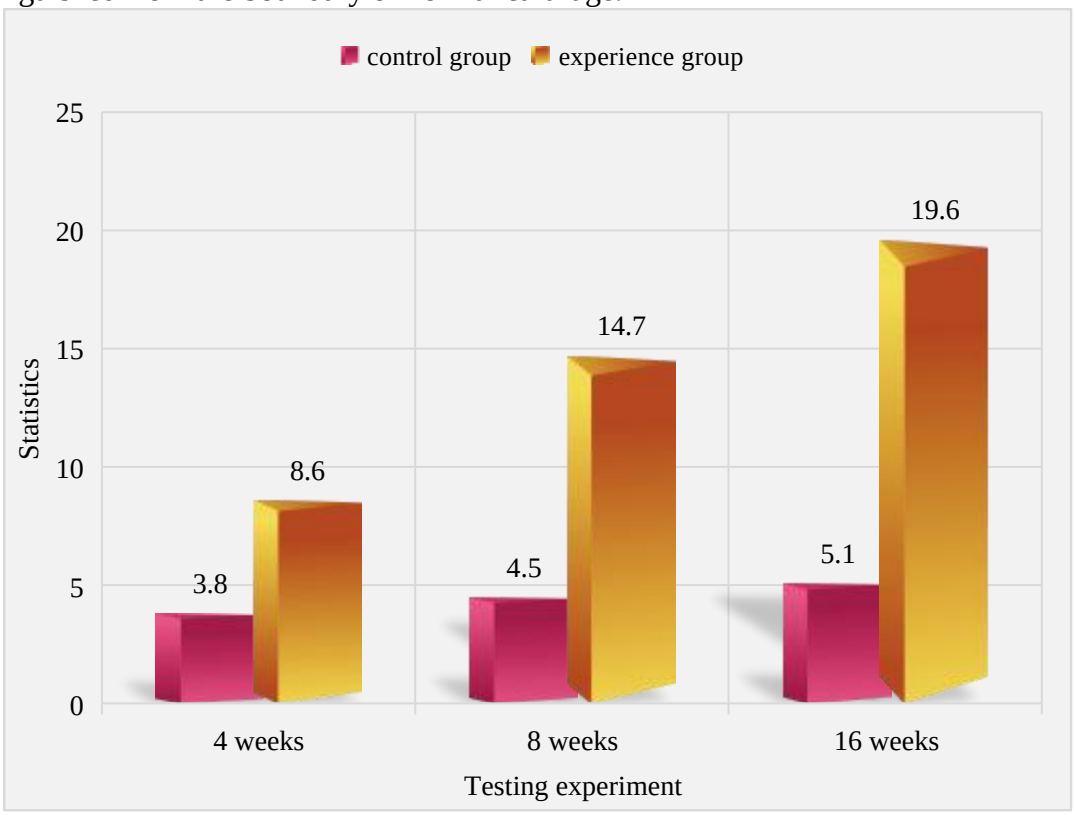

Figure 1. Gross morphological observation and Score statistical analysis of new cartilage

4.2. Analysis of Biomechanical Properties of Cartilage

According to the analysis results in Figure 2, at 4W after operation, the compressive modulus of new cartilage in the experimental group was 0.219 ± 0.0019mpa, and that in the control group was 0.063 ± 0.027mpa, with no significant difference between the two groups. At 8 weeks after operation, the compressive modulus of new cartilage in the experimental group was 0.368 ± 0.0039 MPa, and that of the control group was 0.093 ± 0.028 MPa. At 16 w after operation, the compressive modulus of new cartilage in experimental group was 0.628 ± 0.047 MPa, that of control group was 0.185 ± 0.064 MPa, and that of normal articular cartilage was 0.673 ± 0.041 MPa. The results showed that the compressive modulus of new cartilage in 8W and 16W groups was significantly higher than that in control group ($P < 0.05$), and that in 16W group was significantly higher than that in 12W group ($P < 0.05$).

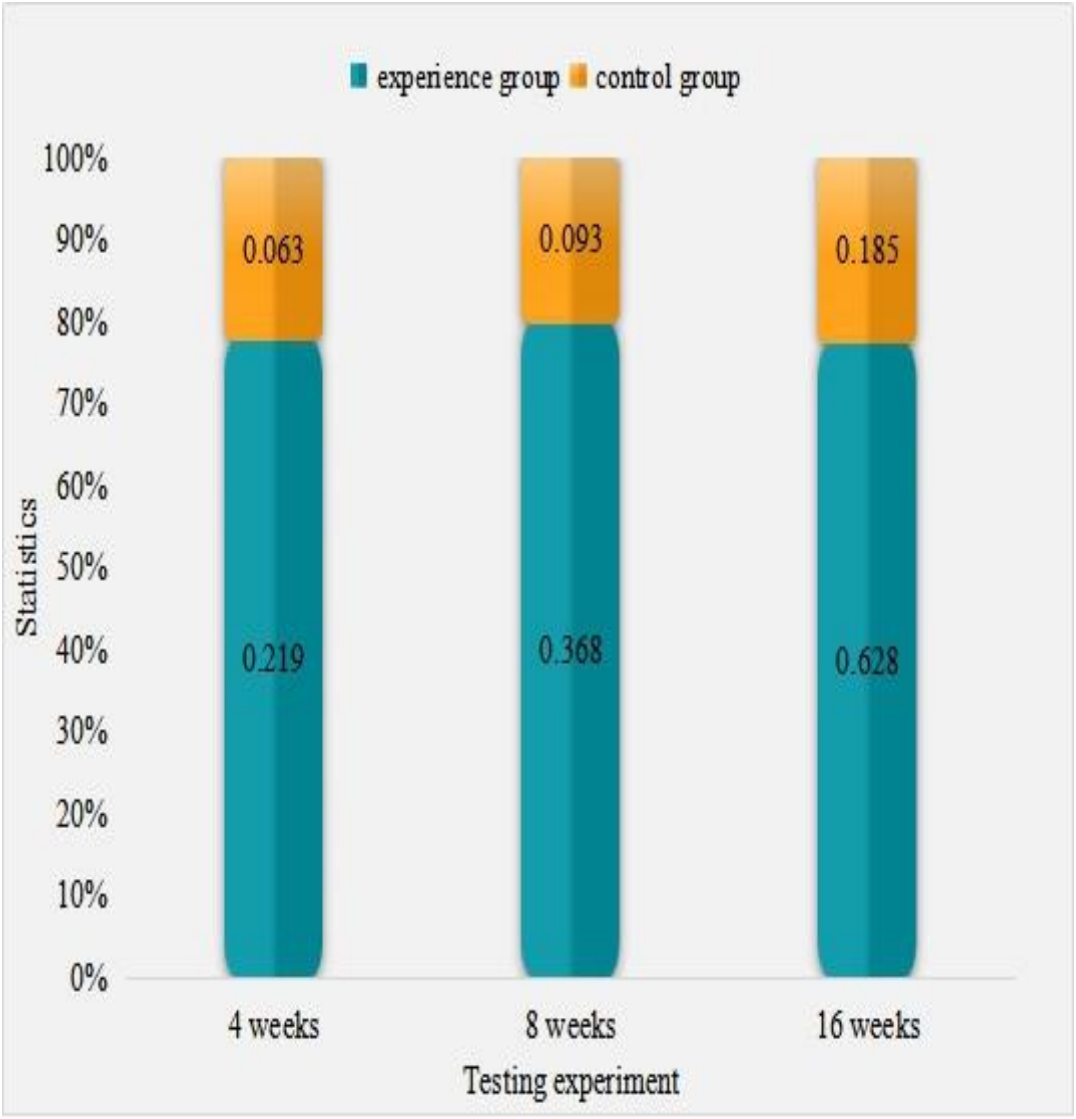

Figure 2. Statistical analysis of biomechanical properties of new cartilage

4.3. Histological Observation of Cartilage

According to the histological score results of the new cartilage in Figure 3, the histological staining of the control group showed that the cartilage was mainly repaired by fibrous tissue at the end of the experiment, and a large number of fibroblasts were seen, showing long spindle shape, and chondrocytes were rare. The immunohistochemical staining of safflower 0, toluidine blue and type I collagen were weakly positive. Histological staining showed that the thickness of new cartilage increased and typical cartilage layered structure appeared in the experimental group. The new cells are round or polygonal, large and stacked with each other. They are columnar and vertically arranged on the surface of cartilage. Toluidine blue staining, saffron 0 staining and type I collagen immunohistochemical staining were strong positive, there were significant differences between the two groups, which had a comparative significance.

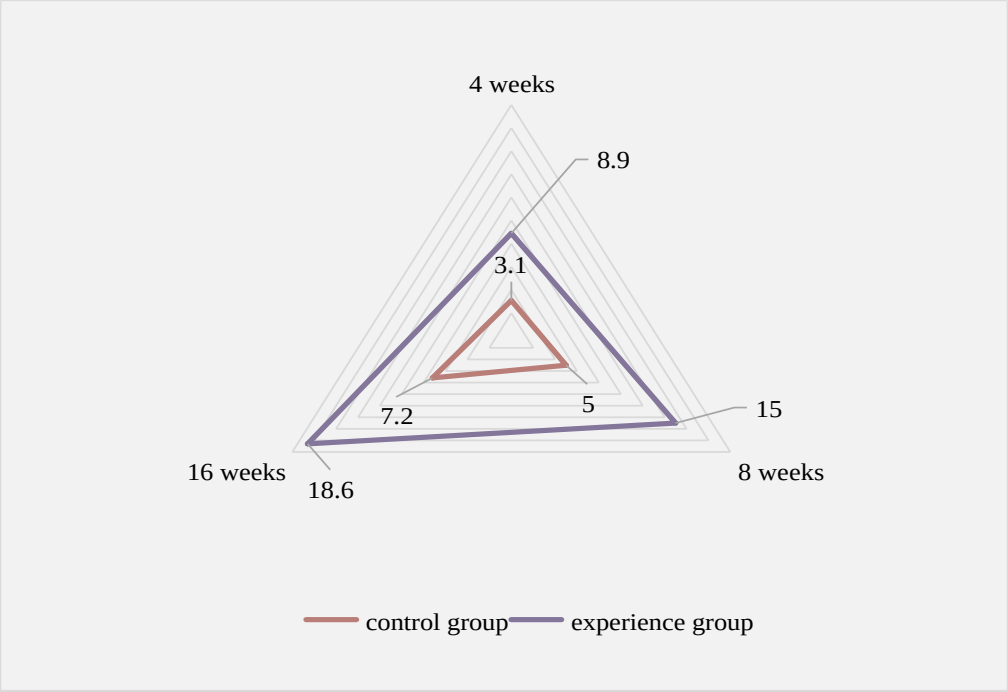

Figure 3. According to the histological score results of the new cartilage

4.4. Expression of Type II Collagen

Western blot was used to detect the expression of collagen in the new cartilage. The results in Figure 4 showed that the expression of medium collagen in the new cartilage in 4W, 8W and 16W groups was significantly higher than that in the control group ($P < 0.05$). The expression of type II collagen in 16W experimental group was significantly higher than that in 8W experimental group ($P < 0.05$). Experimental data show that platelet rich plasma combined with collagen matrix can significantly promote the regeneration of articular chondrocytes.

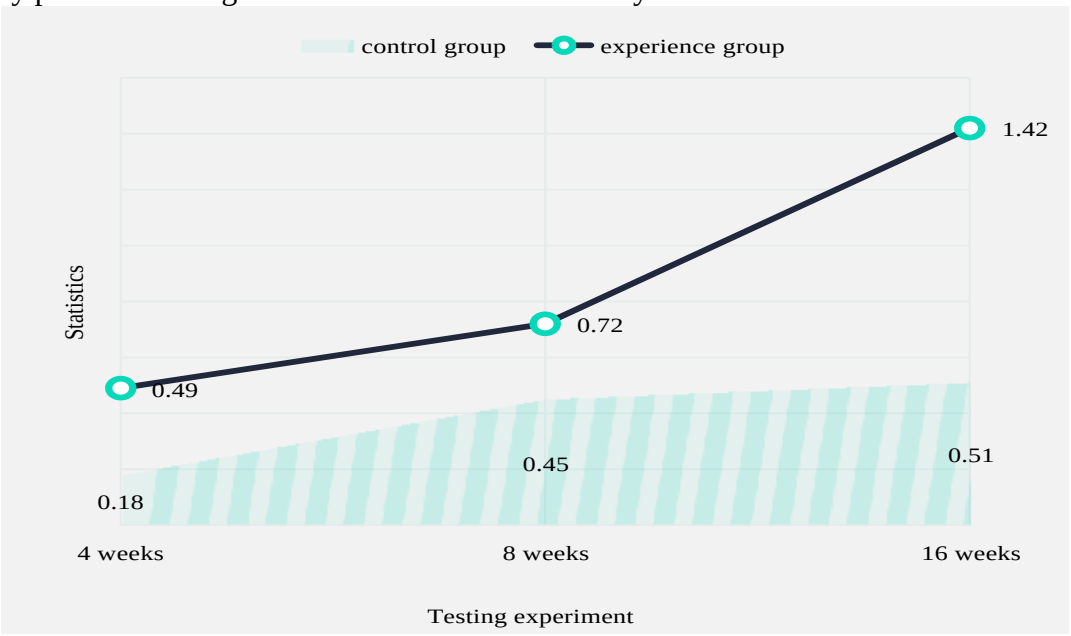

Figure 4. Statistical analysis of type II collagen expression in new cartilage tissue

5. Conclusion

With the deepening understanding of the function and biological characteristics of articular cartilage, the repair of cartilage damage caused by arthritis has also been a technical breakthrough. However, the current mainstream program is still focused on external forces, the use of instruments to intervene in cartilage repair. Although the effect of traditional drug therapy has been significantly improved, it is still difficult to fundamentally solve this problem. To this end, this paper proposed the treatment scheme of PRP combined with collagen matrix on the regeneration of articular cartilage repair cells, which filled the blank of research in this field to a certain extent. The core of this study is to use the characteristics of PRP containing various growth factors to promote the repair of cartilage tissue. At the same time, collagen matrix, combined with its strong tissue repair properties, can accelerate the cell proliferation of arthritis cartilage tissue. After understanding the treatment of cartilage injury, the corresponding animal experimental model was established. The model has the advantages of high simulation, simple operation and theoretical knowledge support. This research has achieved ideal results and made a contribution to the research in this field.

References

[1] Dequeker, J., Mokassa, L., Aerssens, J., & Boonen, S. (2015). Bone density and local growth factors in generalized osteoarthritis. Microscopy Research & Technique, 37(4), 358-371.https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<358::AID-JEMT10>3.0.CO;2-L

[2] Oegema, T. R., Carpenter, R. J., Hofmeister, F., & Thompson, R. C. (2015). The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. Microsc Res Tech, 37(4), 324-332.https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<324::AID-JEMT7>3.0.CO;2-K

[3] Cheng, Y., Macera, C. A., Davis, D. R., Ainsworth, B. E., & Blair, S. N. (2015). Physical activity and self-reported, physician-diagnosed osteoarthritis: is physical activity a risk factor. Journal of Clinical Epidemiology, 53(3), 315-322.https://doi.org/10.1016/S0895-4356(99)00168-7

[4] Zhen, G., Wen, C., Jia, X., Li, Y., Crane, J. L., & Mears, S. C., et al. (2016). Inhibition of tgf-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nature Medicine, 19(6), 704-712.https://doi.org/10.1038/nm.3143

[5] Blaney Davidson, E. N., & Van, d. K. P. M. (2015). A role for tgf-β in osteoarthritis-related pain? International Journal of Clinical Rheumatology, 10(5), 309-311. https://doi.org/10.2217/ijr.15.28

[6] Weinberger, M., Tierney, W. M., Booher, P., & Hiner, S. L. (2016). Social support, stress and functional status in patients with osteoarthritis. Social ence & Medicine, 30(4), 503-508. https://doi.org/10.1016/0277-9536(90)90353-T.

[7] Baselga Garc á-Escudero Jaime, Miguel Hern ández Trillos Pedro, & Shervin, A. Treatment of osteoarthritis of the knee with a combination of autologous conditioned serum and physiotherapy: a two-year observational study. Plos One, 10(12), 4395-4402.https://doi.org/10.1371/journal.pone.0145551

[8] Livne, E. (2015). Osteoarthritis in the temporo-mandibular joint (tmj) of aged mice and the in vitro effect of tgf-beta 1 on cell proliferation, matrix synthesis, and alkaline phosphatase activity. Microscopy Research & Technique, 37(4), 314-323.https://doi.org/10.1002/(SICI)1097-0029(19970515)37:4<314::AID-JEMT6>3.0.CO;2-K

[9] Sánchez, Andrés R, Sheridan, P. J., & Kupp, L. I. (2015). Is platelet-rich plasma the perfect enhancement factor? a current review. Int J Oral Maxillofac Implants, 18(1), 93-103.

[10] Pradeep, A. R., Pai, S., Garg, G., Devi, P., & Shetty, S. K. (2015). A randomized clinical trial of autologous platelet-rich plasma in the treatment of mandibular degree ii furcation defects. Journal of Clinical Periodontology, 36(7),

581-588.https://doi.org/10.1111/j.1600-051X.2009.01428.x
[11] Takikawa, M., Nakamura, S., Nakamura, S., Ishirara, M., & Kiyosawa, T. (2015). Enhanced effect of platelet-rich plasma containing a new carrier on hair growth. Dermatologic Surgery, 37(12), 1721.https://doi.org/10.1111/j.1524-4725.2011.02123.x
[12] Chamberlain, L. J., Yannas, I. V., Hsu, H. P., Strichartz, G. R., & Spector, M. (2015). Near-terminus axonal structure and function following rat sciatic nerve regeneration through a collagen-gag matrix in a ten-millimeter gap. Journal of Neuroence Research, 60(5), 666-677. https://doi.org/10.1002/(SICI)1097-4547(20000601)60:5<666::AID-JNR12>3.0.CO;2-0
[13] Van Wachem, P. B., Van Luyn, M. J. A., & Ponte, d. C. M. L. (2015). Myoblast seeding in a collagen matrix evaluated in vitro. Journal of Biomedical Materials Research Part B Applied Biomaterials, 30(3), 353-360. https://doi.org/10.1002/(SICI)1097-4636(199603)30:3<353::AID-JBM9>3.0.CO;2-P
[14] Miranda, S., Costa, M. F. M., Natália Rebouas, Márcia T. Ramos, & Alencar, N. X. (2019). Protocols for preparation of platelet rich plasma (PRP) in quarter horses. Pesquisa Veterinária Brasilra, 39(8), 614-621.https://doi.org/10.1590/1678-5150-pvb-5883
[15] Hoareau, G. L., Jandrey, K. E., Burges, J., Bremer, D., & Tablin, F. (2015). Comparison of the platelet-rich plasma and buffy coat protocols for preparation of canine platelet concentrates. Veterinary Clinical Pathology, 43(4), 513-518.https://doi.org/10.1111/vcp.12195