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Medium and Long-Term Animal Experiment Study on Replacement of Allogeneic Meniscus Tissue for Repairing Cartilage Defects

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Abstract

Objective: To explore the repair effect of allogeneic meniscus replacement cartilage graft on animal articular cartilage.

Methods: The rabbit cartilage defect model was constructed, and allogeneic meniscus tissue replacement transplantation was performed. Gross observation, cytological observation, and histomorphological score were performed on the knee joint 2 months after operation, and the biomechanical properties were analyzed. Furthermore, Western blotting and ELISA were used to determine the expression of key proteins, Real-time RT-PCR technology was used to detect the expression of key genes, and logistic regression analysis was used to screen out the time points and key genes/proteins that affect the efficacy.

Results: Through gross observation, it was found that the height, texture and color of cartilage tissue repaired by meniscus transplantation were closer to normal cartilage, and the repair degree was higher than that of cartilage transplantation group. At the same time, the analysis of biomechanical properties showed that the ultimate stress, ultimate strain, compressive modulus and tangent modulus of the meniscus transplantation group were significantly higher than those of the cartilage transplantation group at December and 18 months ($P<0.05$). The key protein level measurement results showed that from 1 week to 18 months after surgery, collagen type II protein expression first increased and then decreased. Among them, the average collagen type II protein expression levels of 1 week, 2 weeks, and January were compared, half a month the plate transplantation group was significantly higher than the cartilage transplantation group ($P<0.05$). From 1 week to 18 months after surgery, the protein content of MMP3 and CTX-II in the two groups showed a downward trend. At 12 and 18 months, the MMP3 protein content of the meniscus transplantation group was significantly lower than that of the cartilage transplantation group ($P<0.05$). The key gene expression level also showed the same result. Logistic regression analysis was further used, and the results showed that 1 month after surgery was the time point that significantly affected the efficacy, and collagen type II and col2a1 were key proteins and genes that significantly affected the efficacy.

Conclusion: Allogeneic meniscus tissue replacement transplantation is effective in repairing cartilage defects. Collagen type II may be the key to the effect in 1 month after surgery.

Keywords: meniscus transplantation; cartilage defect; medium and long-term animal experiment

Preface

The Cartilage defect refers to the damage of human articular cartilage during exercise or other factors, resulting in cartilage thinning or even ruptures and wear, and its clinical manifestations are joint pain and limited mobility [1]. According to statistics, among patients who need to undergo a knee joint examination, more than 60% of cases are

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found to have cartilage defects [2]. In the early stage of cartilage defect, patients often have no obvious symptoms due to the small defect area. As the disease progresses, the defect area increases, and the patient will experience symptoms of joint swelling and pain. If the lesions cannot be treated in time and effectively, osteoarthritis may occur [3]. At present, effective repair of cartilage defects is the current research focus.

1. Introduction

In recent years, research on MAT has provided new solutions for cartilage defect repair. Previously, some researchers also confirmed that MAT has a good and reliable repair effect on focal cartilage defects on the weight-bearing surface of rabbit knee joints [4]. However, the medium and long-term efficacy of MAT is not yet clear. After MAT, whether it will change the weight-bearing capacity of articular cartilage, and how cartilage cells and matrix have changed at the nucleic acid and protein molecular level during the damage and repair of the medium and long-term cartilage tissue, thereby inducing the repair or degeneration of cartilage tissue? It is a key point worth investigating. Therefore, in this study, frozen tissues were used for MAT and OAT respectively, and it was planned to follow up and observe the changes in the cell morphology and biomechanical properties of articular cartilage and the transcription and translation levels of cartilage-related characteristic proteins during the medium and long-term process of damage repair. Through the above research, the biomarkers of regeneration/degradation of cartilage tissue in the middle and long-term damage repair process are discovered to guide the adjustment of treatment strategies and extend the normal use time of articular cartilage after transplantation.

2. Methods and materials

2.1 Experimental animal grouping

80 New Zealand rabbits (SPF grade) either male or female, weighing about 2.5 kg. The animals were randomly divided into MAT group and OAT group, 40 per group, MAT group is given meniscus transplantation, OAT group is given cartilage transplantation.

2.2 MAT/OAT

After 80 New Zealand rabbits were given intravenous anesthesia, the rabbits' knees and surrounding hair were shaved, and the rabbits were fixed in the supine position on the operating table and then the knees were disinfected. Under aseptic conditions, drill a circular 5mm×5mm×3mm full-thickness articular cartilage from the articular surface of the femoral condyle and trochlear defect. The subchondral layer of the defect shows micro-hemorrhage and no cartilage remains. The defect is as required the meniscus or cartilage is implanted, specifically, the cryopreserved meniscus/cartilage tissue is re-warmed and trimmed to the size of the drilled hole. The bone hole in the receiving area is slightly expanded with a dilator, and then trimmed with an ejector. The finished allomeniscal/cartilage tissues are embedded into the defect one by one and glued with protein gel. After implanting the graft, the patella was reset, and the knee joint capsule and skin were sutured layer by layer. Penicillin was given intramuscular injection every day for 3 days after operation to prevent infection. The limbs were not fixed and were free for movement. In the first week, 2 weeks, 1 month, 2 months, 6 months, 12 months and 18 months after the operation, each group of rabbits were Put the to death and materials were collected.

2.3 Analysis of biomechanical properties

In December the specimens were obtained after 18 months, the mechanical properties were tested on the Instron 5544 material testing machine [7]. The cartilage ultimate stress, ultimate strain, compressive modulus and tangent modulus of cartilage tissue are calculated according to the parameters of cartilage tissue height, diameter, cross-sectional area, load increment and material deformation.

2.4 Determination of key protein content

After obtaining tissue samples, homogenize or grind with liquid nitrogen, CTX-II antibody ELISA kit (Haring Biotechnology, HL95014) were used to detect CTX-II expression, rabbit matrix metalloproteinase ELISA kit (Jianglai Biotechnology, JL17211-48T) was used for the determination of MMP3, refer to the instructions for the specific method. Type collagen II and collagen type X were measured by immunoblotting method [8] to determine their average gray values.

2.5 Real-time RT-PCR technology detects the expression of key genes

The total RNA of tissue samples was extracted, and the expression of col2a1, col10a1, mmp3, and CTX-II genes were detected after reverse transcription. The primers were synthesized by Shanghai Shenggong Co., Ltd., the probes used Roche's Universal Probe Libreary probe for real-time PCR detection, the FAM channel detects the

signal, and β-actin was used as the internal standard gene. The relative expression rate (RE) of the target gene of the sample is calculated using the ΔΔCT method, RE=2-ΔΔCt (Ct represents the number of cycles when the reaction fluorescence intensity is significantly greater than the background value).

2.6 Statistical methods

Measurement data are expressed as "mean ± standard deviation" and analyzed by t test. The time points and key genes/proteins that affect the efficacy were screened by Logistic regression analysis.

3. Results

3.1 Histomorphology score

From 1 week to 6 months, the histomorphology score of the MAT group was slightly higher than that of the OAT group, and the difference was not statistically significant (P>0.05). At the time points of 2 weeks, January, December, and 18 months, the histomorphology score of the MAT group was significantly higher than that of the OAT group (P<0.05).

Table 1. **Morphological scores of the two groups**

Point in time	MAT group	OAT group
1st week	7.84±0.86	7.52±0.84
2nd week	10.75±0.93	9.05±0.91
1st month	13.93±1.04	12.87±1.07
2nd month	15.81±1.03	14.75±1.06
4th month	17.56±1.17	16.84±1.20
6th month	20.54±1.12	19.53±1.17
12th month	22.59±1.08	21.34±1.12
18th month	23.63±1.02	22.16±1.08

P<0.05, compared with MAT group.

3.2 Analysis of biomechanical properties

In December and 18 months, the ultimate stress, ultimate strain, compressive modulus and tangent modulus of the MAT group were significantly higher than those of the OAT group (P<0.05).

Table 2. **Analysis of the biomechanical properties of the two groups**

index	MAT group		OAT group	
	12th month	18th month	12th month	18th month
Ultimate stress/Mpa	0.0208±0.003	0.0210±0.002	0.0203±0.002*	0.0205±0.004*
Ultimate strain/%	44.37±6.32	46.58±5.13	40.21±5.26*	42.08±5.26*
Compressive modulus/Mpa	0.541±0.035	0.568±0.024	0.528±0.031*	0.547±0.029*
Tangent modulus/Mpa	0.027±0.002	0.028±0.001	0.025±0.001*	0.026±0.001*

3.3 Determination of key protein levels

It can be seen from the changes in the average expression of collagen type II protein at different time points in the two groups that there was an upward trend within 1 month after surgery, and the average expression of collagen type II protein showed a downward trend from 1 month after surgery to 18 months after surgery. Among them, when comparing the average values of collagen type II protein expression in the two groups at 1 week, 2 weeks, and January, the MAT group was significantly higher than the OAT group (P<0.05). It can be seen from the average expression of collagen type X protein at different time points of the two groups that it showed an upward trend within 1 month after surgery, and the average expression of collagen type X protein showed a slow downward trend from 1 month after surgery to 18 months after surgery. There was no statistically significant difference in the mean value of collagen type X protein expression between the two groups (P>0.05). From 1 week to 18 months after operation, the protein content of MMP3 and CTX-II in the two groups showed a downward trend. At 12 and 18 months, the protein content of MMP3 and CTX-II in the MAT group was significantly lower than that in the OAT group (P<0.05). See Figure 2 for details.

3.4 Determination of key gene expression

From the changes in the expression of col2a1 at different time points in the two groups, it can be seen that the expression of col2a1 showed an upward trend within 1 month after surgery, and the expression of col2a1 showed a downward trend from 1 month after surgery to 18 months after surgery. From February to 18 months after surgery, the expression of col2a1 decreased. Among them, the two groups of 1 week, 2 weeks, and January col2a1 expression level, the MAT group was significantly higher than the OAT group (P<0.05). It can be seen from the expression of col10a1 at different time points in the two groups that there was an upward trend within 1 month after surgery, and the expression of col10a1 did not change much

from 1 month after surgery to 2 months after surgery. From 2 months after surgery to 18 months after surgery, the expression of col10a1 showed a downward trend. The col10a1 expression value of the two groups was compared, and the difference was not statistically significant (P>0.05). The expression levels of mature cartilage matrix synthesis-related genes are consistent with the changes in key protein expression levels. From 1 week to 18 months after surgery, the expression of mmp3 in the two groups showed a downward trend. Among them, the expression of mmp3 between the two groups was compared at 12 and 18 months, and the difference was statistically significant. The expression of key proteins related to cartilage degeneration is consistent with the change trend of key protein expression. See Figure 3 for details.

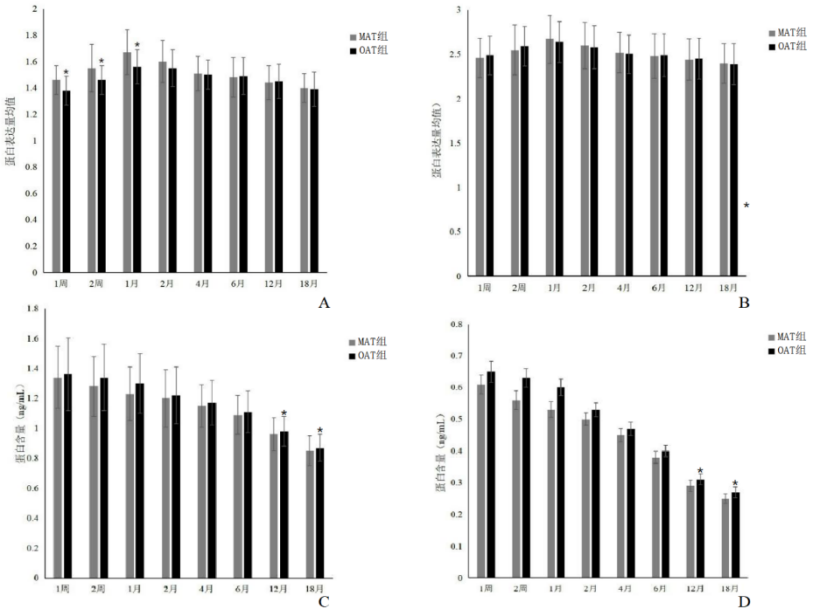

Figure 2. **mean expression of key proteins at different time points， A:collagen type II ;B:collagen type X;C:MMP3;D:CTX-II。*P<0.05, versus MAT group comparison**

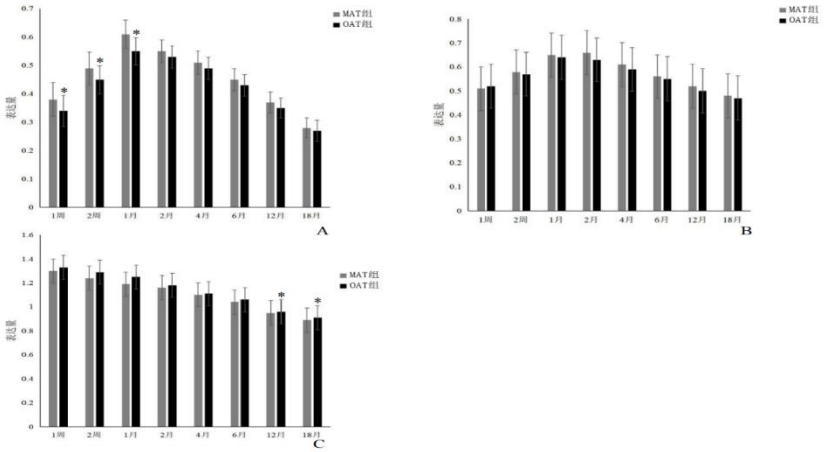

Figure 4. **key gene expression at different time points， A:*col2a1*;B:*col10a1*;C:*mmp3* *P<0.05,versus MAT group comparison**

2.5 Logistic regression analysis

According to the expression results of key proteins and genes, logistic regression analysis was performed, and the results showed that one month after surgery was the time point that significantly affected the efficacy，collagen type II、col2a1 for the key proteins and genes that significantly affect the efficacy

Table 3. **Logistic regression analysis screening time points**

Factor	B	SE	Wald	df	Sig.	Exp	95.0% CI for Exp(B)	
							Lower	Upper
2 weeks after surgery	-0.430	0.395	1.201	1	0.268	0.650	0.300	1.405
1 month after operation	1.129	0.416	7.293	1	**0.007**	3.094	1.361	7.026
12 months after surgery	0.395	0.232	2.898	1	0.089	1.485	0.942	2.34
18 months after surgery	0.432	0.362	1.423	1	0.233	1.54	0.758	3.132

Table 4. **Logistic regression analysis to screen key proteins/genes**

Factor	B	SE	Wald	df	Sig.	Exp	95.0% CI for Exp(B)	
							Lower	Upper
collagen type II	-1.182	0.521	5.154	1	**0.023**	0.307	0.111	0.851
MMP3	0.306	0.433	0.5	1	0.48	1.358	0.581	3.175
CTX-II	0.473	0.371	1.633	1	0.201	1.606	0.777	3.319
col2a1	-1.435	0.516	7.730	1	**0.005**	0. 238	0.087	0.655

4. Discussion

Knee cartilage injury is a problem frequently encountered by orthopedic surgeons. According to statistics, 4%-10% of young people aged 15-24 in my country have cartilage disease, and about 80% of people over 55 have cartilage Lesions [3]. The regenerative capacity of articular cartilage defects is limited, which may lead to early-onset osteoarthritis. Cartilage defects can simultaneously make the patient very weak, resulting in a quality of life similar to that of patients with severe knee osteoarthritis waiting for total knee replacement. Currently, there are limited clinical methods for repairing articular cartilage damage. Non-steroidal drugs, hormones and other drugs can only relieve pain but cannot slow down joint degeneration. Surgical treatment is the main option for this disease. Clinically, the surgical treatment of cartilage defects includes autologous osteochondral transplantation, allogeneic osteochondral transplantation, etc., and they have achieved certain effects [9].

The meniscus is the fibrous cartilage in the knee joint. Its main function is to make the joint fluid easily diffuse and reduce local friction. MAT was originally a mature surgical treatment suitable for partial or total removal of the meniscus [10-11], which can relieve pain and improve function [12-14]. In addition, there are reports that MAT can alleviate the progression of osteoarthritis [15]. Research on the replacement of allogeneic meniscus transplantation to repair cartilage defects is currently rare. Therefore, this study first applied allogeneic meniscus replacement transplantation to repair cartilage defect tissue in an animal model. The results found that the height, texture, and color of the repaired tissue in the MAT group were close to normal cartilage at 2 months after the operation, and the surface had no depression and Smooth, HE and toluidine blue staining microscopic examination showed that the number of new chondrocytes was higher than that in the OAT group, and the arrangement was similar to normal cartilage. The above results suggested that the replacement of allogeneic meniscus transplantation to repair cartilage defects has a definite repair effect.

At present, frozen osteochondral grafts are more commonly used in allogeneic transplantation. The reason is that compared with fresh cartilage, frozen osteochondral grafts can be operated on an elective basis and have ample time for multiple tests to prevent viral or bacterial infection and spread. It has advantages such as low immunity. However, long-term studies have confirmed that various aspects of the freezing process, such as the cooling rate, the type of cryoprotectant, and other factors, have varying degrees of influence on the cartilage tissue. The elastic coefficient and viscosity coefficient of the cartilage tissue after freezing allograft cartilage have obvious effects. It changes the degradation of cartilage matrix appear [16]. Therefore, the allogeneic osteochondral transplantation technique may cause pathological changes such as the degeneration of the transplanted cartilage and the decrease in load

capacity in the middle and long-term clinical observation. Some key proteins can indirectly reflect specific conditions and help us investigate the medium and long-term effects of allogeneic meniscus replacement transplantation in repairing cartilage defects. (Type collagen II) it is one of the characteristic signs of articular cartilage. The repair of cartilage tissue defect after transplantation will initiate the proliferation of chondrocytes in the early stage, and chondrocytes often secrete a large amount of type collagen II [17]. Over time, hypertrophic chondrocytes in the growth phase synthesize type collagen X (type collagen X) and secrete it into the extracellular matrix, forming the main component of the mature cartilage matrix [18]. However, in the medium and long-term observation after transplantation, chondrocyte apoptosis may gradually appear, resulting in the weakening of the ability of chondrocytes to secrete type collagen II. At the same time, cartilage matrix appears under the action of proteolytic enzymes such as matrix metalloproteinases (MMPs). Degraded, type collagen II is decomposed into CTX-II [19], resulting in cartilage degeneration, cartilage fibrosis and calcification. Researchers believe that this pathological process may be one of the main reasons that cartilage transplantation has a better effect in the early stage, but changes in mechanical properties, decreased load capacity, and unsatisfactory efficacy in the middle and late stages [20-21]. Many surgical methods for the treatment of articular cartilage defects have also reported poor long-term efficacy and changes in mechanical properties after transplantation [22-23].

In this study, the histomorphological score of the MAT group was significantly higher than that of the OAT group at the time points of 2 weeks, January, December, and 18 months ($P<0.05$), suggesting that MAT has a better effect in repairing cartilage defects in the early, middle and late stages. OAT is good, and there is not much difference in mid-term efficacy. In December and 18 months, the ultimate stress, ultimate strain, compressive modulus and tangent modulus of the MAT group were significantly higher than those of the OAT group ($P>0.05$), indicating that the weight-bearing capacity of MAT in the middle and late stages was higher than that of OAT, further indicating that in the middle and late stages MAT repairs cartilage defects better than cartilage transplantation. It can be seen from the changes in the average expression of collagen type II protein at different time points in the two groups that there was an upward trend within 1 month after surgery, and the average expression of collagen type II protein showed a downward trend from 1 month after surgery to 18 months after surgery. Among them, when comparing the average values of collagen type II protein expression in the two groups at 1 week, 2 weeks, and January, the MAT group was significantly higher than the OAT group ($P<0.05$). From 1 week to 18 months after surgery, the protein content of MMP3 and CTX-II in the two groups showed a downward trend. At 12 and 18 months, the protein content of MMP3 in the MAT group was significantly lower than that in the OAT group ($P<0.05$). The key gene expression level also showed the same result. The above results suggest that MAT repairs bone defects and can more significantly regulate the level of key proteins/genes. The logistic regression analysis was further used, and the results showed that the first month after surgery was the time point that significantly affected the efficacy. Collagen type II and col2a1 were key proteins and genes that significantly affected the efficacy and the key to the curative effect of repairing cartilage defects.

To sum up, the MAT technique has a good effect in repairing cartilage defects in the early and middle and late stages, and the key to screening the effect of MAT in repairing cartilage defects may be the expression level of collagen type II in January after surgery, confirming that the meniscus repairs cartilage defects and provide new predictive indicators for efficacy monitoring.

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