Influence of Nicotine on Evaluation Indexes of Volleyball Players' Physical Training

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Objectives: This paper studies the effect of nicotine on the recovery of articular cartilage injury and physical training of volleyball players. Methods: In this study, a rat model of cartilage defect was established, and BMSCs sodium alginate gel was used to repair and give nicotine treatment. On the other hand, 20 volleyball players in our school were taken as the experimental objects. Group A is the experimental group and group B is the control group, with 10 people in each group. The experimental group was tested in smoke-free environment for one week. The control group was tested in smoke-free environment for one week. The physical differences between the two before and after the experiment were compared. Results: BMSCs composite alginate gel can repair cartilage defects well, and nicotine has an adverse effect on its defect repair effect. The repair tissue produced by BMSCs composite alginate gel is similar to normal cartilage. Nicholas Ding Ke affects the morphology of chondrocytes and proteoglycan synthesis in matrix. After 10 weeks of physical exercise function training, the total score of functional movement screening (FMS) index of the experimental group in smoke-free environment showed significant difference compared with that before the experiment. It shows that after 10 weeks of physical exercise function training, the injury degree of athletes in the experimental group in smoke-free environment has been significantly improved, and their physical fitness has also been relatively improved. Conclusion: Nicotine has a certain impact on the recovery of bone and joint injuries and physical training of volleyball players. Volleyball players should smoke less or even no smoking in order to improve their performance. Non smoking volleyball players should not be in a passive smoking environment for a long time.

Key words: nicotine, smoke-free environment, volleyball training, recovery after cartilage injury
Tob Regul Sci.™ 2021;7(4): 353-363
DOI: doi.org/10.18001/TRS.7.4.12

China is the country with the largest smoking population base in the world. The smoking population is increasing at a rate of 2% every year[1]. This kind of population is often nicotine dependent. According to relevant statistics, in addition to active smokers, the rate of passive smoking in China has also reached more than 70%. The study found that nicotine is one of the most harmful substances to human body among more than 3800 known toxic chemicals released by tobacco combustion[2-3]. Nicotine is known to be a risk factor for a variety of diseases, including osteoporosis, cancer and periodontal diseases, which has an adverse effect on cell proliferation and differentiation. Studies have found that smoking can up

regulate the synthesis of Glucosaminoglycan (rather than collagen) in articular chondrocytes, accelerate cartilage loss and aggravate joint pain in people with osteoarthritis. However, some studies have shown that smoking can affect the endochondral osteogenesis of fractured mice and lead to delayed fracture healing[4-5]. Nicotine exposure in vitro can inhibit matrix synthesis of chondrocytes and lead to the loss of phenotype of chondrocytes[6]. In addition, nicotine also significantly inhibited the proliferation and differentiation of BMSCs into osteoblasts. However, there are few reports on the effect of nicotine (tobacco) widely existing in the environment on the chondrogenic differentiation of BMSCs.

On the other hand, the development trend of modern volleyball is "strength", "height" and "speed"[7-8]. Modern high-level volleyball needs comprehensive physical quality, especially explosive strength and jumping quality. High level volleyball players in Colleges and universities in China are the reserve force of national men's volleyball. At present, more and more men's volleyball matches in Colleges and universities adopt the attack mode of the combination of the back row and the front row, which puts forward higher requirements for the athletes' physical fitness and jumping ability[9]. The jumping ability is also directly related to the success or failure of blocking. Male volleyball players generally have weak upper limb strength, but the whip ability of upper limb is also an important physical element of volleyball players. However, in volleyball competition, they mainly rely on the spiking of players for attack. The greater the strength of the player, the faster the swing speed, and the higher the score rate of the attack. If a volleyball team wants to achieve excellent sports results, it must achieve a highly coordinated development of competitive ability elements such as physical fitness, intelligence, tactics, technology and psychology[10]. Physical fitness is the basis of the five competitive ability elements. Good physical fitness will provide the premise and possibility for the full play of skills and tactics. From the High-level Men's volleyball competition in Colleges and universities, it can be seen that the technical and tactical level and athletes' physical fitness directly affect the outcome of the competition[11]. Athletes with abundant physical energy and appropriate technical and tactical sports have a greater chance of winning the competition. Some athletes have the habit of smoking for a long time or in a passive environment, which requires us to study the impact of tobacco and nicotine on Volleyball Players' physical training. So it can better guide the physical training of volleyball players[12].

METHODS

Basic Information

The development of tissue engineering technology has significantly improved the clinical effect of cartilage defect. BMSCs are used as seed cells of cartilage tissue engineering for replantation[13-14], induction and differentiation to repair articular cartilage defect, which has achieved good clinical effect and made great progress in the treatment of clinical cartilage defect. Since it was first reported in 2002 that BMSCs was successfully used to repair human femoral condylar cartilage defects[1]. BMSCs has gradually become the mainstream method for clinical treatment of cartilage defects, and relevant basic and clinical research applications have been more and more extensive. However, most of the current studies focus on how to optimize the environmental conditions in the process of cartilage differentiation of BMSCs to complete high-quality differentiation, how to improve the clinical efficacy of cartilage defect repair, and there are few studies on the impact of adverse factors in the process of differentiation.

At present, China is the world's largest tobacco manufacturing and marketing country, and the smoking population ranks first in the world. Active and passive tobacco exposure leads to the occurrence of a variety of diseases. Nicotine is one of the main harmful components of tobacco. Epidemiological studies have found that nicotine abuse can have an adverse impact on the clinical effect of autologous cartilage transplantation to repair cartilage defects, but so far there is no report on the impact of nicotine on BMSCs transplantation to repair cartilage defects. The aim of this study was to establish a rat model of cartilage defect, to repair the nicotine with BMSCs sodium alginate gel and observe its effects on cartilage repair defects and possible mechanisms.

Drugs and Reagents

1) Nicotine, sigma company, origin in the United States;
2) Safranine O and solid green dyes, Shanghai Hengyuan Biotechnology Co., Ltd., origin in China;
3) Hematoxylin and eosin dyes, Shanghai Hengyuan Biotechnology Co., Ltd., origin in China;
4) Chloral hydrate, Wuhan Hechang chemical industry, origin in China;
5) Penicillin, Harbin Pharmaceutical Group Pharmaceutical Co., Ltd., origin in China;
6) Low viscosity sodium alginate, sigma company, origin in the United States;
7) CaCl2, sigma company, origin in the United States;
8) 1 × PBS, Wuhan Kerui Technology Co., Ltd., origin: China;
9) DMEM / F12 medium, hyclone, USA;
10) Fetal bovine serum, trypsin, GIBCO, USA;
11) Trizol, Invitrogen, China;
12) Reverse transcription kit, Dalian Bao Bioengineering Co., Ltd., origin: China;
13) Takara RT-PCR kit, Dalian Bao Bioengineering Co., Ltd., origin: China;
14) Y chromosome sex determining domain transcription factor (SRY type high mobility group box9, Sox9), type II collagen （ α L chain of type collagen gene, COL2A1) polyclonal antibody, Santa Cruz biotechnology, USA;
15) Secondary antibody (Sheep anti rabbit), pierce biotechnology, USA;
16) DAB chromogenic kit, Shanghai Tianqi Biotechnology Co., Ltd., origin: China;
17) Other reagents are domestically produced.

Experimental Object

4-week-old and 12-week-old healthy female (280-300g) and male (350-370g) Wistar rats, and obtained the use license of experimental animals. The experimental scheme was approved by the animal ethics committee and animals were fed in strict accordance with the relevant treatment guidelines of the International Committee for experimental animal welfare. The experiment was completed in A3 animal experiment center.

Experimental Steps

Healthy Wistar rats aged 4 weeks were anesthetized by intraperitoneal injection of 10% chloral hydrate (3.5ml / kg), decapitated and killed, and soaked in 75% alcohol for 10 minutes. The rats were moved to a sterile tray, and the bilateral femurs and tibias of the rats were separated and dissected under sterile conditions with sterile gloves. After washing with normal saline, they were transferred to an ultra clean workbench. Replace sterile gloves and completely remove fascia, muscle and other soft tissues attached around femur and tibia. After cutting the proximal and distal metaphysis, F12 / DMEM (containing

10% fetal bovine serum, streptocyanine double antibody, vitamin C, 0.4mm proline, insulin and 1mm non essential amino acids) was extracted with a 5ml syringe.

Wash the bone marrow cavity with the culture medium in a sterile glass dish for 3-5 times, and try to flush out the bone marrow in the bone marrow cavity until the color of the bone marrow cavity turns right red to white. The washed F12 / DMEM medium containing red bone marrow was gently blown and mixed. After the sterile filter, it was transferred to a 15 ml centrifuge tube and centrifuged at 1000 rpm for 5 minutes. Discard the supernatant, add 6ml F12 / DMEM medium, gently blow and mix, and inoculate the cells into T25 cell culture bottle (3ml / bottle). Each bottle of cells was added with 2ml medium and cultured in a incubator with constant temperature at 37 ℃, 5% CO2 concentration and saturated (95%) humidity for monolayer adherent culture of primary cells.

The culture medium was changed every 2 days, and the cell adhesion and growth were observed under an inverted microscope. The cells were subcultured when the cell growth reached 85-90%. During passage, the culture medium was removed from each bottle of cells. Use 1 × The cells were washed twice with 5 ml PBS to remove the residual medium. Then add 1 ml of trypsin without EDTA and place it in the cell incubator to digest the cells for 6-7 minutes. Under the inverted microscope, the chondrocytes were observed to shrink and round, and the cell space became larger. After that, 9 ml F12 / DMEM medium was added to terminate the digestion. Gently tap the side of the culture bottle several times and observe under the inverted microscope again. After most of the cells fell off, gently blow the cells at the bottom of the bottle repeatedly with a 5ml pipette. Make all cells fall off the bottom wall of the culture bottle and sub pack them into two new culture bottles. Continue to culture in the cell incubator. This is the P1 generation. BMSCs were transferred to P3 generation by the same method, and subsequent experiments were carried out.

Weigh 320 mg of low viscosity sodium alginate on an electronic balance and place it in a 100 ml clean glass bottle. Put in a 10 mm magnetic stirrer and add 20 ml of normal saline. After sealing the bottle mouth with 3M film, it is sterilized under high temperature and high pressure. With the bottle mouth sealed, place the glass bottle on the magnetic stirrer and stir it at the lowest speed for 2 ~ 3 days until the liquid in the bottle becomes a uniform and transparent colloidal solution. Add the colloidal solution with 20 μ M sterile filter, and store it in 4 ℃ refrigerator for standby. Weigh 750mg anhydrous CaCl2 powder and put it into a clean beaker. Add 50ml distilled water, stir and mix until the powder is completely dissolved, and prepare a 102mm CaCl solution. 20 μ M sterile filter was used, and it was stored in 4 ℃ refrigerator for standby.

Cartilage Defect and Repair

Remove alginate gel and CaCl2 solution to reheat. Take several bottles of BMSCs from P3 generation rats with good growth and uniform distribution. Take 1 bottle of cells and add the cells twice with 5 ml PBS to remove the residual medium; Then add 1 ml of trypsin without EDTA. The cells were digested in a cell incubator for 6-7 minutes. Under the inverted microscope, the chondrocytes were observed to shrink and round, and the cell space became larger. After that, 5 ml F12 / DMEM medium was added to terminate the digestion. Gently tap the side of the culture bottle several times and observe under the inverted microscope again. After most of the cells fall off, gently blow the cells at the bottom of the flask repeatedly with a 5ml pipette to make all the cells fall off from the bottom wall of the culture flask. Remove 10 μ Put the cell suspension into a 1.5 ml sterile EP tube

and add 10 ml μ L trypan blue dye solution (final concentration is 0.04%). After fully mixing, take 7 with a pipette μ On the cell counting plate, read and calculate the total number of cells under the microscope. According to the counting results, the total number of required cells was calculated. The cells were digested as described above, collected and cultured. The cells were collected by centrifugation at 1000 rpm based on 50 ml centrifuge tube. Press 6 × The density of 10 degree cell/mL was uniformly mixed into alginate gel.

Wistar rats were randomly divided into 4 groups:

(1) Defect control group (non treated); The cartilage defect was not repaired after modeling;

(2) Gel repair group (Alginate): cartilage defect modeling and alginate gel repair;

(3) Gel + stem cell repair group (BMSC): cartilage defect modeling and BMSCs alginate gel repair.

(4) Gel + stem cell repair + nicotine treatment group (Nicotine): cartilage defect modeling and compound BMSCs alginate gel repair, postoperative daily total 2 mg/kg (interval 12 h 2 times given) subcutaneous injection of nicotine.

Operation procedure: the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (dose: 3.5 ml / kg). After successful anesthesia, the rats were fixed on the rat fixator in the supine position. Prepare the skin in the operation area of the right lower limb, disinfect it with active iodine and 75% ethanol for 3 times, and lay a sterile hole towel. Fix the right lower limb in the knee extension position, take the medial femoral condyle of the right knee joint as the midpoint, and make a straight incision with a length of about 2.0 cm. The skin, fascia and some medial femoral muscles were cut successively, and then separated to the distal femur. The joint capsule was cut to expose the femoral condyle and femoral trochlea.

The patella was dislocated laterally, and a full-thickness cartilage defect was made at the femoral intercondylar trochlea with self-made surgical instruments. The diameter of the defect was 3.0 mm and the depth was 1.5 mm. The degree of blood leakage at the bottom of the defect was taken as the degree. The articular cavity was repeatedly rinsed with normal saline to remove the cartilage debris formed during the fabrication of defect. According to the grouping condition, the corresponding repair treatment was given. The repair group was injected with compound or non BMSCs alginate gel into the defect, and then injected 102 mM CaCl2 solution. After the gel was fixed, the patella was restored and the muscles, fascia and skin were sutured by 5-0 threads. After operation, the rats were injected intraperitoneally for 4 hours × 105u penicillin was put into the incubator for anesthesia and resuscitation. After the rats woke up from anesthesia, they were put back into the cage for normal feeding. Penicillin 4 was injected intraperitoneally for 7 days × 10°U。 The limbs on the operation side were not fixed, fed with normal water, and the rats were allowed to move freely in the cage. The nicotine treatment group was subcutaneously injected twice a day, with a total amount of 2.0 mg / kg nicotine.

Score of Repair Effect of Sports Cartilage Injury

Three months after operation, the rats were decapitated and killed after anesthesia. The right medullary and knee joints were severed respectively. The muscle and fascia attached to the femur were removed, the femur was cut above the femoral trochlea, only the femoral condyle and trochlea at the distal femur were retained for photography, and the repair of cartilage defects was evaluated according to the general scoring standard of cartilage repair formulated by the

International Cartilage Repair Society (ICRs) (Table 1).

Table 1
Gross Score of Cartilage Repair

ICRs cartilage repair evaluation items	Score
ICRs cartilage repair evaluation items	
The degree of defect repair was flush with the surrounding cartilage	4
Repair defect 3 / 4 depth	3
Repair defect 1 / 2 depth	2
Repair defect 1 / 4 depth	1
Not repaired	0
Repair area	
Complete connection and healing with surrounding cartilage	4
The distance to the surrounding cartilage is < 1mm	3
3 / 4 were completely connected with the surrounding cartilage, and 1 / 4 were not connected with the cartilage	2
1 / 2 completely connected with the surrounding cartilage, and the distance between the unconnected 1 / 2 and the cartilage is > 1mm	1
Complete connection with surrounding cartilage < 1 / 4	0
General view of repaired cartilage surface	
Smooth surface	4
Fibrocartilage	3
Scattered small cracks	2
Scattered large cracks	1
Obvious defect or ulcer	0
Overall rating	
Grade I: normal	12
Grade II: close to normal	11-8
Grade III: abnormal	7-4
Grade IV: obvious abnormality	3-1

Note.
ICRS-International Cartilage Repair Society

RESULTS

Pathological Observation on Repair of Sports Cartilage Injury in Volleyball Players

Three months after operation, the knee joint was cut and observed. The surface of femoral cartilage of knee joint in each group was smooth without joint adhesion and osteophyte formation. No obvious effusion is found in the articular cavity, and no edema is found in the synovial tissue.

The repaired cartilage tissue was found in the defect control group, and the repair depth was basically flush with the surrounding cartilage; The repair area is small, which can be connected with the surrounding cartilage. Obvious defects and ulcers can be seen on the surface of cartilage. In the gel repair group, part of the repaired cartilage tissue was visible,

and the depth of repair was close to the surrounding normal cartilage. The repair area can reach more than 1 / 2 of the defect area. It is completely connected with the surrounding cartilage by more than 1 / 2, and the distance between the unconnected part and the cartilage is > 1mm. Large cracks can be seen on the surface of cartilage. The cartilage defects were repaired well in gel + cell repair group. The repair depth was flush with the surrounding cartilage; Complete healing with surrounding cartilage tissue. A few can be seen with a distance of < 1 mm from the surrounding cartilage. The cartilage is smooth. Gel + cell repair + nicotine treatment group showed obvious cartilage defect repair. The repair depth was basically flush with the surrounding cartilage. Completely connected with the surrounding cartilage 3 / 4. The distance between unconnected 1 / 4 and cartilage > 1 mm. A few can be seen with a distance of < 1 mm from the surrounding cartilage. Cartilage showed no obvious defect. Some of them showed fibrocartilage like changes. The above results showed that BMSCs composite sodium alginate gel could repair cartilage defects well, and nicotine had a negative effect on the defect repair effect.

Histological Observation on Repair of Sports Cartilage Injury in Volleyball Players

In the defect control group, he and safranine fast green staining showed that most of the repaired tissue cells were long spindle and fibrocartilage like tissue. The staining is obviously lighter. The cartilage surface is seriously irregular. The repair depth of cartilage can reach more than 2 / 3 of the defect and combine with the surrounding cartilage tissue. The repaired tissue was non cartilaginous tissue. Cartilage matrix was not stained. The cartilage surface is irregular. The repair depth of cartilage can reach more than 2 / 3 of the defect and combine with the cartilage tissue on both sides. Gel + stem cell repair group HE and red green green staining showed that cells were round or round like. Lacunae like structures were formed around some cells, which were hyaline cartilage. There was no difference between cartilage matrix staining and surrounding normal cartilage. The depth of cartilage repair was flush with the surrounding cartilage tissue, and healed with the cartilage tissue on both sides. Gel + stem cell repair + nicotine treatment group HE and reddish green staining showed that cells were round or round like, and cells arranged in disorder. No lacuna like structure was found, but most of them were hyaline cartilage like tissue. The staining of cartilage matrix was lighter than that of surrounding normal cartilage. The depth of cartilage repair was flush with the surrounding cartilage tissue and combined with the cartilage tissue on both sides. These results suggest that the repair tissue produced by BMSCs composite alginate gel is similar to normal cartilage. Nicotine can affect the morphology of chondrocytes and the synthesis of proteoglycan in matrix.

Physical Training Experiment and Results

Twenty volleyball players in our school were the experimental subjects. Group A is the experimental group and group B is the control group, with 10 people in each group. The basic information of the subjects is shown in Table 2. As shown in Table 2, the average age of the experimental group was 19.10 ± 1.45 years, the average height was 179.90 ± 5.04cm, and the average weight was 71.61 ± 7.85kg ($P > 0.05$). The average age of the control group was 19.00 ± 1.49 years, the average height was 180.00 ± 1.88cm, and the average weight was 71.87 ± 2.83kg ($P > 0.05$). Therefore, there is no significant difference in average age, height and weight between the experimental group and the control group, that is, the physical conditions of the two groups are tested before the experiment. There was no significant difference.

The special physical fitness test indexes of women's volleyball players selected in this study are based on a large number of reading literature and discussed by experts and volleyball coaches. Seven indexes are selected as the test contents, as shown in Table 3. They are arm flexion support, back throwing solid ball, run-up touch height, 30m acceleration run, Mizi movement, double swing rope skipping and 20m multi-level turn back run. The evaluation indexes of strength quality mainly include: bending arm support, throwing back solid ball, running up and touching high, double swing rope skipping test. Among them, the arm flexion support mainly evaluates the strength of the athlete's trunk pillar, the back throwing solid ball mainly evaluates the explosive power and coordination of the athlete's whole body, the run-up touch highly evaluates the athlete's bouncing power, and the double swing jump rope mainly evaluates the athlete's lower limb strength and coordination. The evaluation indexes of speed quality mainly include: 30 meter accelerated running to evaluate the athletes' accelerated running ability. The evaluation indexes of sensitive quality mainly include: meter character movement. Meter movement mainly evaluates athletes' sensitivity. The main evaluation indexes of aerobic endurance are: 20m multi-level turn back run test.

SPSS18.0 statistical software is used to sort out and statistically analyze the relevant data of the experiment. The descriptive statistical analysis method is used to count the mean standard deviation of each test index, the paired t-test is used to analyze the changes of each test index in the two groups before and after the experiment, and the independent sample t-test is used to compare the values of each test index between the two groups.

One week before the start of the training program, the two groups of athletes were first in a smoke-free environment and a smoke-free environment. The two groups of athletes were tested by functional movement screening (FMS) and volleyball special sports quality, which was called pre experiment test, and the test data were recorded. Then the training team of the experimental group was trained for 10 weeks. After the training, the two groups of subjects were tested again, which is called post experiment test. The test data were recorded and compared with the data before the experiment. Aerobic endurance refers to the ability to supply aerobic energy for a long time. The physiological factors that determine the body's aerobic endurance are mainly the supply of oxygen during exercise and the content of glycogen as an energy substance. The training methods are shown in Table 4.

The comparison of FMS scores between the two groups before the experiment is shown in Table 5. The average score of FMS is shown in Figure 1. It can be seen from table 5 that before the experiment, the total score P of functional movement screening (FMS) between the experimental group and the control group was greater than 0.05, indicating that there was no significant difference in functional movement screening between the two groups before the experiment. The comparison of FMS scores of the experimental group after the experiment is shown in Table 6. As shown in Table 6, before and after the experiment, the total score of functional movement screening (FMS) index of the experimental group in smoke-free environment increased significantly. Through the t-test, the p value is less than 0.05, indicating that after 10 weeks of physical exercise function training, the total score of functional movement screening (FMS) index of the experimental group in smoke-free environment is significantly different from that before the experiment. It shows that after 10 weeks of physical exercise function training, the injury degree of athletes in the experimental group in smoke-free environment has been significantly improved, and their

physical fitness has also been relatively improved.

Table 2
Basic Information of Subjects

	Experience Group	Control Group	P Value
Age	19. 10±1.45	19.00±1.49	0.888
Height (cm)	179. 90±5. 04	180. 00±1. 88	0. 945
Weight (Kg)	71. 61±7.85	71. 87±2. 83	0. 922

Table 3
Test Index

Type	Index
Power class	Bent arm support, back throw solid ball, run-up touch high, double swing rope skipping
Speed class	30 meter acceleration run
Sensitive class	Meter word movement
Aerobic endurance	20m multi-level turn back run

Table 4
Analysis and Comparison of Functional Movement Screening (FMS) Test Scores Before Experiment

Practice form	Project content	Load size	Intermittent time
Aerobic Endurance Training	Variable speed running (100m)	6 turns / group * 2 groups	5 min
	1500 meter run	2 groups	5 min
	15 minute run	1 groups	
	Chase interval	20 minutes / group * 1 group	
	20m multi-level turn back run	limit	

Table 5
The Fit Indicators of CFA for the SMRQ Data

	Experience Group	Control Group	P Value
Squat	2. 00±0.94	1.90±0. 58	0. 758
Hurdle step	1. 70±0. 67	1.80±0. 63	0. 758
Split squat	2. 10±0. 57	2. 00±0. 67	0. 758
Shoulder range of motion	2. 70±0. 48	2. 80±0. 42	0. 678
Straight leg active lifting	2. 80 ±0. 42	2. 90±0. 32	0. 591
Trunk stable push ups	1. 50±0. 97	1.60±0. 97	0. 823
Rotational stability	1. 40 ±0. 97	1.70±0. 67	0. 496
Total score	14. 20±2. 35	14. 70±1, 49	0. 575

Table 6
Analysis and Comparison of Functional Movement Screening (FMS) Test Scores After Experiment

	Experience Group	Control Group	P Value
Squat	2. 00±0.94	2.80±0.42	0. 022
Hurdle step	1. 70 + 0. 67	*2.* 30±0.48	0. 051
Split squat	2. 10±0. 57	2.80±0.42	0.010
Shoulder range of motion	2. 70±0. 48	2. 70±0.95	1.00
Straight leg active lifting	2. 80±0, 42	2. 70±0.48	0. 591
Trunk stable push ups	1. 50±0. 97	1.40±1.07	0. 726

Rotational stability	1. 40±0. 97	2.00±0.00	0. 081
Total score	14. 2±2, 35	16. 7±2. 21	0. 000

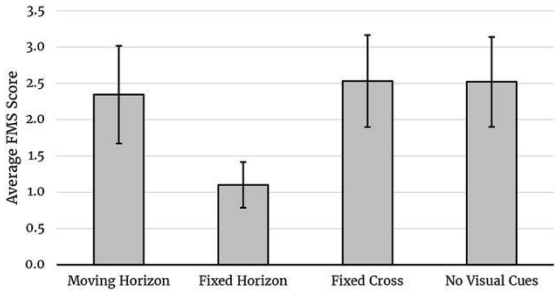

Figure 1 The average score of FMS

DISCUSSION

The dose of nicotine exposure was a key factor in this study. In previous studies, the maximum dose of nicotine exposure was 6-9 mg / kg. D, and the common study dose was 2-3 mg / kg. D. Studies have found that the average nicotine content of each cigarette in China's regular tobacco is about 2mg. The dose conversion relationship between human and rat was human: rat = 1 ∶ 6.1767. According to the above dose conversion ratio, the nicotine exposure dose of rat cartilage defect model in this study is 2mg / kg. D, which is equivalent to 11.3 cigarettes a day for an adult with a weight of 70kg (the calculation process is as follows: 2mg / kg. D + 6.17) × 70kg ÷ 2mg / cigarette = 11.3 cigarettes / day). Research shows that the essence of smoking addiction is nicotine dependence. The definition standard of smoking addiction is that the amount of smoking is equivalent to more than 10 cigarettes a day, and the nicotine content of each cigarette is at least 0.5mg. Therefore, the dosage of nicotine in this study is in line with the actual exposure of nicotine in smoking addicted people in real life. The experimental results obtained are more convincing and practical significance, and can provide guidance for clinical nicotine dependent people to accept the treatment of cartilage defects.

The clinical effect of articular cartilage defect repair has a significant impact on the prognosis and long-term life of volleyball injured athletes. It is known that there are many factors affecting the clinical effect of autologous cartilage transplantation in repairing cartilage defects, including nicotine abuse. However, relevant studies only analyzed the correlation from the perspective of epidemiology, and did not elaborate on the causes and specific manifestations of its impact. This study showed the adverse intervention effect of nicotine on BMSCs in repairing cartilage defects from the aspects of cartilage repair, histopathology and cartilage phenotype molecular biology. It mainly inhibits the expression of aggrecan. The main components of articular cartilage matrix, and then affects the quality of repaired cartilage tissue, so that the repair effect is not ideal. Further studies found that the effect of nicotine on cartilage repair may be mainly achieved by inhibiting the expression of Sox9, the key initiation transcription factor of chondrogenesis. The process of BMSCs implanted into cartilage defects for repair is actually equivalent to the process of BMSCs differentiating into chondrocytes. At present, the existing research mainly focuses on how to optimize various influencing factors in the differentiation process to achieve high-quality

differentiation, such as compound growth factors, selecting cell scaffolds with good biocompatibility, improving cell inoculation density, controlling oxygen concentration, regulating stress and microgravity, etc. there is no research on the exposure of common adverse environmental factors, Therefore, based on this animal experiment, this study further studied the effect of nicotine on the directional differentiation of BMSCs cartilage and explored its possible mechanism.

In addition, after 10 weeks of physical exercise function training, the injury degree of athletes in the smoke-free environment was significantly improved, and their physical fitness was also relatively improved. This also shows that nicotine has a certain impact on the recovery of bone and joint injuries and physical training of volleyball players. Volleyball players should smoke less or even no smoking in order to improve their performance. Non smoking volleyball players should not be in a passive smoking environment for a long time.

ACKNOWLEDGEMENTS

This research was supported by 2021 education and teaching reform research project of Zhoukou Normal University (No: J2021013); and this research was supported by General topics of the "14th five year plan" of Educational Science in Henan Province in 2021 (No. 2021YB0212).

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