RESEARCH ARTICLE

Influence of tobacco usage on physical recovery after exercise

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Tobacco has an impact on the respiratory system and cardiovascular system, especially in the recovery stage after exercise. This study explored the effects of tobacco on physical training ability and physical recovery by analogy with mice experiments. 30 mice were randomly divided into experimental group I, experimental group II, and control group. Experimental groups I and II were exposed to different smoke concentrations for different durations. The smoke concentration and exposure time of experimental group II were twice of that of experimental group I. After tobacco exposure, the experimental animal's time of exhaustive swimming, the activity of superoxide dismutase (SOD), and the content of malondialdehyde (MDA) in serum were used as evaluation indexes. The results showed that the swimming time, serum SOD activity, and MDA content in the two experimental groups were significantly lower than that in the control group. Both experimental groups also demonstrated significant differences in various indicators with statistical significance ($P < 0.05$). The results suggested that smoking could significantly reduce the aerobic training and anti-fatigue ability of mice, which was related to the decrease of SOD activity and the increase of MDA content induced by passive smoking. The longer the smoking time, the more serious the adverse reactions. Therefore, smoking and nicotine usage could damage the aerobic training ability of experimental animals, and had a negative impact on the physical recovery after exercise. The results of this study provided a scientific basis for further research on the effect of tobacco on human exercise ability.

Keywords: tobacco; nicotine; sports; physical fitness; physical recovery.

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Introduction

Smoking can damage all organs of the human body and tobacco has become the second largest global killer after hypertension. According to statistics from the World Health Organization, there are approximately 1.3 billion smokers worldwide, and 5 million people die annually from diseases related to smoking [1]. There are approximately 350 million smokers in China with a male smoking rate of over 60%. At the same time, 5.4 billion nonsmokers worldwide have been exposed to second-hand smoke. However, the harm to passive smokers has not yet received sufficient attention. More than 90% of the smoke generated during smoking containing tar, nicotine, carbon monoxide, and other harmful substances is directly dispersed in the air [2, 3]. People have clearly realized the harm of active smoking to their health, but the harm of smoking to other passive groups should also be brought to their attention [4].

As early as 1985, McMurray, *et al*. through exercise tests showed that passive inhalation of smoke could adversely affect exercise [5]. For children and adolescents, passive smoking significantly reduces lung function [6]. Renaud

and Cormier recruited 15 heavy smokers who smoked for more than 20 years and smoked more than 20 cigarettes daily in their study. The resting pulmonary function tests and electrocardiograms of those heavy smokers showed no cardiovascular and pulmonary diseases and were in a sedentary and inactive lifestyle. A baseline cardiorespiratory endurance test was then performed on 15 subjects, and their peak oxygen uptake was significantly lower than that of nonsmokers, while other measurements such as anaerobic threshold, oxygen pulse, *etc*. were also lower than that of nonsmokers. The results suggested that overall effects of smoking led to a decrease in aerobic capacity [7]. Smoking leads to a decline in pulmonary function and impairs the lungs' ability to absorb oxygen, while also causing damage to the cardiovascular system and increasing the risk of heart disease, hypertension, and other related illnesses. Despite the multitude of hazards associated with smoking, approximately 42% of college students choose to become smokers, and 24% of faculty and staff are also smokers. Among the college student population, around 25% of students engage in physical exercise during their leisure time. Therefore, conducting a thorough investigation into the effects of smoking on the body's exercise recovery can serve as compelling evidence to discourage smoking among college communities.

This study explored the effects of tobacco on physical training ability and physical recovery by analogy with mice experiments. The results of this study could provide a scientific basis for further research on the effect of tobacco on human exercise ability and the impact of smoking on the body's physical recovery capabilities.

Materials and Methods

Experimental animals

Thirty (30) Kunming mice (The Animal Feeding Center, Medical College, Zhengzhou University, Zhengzhou, Henan, China), 15 male and 15 female, weighing at 24±3 g were employed in this study for smoking experiments. The animals were raised in separate cages and fed freely in a well-ventilated room with the room temperature controlled at about 24°C. The animals were randomly divided into three smoking experimental groups including passive smoking group I, passive smoking group II, and non-smoking group (control) with half male and half female. Additional 40 Wistar rats with 20 males and 20 females, aged from 4 to 12 weeks with the bodyweight of 280-300 g for females and 350-370 g for males were obtained from the A3 animal experiment center in Wuhan University (Wuhan, Hubei, China) for cartilage repairing experiments. The animals were fed in strict accordance with the relevant treatment guidelines of the International Committee for experimental animal welfare and were randomly divided into four groups including (1) control group with no repair treatment for cartilage defects after modeling; (2) gel repair group (Alginate) with sodium alginate gel repairing after cartilage defect modeling; (3) gel + bone marrow stem cell (BMSC) repairing group (BMSC) with alginate gel repairing combined with BMSCs after cartilage defect modeling; and (4) gel + BMSC repairing + nicotine treatment group with 2.0 mg/kg nicotine injection subcutaneously twice a day after the repairing with sodium alginate gel and BMSCs. All animal experimental procedures were approved by the animal ethics committee of Wuhan University (Approval No. 14016),

Passive smoking experiments

A VC100 glass mobile air poisoning cabinet (Beijing Yuansen Kaide Bio-technology Co., Ltd, Beijing, China) with the dimensions of 100 × 100 × 70 cm^3 was employed for smoking poisoning experiments and connected to an in-house made smoking device with a cigarette holder, an outer cover, and a fan. The animal in the passive smoking group I was treated by 6 Hongqiqu brand cigarettes (Henan China Tobacco Industry Company, Anyang, Henan, China) with 15 mg tar and 1.2 mg nicotine per cigarette for 1 hour twice a day at 8 am and 4 pm for 14 consecutive days. Briefly, the animal was put into the glass mobile

air poisoning cabinet. A cigarette was put into the smoking device and fixed on the cigarette rack. After lighting, the cigarette was quickly put into the passive smoking poisoning cabinet until the cigarette burned out naturally before another lighted cigarette was put in. After burning out the sixth cigarette naturally in turn, the animal would continue staying in the poisoning cabinet up to an hour before moving out. The animal in the passive smoking group II was treated with the same conditions of smoking starting times and smoking duration except the time of each smoking was prolonged to 2 hours and the number of cigarettes was doubled to 12 cigarettes each time. The animals in the control group experienced the same conditions without passive smoking. During the passive smoking experiments, 90% and 10% of the main side stream smoke were controlled with the concentration of total suspended particulates (TSP) at 120 mg/m^3, while the concentrations of CO, CO_2, and O_2 supplied from the oxygen tank at a flow rate of 0.5 L/min were monitored by using LK-3 gas detection alarm monitors (Meker Technology Co., Ltd, Suzhou, Jiangsu, China). Both TSP and CO contents were used as smoking exposure indicators.

Swimming experiments

After the completion of passive smoking treatment, the animals in all groups were trained for weight-free adaptive swimming together for 6 days with the swimming time gradually increased from the beginning of 20 minutes. The swimming experiment was carried out in a 100 × 50 × 50 cm^3 swimming pool with a water depth of 50 cm, which was more than twice the length of animal from the head to the tail tip, and the water temperature of 28 ± 2°C. After 1 day rest from the training session, the exhaustive swimming experiment was conducted, and the animal swimming time for the continuing swimming until the head sinking under the water for 10 seconds without exposing to water surface was recorded.

Tissue sampling and SOD, MDA measurements

After 24 hours of recovery from exhaustive swimming, all animals were sacrificed by cervical dislocation with one eyeball of the animal was removed, and 1.5-2.5 mL of blood was taken. The blood was kept in refrigerator at 4°C overnight before centrifugation at 3,000 rpm for 10 minutes at room temperature. The activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) were detected through xanthine oxidase and thiobarbituric acid methods by using SOD and MDA kits (Jiancheng Bioengineering Technology Company, Nanjing, Jiangsu, China) following the manufacturer's instructions.

Construction of animal cartilage defect model

The knee joint cartilage defect animal model was constructed. Briefly, the animal was anesthesia with 10% chloral hydrate at the dose of 3.5 mL/kg weight intraperitoneally. The right lower limb was fixed in the extensor knee position and the femoral condyle and trochlea were exposed. The cartilage full-thickness defect was made at the intercondylar trochlear of the femur in a diameter of 3.0 mm and a depth of 1.5 mm until the bottom of the defect oozed blood. The joint cavity was rinsed repeatedly with sterile saline to remove cartilage debris. The animals in repair groups were injected with alginate gel with or without composite BMSCs at the defect locations, and then, 102 mM $CaCl_2$ solution before closing the articular capsules. The animals were given intraperitoneal injection of 4×10^5U penicillin for 7 consecutive days after the surgery and were fed with normal food and water in a free move cage.

Gross score of cartilage defect repair

Three months after surgery, the animals were sacrificed by decapitation after anesthesia. The right femur and knee joint were separated with the femur cut above the femoral pulley. Only the distal femoral skeleton and pulley were retained for observation and photography by using Nikon H55oS Light Microscope (Nikon Optics, Tokyo, Japan). The repair of cartilage defects was evaluated according to the general scoring standard of cartilage repair formulated by the

Table 1. The index comparison after smoking experiment.

Groups	Number of cases	Exhaustive swimming time (min)	Serum MDA (mol/mL)	Serum SOD (U/mL)
Control group	10	276.57 ± 45.97	16.41 ± 3.47	216.46 ± 15.95
Experimental group I	10	215.57 ± 29.49	22.31 ± 3.31	169.16 ± 24.80
Experimental group II	10	176.54 ± 21.79	23.55 ± 2.37	154.91 ± 36.37

International Cartilage Repair Society (ICRs) (https://cartilage.org/society/publications/icrs-score).

Statistical analysis

SPSS 13.0. (IBM, Armonk, New York, USA) was employed in this study for statistical analysis. All data were presented as mean ± SD. The differences between groups were tested by using t-Test with $P < 0.05$ as significant difference, and $P < 0.01$ as very significant difference.

Results

The effects of passive smoking on the exercise ability

The data of exhaustive swimming time, blood serum SOD, and MDA were listed in Table 1. The results showed that there were significant differences between experimental group I and control, and experimental group I and II ($P < 0.05$) in all three tested factors. The exhausted swimming time and serum SOD activity in the experimental groups were lower than those in the control group after smoking experiment, while the contents of MDA in both smoking experimental groups were increased significantly comparing to that in control group ($P < 0.05$). There were also significant differences between the experimental groups. The results confirmed that passive smoking had an adverse effect on the aerobic and antioxidant capacity of healthy mice.

The impact of smoking on the body's recovery ability

Three months after surgical operation, the animal femoral cartilage surface of knee joint in each group was smooth without joint adhesion and osteophyte formation. No obvious effusion was found in the articular cavity, and no edema was observed in the synovial tissue. In the defect control group, the repaired cartilage tissue could be seen with the repair depth basically flushed with the surrounding cartilage. The repair area was small and could be connected with the surrounding cartilage, while obvious defects and ulcers were on the cartilage surface (Figure 1A). In the gel repair group (Alginate), part of the repaired cartilage tissue was visible with the depth of repair close to the surrounding normal cartilage. The repaired area reached more than 1/2 of the defect area, and more than 1/2 was completely connected with the surrounding cartilage. The distance between the unconnected part and the cartilage was more than 1 mm, and large cracks were observed on the cartilage surface (Figure 1B). The gel + stem cell repair group (BMSC) had good repair of cartilage defects. The depth of repair was neat with the surrounding cartilage and completely healed with the surrounding cartilage tissue. A few samples showed the distance from the surrounding cartilage less than 1 mm, and the cartilage was smooth (Figure 1C). In Gel + stem cell repair + nicotine treatment group, obvious cartilage defect repair was also observed. The depth of repair was basically aligned with the surrounding cartilage with 3/4 completely connected with the surrounding cartilage. The distance between the unattached 1/4 and the cartilage was larger than 1 mm. A few samples could see the distance from the surrounding cartilage less than 1 mm (Figure 1D). The cartilage showed no obvious defect, and some demonstrated fibrocartilage-like changes. The results confirmed that BMSCs composite sodium alginate gel could repair cartilage defects from a

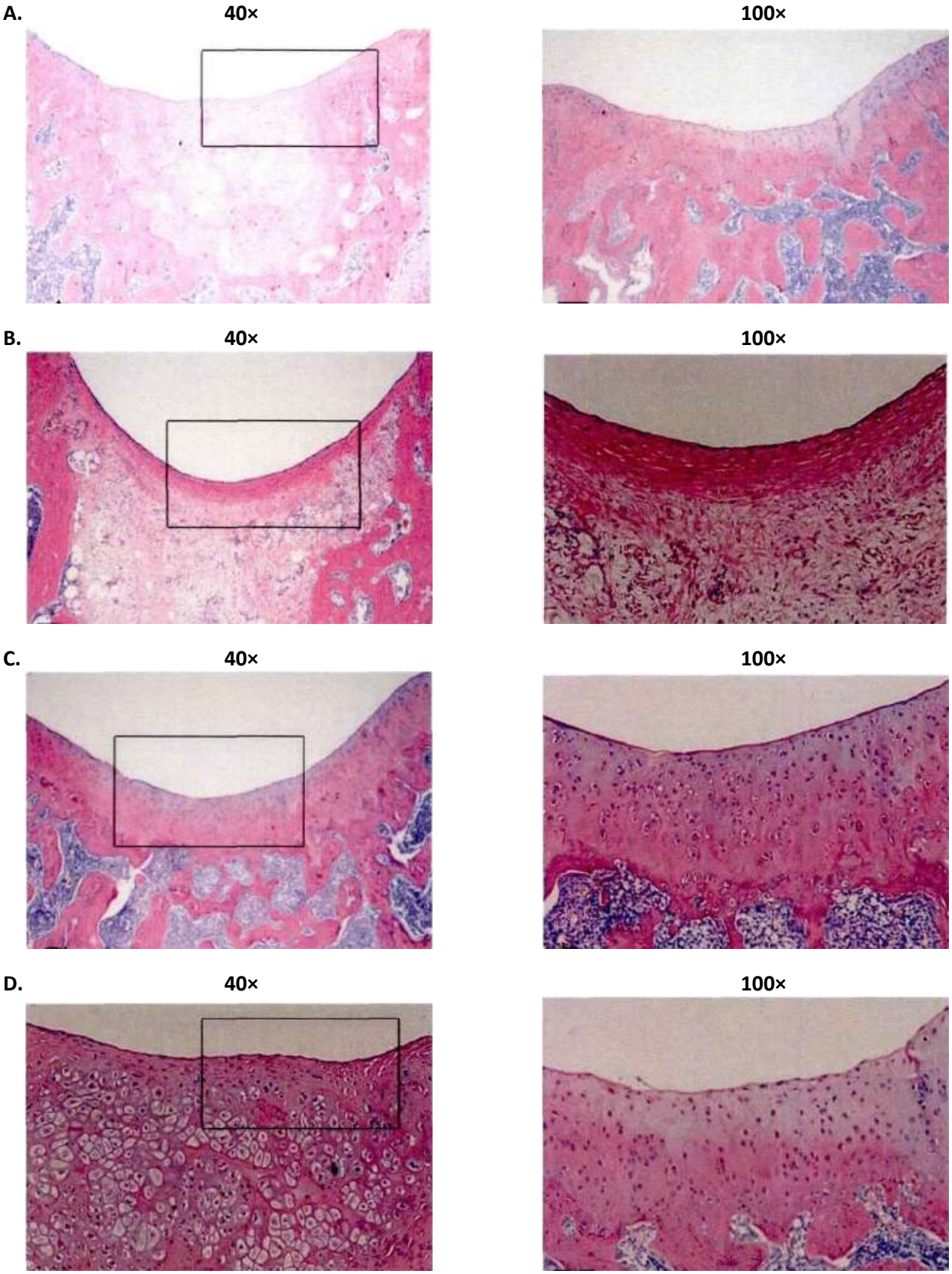

Figure 1. Cartilage defect repair. **A.** Control. **B.** Alginate. **C.** BMSC. **D.** BMSC + nicotine.

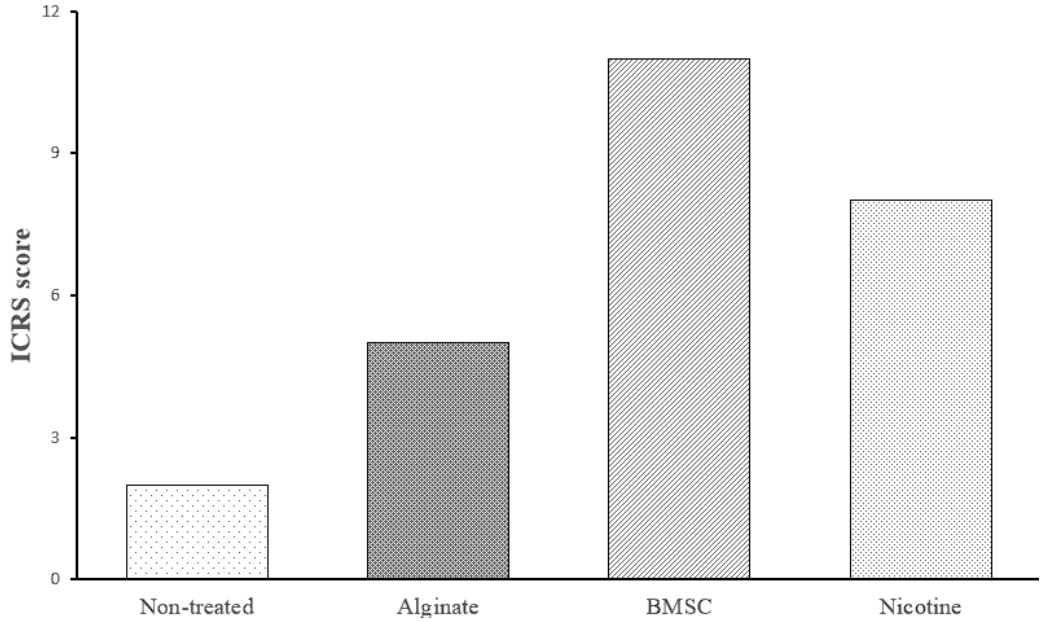

Figure 2. Cartilage defect repair score. A rank sum test was used, and groups were compared to each other.

general perspective, and nicotine had adverse effects on the cartilage defect repair. Histological ICRs scores for cartilage defect repair demonstrated that the animal group of non-treated (Control) had the lowest scores with the worst repair effect. Alginate group scored better than control group ($P < 0.05$), but lower than that of BMSC group ($P < 0.01$). The BMSC group scored the highest numbers and had the best repair effect, while nicotine treated group scored lower than that of BMSC group ($P < 0.05$) (Figure 2).

Discussion

Aerobic exercise ability refers to the body's ability to exercise for a long time based on aerobic metabolism. The factors that determine and affect aerobic exercise ability include cardiopulmonary function, muscle oxygen utilization ability, gender, and age. Low load or no-load exhaustive exercise time is the main method to judge the aerobic exercise ability of experimental animals [8, 9]. Pastr studies confirmed that tar and other harmful substances in smoke could adhere to the surface of trachea, bronchi, and alveoli of passive smokers and inhibit ciliary movement, which increased the releasing of oxygen free radicals by alveolar macrophages and blood neutrophils, and then increased the adhesion between leukocytes and endothelial cells, causing inflammatory reaction, and finally leading to the destruction and dysfunction of lung tissue [10-13]. Nicotine can cause the increase of blood pressure, acceleration of heartbeat, decline of cardiac pumping function, increase of oxygen consumption, and decline of aerobic exercise ability. The results of this study were consistent with the previous reports. After 14 days of passive smoking, the lung tissue of passive smoking mice might be damaged to varying degrees, and therefore, the functions of heart and muscle declined, resulting in the reduction of continuous aerobic exercise ability and anti-fatigue ability as the shortening of exhaustive swimming time. The changes of SOD activity and MDA content in the serum of experimental animals were the strong evidence to explain the observed phenomena. In recent years, the free

radical theory has provided a new theoretical basis for the generation and recovery of exercise-induced fatigue and received more and more attention. Oxygen free radicals and lipid peroxidation have strong toxic effects on living cells with the alternation of the lipid microenvironment of membrane receptors, ion channels, and membrane proteins, and further affecting the cell membrane functions. Malondialdehyde (MDA) is the ultimate breakdown product of lipid peroxidation and the opposite of lipid peroxidation in the human body. Therefore, MDA has usually been used as an index to evaluate the degree of free radical lipid peroxidation. In addition, there are enzymes and non-enzyme molecules that scavenge oxygen free radicals, mainly including superoxide dismutase (SOD), catalase (CAT), and vitamin E, which maintain the dynamic balance of free radical production and elimination in the body through their own pathways. Therefore, serum SOD activity and MDA content are the reflection of the overall oxidation and antioxidant state of the body. The results of this study showed that the blood MDA of experimental animal was significantly higher than that in the control group after 14 days of passive smoking, while the activity of SOD scavenging free radicals was significantly lower than that in the control group. The results suggested that passive smoking promoted lipid peroxidation *in vivo*, consumed a large amount of antioxidant enzymes or inhibited the activity of antioxidant enzymes, and affected the functions in many parts of the cells [14]. Therefore, the working abilities of blood, heart, lungs, and muscles decreased, and the development of the fatigue process was accelerated, which was consistent with the findings of previous studies [15-18]. The lipid hydroperoxides (LPO) of smokers is significantly higher than that of non-smokers, while SOD activity is significantly lower than that of non-smokers. Smoking exacerbates lipid peroxidation, oxidative and antioxidant imbalances, and increases MDA, which affects the normal physiological function of cells. The SOD activity and MDA content measured in this study could simulate the recovery period of 24 hours after exhaustive swimming, indicating that the harmful substances in cigarette smoke could not volatilize from the body in a short time. Therefore, smoking or passive smoking not only affected the exercise capacity, accelerated the development of fatigue, but also affected the elimination of fatigue and functional recovery after exercise. The results of this study suggested that cigarette smoke could reduce the activity of serum SOD and increase the content of MDA in healthy experimental animals, which was an important reason of reduced ability of aerobic exercise and antifatigue, delayed elimination of fatigue and functional recovery. The longer the smoking time was, the more serious the adverse effects were.

The clinical effect of articular cartilage defect repair has a significant impact on the prognosis and long-term life of patients. There are many known factors affecting the clinical effect of autologous cartilage transplantation in repairing cartilage defects. The specific causes and effects of nicotine abuse have not been analyzed from the perspective of epidemiology. This study showed the adverse intervention effect of nicotine on BMSCs in repairing cartilage defects. The process of BMSCs implanted into cartilage defects for repair was equivalent to the process of BMSCs differentiating into chondrocytes. Current studies mainly focus on how to optimize various influencing factors in the process of differentiation to achieve high-quality differentiation. There is lack of studies on exposure to common adverse environmental factors including compound growth factors, selection of biocompatible cell scaffolds, increasing cell inoculation density, controlling oxygen concentration, and regulating stress and microgravity. Therefore, this study investigated the effect of nicotine on the directional differentiation of BMSCs cartilage and explored the possible mechanism. The results showed that BMSCs as seed cells and sodium alginate as scaffold materials could achieve good results in cartilage defect transplantation and repair. A dose of nicotine exposure equivalent to that of

daily nicotine addicts could adversely affect the repair effect of cartilage defects.

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