

# *Cell Biological Characteristics by Fluorescence Microscope and Its Application in Animal Models of Muscle and Tendon Injuries*

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**Abstract:** With the progress and development of medical science, fluorescence microscope has been widely used in biological research. Muscle and tendon injuries, as common sports injuries, bring a lot of inconvenience to people's daily life and work. At present, the commonly used methods of repairing muscle and tendon injury have certain curative effect, but the treatment cycle is long and the recovery degree is low. In order to better detect the biological characteristics of cells, design muscle and tendon injury model, and explore the treatment of muscle and tendon injury, fluorescence microscope was introduced into the experimental process. Firstly, the muscle satellite cells and tendon stem cells of mice were cultured and passaged. After cryopreservation and resuscitation, the biological characteristics of the cultured cells were detected. The proliferation and cloning of the cells were observed by fluorescence microscope, and the growth curve was drawn. Then the muscle injury model and tendon injury model were established by cardiotoxin and collagenase type I respectively. Paraffin section was made for observation and serum CK index was detected. The experimental results show that fluorescence microscope can effectively observe the biological characteristics of cells and help to establish muscle tendon injury model. It is concluded that muscle satellite cells and tendon stem cells can recover the injury. This provides experimental basis for the clinical application of cell therapy in the treatment of muscle tendon injury.

## **1. Introduction**

### **1.1. Background Significance**

Muscle and tendon injury is the main type of sports injury, these injuries will not only affect the health of the body, but also bring great impact on daily life [1]. Research on the repair and treatment of muscle and tendon injury has become a hot topic in the medical field. Fluorescence microscope can effectively study biological tissue and cell structure, which is a necessary large-scale scientific

instrument in the field of biological research, and has been widely used [2]. It is very important to study the repair therapy of muscle and tendon injury by observing cells with fluorescence microscope and detecting their biological characteristics.

## 1.2. Related Work

At present, there are many researches on the repair of muscle injury and tendon injury. Suzuki, M. stained the gastrocnemius muscle of 24 male Sprague Dawley rats with Fluorogold. The right gastrocnemius muscle of the hind limb was injured by dropping block method. Anti NGF antibody (50  $\mu$  L) was injected intramuscularly to 12 rats. The percentage of FG positive cells in bilateral dorsal root ganglia was measured to study the effect of anti NGF antibody on sensory innervation in muscle injury model of rats to study the effect of anti NGF antibody on myogenic pain [3]. His research methods are complex, and the required experimental specimens are expensive and difficult to implement. Gaut, C. injected [99mTc] MDP into the muscle injury model of mice. Static whole-body single photon emission computed tomography / computed tomography (SPECT) was performed in two groups of animals with different parts: a group of mice aged 6, 15 and 19 weeks, and a separate cohort of 16 weeks old, and the second group was scanned with high-resolution CT at 8 weeks to explore whether [99mTc] MDP can be used for Duchenne muscular dystrophy MUs cle injury in mdx mice with DMD [4]. His research is not appropriate in the operation steps, which may affect the experimental results. Freeberg, M. A. T. used knockout (KO) mice and local Nanoparticles Mediated siRNA transmission to evaluate the inhibitory effect of PAI-1 on flexor tendon injury in region II of mice [5]. His research only focuses on flexor tendon injury, which is special and not representative.

## 1.3. Innovative Points in this Paper

In this study, muscle satellite cells were successfully isolated, cultured and passaged successfully. Their biological characteristics were detected by fluorescence microscope. By observing their growth curve and colony forming ability, it was found that they had good self-renewal and in vitro proliferation ability. The muscle injury models induced by cardiotoxin drugs and tendon injury induced by collagenase injection were established. The muscle satellite cells and tendon stem cells were stained. The paraffin section and frozen section were observed by fluorescence microscope, and the serum indexes were detected. The experimental data showed that muscle satellite nuclear tendon stem cells transplantation had therapeutic effect on muscle and tendon injury.

## 2. Observe Cells with Fluorescence Microscope

### 2.1. Fluorescence Microscope

The fluorescent microscope uses ultraviolet as light source to irradiate the tested object, and then observe the shape and position of the object under the microscope. It is generally used to study the absorption, transportation, distribution and location of chemical substances in cells.

#### (1) Working principle

The light source is usually a 200W ultra-high pressure mercury lamp. The discharge between two electrodes in the working room makes the mercury molecules in the lamp dissociate and reduce continuously, thus emitting light quantum [6]. Generally, it is strong ultraviolet light and blue violet light, which can excite all kinds of fluorescent substances. The new type of ultra-high pressure mercury lamp can be turned on without high pressure at the beginning of use. After using for a period of time, it needs to start with high voltage. The average life of 200W ultra-high pressure

mercury lamp is about 200 hours when it is used for 2 hours each time. The shorter the service time is, the shorter the life is. Therefore, it is necessary to control the starting times to a minimum. In the process of using the bulb, its light efficiency will gradually decrease. After the lamp goes out, it must be allowed to cool down before restarting. If it is turned off immediately after lighting, incomplete evaporation of Mercury will cause damage to the electrode.

The color filtering system composed of excitation filter plate and pressing filter plate is an important part of fluorescence microscope. According to the characteristics of light source and fluorescent pigment, the excitation filter plate can be divided into the following three categories: ultraviolet excitation filter plate, ultraviolet blue light excitation filter plate and violet blue light excitation filter plate. The thickness of the excitation filter plate depends on the brightness of the field of vision, and the darker the plate, the thinner it is. The pressing filter plate is used to prevent the excitation light from passing through. The corresponding excitation filter plates are ultraviolet light pressing filter plate, violet blue light pressing filter plate and ultraviolet violet light pressing filter plate.

The reflector should use the aluminized reflective layer with more than 90% reflection, and the condenser should use quartz glass or other UV transparent glass. The objective lens with achromatic function and large aperture ratio should be used together with low power eyepiece.

#### (2) Requirements for specimen preparation

The characteristics of fluorescence microscope make it have different requirements for specimen making. First of all, the thickness of the slide should be between 0.8-1.2mm, which should be smooth, uniform and without obvious spontaneous fluorescence. Secondly, the cover glass should be about 0.17mm. It is better to use interference cover glass, which can strengthen the excitation light and stimulate the specimen more effectively. The specimen should not be too thick, otherwise it can not be fully excited, and overlapping cells will affect the judgment. The mounting agent must be glycerin without autofluorescence and colorless and transparent. When the field of vision is dim, it is necessary to use non fluorescent lens oil.

#### (3) Usage and precautions

Fluorescence microscope is a kind of optical microscope, but the excitation wavelength is different, so their structure and use method are also different. First, open the lamp source to preheat for 15 minutes to make the ultra-high pressure mercury lamp reach the hottest spot; then install appropriate excitation filter and pressing filter; if it is a drop emission fluorescence microscope, it is also necessary to insert a two-color beam separator; use a low-power microscope to observe, constantly adjust the light source center to make it in the middle of the light spot; place the specimen and adjust the focal length to observe [7].

When using fluorescence microscope, the following matters must be paid attention to, so as to avoid damage to microscope and human body. The operation must be carried out in strict accordance with the instructions, and the steps should not be changed at will. Observe in the dark room, connect the power supply to light the ultra-high pressure mercury lamp, wait for its light source to stabilize before starting the observation. Wear protective glasses when adjusting the light source to avoid eye damage. The observation time should be more than 1 hour but not more than 3 hours. The specimens should be concentrated during observation to save time and protect the light source. The specimen should be observed immediately after staining to avoid the effect of fluorescence weakening.

## 2.2. Muscle Injury

### (1) Structure and function of muscle

Most of the muscle fibers are combined by connective tissue to form muscle bundles, and

thousands of muscle bundles are arranged neatly to form skeletal muscle. There are abundant capillaries and capillaries in skeletal muscle, and the blood flow of these vessels is more than 100 times as much as that in the static state [8]. Under a high magnification microscope, we can see that the muscle fibers have different depth of transverse stripes, so skeletal muscle belongs to transverse muscle. Sarcomere is the basic unit of muscle contraction, which is composed of myosin microfilaments and actin microfilaments. The outer side of myofibrils is surrounded by sarcoplasmic reticulum and T-tubules.

Both ends of skeletal muscle connect with tendon, directly or indirectly connect bone to form motor system. The main function of muscle is to produce strength through contraction, and complete movement in cooperation with bones and joints. According to the myofilament gliding Theory: due to the participation of ATP, the combination of  $Ca^{2+}$  and troponin, myosin changes, and actin is exposed at the junction of transverse bridge. Through the circulation of the transverse bridge, the thin muscle filament will stretch to the M line of the sarcomere center, and the thin muscle filament in the sarcomere will slide to the thick muscle filament, and finally realize the muscle contraction.

### (2) Mechanism of muscle injury

Overload and repeated exercise is generally considered to be the cause of muscle injury. Scientific training methods and moderate load stimulation can effectively increase the cross-sectional area of skeletal muscle, thus increasing muscle strength. Wrong method and overload exercise, easy to cause muscle pain, swelling, contractile force drop, resulting in muscle damage.

The physiological and pathological mechanism of exercise-induced muscle injury is similar to other soft tissue injury, which is the process of repairing and reconstructing the damaged part. Injury causes vascular rupture of muscle tissue, resulting in hematoma and injury. Hematoma then causes severe ischemia, hypoxia, necrosis and inflammation of muscle cells to transfer to the injured site. Phagocytes phagocytize the necrotic tissue, and then slowly form scar tissue to repair the damaged site [9]. Scar tissue is more likely to be damaged than normal muscle tissue. The tensile strength of the scar tissue is weak, and it is easy to cause more serious muscle laceration and scar formation under external force, which has become a common complication in muscle injury.

At present, there are several views on the mechanism of muscle injury. Energy metabolism disorder said: in the process of muscle contraction, high phosphate compounds and muscle glycogen decompose, producing energy at the same time, the accumulation of metabolites will break the stable state of local internal environment, cause slight damage to muscle cells, and affect the working ability of muscle. Calcium imbalance said: calcium ion as an indispensable part of muscle contraction, if the recovery is not smooth after the completion of the task of information transmission, it will break the balance of sarcoplasmic reticulum and intracellular calcium concentration, leading to the apoptosis of some healthy muscle cells. Free radical oxidation theory: the oxidation and decomposition of free radicals will produce harmful substances, destroy the cell membrane structure, the production and supply of ATP will also be affected, leading to energy metabolism disorder. Mechanical injury theory: skeletal muscle in mechanical movement, muscle cell membrane will suffer from varying degrees of damage, muscle enzyme activity increased, and release of inflammatory cytokines, resulting in muscle injury.

### (3) Treatment of muscle injury

There are various ways and degrees of muscle injury, which makes clinical research more difficult. The main forms of muscle injury include contusion, strain, tear and atrophy. At present, the main treatment methods of muscle injury include surgical treatment, drug therapy, acupuncture treatment with traditional Chinese medicine, physical therapy, growth factor, gene therapy, stem cell therapy, etc.

In the early stage of treatment of muscle injury, people usually agree with the principle of rice (rest, ice, compression, elevation). This refers to the injury immediately stop movement, rest, pressure bandage, lift the injured limbs. The current drug therapy generally uses non steroids and adrenocortical hormone anti-inflammatory and analgesic drugs, short-term use has therapeutic effect, but long-term use will have side effects on bone and muscle. Surgical treatment is usually used when the pain remains and the range of motion can not reach the normal value after the treatment. However, the fixation of shortening position and stretching position will cause atrophy of connective tissue and muscle fiber. Growth factors can promote the growth of muscle cells, accelerate the growth and proliferation of cells, so as to accelerate the repair of damaged sites. The commonly used factors include transfer growth factor, hepatocyte growth factor, fibroblast growth factor, etc., but the growth factors are not specific and can not be accurately applied to the injured site. If too many growth factors are used, muscle cell fibers may be hard. Massage, ultrasound and fire therapy are widely used in physical therapy. The principle of genetic factor therapy is to search for genetic factors that may restore muscle, so as to clone them. The target gene reaches the injured site, plays a corresponding role in the target tissue, repairing and improving the damaged tissue [10].

In addition, the prevention of muscle injury is also very important in sports training and daily life. Moderate warm-up and stretching before formal training or exercise, increasing the temperature of muscles, increasing the mobility of joints, reducing the viscosity of muscles and increasing the contractile elasticity of muscles, can avoid sports injuries to a certain extent.

### 2.3. Tendon Injury

#### (1) Tendon structure

Muscles and tendons are important parts of the motor system. Tendon is a tight collagen connective tissue fiber bundle connecting muscle belly and bone. It has strong pressure resistance, tension resistance and friction resistance, but no shrinkage capacity. The transmission of skeletal muscle contraction and relaxation force, can maintain the stability of the joint, so as to be able to move. It is possible to cause tendon injury if the mechanical force is overloaded frequently. Understanding the basic anatomical structure and mechanical characteristics of tendon is helpful to further investigate the causes of tendon injury.

Tendon is mainly composed of matrix such as collagen fiber, protein, elastin and a few cells. Collagen fiber is the most important component of tendon. Its main function is to bear and transmit different tensile loads and respond to it. 95% of adult tendons are type I collagen, which plays a role in tissue stabilization. Type III and type IV collagen fibers account for 5% of the total, and mainly exist in immature tendons and injured tendons. Different types of collagen fibers have different functions in tendon tissue [11]. Fibroblasts and tendon cells are the basic functional units of tendon, which synthesize extracellular matrix such as collagen and maintain the metabolism of tendon tissue. Tendon cells have a high degree of differentiation, and the phenotype of tendon cells will change after many passages in the test tube, and the secretion of collagen and proteoglycan will be reduced, and then the function of tendon will be affected. Tendon matrix is closely related to tendon cells. Tendon cells synthesize and secrete collagen, absorb and decompose metabolites in tendon matrix, and adjust the dynamic balance of microenvironment. Meanwhile, tendon matrix has effects on proliferation, growth, differentiation and metabolism of tendon cells. The change and destruction of matrix components will affect the normal function of tendon cells and reduce the mechanical strength of tendon. At the same time, due to insufficient vascularization of tendon tissue, energy supply mainly depends on anaerobic metabolic system, which is easy to cause injury accumulation.

#### (2) Pathology and pathogenesis of tendon injury

Tendon injury can be divided into two types according to the length of the disease: acute tendon injury and chronic tendon injury. Chronic tendon injury is caused by excessive use or improper training during training. The symptoms include tingling of tendon, swelling, dysfunction, etc. In the paraffin pathological tissues of tendon injury athletes, we found that inflammatory cells increased, blood vessels ruptured, collagen fibers decomposed, and various substances in the cell matrix changed. The light microscope showed that there were calcium deposits on the surface of all tendons. The collagen fibers were loose and some of them were damaged. Moreover, the content of aminoglycan in extracellular matrix increased significantly. It is difficult to distinguish between the normal tissues and the smooth ones. The length of the course of chronic tendon injury is different in different stages, the number of inflammatory cells increases, the distribution of collagen fiber cells is different, and accompanied by protein like mucus. An abnormal increase in tendon cells was also observed somewhere in the tendon. In the hyperfine structure, due to the increase of proteoglycan and protein content, the nucleus becomes oval, the tendon appears cartilage like appearance, and the size and shape of mitochondria and nucleus change.

The pathological changes under electron microscope showed that: hypoxic degeneration, hyaline degeneration, mucoid degeneration, cellulose degeneration, fat degeneration and calcification. The above pathological changes do not exist alone, and the occurrence conditions are different: tendon degeneration, tendinitis, scapulohumeral periarthritis and tendon degeneration.

The period of self-healing and repair of tendon usually goes through the period of inflammation and repair. Red blood cells and neutrophils are the first to be mobilized in the early stage of inflammation. On the second day of injury, macrophages and monocytes in the tissue were also activated and gathered to phagocytize necrotic cells. In this process, various types of tendon factors promote tendon cell proliferation and vascular repair. Bone collagen III can promote the healing of injured sites. One week after the inflammatory phase, edema began to decrease and generally entered the recovery period of 40-50 days. The more severe the local injury, the more collagen fibers and polysaccharides. The difference in the physiological function of the tendon during about 70 days is based on the individual physiological recovery period. During this period, tendon cells, collagen and polysaccharides gradually returned to normal values, the content of type I collagen increased, and the tendon became smooth and neat. At this time, the boundary of collagen fiber becomes clear, and tendon tissue changes gradually from fibrous tissue to scar tissue.

### (3) Treatment of tendon injury

Due to the incomplete understanding of the pathogenesis of tendon injury, most of the current treatments are palliative rather than permanent, including cryotherapy, NSAID, low intensity pulsed ultrasound stimulation, surgical treatment and massage recovery therapy. Generally speaking, acute tendon injury needs surgical treatment, but surgical treatment can not restore the original structure of the tissue, only the damaged part is reconnected, after recovery, if high-intensity exercise will be broken again. If multiple operations are performed at the same point, the injured tissue will calcify and lose its original ability to move. Chronic tendon injury is usually treated with conservative methods. For example, combined with medical pain relief and massage techniques, shock wave therapy. The treatment cycle is long, the effect is not very obvious, only temporarily relieve pain, can not completely recover [12].

Surgical treatment can be divided into two types. One is self transplantation that does not rely on biomaterials or enhances the repair of injured sites. The second is to use biomaterials instead of tendon tissue to repair it. These two methods have their own shortcomings and problems, patients can not return to normal, and their clinical application is also questionable. For example, autologous transplantation can only restore the physiological structure of tendon, but can not promote the normal healing of tissue. The surgical junction is easy to damage or form calcified lesions, which may lead to the decline or loss of motor ability. In allogeneic transplantation, a part of the tendon is

replaced by biomaterials. However, the aging of the material, the rejection of the tendon itself, and the decrease of strength and elasticity lead to necrosis or rupture of the transplanted tissue. In recent years, the synthetic materials which have been paid attention to have been questioned by many patients.

Conservative treatment includes drugs, regular exercise therapy, rehabilitation training and so on. The drugs are basically steroid drugs and non autogenous anti-inflammatory drugs. Regular exercise therapy can promote the metabolism of tendon tissue, which is beneficial to the formation of early injured blood vessels and promote the repair and regeneration of damaged tendon. Physical therapy mainly uses extracorporeal shock wave therapy and centrifugal training to promote tendon repair. The advantage of conservative treatment is that the pain can be greatly reduced. However, some studies suggest that immobilization, rehabilitation training, shock wave therapy, NSAIDs and steroid injection have no good therapeutic effect on chronic tendinitis.

### 3. Experiments on Detection of Cell Biological Characteristics and Animal Model of Muscle and Tendon Injury

#### 3.1. Study on Cell Biological Characteristics

##### (1) Subjects and materials

The experimental animal was small tail Han sheep embryo, which was provided by the animal husbandry and veterinary research experimental base of Chinese Academy of Agricultural Sciences.

The main reagents include: DMEM/F12 cell culture medium, phosphate buffer salt solution (PBS), 0.25% pancreatin, 0.3% type I collagenase, 0.1% bovine serum albumin, muscle satellite cell complete medium, digestive termination fluid, cell cryopreservation liquid and other cell separation and culture reagents; DEPC water, 75% DEPC alcohol, 2% agarose gel and other reverse transcription agents; and induced differentiation media, including IBMX, indomethacin,  $\beta$ -mercaptoethanol, sodium pyruvate concentrate, adipogenic differentiation medium, osteogenic differentiation medium, chondrogenic differentiation medium and myoblasts differentiation medium; immunohistochemistry and flow cytometry detection reagents.

The main experimental equipment includes fluorescence microscope, high-speed centrifuge, electronic analysis platform, digital display constant temperature water bath pot, automatic electric pressure steam sterilizer, PH033A incubator, ultra-low temperature refrigerator, term technology office, liquid nitrogen storage tank, ND-1000 spectrophotometer, etc.

##### (2) Experimental methods

Muscle satellite cells and tendon stem cells were isolated, passaged, frozen and resuscitated. Muscle satellite cell line was obtained and its biological characteristics were studied.

The growth of muscle satellite cells. The muscle satellite cells of passage P3, P8 and p12 were taken as the research objects. The adherent cells were digested by conventional method, and the blood cell counting plate was counted and diluted with the growth medium to make the density reach  $1.0 \times 10^4$  cells / ml. the cells were placed in 24 well culture plate. From the second day to the seventh day, three holes were randomly selected and observed under the fluorescence microscope to calculate the average value. The calculation formula is shown in Formula 1:

$$PDT = \frac{(t - t_1) \times \lg 2}{\lg N_e - \lg N_1} \quad (1)$$

Among them,  $t$  is the termination time,  $t_1$  is the start time,  $N_e$  is the number of cells at termination, and  $N_1$  is the number of cells at the beginning.

The ability of clone formation was tested. The P5 and p15 passage cells were centrifuged, suspended in complete medium, inoculated in six well plates with a cell density of 50 cells / well,

and then continuously cultured in the incubator. When a large colony was formed, it was removed from the medium, washed with PBS for three times, and then fixed with paraformaldehyde for 45 minutes. After washing with PBS for three times, staining with Giemsa working solution for 45 minutes, washing and drying at room temperature, the clones were observed under fluorescence microscope, and the clone formation rate was calculated. The calculation method is shown in formula 2:

$$v = \frac{n}{N} \times 100\% \quad (2)$$

Where n is the number of clones and N is the number of cells inoculated.

### 3.2. Animal Model of Muscle Injury

#### (1) Experimental objects and materials

Male ICR strain mice, 6 weeks old, muscle satellite cells were used.

The main instruments include forceps, needle holder, tissue scissors, scalpel, culture dish and culture bottle. The main reagents include 0.3% collagenase, 70% absolute ethanol, 4% paraformaldehyde, etc.

#### (2) Experimental methods

The mice in the experimental group were intraperitoneally injected with 0.25 mg / ml cyclosporin A to carry out cardiac toxin induced muscle injury model, while the control group was not treated. The abdominal muscle tissue of mice was dissected and fixed with paraformaldehyde. The pathological changes were observed under fluorescence microscope after staining.

Muscle satellite cells were implanted into the injured site of the mouse model, and then the serum CK index was detected.

### 3.3. Animal Model of Tendon Injury

#### (1) Experimental objects and materials

Male ICR mice of 6 weeks old were used. Tendon stem cells.

The main instruments and reagents were consistent with those in the animal model of muscle injury.

#### (2) Experimental methods

The Achilles tendon of the experimental group was injected with 0.3 mg / ml collagenase I, while the control group was not treated. The Achilles tendon and its surrounding tissues were dissected and taken out. The Achilles tendon was fixed with paraformaldehyde and made into paraffin section. The pathological changes were observed under fluorescence microscope.

Tendon stem cells were transplanted into the injured site of mouse model, and the serum CK index was detected.

## 4. Discussion of Cell Biological Characteristics and Experimental Results of Muscle and Tendon Injury Animal Model

### 4.1. Research Results of Biological Characteristics

#### (1) Cell growth curve drawing

The count of P3, P8 and P12 cells was counted, and the population doubling time of different generations of cells was recorded. The results were as follows:

Table 1. Cell population doubling time

Cell passage	Population doubling time
P3	33.6
P8	36.8
P12	39.9

As shown in Table 1, although the doubling time of three generations of cells was different, they were all on the second day. With the increase of passage, the doubling time of cell population also increased. And draw its growth curve, as shown in the following figure:

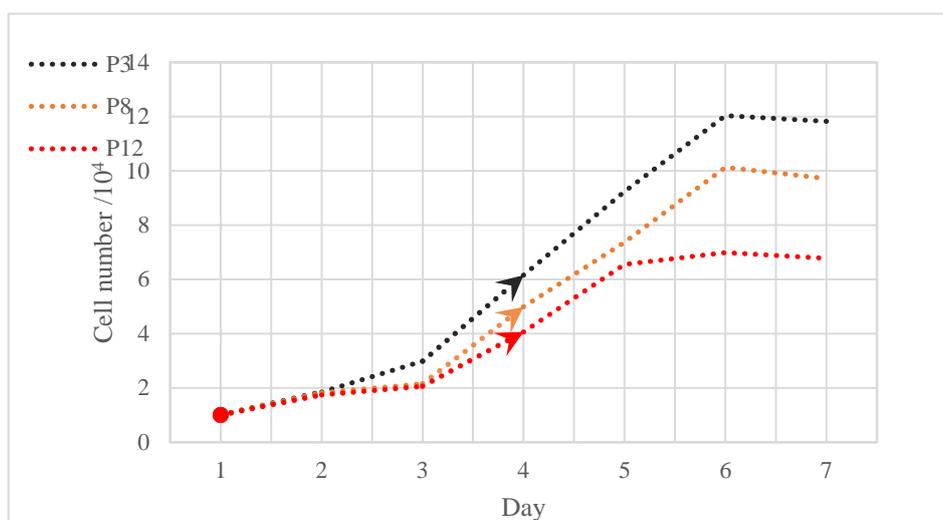


Figure 1. Growth curve of muscle satellite cells

As shown in Figure 1, the growth curves of the three generations were similar, showing S-shape. It has gone through latent period, logarithmic period and plateau period. The first three days are the incubation period. The P3 and P8 generations enter the platform stage on the sixth day, while the P12 generation enters the platform stage on the fifth day. After entering the plateau phase, the cells grew slowly and began to senescence and apoptosis. This indicated that the ability of self-renewal and proliferation of cells decreased after passage.

#### (2) Analysis of clone forming ability

The colony forming ability of P3, P8 and P12 cells was detected. The results were as follows:

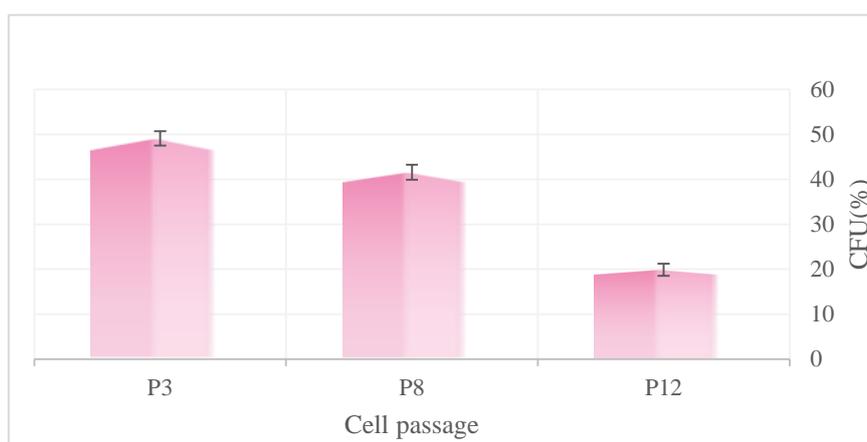


Figure 2. Cell clone formation rate

As shown in Figure 2, with the gradual increase of cell passage times, the ability of cell clone formation decreased and the clone formation rate decreased. The clone formation rate of P12 generation was the lowest, which was 19.89%.

#### 4.2. Experimental Results of Muscle Injury Animal Model

Through the observation of paraffin section and frozen section of mouse muscle tissue, the muscle fiber of the control group was more complete, and the muscle fiber of the experimental group was broken. The degree of injury increased with the increase of injury time. In this study, a mouse model of muscle injury was established by cardiotoxin, and serum CK was detected. The results were as follows:

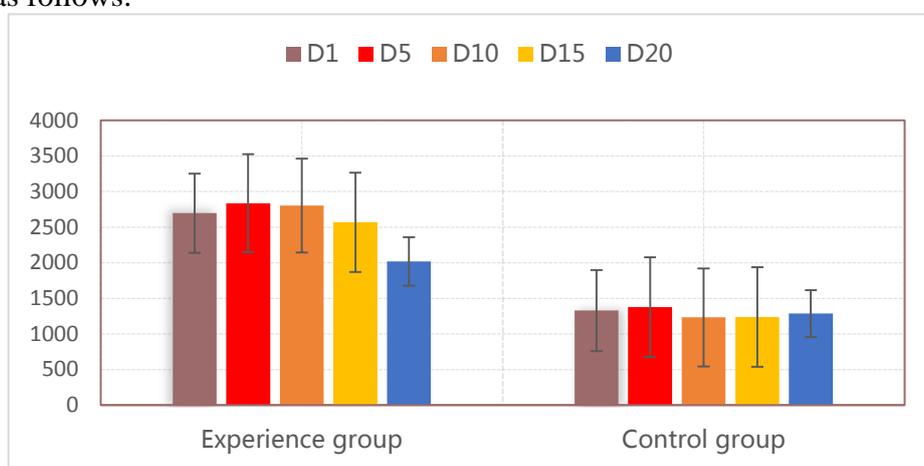


Figure 3. Relationship between CK index and muscle injury time in two groups

As shown in Figure 3, it can be clearly seen that no matter which day the test is carried out, the serum CK value in the blood of the experimental group is higher than that of the control group. Through observation, it is found that the muscle tissue has certain self-healing ability.

#### 4.3. Experimental Results of Tendon Injury Animal Model

24 hours after collagenase injection, the injection site of the experimental group mice showed obvious swelling, the tendon color became dark, and the tendon edge was rough. In this study, collagenase was used to establish tendon injury model. The results of CK detection were as follows:

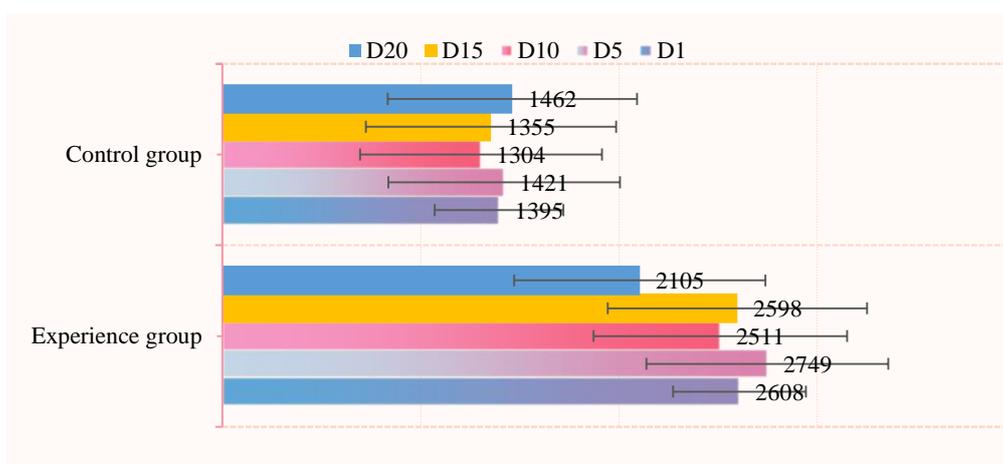


Figure 4. Relationship between CK index and tendon injury time in two groups

As shown in Figure 4, the detection results clearly show that no matter which time period is tested, the CK value in the control group is significantly lower than that in the experimental group, and cell therapy will reduce the serum level.

## 5. Conclusion

Through the observation of fluorescence microscope, muscle satellite cells and tendon stem cells can still maintain a strong proliferation ability after passage. Even after cryopreservation and resuscitation, it still has high activity. The biological characteristics of the cells showed that it had good growth ability and clone formation rate.

Cardiac toxin was used to establish the animal model of muscle injury, and collagenase was used to establish the animal model of tendon injury. The distribution and serum CK value of muscle and tendon injury model were observed by fluorescence microscope. The results showed that cell transplantation could repair the injury to a certain extent and promote the regeneration and repair of muscle and tendon tissue.

Due to the limited time and knowledge, the biological characteristics of cells were not fully detected in this study, and the establishment process of animal models of muscle and tendon injury was not described carefully. Moreover, the detailed mechanism of stem cell therapy needs further study. In the next research work, efforts will be made to improve the above aspects and contribute to the clinical application of stem cell therapy.

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