



# Study on Immune Rejection of Xenotransplantation of Bone Fracture Hematoma Cells in Rabbits

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DOI: 10.32629/jcmr.v3i2.818

**Abstract:** Purpose — To explore the immune rejection of xenotransplantation of bone fracture hematoma cells in rabbits. Way — Lan Tu was used as the donor for allogeneic transplantation of fracture hematoma cells. Twelve New Zealand white rabbits were selected to establish the fracture model. The fracture hematoma cells of Lan Tu were transplanted to the broken end of the experimental group of New Zealand white rabbits, while the rabbits in the control group were not transplanted with allogeneic fracture hematoma cells. To observe the expression level of allogeneic fracture hematoma cells in the fracture site, and to analyze the rejection caused by xenotransplantation by observing the number of macrophages in the fracture model section and lymphoid follicles in the recipient spleen. Result — On the 7th and 12th day after transplantation, the hematoma cells in the fractured end of rabbits in the experimental group showed a decreasing trend, but their survival still met the needs of treatment. The results of immunohistochemical examination on the fractured bone tissue and spleen of the two groups of rabbits showed that there was no significant difference between the macrophage content in the fractured bone tissue and the lymphocyte follicular count in the spleen tissue of the two groups of rabbits in different transplantation time ( $P > 0.05$ ). Conclusion — Using allogeneic hematoma cell transplantation to treat fractures will not cause excessive rejection of receptors, which is worthy of further clinical discussion and provides a favorable scientific reference for its application in clinical treatment of fractures.

**Keywords:** fracture, hematoma cells, xenotransplantation, immunology, rejection reaction, rabbit

## Introduction

Fracture is a common bone injury disease, which refers to a phenomenon that the integrity of bone or cartilage tissue is destroyed when the human body is impacted by external force, resulting in interruption, loss or deformity. Typical symptoms of patients after fracture include severe pain, tenderness, local swelling, ecchymosis, and in severe cases, complications such as temperature rise and shock. The fracture site is damaged due to the coherence of bone tissue, which will lead to certain dysfunction or local deformity of patients, so corresponding treatment measures should be taken in time. At present, with the continuous improvement and progress of medical technology, rich treatment experience has been summarized for fracture clinic. Some studies have found that after a fracture, the hematoma cells at the broken end of the fracture have a positive influence on the healing of the fracture, and the promotion of hematoma in the healing of the fracture has long been a clinical consensus.

## 1. Data and methods

### 1.1 Basic information

In this study, the fracture hematoma cell donor rabbits and fracture model rabbits were provided by the Animal Experimental Center of our region. A healthy male Lan Tu, weighing 3kg and 4 months old, was selected as the donor of fracture hematoma cells. Eight New Zealand white rabbits were selected to establish the fracture model. In order to realize the comparative analysis of the experiment, they were randomly divided into control group and experimental group, with 4 rabbits in each group. After the establishment of the rabbit fracture model in the control group, allogeneic fracture hematoma cells were not transplanted, while the experimental group rabbits were transplanted with bruised Lan Tu fracture hematoma cells. The basic situation of rabbits in each group is as follows: in the control group, there are 2 female rabbits and 2 male rabbits, weighing 2.3-2.6kg, with an average of 2.5kg, and the age of 4-6 months, with an average of 4.5 months; In the experiment group, there were 1 female rabbit and 3 male rabbits, weighing 2.4-2.6kg, with an average of 2.5kg, and aged 4-6 months, with an average of 4.8 months.

## 1.2 Method

### 1.2.1 Preparation of rabbit fracture hematoma cells

(1) Fix the cyanotic Lan Tu, remove the hair from the right leg, and then inject 3% and 120mg pentobarbital sodium into the vein of the ear margin of cyanotic Lan Tu for local anesthesia. After the anesthesia takes effect, cut off the tibia and fibula with a sterile hacksaw, sew up the leg muscles and skin, bandage the wound after disinfection, and inject antibiotics into cyanotic Lan Tu by intramuscular injection. On the 4th day after the fracture, the same anesthetic measures were taken. After the fracture site was disinfected routinely, the hematoma cells were extracted from the broken ends of tibia and fibula fractures, placed in a disposable automatic quantitative intravenous catheter of heparin sodium, repeatedly shaken to prevent blood coagulation, then 2 ml of DMEM culture solution was added, and the single cell suspension was made by repeated suction with a disposable syringe. Then, centrifuge at 1000r/min for 7 minutes, suck the supernatant and fat clean, then remove 2ml of cell components from the lower layer, put it in 50ml DMEM culture solution, put it in 37°C, 5%CO<sub>2</sub> environment, change the liquid completely after one week, and remove the hematopoietic hepatocytes, then change the liquid once every 3-4 days, and observe the growth of fracture hematoma cells with inverted microscope.

(2) Subculture of fracture hematoma cells. When the primary fracture hematoma cells converge and spread all over the bottom of the culture flask, subculture is carried out, all the original culture solutions are sucked out, then the primary cultured cells are washed with phosphate buffer (PBS) for three times, after the supernatant is cleared, the 1:2 ratio of 0.25% trypsin and 0.02%EDTA mild digestive solution is added into the culture flask, and after the cells are found to shrink and separate under microscope, the conventional culture solution containing serum is added to stop digestion. Suck up the culture solution and blow the bottom wall of the culture bottle repeatedly, so that the digestible cells can be detached from the wall to form cell suspension. The enzyme solution of hematoma cells was collected in a centrifuge tube, centrifuged at 1000r/5min for 5 minutes, and after the supernatant containing enzyme solution was completely absorbed, the conventional culture solution was added, fully blown to form a single cell suspension, which was divided into bottles and cultured at 37°C and 5%CO<sub>2</sub>. Every day, observe the growth of cells under the microscope. When the second generation cells grow to 50% confluence, add 10mmol/L BrdU labeling solution to incubate for 24 hours, and then wash with DMEM serum-free medium for 5 times. After clearing the supernatant, add 1:2 mixed digestive juice of 0.25% trypsin and 0.02%EDTA to prepare 1×10<sup>8</sup>/ml cell suspension for cell transplantation.

### 1.2.2 Establishment of rabbit fracture model

Eight selected New Zealand white rabbits were anesthetized by intravenous injection of 3% and 30mg/kg pentobarbital sodium into the ear margin. After the anesthesia took effect, they were fixed, and then the leg hair was removed. Under sterile conditions, the skin tissue was separated to expose the tibia and fibula. After sawing the tibia and fibula with sterilized hacksaw, the muscles and skin were sutured. After routine disinfection, the wound was bandaged and intramuscular antibiotic intervention was given.

### 1.2.3 Hematoma cell transplantation of heterogeneous fracture

Four New Zealand white rabbits in the control group were not transplanted with allogeneic bone fracture hematoma cells, while four New Zealand white rabbits in the experimental group were transplanted with blue and purple bone fracture hematoma cells. Operation is as follows: After the fracture of New Zealand white rabbits, the above-mentioned bruised Lan Tu fracture hematoma cells marked by BrdU are transplanted to the fracture of New Zealand white rabbits. The specific implementation method is as follows: No.4 needle sucks 10ul BrdU-labeled bluish Lan Tu fracture hematoma cell suspension, and the needle is inserted vertically to the tibia and fibula fracture cross section of New Zealand white rabbit, and 5ul cell suspension is injected at the depth of 1cm and 1.5cm respectively, and xenograft fracture blood cells are transplanted. In order to prevent the fracture hematoma cell suspension from fully acting on the bone injury site, the needle is slowly pulled out for about 10 minutes.

## 1.3 Observation indicators

Two rabbits in each group were killed on the 7th and 12th day after the transplantation of fractured hematoma cells, and then the tibia and fibula were dissected out quickly. After being fully washed with normal saline, they were put into 4% formaldehyde solution, decalcified with 15%EDTA for 60 days, and then gradient dehydration, paraffin embedding and 4-5um tissue sections were made, which were developed with anti-BruD monoclonal antibody ABC DAB method. Microscopically, the nucleus is yellow and BrdU positive. The above sections were examined by S-P method for CD68 staining of mononuclear macrophages at the broken end of fracture, and the cells contained fine brown particles which were CD68 positive cells. When the total number of brown granulosa cells exceeds 10%, it is positive. The spleen of the above-mentioned killed rabbits was washed with normal saline,

then placed in 4% neutral formaldehyde solution, then subjected to gradient dehydration, paraffin embedding and HE staining, and 5-6 visual fields were selected at the largest section to count its lymphoid follicles.

## 2. Results

### 2.1 The content of allogeneic hematoma cells in broken end of New Zealand rabbit fracture

According to the immunohistochemical detection of the tissue end of the broken section of the recipient fracture on 7th and 12th days after transplantation of allogeneic fracture hematoma cells, the expression level of BrdU positive cells in the broken section of the tibia and fibula of the recipient rabbit was high on 7th and 12th days after transplantation, among which the expression level was (52.16 4.721) on 7th day and (39.67 5.126) on 12th day after transplantation. It shows that the expression level of allogeneic fracture hematoma cells in the broken end of the recipient fracture is higher at the initial stage of transplantation, and its expression level tends to decrease with the passage of time.

### 2.2 Positive expression level of spleen lymphoid follicles and CD68 in three groups of rabbits

7 days and 12 days after transplantation, the results of immunohistochemical detection on the broken ends of New Zealand white rabbits showed that different numbers of CD68 positive cells were found in different groups of rabbits, which did not constitute a positive standard according to the diagnostic criteria of 1.3. In addition, five observation fields of the maximum section of rabbit spleen were selected, and there was no significant difference among and within the groups at different times, suggesting that there was no obvious proliferation of splenic lymphoid follicles, as shown in Table 1.

Table 1. Comparison of spleen lymphoid follicles and CD68 positive expression levels among three groups of rabbits (±s)

group	CD68 (Number of slices=30)		Lymphoid follicles of spleen	
	transplant7d	transplant12d	transplant7d	transplant12d
control group	4.5±1.7	5.2±0.6	17.9±0.51	18.1±0.36
Experimental group	5.1±0.9	5.3±1.0	18.2±0.37	18.5±0.39
P	>0.05	>0.05	>0.05	>0.05

## 3. Discussion

Medical scholars at home and abroad have found that hematoma caused by fracture has an irreplaceable role in promoting fracture healing. Therefore, accelerating the healing of bone injury with autologous fracture hematoma cells has become another new idea for clinical treatment of fractures. However, factors such as long formation time, unstable output and long culture time in vitro restrict its practical application in fracture clinic. However, in view of the remarkable role of fracture hematoma cells in promoting fracture healing, more and more medical scholars have been exploring in this field, trying to find a method that can break through the insufficient output of autologous fracture hematoma cells and fail to achieve the therapeutic effect.

The results showed that a large number of surviving allogeneic fracture hematoma cells marked by BrdU were detected in the fracture end of rabbits in the experimental group on 7th and 12th days after transplantation. Although the number of allogeneic fracture hematoma cells in the fracture end of recipients decreased with the increase of transplantation time, the survival rate could still meet the treatment needs. The results of immunohistochemical examination on the fractured bone tissue and spleen of the two groups of rabbits showed that there was no significant difference between the macrophage content in the fractured bone tissue and the lymphocyte follicular count in the spleen tissue of the two groups of rabbits in different transplantation time ( $P > 0.05$ ). It shows that allogeneic haematoma cell transplantation in the treatment of fractures will not cause excessive rejection of receptors, which is worthy of further clinical discussion and provides a favorable scientific reference for its application in the clinical treatment of fractures.

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