Using Xenogenic (Calf Foetal) Osteochondral Transplantation for Articular Cartilage Defect in Rabbit Model

Abstract

Background: The destruction of articular cartilage is the major cause of articular problems. The articular cartilage has little repair postertial due to lack of perichondrium and direct blood circulation. It is, therefore important to consider this phenomena in surgical treatments. One of the articular cartilage reconstructive surgeries is using Osteo-Chondral graft. The main purpose of this research was to investigate the use of Xenogenic (calf foetal) Osteo-Chondral graft in repairing articular cartilage defect on Rabbit's model.

Methods: Osteo-Chondral pieces were prepared under aseptic condition from the joints by skin punch device and kept at a temperature of 70ºc below zero. Ten male New Zealand rabbits of one year old were randomly divided into two groups of five, as control and transplantation groups calf's fetal. The skin and joint capsule were opened by surgery and articular cartilage was exposed. After defect creation by drill, in the transplanted group an Osteo-Chondral piece was inserted in the defected area; however, in the control group the defect was created but left empty. Joint capsule and skin were sutured in both groups. During 60 days of study, radiographs were taken from rabbits of each group randomly to evaluation of osteoarthrits signs on days 14, 28 and 42. Finally all rabbits were euthanized for histopathological sampling and evaluated on day 60.

Results: The result of the clinical evaluations did not show any sing of inflammation nor limping. In radiological evaluation there was no evidence of arthritis complications but showed defect filling signs in experimental group. In the histopathologic evaluations, the defect of transplanted group was filled with fibro-cartilage tissues and without any signs of graft rejection. In two samples of five specimens of transplanted group fibrous tissue was the dominant tissue and in other two as the dominant tissue. Only in one sample of this group the integrity of the cartilage tissue was completely formed. But in the control group, the lesions were observed without any restorative tissue and only filled by red blood cells.

Conclusion: The study suggests that Xenogenic Foetal Osteo-Chondral tissue is an effective tissue for repairing articular cartilage defects.

Key words: Articular cartilage, Xenograft, Biomaterial

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Introduction

The most common cause of joint problems is the destruction of articular cartilage, which is generally caused by abrasion, arthritis, or pressure during severe loading. beginning of the destruction process of the articular cartilage, normal joint function is also affected, and in the absence of treatment, the cartilage of the joint is destroyed and the joint is susceptible to osteoarthrits; on the other hand, the lack of pericardium on the articular cartilage, as well as the lack of vascularity, make repairing of the articular cartilage suffer from lots of limitations (1). Surgical procedures are considered as the most effective treatment for healing of articular cartilage, among which the osteochondral autologous transplantation procedure provides the most similar tissue to the primary cartilage in terms of tissue healing (2). Due to the limited amount of harvestable osteochondral autologous bone-cartilage tissue, as well as incidence of complications resulting from the area of harvesting bone-cartilage grafts, this procedure also has limitations in implementation (3,4).
With high concentrations of growth factors, embryonic tissues can accelerate the tissue healing process; on the other hand, due to the presence of alpha-phytoprotein isomers, as the dominant protein, they can suppress the immune system and since it is not detected and rejected by the maternal immune system, they also can prevent the graft rejection after grafting (5).

Choosing an animal model requires several backgrounds. First, the animal model should be sufficiently evaluated in studies and tailored to the desired hypothesis. After that, it must be economically cost effective, and animal protection rights must be observed. Finally, there must be the ability to perform different actions on the selected animal. Rabbit is one of the common animal species used in musculoskeletal research. The average size of this animal allows the bone and joint surgery easily be manipulated and performed (5, 6).

The ultimate goal of this project is to investigate the ability of xenogeneic cartilage-bone graft from fetal calf in healing articular cartilage defect on a rabbit animal model.

**Methods**

The present study was conducted in the department of surgery and radiology of faculty of veterinary medicine of Shahrekord University in spring of 2018. This study was approved by the university's ethics committee, and during the study, all animal rights were observed and treated according to the principles of working with animals.

**Cartilage-bone tissue preparation**

The fetal calf was transferred from the slaughterhouse to the surgical ward, and articular cartilage was exposed under aseptic conditions. Harvesting of the bone graft, including part of the articular cartilage with its lower bone, was performed by a biopsy punch of 3 mm as cylindrical shape (Fig. 1).

**Maintenance of bone graft**

After harvesting of bone grafts, they were covered by sterile gas impregnated with normal saline and stored in a freezer at a temperature of 70 ° C until grafting time (8).

**Defrosting specimens**

One hour before surgery, specimens were removed from the freezer and placed inside a nylon lid in a 60 ° C water container until they defrost.

![Figure 1. Cylindrical bone-cartilage of bone graft](image)

**Preparing rabbits and method of maintenance**

Ten male New Zealand one-year-old rabbits were purchased from an animal breeding laboratory center in Shiraz, and kept at the Animal Hospital of Shahrekord University for 15 days to adapt to nutritional, water and light conditions. During all maintenance, rabbits were fed with dry alfalfa.

**Surgical procedure**

The rabbits were randomly divided into two groups (quintuplet) of graft and control. In order to perform grafting, the knee joint of the rabbits was shaved and the surgical procedures were done aseptically.

Anesthesia induction was performed by intramuscular injection of ketamine (30mg / kg) with aspromazine (0.2mg / kg) and then, in order to continue the anesthetic process, the rabbit was attached to the isoflavin by a mask.

After induction of anesthesia, the lateral surface of knee joint was cut and the articular capsule released from the medial ligament and the articular cartilage was exposed. The cylindrical defect of 3 mm in diameter at the non-weight bearing level of joint was created as the same size of bone graft. After the creating of defect in grafting group, the prepared bone-cartilage was placed in defect area, but in the control group, the defect was left without any manipulation. Suture of the joint capsule and skin was conducted by Vicryl thread No. 0-2 with a simple continuous pattern. 3 days after surgery, rabbits received antibiotics injectable of 10% (10 mg / kg) enrofloxacin as muscle administration (5, 6).
Clinical evaluation
The rabbits were examined daily in terms of inflammation of the surgical area, the amount of wound healing, existing infections in area and how they were weighing on the organs.

Radiological evaluation
On days 14, 28 and 42, after the surgery, the rabbits were subjected to X-ray imaging to examine the incidence of arthritis responses. The film distance from the X-ray source was 40 cm, and imaging was done with 40 kV and 6.8 mAs.(8)

Histopathological evaluation
On the 60th day after surgery, the rabbits were first anaesthetized by injection of ketamine and aspromazine, and then they euthanized by injection of copper sulfate solution into the heart, then specimens from graft area were harvested for histopathological evaluation. Specimens were placed in 10% formalin for one week to generate tissue stability. Subsequently, they were demineralized with 5% acid during 10 days. After this stage, the specimens were washed with water and treated with an autotechnicon. The stages for preparing microscopic slides were as follows:

At the treatment stage, the tissues of the specimens were passed through varying degree of alcohol. First, they were placed in 70% and 80% alcohol for 1 hour and then were placed in three 95% alcohol containers, respectively, 2 hours in the first container and 1 hour in the second and 1 hour in third container. At the end, they were placed inside 3 containers of 100% alcohol at the same time as 95% alcohol. At the clearing stage, the specimens were first placed in a container containing xylene solution for 45 minutes and then again placed in another container containing the above solution for 45 minutes.

At paraffinization stage, the specimens were first placed in a molten paraffin container for 2 hours. This task was conducted to completely isolate the xylene solution from the tissue. In next step, in order to infiltrate of paraffin into the pores of tissue, the specimens were placed in a paraffin container for 3 hours. In the molding stage, the specimens were placed in metal cubic molds and the molds were completely filled with molten paraffin. Paraffin was then allowed to freeze at normal temperature. The molded sample was taken out from paraffin mold and placed in a refrigerator.

The microtome was tuned to cut tissue cross sections with 5 micron thickness. Next, the slices were placed on an aqueous bath at 45 degrees Celsius to remove their wrinkles. Then, the lamella was slowly immersed in the water at 45 degree angle until the section was removed and placed on a slide. After doing this, the specimens were transferred to the warm house to allow the extra paraffin to be melted and tissue to be stuck to the lamella. After placement of the slices on the lamella, they were stained in a hematoxylin and eosin manner, which took place in the following stages: The lamellas were respectively placed in xylene solutions for 10 min, 100% alcohol for 1 minute, 95% alcohol for 1 minute, 70% alcohol for 1 minute, distilled water for 1 minute, hematoxylin color for 10-8 minutes, acid alcohol for seconds, distilled water for minutes, eosin for 30 seconds, Distilled water for 1 minute, 70% alcohol for 1 minute, 95% alcohol for 1 minute, 100% alcohol for 1 minute, and after staining, lamella was stuck using ethylene glue. After conducting the above stages, the lamellas were examined by optical microscopy.(9, 10)

In this type of evaluation, healing study is conducted by a descriptive study method.

Findings

Clinical evaluation
The recovery from anesthesia stage of all rabbits occurred very slowly and without any emotional reactions. After surgery, swelling and inflammation appeared in the surgical area, and it were observed by complete recovery of the consciousness of the rabbits, unwillingness to move, non-weight bearing on the surgical foot and lameness in motion. Swelling, immobility and lameness disappeared completely in the first week after surgery.

It should be noted that during the operation and the two-month period of keeping rabbits, no illness or death occurred.

Radiological evaluation
In radiographs of 14th, 28th and 42th days that were randomly prepared from one rabbit of each group, no signs of arthritis symptoms were observed. In the graft group, observing the obtained radiographs, filling the defect can be seen over time; this event was not seen in the control group during this time interval (Fig. 2a, A, B, C and D).
Figure 2: Radiographs of day 14 of graft group (a), radiograph of day 42 of graft group (B), radiograph of day 14, control group (C) and radiograph of day 42 control group (D). In all images, yellow arrow shows the defect area in graft group and control group.

**Histopathological evaluation**

The results of the study of the 5 lamellas prepared from the harvested specimen of the rabbits in the graft group are as follows: In two specimens of this group, the predominant tissue obtained from post-transplantation was fibrosis-cartilage, with a fibrosis dominance. In two other specimens of this group, the graft area had been filled with fibrosis-cartilage tissue with a cartilaginous dominance. In last specimen of this group, the predominant tissue was cartilaginous. In all of these five specimen, the bone precursor structures, such as osteoid, sponge bone and even bone blade, could be seen in some places from the graft area. It should be noted that in none of the specimens in this group, there was no evidence of graft rejection (Fig. 3).

In the control group, none of the 5 prepared lamella had not filled any tissues of the defect area, and the defect area had no tissue structure and only filled with red blood cells (Fig. 4).

Figure 3: Histopathologic examination of the graft area in the graft group represented the formation of the trabecular bone at the defect area, which the formation region is shown by a blue-arrow (eosin and hematoxylin staining with a magnification of 10 times (A) 40 times) B and 100 times (c).
Figure 4: Histopathologic examination of graft area in the control group represented that the bone tissue was not formed and it was filled with red blood cells in the defect area staining of eosin and hematoxylin with a magnification of 4 times (A) and 10 times (B).

Discussion

The graft and control group did not show any lameness and inflammation in the surgical procedure in clinical evaluations. In radiological evaluations was not observed any complications of arthritis in either of the two groups. The Ferreira et al. overview study of osteochondral autologous bone-cartilage in curing of Talus Bone-cartilage lesions which occurred between 2005 and 2016 suggests that this surgical procedure, although involve complications caused by harvesting of graft tissue, has a good result in reducing the symptoms of patients with Talus bone-cartilage lesions. In our study, despite the fact that defect creation and transplantation operating was made in the Non weight bearing of joint area, and in the control group, like the graft group, no lameness and pain in the joint area was observed during 60 days of maintenance, which was a prove of reduction of the symptoms by the graft; but the lack of the incidence of arthritis complications in radiological studies in the graft group, like the control group, can compensate for the lack of sufficient reason regarding the decline of the symptoms of cartilage defect by xenogeneic bone-cartilage transplantation.

The obtained results of histopathologic studies suggest that the cartilage defect of the graft group after the transplantation has been filled by repairing tissue with cartilaginous dominance; while, 60 days after the occurrence, the defect of control group was not observed in the histopathologic cross-sections. Horas et al. 2003, while expressing the obtained results of their prospective studies over examining the two methods of autologous chondrocyte implantation and cylindrical bone-cartilage transplantation in knee articular cartilage repair, they suggested that both methods have a useful performance in reducing the symptoms resulting from articular cartilage injury; The difference is that the retrieval of daily activities in the autologous chondrocyte implantation occurs more slowly than the bone-cartilage transplantation, and on the other hand, the recovered tissue in the chondrocyte implantation method is made of fibrocartilige and the bone-cartilage transplantation tissue, is similar to the initial cartilage and adjacent tissues. Another point mentioned in this study is the existence of a distinct boundary between the adjacent cartilage areas in the bone-cartilage transplantation method, which was also diagnosable after two years of surgery. Comparing the results of our study with this study, the similarity of the tissue from the healing tissue to the initial cartilage, similar histopathologic observations, and even the existence of a distinct boundary between the graft area with the adjacent tissue, as well as the above study, somewhat proves that bone-cartilage tissue of the embryo calf can serve in repairing of the rabbit bone-cartilage defect as autologous tissue.

Although autologous transplantation take priority in terms of transplantation quality, but due to incidence of complications resulting from the area of harvesting graft tissue and the limited amount of harvesting, it cannot always be applicable. Allograft transplantation, which take priority as a substitute for autologous transplantation, also has a disadvantage due to the high cost and limited transplantable tissues, which genetically have some issues, limits its usage (13).

Takahashi et al. 2018, published their results on the repairing of the rabbit bone-cartilage defect by a xenogeneic transplantation of human chondrocytes to indicate that the defect has been repaired while rabbits would have received a weaker immune system drug, but 8 weeks after discontinuation of the drug, the incidence evidence of transplant rejection has seen (14). Regarding with the cartilage transplantation among species, few
quantitative studies is taken in which in most of them, rejection of the transplant has been introduced as the main limiting factor of project\(^\text{14,15}\). Given the above and the evidence regarding that embryo tissue is not rejected, so this tissue can be considered as an appropriate substitute for autologous tissue. The purpose of this research, which was conducted for the first time in the world, was to introduce a proper tissue for healing of articular cartilage defects without any disadvantages of current methods. Due to the limitations of this plan, including the short duration of maintenance, the small number of tested specimens and also non-use of autologous transplantation in rabbits for comparisons with xenogeneic transplantation, has not been fully proven. It should be noted that there is a long way of treatment process from proof until they are implemented, which requires more extensive research and more precise testing to conduct experimental treatment on human.

**Conclusion**

Comparison of the evaluations of this study with the results of previous studies shows that fetal calf bone-cartilage piece in the repairing of joint articular cartilage can participate as an autologous transplant without graft rejection and create a tissue similar to that of the original cartilage.

**Conflict of interest**

All writers hereby confirm that there are no conflicts of interest in this study.

**References**