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Histologic and Biomechanical Studies of Tendon-To-Bone Healing After Autologous and Allogeneic Bone Transplants

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Abstract

Objectives: **Compare histologic and biomechanical differences of tendon-to-bone healing between autologous and allogeneic bone transplants.**

Materials and Methods: **Adult, healthy, New Zealand white rabbits were used to establish the extra-articular tendon-to-bone healing model with the left hind limb transplanted with allogeneic bone and the right hind limb transplanted with autologous bone. After 3, 6, and 12 weeks after the transplant, the rabbits were killed to collect tendon-to-bone specimens, and then the healing processes in tendon-to-bone interfaces were examined.**

Results: **All rabbits grew well after incision without infection and can freely move. Histologic observations 3 and 6 weeks after surgery and biomechanical test results 6 weeks after surgery were statistically different between the autologous and the allogeneic transplants ($P < .05$). After 12 weeks, histologic observations and biomechanical test results showed no difference between the 2 transplants ($P > .05$).**

Conclusions: **Allogeneic bone transplant has a relatively slower tendon-to-bone healing than does autologous bone transplant, but finally allogeneic and autologous bone transplants have the same extent of tendon-to-bone healing.**

Key words: *Autologous bone, Allogeneic bone, Tendon-to-bone healing, Histology, Biomechanics*

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Introduction

Treatments of bone defects mainly include autologous bone transplant, allogeneic bone transplant, and artificial prostheses. Successful fixation of self-tendon to grafts is pivotal for recovery of joint activity in bone defects close to joints. Prostheses implantation can be applied to repair bone defects, but its wide application has been impeded by the difficulty of tendons to be biologically well fixed to the transplanted prosthesis. Tendons can be well fixed to autologous transplanted bones, but the limitation of autologous bone graft source impedes its wide application.[1] Allogeneic bone transplant has been used widely to treat bone defects because of its ease of access to allogeneic bone grafts from tissue banks. However, the clinical application and laboratory research of the fixation of self-tendon to allogeneic transplanted bone has been rarely reported. In this study, we examined tendon-to-bone healing after fixation of self-tendon to allogeneic or autologous transplanted bone in a rabbit model.

Materials and Methods

Experimental animals

New Zealand white rabbits were used. Rabbits were purchased from Qinglongshan Animal Breeding Ranch in Nanjing of China, and they weighed 2.5 to 3.0 kg. All animal protocols were in conformity with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health 86-23, revised in 1985.

Experimental procedures

Donor rabbits were killed by 15 mL of air injected into the ear vein. Bones with cancellous bones were flushed with saline to remove the canal contents, and flushed again with 70% ethanol, and then

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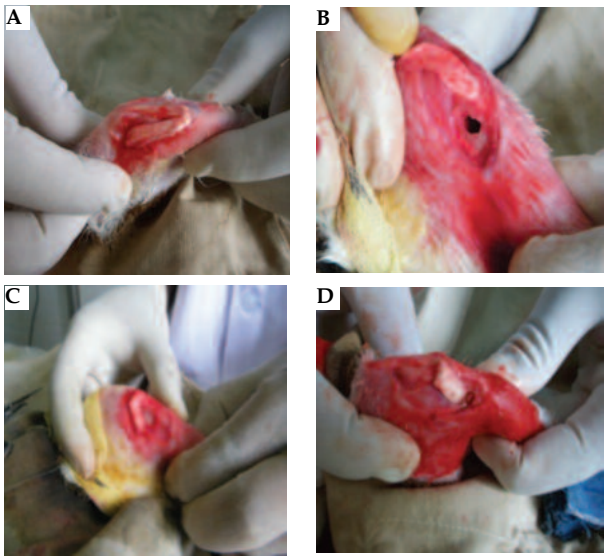
placed in 95% ethanol for 2 weeks. The donor bones were transferred in 70% ethanol, and stored in a 4°C refrigerator.

Urethane was injected into the ear vein for anesthesia. Anesthetized rabbits were placed on their backs and fixed to the operating table. An anterior medial incision to the proximal tibia was made to expose the patellar ligament and tibial tubercle. The patellar ligament was separated into 2 parts in the middle, cut proximally, and the medial patellar ligament was made to be a free part (Figure 1A). The tibia was exposed in front and a bone defect was opened about 4 mm × 5 mm deep to the bone marrow cavity in the medial tibial tubercle (Figure 1B). With tibial tuberosity as a fulcrum, the free patellar ligament at the flip was placed in the bone defect (Figure 1C and 1D), and absorbable sutures were used to fix the proximal patellar tendon to the bone defect, and then allogeneic or autologous bone was used to fill the bone defect; the tendon was embedded at the same time.

The left hind limbs of recipient rabbits were transplanted with allogeneic bones while the right hind limbs of the same rabbits were transplanted with autologous bones (ilium). Three, 6, and 12 weeks after surgery, 3 rabbits were killed for histologic examination at each time point. 6 and 12 weeks after the transplant, 12 rabbits were killed for biomechanical examination at each time point.

Figure 1. Experimental Procedures



(A) The anterior medial patellar ligament was cut proximally and made to be free. **(B)** The tibia was exposed in front, and the bone through (about a 4 mm × 5 mm) to the bone marrow cavity, which was opened in the medial tibial tubercle to make a bone defect. **(C)** and **(D)** With the tibial tuberosity as a fulcrum, the free patellar ligament at the flip was placed in the bone defect, and absorbable sutures were used to fix the proximal patellar tendon to the bone defect, and the allogeneic or autologous bone was used to fill in the bone defect, and the tendon was embedded at the same time.

Histologic observation and biomechanical tests

Knee joints were broken and surrounding soft tissues of tendon-to-bone (including periosteum) were removed with the patellar tendons spared. The patellar ligaments were cut off from the endpoint in the tibial tuberosity, and the taluses were amputated about 0.5 cm distant from the bone defects. After specimens were washed with saline, they were fixed with 10% neutral formaldehyde solution and then subjected to hematoxylin-eosin staining for histologic examination.

For biomechanical test, the taluses were amputated at the place approximately 2 cm distant from the bone defect, and then immediately, the maximal pull-out load test was performed. The specimens were fixed on the Instron-8874 computer-controlled universal testing machine (Instron Corp, Canton, MA, USA), and the tendons were pulled with 10 mm/min to measure its pull loads and instantaneous maximum tensile load.

Statistical analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 10.0, IBM Corporation, Armonk, New York, USA). Data were analyzed with the paired *t* test. Values for *P* less than .05 were considered statistically significant.

Results

General observations

All rabbits grew well after transplants, and the incision had no liquid leakage, no irritation, and no infection. Three weeks after surgery, in the autologous transplant group, bone slots and tendons were wrapped around by a large number of new bone tissues, and there was a gap between the tendon and the bone slot (Figure 2A); in the allogeneic transplant group, bone slots and tendons were wrapped around by a small amount of new bone tissues, and there was a clear gap between the tendon and the bone slot (Figure 2B). Six weeks after the operation: in the autologous transplant group, bone slots and tendons were tightly connected by new bone tissues (Figure 3A); in the allogeneic transplant group, bone slots and tendons were loosely connected by new

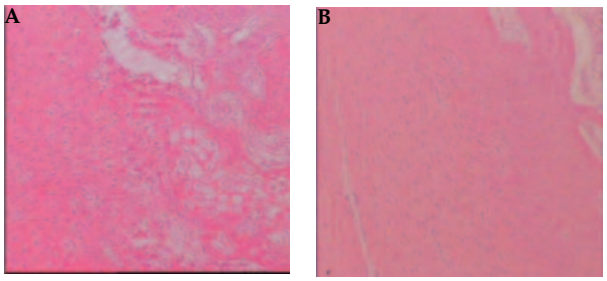
bone tissues, and the gaps between the tendons and the bone slots still existed in some parts (Figure 3B). Twelve weeks after the operation: in both groups, bone slots and tendons were tightly connected by new bone tissues (Figure 4).

Histologic observations

Three weeks after the operation

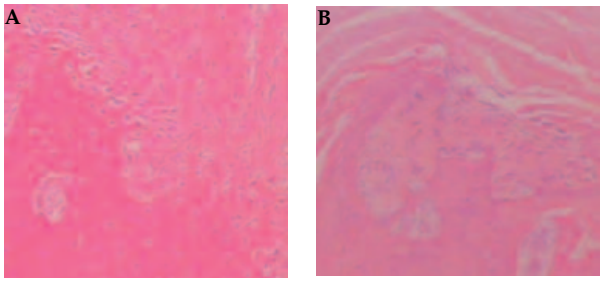
The autologous transplant group: In the interfaces between tendons and bones, fibroblasts actively proliferated with formation of visible collagen fibers; osteoblasts actively proliferated with a large number of new bonelike tissues; and chondrocytelike cells were visible (Figure 2A). The allogeneic transplant group: In the interfaces of tendons and bones, fibroblasts actively proliferated with the formation of visible collagen fibers; osteoblasts actively proliferated without obvious new bonelike tissues (Figure 2B).

Figure 2. Histologic Observations of Tendon-to-Bone Healing 3 Weeks After Bone Transplants



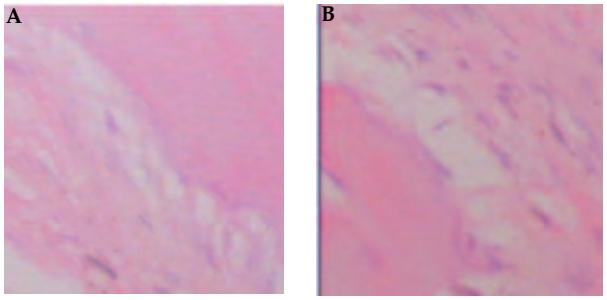
Three weeks after bone transplants, the rabbits were killed to collect tendon-to-bone specimens and then hematoxylin-eosin staining was performed (magnitude, ×100). **(A)** In the interfaces between tendons and autologous transplanted bones, fibroblasts actively proliferated with formation of visible collagen fibers; osteoblasts actively proliferated with a large number of new bonelike tissues; chondrocytelike cells were visible. **(B)** In the interfaces of tendons and allogeneic transplanted bones, fibroblasts actively proliferated with formation of visible collagen fibers; osteoblasts actively proliferated without obvious new bonelike tissues.

Figure 3. Histologic Observations of Tendon-to-Bone Healing 6 Weeks After Bone Transplants



Six weeks after bone transplants, the rabbits were killed to collect tendon-to-bone specimens and then hematoxylin-eosin staining was performed (magnitude, ×100). **(A)** In the interfaces between tendons and autologous transplanted bones, the numbers of osteoblasts and fibroblasts decreased compared with the numbers of those 3 weeks after surgery; a large number of collagen fibers developed connecting closely with new bone tissues; no Sharpey's fibres were visible. **(B)** In the interfaces of tendons and allogeneic transplanted bones, the numbers of osteoblasts and fibroblasts decreased compared to the numbers of those 3 weeks after surgery; a large number of collagen fibers developed connecting loosely with new bone tissues; no Sharpey's fibres were visible; a few osteoclasts existed.

Figure 4. Histologic Observations of Tendon-to-Bone Healing 12 Weeks After Bone Transplants



Twelve weeks after bone transplants, the rabbits were killed to collect tendon-to-bone specimens and then HE staining was performed (magnitude, ×400). **(A)** In the interfaces between tendons and autologous transplanted bones, a few osteoblasts and fibroblasts were seen; a large number of collagen fibers existed fusing with bone tissues; Sharpey's fibres were visible. **(B)** In the interfaces between tendons and allogeneic transplanted bones, a few osteoblasts and fibroblasts were seen; a large number of collagen fibers existed fusing with bone tissues; Sharpey's fibres were visible.

Six weeks after the operation

The autologous transplant group: In the interfaces between tendons and bones, the numbers of osteoblasts and fibroblasts decreased compared to the numbers of those 3 weeks after surgery; a large number of collagen fibers developed connecting closely with new bone tissues; no Sharpey's fibres were visible (Figure 3A). The allogeneic transplant group: In the interfaces between tendons and bones, the numbers of osteoblasts and fibroblasts decreased compared with the numbers of those 3 weeks after the operation; a large number of collagen fibers developed connecting loosely with new bone tissues; no Sharpey's fibres were visible; a few of osteoclasts existed (Figure 3B).

Twelve weeks after surgery

The autologous transplant group: In the interfaces between tendons and bones, a few osteoblasts and fibroblasts were seen; a large number of collagen fibers existed, fusing with bone tissues; Sharpey's fibres were visible (Figure 4A). The allogeneic transplant group: In the interfaces between tendons and bones, a few osteoblasts and fibroblasts were seen; a large number of collagen fibers existed fusing with bone tissues; Sharpey's fibres were visible (Figure 4B).

Biomechanical test (maximal pull-out load)

Six weeks after surgery, the maximal pull-out load in the autologous transplant group was significantly greater than in the allogeneic transplant group (Table 1; $P < .01$). Twelve weeks after the operation, there was no difference in the maximal pull-out load between 2 groups (Table 1; $P > .05$). The results suggest that in the early stage, tendon-to-bone healing in the autologous transplant group is superior to healing in the allogeneic transplant group, but in the later stage, there is no difference in tendon-to-bone healing between the 2 groups.

Table 1. Maximal Pull-Out Load Test at Different Times

Test Time	Number of Cases (n)	Maximal Pull-Out Load (N)		Paired *t* Test
		Allogeneic transplant	Autologous transplant	*P* value
6 weeks	12	53.476 ± 2.840	63.588 ± 1.867	< .01
12 weeks	12	85.353 ± 3.044	86.654 ± 2.850	> .05

Six weeks and 12 weeks after bone transplants, rabbits were killed to collect tendon-to-bone specimens. knee joints were broken and surrounding soft tissues of tendon-to-bone (including periosteum) were removed with the patellar tendons spared. The patellar ligaments were cut off from the endpoint of the patellar ligaments in the tibial tuberosity, and the taluses were amputated at approximately 2 cm from the bone defect. Specimens were fixed on the Instron-8874 computer-controlled universal testing machine, and then the tendons were pulled to measure their pull loads and instant maximum tensile load.

Discussion

Bone healing processes after allogeneic and autologous bone transplants are basically similar, except that allogeneic bone healing is relatively slower probably because there are no viable cells in the transplanted allogeneic bone after freezing process and thus, bone healing relies entirely on the host tissue invading and growing into the transplanted bone, known as *crawling substitution*.[1,2]

The current study examined tendon-to-bone healing after autologous and allogeneic bone transplants, and showed that allogeneic and autologous tendon-to-bone healing processes were basically similar, but allogeneic tendon-to-bone healing was slower. Leung and colleagues found that in the autologous tendon-bone interface, extensive scar tissues were formed to overbridge the healing interface and remodel with healing over time.[3] In the current study, we found the similar phenomenon in allogeneic bone-tendon healing: the tendon and the allogeneic bone were connected with collagen tissues, and over time, collagen tissues were fused with the bone and the tendon, and then remodeled to develop a normal attachment site.

Tendon enthuses can be classified as *fibrocartilaginous* or *fibrous* according to tissues present at the skeletal attachment site.[4] Fibrocartilaginous entheses have a 4-layer transitional structure: fibrous connective tissue, fibrocartilage, calcified fibrocartilage, and bone tissue.[5] A clear, continuous "tide line" develops between the fibrocartilage and the calcified fibrocartilage. Fibrous enthuses are fibrous connective tissues between the fibrous tendon and bone. In the present study, 3 weeks after the operation, fibroblast proliferation activity and the formation of collagen fibers were observed between the tendon-bone interface. Six weeks after the operation, a large number of collagen fibers developed connecting loosely with new bone tissues. Twelve weeks after the operation, a large number of collagen fibers existed, fusing with bone tissues, and Sharpey's fibres were visible. So, fibrous enthuses were observed in our study.

Bone defects caused by trauma are common problematic conditions. Currently, treatments for bone defects mainly include autologous bone transplant, allogeneic bone transplant, and artificial prostheses. Artificial prostheses implantation can be applied to repair bone defects, but it is difficult for tendons to be biologically fixed well to the transplanted prosthesis. Tendons can be fixed well to autologous transplanted bones, but the limitation of autologous bone graft source impedes its wide application. Allogeneic bone transplant has been used widely to treat bone defects because of ease of access to allogeneic bone grafts from tissue banks. However, little is known about the clinical application and preclinical research of the fixation of self-tendon to allogeneic transplanted bone in bone defects close to joints. Our study showed that allogeneic bone transplant had a relatively slower tendon-to-bone healing than did autologous bone transplant in a rabbit model, but ultimately allogeneic and autologous bone transplants had the same level of tendon-to-bone healing, as demonstrated by histologic and biomechanical tests. Our study suggests that it may be feasible to apply allogeneic bone transplant to treating bone defects close to joints and keeping joint activity.

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