ORIGINAL RESEARCH PAPER

Orthopaedics

TREATMENT OF OSTEOCHONDRAL DEFECTS IN KNEE USING IMPLANT MATERIAL OBTAINED FROM BEECH WOOD: AN EXPERIMENTAL STUDY IN SHEEP

KEY WORDS: Osteochondral Defects; Knee; Beech Wood; Sheep

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ABSTRACT

Objective: This animal study examines the macroscopic and microscopic changes occurring after filling the osteochondral defects in the knee with implant material obtained from beech wood.

Methods: The study was conducted on 24 knees of 12 female sheep. An International Cartilage Repair Society (ICRS) type IV defect with 7 mm diameter and 9 mm depth was created on the right medial femoral condyle, and wood grafts with a diameter of 7 mm and depth of 7 mm, which were prepared in conical shape were placed in the defect using press-fit technique, to make it stays at 2 mm depth from the cartilage surface. An ICRS type IV osteochondral defect was created with the same diameter and depth for the control group at the medial condyles of the left knee.

Results: In terms of surface area and subchondral bone; there was a significant difference between 8th week and 16th week in the study group ($p < 0.05$). There was no significant difference between control group and study group at 8th week, or 16th week ($p > 0.05$). In terms of the matrix, viability and mineralization there was no significant difference between 8th week and 16th week in the study group. There was no significant difference between control group and study group at 8th week, or 16th week ($p > 0.05$).

Conclusion: The wood graft that we used in the treatment of osteochondral defect is inexpensive, biocompatible, biomechanically solid and stable, allows attachment of chondrocytes, enables a joint range of motion and bearing of load, can be applied in a single arthroscopic session. Therefore, in the treatment of osteochondral defects, it may be an alternative treatment method.

Introduction

There are many studies related to surgical treatment of osteochondral defects. Biological repair and stimulation by using methods such as periosteal grafts, perichondral grafts, meniscal grafts, pedicled muscle flaps, osteochondral grafts, tendon grafts, chondrocyte cultures, and microfracture are primarily considered for repairing the defects [1-3]. Although autologous chondrocyte implantation is an important method for treatment of such defects, it presents challenges because of its invasive nature, the requirement of two successive surgical sessions, and difficulty of the technique itself [2]. Besides all these difficulties, fibrosis or fibrocartilaginous tissue may develop as a result of the biological repair [3].

An alternative way of treating osteochondral defects is repair of the joint surface with biomaterials that are suitable for the defect size [3]. Biomaterials that can be used for this purpose include calcium salts, phosphate, ceramics, carbon fibers, and polymers, as well as natural materials of bone origin such as cow's bone, nacre, coral, bioactive glass and polymers [4,5]. Biomaterials have to be biocompatible, bioactive and osteoconductive. Wood is a biomaterial that meets all of these requirements. It is a durable and dense material and resembles bone in terms of its structure [6]. As natural materials, wood and bone perform the same tasks of providing mechanical support and aiding in the transport of nutrients. While mechanical support is provided by collagen fibers in the bone, the same function is fulfilled by cellulose in the wood [6].

Beechwood is categorized in the medium-rigidity group, and mechanically it has high-pressure resistance parallel to fibers, high bending strength, pressure resistance, and high elastic module during bending. Biomechanics studies have shown that beech tree has a similar elastic module with the bone [4]. This animal study examines the macroscopic and microscopic changes occurring after filling the osteochondral defects in the knee with implant material obtained from beech wood.

Materials and Method

This study was approved by the institutional review board and the animal ethics committee. The study was conducted on 24 knees of 12 female sheep. The average weight of the sheep was 21.5 kg (18.6 kg-25.4 kg), and mean age was 13 months (11-16 months). Medial condyles of the right knees of the sheep were determined as the study group, while medial condyles of the left knee constituted the control group.

The sheep were not fed for 12 hours prior to the operation. For premedication, Xylazine hydrochloride (Rompun) was administered intramuscularly at 1 mg/kg dose 15 minutes before surgery. Anesthesia was achieved via intramuscular injection of ketamine hydrochloride (Ketalar) at 10 mg/kg dose, 10 minutes before the operation. When necessary, an additional dose of 10 mg/kg ketamine was administered for maintenance of anesthesia. After achieving anesthesia, the knees of the sheep were shaved. The knees were washed with Betadine soap and then dyed with polyvinylpyrrolidone iodine solution. Sterile green surgical clothes were placed, and a sterile environment was achieved. The right knee was operated first. A medial parapatellar incision was made, and the capsule was opened from the medial side of the patella and patellar tendon in order to reach the medial femoral condyle. Using a drill with a diameter of 7 mm and a conical tip, an International Cartilage Repair Society (ICRS) type IV defect with 7 mm diameter and 9 mm depth was created on the medial femoral condyle, paying attention to create it at a weight-bearing area. Wood grafts with a diameter of 7 mm and depth of 7 mm, which were prepared in conical shape and were sterilized before use via incubation at 134 degrees temperature for 1 hour, were placed in the defect using press-fit technique, to make it stays at 2 mm depth from the cartilage surface (Figure 1). The joint was rinsed with saline to remove particles formed during the drilling process. Then the capsule was closed by suturing with 2/0 vicryl, and the skin was sutured with 2/0 silk. The left knee was opened using the same incision method. An ICRS type IV osteochondral defect was created with the same diameter and depth for the control group. After the operation, the sheep were released to their shelter that was prepared appropriately in advance. The knees were not encased in plaster to allow active motion in the joint. Postoperatively, cefazolin sodium 500 mg was administered once a day intramuscular for three days. For analgesia, 1 gr/day paracetamol was added to the drinking water. The wounded side was cleaned with Betadine solution for three days, and no wound dressing was applied except for the first day. The sutures were removed on the postoperative 10th day.

At the postoperative 8th week, six sheep were sacrificed as the first group, by administering ketamine at the lethal 30 mg/kg dose, followed by sacrification of another six sheep at the postoperative 16th week as the second group. The knee specimens were prepared for histological examination by removing muscles and surrounding soft tissues. Then, the femoral area where the wood graft was implanted was reduced to 2x2 cm size and divided into two pieces in diamond cutter (Low-Speed Saw, IsoMet, BUEHLER). Microscopic images of the tissue and the implanted wood graft were obtained. Afterwards, these tissues were placed in 10% Formalin and then decalcified in 5% nitric acid, and they were embedded in paraffin together with the wood graft present inside. The lesion was sliced along with the wood graft inside, with 5 µm thickness, and stained with hematoxylin and eosin. The sections were histopathologically examined under a light microscope by a pathologist blinded to the study. Histological evaluations were made according to ICRS Visual Histological Assessment Scale, which allows evaluation of the surface, matrix, cell distribution, subchondral bone and cartilage mineralization [7].

Statistical Analyses

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) (IBM Corporation, Armonk, NY, USA). Kruskal-Wallis Variance Analysis Test and Mann-Whitney U Test were used to compare the groups. A *p*-value less than 0.05 was considered statistically significant.

Results

At the postoperative period, 5 sheep were observed to have limping. The limping improved in four sheep at the second week, whereas in the other sheep, it improved at the end of the third week. Before they were sacrificed, 24 knees in 12 sheep were physically examined, and none showed any limitation in the range of motion, contracture, or deformity. No anesthesia-related complication or death was observed, and none of the animals developed an infection at the knee.

In the control and study groups sacrificed at the 8th week, the defects were partially filled, the hollowness was still present, and the surface was not smooth. In both control and study groups, no degeneration was observed at the opposing joint surface. In the study group, the wood graft was observed to adhere tightly to the surrounding tissue, and the graft did not fall into the joint space. For sheep sacrificed at 16th week, it was observed that the defect area was not filled and showed hollowness in all 6 knees in the control group, whereas in study group, the surface was completely filled in 4 of 6 knees (66.6%), but was partially filled and was hollower than the cartilage surface in the remaining 2 knees (33.3%). There was no degeneration in the opposing surfaces in control or study groups, and the graft was observed to adhere tightly to the bone in the study group. In both groups, the newly formed tissue had an appearance different than the surrounding cartilage tissue, and its borders were visible (Figure 2).

Histological examination of the specimens obtained at 8th week showed that the healthy joint cartilage was interrupted at the edge of the defect in all cases in the control group, and there was hollowness at the surface of the defect area. In the study group, the surface was also interrupted and irregular in all 6 cases, and there was hollowness at the surface of the defect area. In the control group, the matrix was composed of hyaline in 1 case (16.6%), hyaline/fibrocartilage mixed tissue in 4 cases (66.6%), and fibrous tissue in 1 case (16.6%). In the study group, the matrix was composed of hyaline in 1 case (16.6%), hyaline/fibrocartilage mixed tissue in 4 cases (66.6%), and fibrocartilage in 1 case (16.6%). In control group, the cell distribution was as columnar/cluster mixed in 3 cases (50%), cluster in 1 case (16.6%), and as individual cells in 2 cases (33.3%). Cell distribution in the study group was in the form of clusters in 5 cases (83.3%), and as individual cells in 1 case (16.6%). In control group, subchondral bone showed increased remodeling in 1 case (16.6%), and bone necrosis and granulation tissue in 5 cases (83%). In the study group, 2 cases (33.3%) had increased remodeling, and 4 cases (66.6%) had bone necrosis and granulation tissue. While most cells were viable in both control and study groups, both groups showed normal mineralization. Histologic examination of the knees at the 16th week showed that the surface in the control group was smooth and continuous in 3 cases (50%), whereas the hollowness was still present in the other 3 cases (50%). In the study group, the surface was smooth and continuous in 4 cases (66.6%), whereas hollowness was still present in 2 cases (33.3%). In control group, the matrix was composed of hyaline cartilage in 2 cases (33.3%), hyaline/fibrocartilage mixed tissue in 2 cases (33.3%), and fibrocartilage in 2 cases (33.3%), whereas in the study group, the matrix was composed of hyaline cartilage in 4 cases (66.6%), and hyaline/fibrocartilage mixed tissue in 2 cases (33.3%). In control group, the distribution of cells was columnar in 1 case (16.6%), columnar/cluster mixed in 3 cases (50%), and cluster in 2 cases (33.3%), whereas in study group, cell distribution was columnar in 3 cases (50%), columnar/cluster mixed in 2 cases (33.3%), and cluster type in 1 case (16.6%). In control group, subchondral bone was normal in 1 case (16.6%), showed increased remodeling in 2 cases (33.3%), and showed bone necrosis and granulation tissue in 3 cases (50%), whereas in the study group, it was normal in 1 case (16.6%), showed increased remodeling in 4 cases (66.6%), and showed bone necrosis and granulation tissue in 1 case (16.6%). While most of the cells in both control and study groups were viable, cartilage mineralization was normal in both control and study groups (Figure 3).

Inflammatory cells observed in the wood-bone interface at the early phase are due to the bone healing process. These cells were present at the 8 weeks group but absent in the 16 weeks group.

In terms of surface area; there was a significant difference between 8 weeks group and 16 weeks group ($p < 0.05$). There was no significant difference between 8 weeks control group and 8 weeks study group, or between 16 weeks control group and 16 weeks study group ($p > 0.05$).

In terms of the matrix, there was no significant difference between 8 weeks study group and 16 weeks study group ($p > 0.05$). There was no significant difference between 8 weeks control group and 8 weeks study group, or between 16 weeks control group and 16 weeks study group ($p > 0.05$).

With regard to subchondral bone, there was a significant difference between 8 weeks study group and 16 weeks study group ($p < 0.05$), There was no significant difference between 8 weeks control group and 8 weeks study group or between 16 weeks control group and 16 weeks study group ($p > 0.05$).

In terms of viability and mineralization, there was no significant difference between 8 weeks study group and 16 weeks study group, between 8 weeks control group and 8 weeks study group, or between 16 weeks control group and 16 weeks study group ($p > 0.05$).

Discussion

There are several studies that used wood in the repair of bone defects and osteochondral defects [4,6]. Large animal models including goat, dog, horse, and sheep are used to research cartilage repair. The thickness of cartilage and the number of chondrocytes are greater in such animals. The animal's age also has an influence on healing of cartilage. Younger animals show faster and better cartilage healing [8,9]. In our study, we used sheep with an average age of 1 year as experimental animals.

The primary strategy in the evaluation of osteochondral defects is to describe the defect based on the degree of cartilage restoration, size, integration, surface smoothness, and matrix morphology. Mechanical evaluation is made by comparing the newly formed cartilage to normal cartilage in terms of elasticity and permeability [10]. In the present study, histological examination was made according to ICRS Visual Assessment Scale, but lack of mechanical evaluation is a limitation of the present study.

The proliferation of neighboring chondrocytes in the experimentally induced defects is a general finding. However, it is

known that this proliferation does not contribute to the healing of the cartilage. In a study, Convery et al. [11] found that healing was adversely affected when the defect was larger than 4 mm. In our study, we created defects with a diameter of 7 mm, because sheep knee had a large size. There are studies in the literature which created defects with similar size [12].

In their study, Gross and Ezerietis [6] performed hemiarthroplasty using implants prepared from juniper tree to rabbits whose hip joints were dislocated and femoral heads were resected. They observed that a tight adherence started to form between wood and bone at the sixth month, and the bone started to replace the wood. At the end of the third year, the wood was osseointegrated so that only a thin strip of wood was left within the bone. Although our study was much shorter than their study, we observed the presence of tight connections between wood and bone at the end of the 4th month.

In a study, Horsky et al. [13] used wood plates prepared in 20 mm length and 3 mm thickness in rabbit femoral fractures. Wood material was tolerated well by the tissues, and there was initially an acute inflammation period characterized with polynuclear leukocytes (PNL) and macrophages. In our study, we also observed an inflammation period with an abundance of macrophages and PNL's in the study group examined at the end of 2 months, but we did not observe macrophage or PNL in the study group examined at the 4th month.

In terms of relaxation, it is important that implanted materials have elastic modulus close to that of the bone. Limited flexibility at the bone-implant interface allows bone remodeling and provides grounds for tight connection [14]. Ashby et al. prepared a chart showing elastic modules of materials used in orthopedic surgery. According to this chart, wood shows a similar elastic module with the trabecular bone [15]. This property allows the wood to effectively transfer the load to the bone tissue. It can be concluded from here, that there would be less relaxation with implants prepared from wood material. Our results support this conclusion since we also observed that the wood implant did not become loose in any of the animals.

Previous experimental studies have shown that postoperative immobilization of the knee often resulted in limitation in joint's range of motion, contracture or deformity, whereas animals that were allowed to move actively preserved their knee functions [16,17]. Rubak et al. [18] reported that movement of the joint enabled development of a hyaline cartilage-like tissue in the defect area, whereas, in immobilized animals, the defect area was replaced by only fibrous tissue. They stated that the extent of collagen tissue formation was in parallel with the mobilization of the joint [19-21]. In their experimental studies, Mooney et al. and Hall [22,23] applied immobilization to the joints and eliminated the mechanical factors, and they observed atrophy and degeneration in the cartilage, and invasion of the cartilage by the surrounding synovial tissue. In the present study, the sheep were allowed to move freely in their shelter during the postoperative period, and we did not observe limitation of movement, contracture or deformity at the knee, and at the end of the study, we observed the development of hyaline cartilage-like tissue. Furukawa et al. and De Palma [24,25] reported that the area with cartilage defect was initially filled with fibrous tissue, but this tissue transformed over time to hyaline cartilage due to mechanical effects. In our experimental study, we created the defects particularly on the weight-bearing joint surface in order to subject the newly formed tissue to mechanical load. At the end of the experiment, we observed the formation of a hyaline cartilage-like tissue with 16.6% rate in the 8 weeks study group, and with 66.6% rate in the 16 weeks study group.

Some of the polymers used as biomaterials have side effects such as inflammatory tissue edema and synovitis. In one rabbit study conducted by Matsusue et al. [26], polylactide rod was implanted intramedullary into the rabbit femur, it was observed that polylactide had toxic effects on bone marrow, and a thin fibrous tissue developed around the implant at the early period. The lack of toxicity, reduced risk for development of synovitis at the joint, and the absence of signs of relaxation are some feature of wood graft that makes it superior to the polymers.

Ceramics can be used alone or in combination with other materials to enhance bone healing. They can be used alone to make use of their osteoconductive properties, or they can be used as carriers for drugs and cells as well [27]. The host tissue that interacts with the ceramic gradually replaces the ceramic with the bone tissue. The wood implant used as filling material is also osteoconductive [4], and may undergo biological interaction with the host tissue [6]. Besides these properties, other advantages of wood over ceramic are that it is easily implantable and cheap.

In a study by Heikkila et al. [28], implants prepared from hydroxyapatite or bioactive glass were implanted into the defects created in the rabbit femur. They observed near-complete osteointegration with both implants at the end of 12 weeks. In our study, we observed complete integration with the bone tissue in all groups, which suggests that wood graft, bioactive glass and hydroxyapatite yield comparable osteointegration.

In conclusion, the wood graft that we used in the treatment of osteochondral defect is inexpensive, biocompatible, biomechanically solid and stable, allows attachment of chondrocytes, enables a joint range of motion and bearing of load, can be applied in a single arthroscopic session. Therefore, in comparison to other biomaterials that are used in the treatment of osteochondral defects, it may be an alternative treatment method.

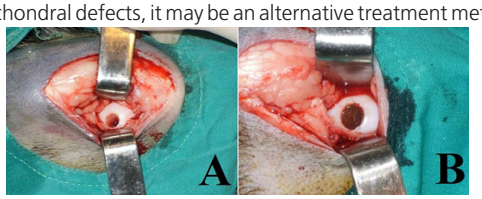

Figure 1: Image of the defect created on medial femoral condyle (A). Image of the defect after implantation of the wood graft (B).

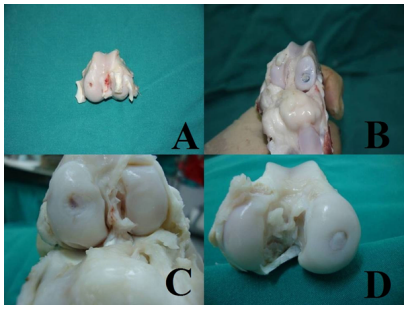

Figure 2: Control group (A) and wood-implanted knee (B) at the end of 8th week. The control group (C) and wood-implanted knee (D) at the end of 16th week.

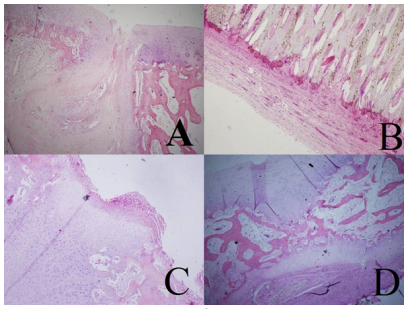

Figure 3: Image showing the defect area in control group is filled with granulation tissue at the end of 8th week (A) (Hematoxylin and eosin stain (HE) x 40). Image showing the absence of chondrocytes and formation of inflammatory tissue on the wood graft in the study group at the end of 8th week (B) (H.E x 100). Image showing the defect is in the control group is filled with cartilage tissue resembling hyaline at the end of the 16th week;

note that there are patches of empty spaces, and hollowness at the surface continues (C) (H.E x 100). Image showing the formation of hyaline-like cartilage on the wood graft in the study group at the end of the 16th week; note that the surface is smooth and uninterrupted (D) (H.E x 40).

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