Repair of Large Osteochondral Defects With Mix-Mosaicplasty in a Goat Model

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abstract

Osteochondral defects in weight-bearing regions must be repaired with cartilage and subchondral bone support simultaneously, as well as the integration between the 2, particularly in young, active patients. In this study, a new method called mix-mosaicplasty was used to reconstruct large osteochondral defects (6-mm diameter) in the weight-bearing region of the femoral condyle of goats. Two periosteum-bone plugs and 1 osteochondral plug harvested from the proximal tibia and intertrochlea groove were assembled to fill the defects in a mosaic mode. The goats were euthanized 16 weeks postoperatively, and the result of the repair process was assessed using macroscopy, morphologic analysis, electron microscope observation, glycosaminoglycan assay, and magnetic resonance imaging. Sixteen weeks postoperatively, the superficial surface of the defective region was covered with regenerated cartilage, and the periosteum-bone plugs were combined with each other. However, cleavage between cartilage plugs was noted. The donor site, which was filled with periosteum-bone plugs, was regenerated with fibrocartilage-like tissue. The repaired tissue was composed of small chondrocyte-like cells arranged tightly within an evenly distributed extracellular matrix containing type II collagen. Cells of the regenerated tissue in periosteum-bone plugs were smaller and distributed more densely. Electron microscopy demonstrated regular matrix fibers and abundant organelles within the repaired tissue. No significant differences of glycosaminoglycan content were observed between reconstructed tissue and normal hyaline cartilage. Magnetic resonance imaging revealed the healing process between plugs other than the control group. The new technique of mix-mosaicplasty can reconstruct full-thickness osteochondral compound defects in the weight-bearing region of the femoral condyle.
Treatment for chondral or osteochondral defects in weight-bearing regions of joints is a clinical challenge, particularly in young, active patients who wish to maintain a high level of activity and avoid being restricted by osteoarthritis.1,2 The goal of any technique for cartilage repair is to regain the integrity of the cartilage surface while maintaining sufficient biomechanical properties to achieve pain-free range of motion.3 Therefore, the osteochondral defects, which include both cartilage and subchondral bone loss, need to be filled with a smooth surface and rigid bony support.

Mosaicplasty is a surgical technique designed to repair focal lesions by using cylindrical osteochondral plugs harvested from lesser load-bearing regions in the knee.4,5 A successful mosaicplasty procedure results in a graft fixation capable of full weight bearing once the cancellous bone of the donor graft heals with that of the host bone.6 However, osteochondral grafting is limited by the number of potential donor sites.7 Regarding donor-site complications, the larger the defect, the higher the morbidity. When repairing osteochondral defects in other joints, such as the ankle, the osteochondral plugs must be harvested from the healthy knee.8 This method may be unacceptable to patients. Although the donor sites can be secondarily filled by fibrocartilage tissue, the surface often remains irregular, which can increase the risk of further degeneration.

The goal of this study was to propose a rational and original therapeutic strategy combining periosteum-bone plugs and cartilage bone plugs to reconstruct and repair damage caused by large osteochondral defects.

**Materials and Methods**

**Reconstructing the Osteochondral Defects In Vivo**

Sixteen adult male Chuandong white goats (weight range, 18-25 kg; age range, 1.5-2 years) were randomly divided into 2 groups. The mix-mosaicplasty group was treated with mix-mosaicplasty to repair the osteochondral defects, and the control group received no treatment to repair the osteochondral defects. The research conducted for this study was done in accordance with the authors’ institution’s guide for and the national law on the care and use of laboratory animals.

General anesthesia was administered using an intravenous pentobarbital sodium solution (30 mg/kg body weight). The animals were placed in the supine position, and surgery was performed on both knees. A full-thickness cylindrical osteochondral defect (6 mm in diameter and 5 mm in depth) was made at the weight-bearing region of the femoral condyle using a custom trepan. One cylindrical osteochondral graft (2 mm in diameter and 6 mm in depth) was obtained perpendicularly from the minimal weight-bearing periphery of the femoral condyle at the level of the patellofemoral joint with a small trepan and pushed out into saline using a small pusher for further application. Three periosteum-bone plugs were harvested in the same manner from the proximal tibia. Two periosteum-bone plugs and 1 osteochondral graft were implanted in a perpendicular fashion into the trimmed defective area (Figure 1). One of the periosteum-bone plugs was filled into the donor site where the osteochondral column was removed. When the defective areas were filled, the knee was put through a range of motion to ensure their press-fit stability, and then the incision was closed by layering and bandaged.

The knees were not immobilized postoperatively, and the animals were allowed to move freely in their cages. All animals were given access to food and water. At 16 weeks postoperatively, the animals were euthanized by overdose of pentobarbital intramuscularly, and the knees were examined grossly for signs of infection, degenerative changes, and evidence of repair.

**Morphologic Analysis**

The condyles were harvested and fixed in 4% paraformaldehyde for 12 hours and embedded in paraffin. Sagittal 4.5-µm sections of the samples were cut and stained with hematoxylin-eosin, toluidine blue, and Masson’s trichrome. To perform the immunochemistry assay, the sections were deparaffinized in xylene, passed through decreasing concentrations of ethanol, washed in phosphate-buffered saline, submerged in phosphate-buffered saline, and pretreated with 33 U/mL hyaluronidase in phosphate-buffered saline (pH, 5.0) for 60 minutes at 37°C. After washing with phosphate-buffered saline and incubation with 1% bovine serum albumin, the sections were incubated overnight at 4°C with a primary rabbit anti-type II collagen immunoglobulin G (IgG) diluted at a ratio of 1:500. After incubation, the sections were washed and then treated with a 1:1000 dilution biotin-conjugated secondary IgG solution for 1 hour. After secondary antibody treatment, the sections were washed, incubated for 30 minutes with avidin-biotin-peroxidase reagent, and then washed again and treated with diaminobenzidine. The nuclei of the
cells were counterstained with hematoxylin-eosin. As controls for nonspecific binding, primary antibodies were used to treat bovine articular cartilage, and sample sections were treated with secondary IgG without primary antibodies.

**Electron Microscopy**

Sixteen weeks postoperatively, the repaired tissue was fixed in 2% glutaraldehyde solution containing 0.1-molar sodium cacodylate buffer (pH, 7.4) for 4 hours and postfixed in 1% osmium tetroxide for 2 hours. The samples were dehydrated in a graded series of increasing concentrations of ethanol, beginning at 30% (v/v), and embedded in Epon 812 (Shell Chemical Co, Houston, Texas). Ultrathin (40-60 nm) sections were cut with an ultramicrotome and stained with uranyl acetate and lead citrate. Transmission electron microscopy was performed with an electron microscope (7100-B; Hitachi, Tokyo, Japan).

Samples for scanning electron microscopy were prefixed, postfixed, and dehydrated. The samples were exsiccated with the critical-point drying method, coated with ions via an ion beam device (BalTec, Pfäffikon, Switzerland), and observed on a scanning electron microscope (Quanta-200; Philips, Zaterdag, The Netherlands).

**Glycosaminoglycan Assay**

Normal cartilage tissue from a healthy goat condyle and repaired tissue 16 weeks postoperatively were harvested for glycosaminoglycan assay. Fifty milligrams of the tissue was digested with lysate solution, freeze-dried, and dissolved in 1 mL phosphate-buffered saline solution. A 50-μL sample was then added into a 750 μL dimethylmethylene blue solution, and the optical density was determined at 525 nm. A mixed isomer shark chondroitin sulfate was used to create the standard curve.

**Radiography and Magnetic Resonance Imaging**

Sixteen weeks postoperatively, the rear limbs of the goats were harvested for radiograph and magnetic resonance imaging (MRI) evaluation. The knees were wrapped in a moisture-proof plastic bag, extended, immersed in a saline bath, and placed in a standard knee imaging coil. Radiograph assay was performed routinely with a radiography machine. Magnetic resonance imaging was performed at a high resolution using a 1.5-T scanner (Magnetom Vision; Siemens, Iselin, New Jersey). Magnetic resonance imaging results were reported by a radiologist experienced with knee MRI who was blinded to the groups.

**Statistical Analysis**

Differences between the glycosaminoglycan content were determined with 1-way analysis of variance. Data were expressed as mean±SD of separate experiments. Differences with a P value less than .05 were considered significant.

**RESULTS**

**Macroscopic Evaluation**

No infections occurred postoperatively, and all incisions healed well. Animals could walk with weight bearing 4 days postoperatively.

Sixteen weeks postoperatively, plugs in the mix-mosaicplasty group were still in situ, with no evidence of subsidence or loosening, and margins were still discernible. The defective areas appeared to be filled by the reconstructed tissue, which was similar to results observed in the cartilage. The surface of the treated area was relatively smooth, and the curvature was similar to the adjacent condyle (Figure 2).

In the control group, the defective areas were filled with a red, soft fibrous tissue, with the margins clearly defined. Cartilage around the defective area appeared pressed and softened (Figure 3). Abrasion of the articular surface in the tibial plateau opposite the defects appeared to have been aggravated, and some degenerative change was observed. Some goats in this group walked with a limp. The tissue regenerated by the periosteum-bone plugs filling the donor site was like cartilage in texture and color, but margins with adjacent cartilage were noted.

**Histological Observation**

Transplanted osteochondral plugs were intact at 16 weeks postoperatively in the mix-mosaicplasty group, and a smooth surface was parallel to the surrounding recipient site. Distribution of the chondrocytes was similar to normal hyaline cartilage. Cells of the regenerated tissue in periosteum-bone plugs were smaller and distributed more densely compared with normal hyaline cartilage.
Regenerated tissue present in the defective area consisted of cells embedded in a matrix containing type II collagen (Figure 5). Cartilage-specific staining revealed a uniform distribution in the matrix of transplanted cartilage and deeper than that in the periosteum-regenerated tissue. The surface of the defective area was smooth. The red arrows point to the interphase between plugs.

In the control group, the defective tissue remained in situ with no evidence of new cartilage generation. Thin, fibrous tissue covered the bottom of the defective area, and no metachromatic matrix was present. Attenuated staining of the cartilage matrix and local fissures were observed primarily along the periphery (Figure 6).

Glycosaminoglycan Assay
Mean amount of glycosaminoglycan was 16.36±1.38 mg/g in the control group and 15.92±1.46 mg/g in the mix-mosaicplasty group. No significant differences were observed between the groups (P>.05).

Electron Microscopy Observation
In the mix-mosaicplasty group, scanning electron microscopy demonstrated that regular parallel fibers with similar diameters were localized to the smooth superficial region of the repaired tissue (Figure 7). Transmission electron microscopy analysis confirmed the presence of round/oval homogenous cells in nonparallel fibers. The cells had multiple cellular projections, within which abundant rough endoplasmic reticulum and ribosomes were also detected (Figure 8).

Radiography and Magnetic Resonance Imaging Observation
In the control group, radiographs revealed subchondral bone defects at 16 weeks postoperatively and osteosclerosis in the articular surface opposite the defective area. In the mix-mosaicplasty group, healed subchondral bone and a smooth surface on the condyle and the tibial plateau were observed.

In the control group, sagittal turbo T2-weighted MRI demonstrated bulk local high signal in the subchondral bone of femoral condyles without discernable cartilage layers, and attenuated cartilage was seen in the adjacent region (Figure 9A). In the mix-mosaicplasty group, the surface of the repaired cartilage appeared smooth and continuous. Fewer irregular signals existed in the reconstructed carti-
lage surface, and the signal detected in the column was almost uniform to adjacent bone (Figure 9B).

DISCUSSION

In contrast to injuries to the skull that require only bony repair, injuries to the knee or other joints often involve damage to the subchondral bone and the overlying hyaline cartilage. Repairing such osteochondral compound defects requires that the bone be repaired properly to serve as a suitable base for regeneration of the cartilage. Whether done simultaneously or sequentially, repairing the subchondral bone always requires suitable primary mechanical properties. Traditional resurfacing techniques, such as subchondral bone drilling, debridement, and microfracture, and advanced methods, such as autologous chondrocyte implantation, have been shown to have limited results in such compound defects because of the poor biomechanical characteristics of the regenerated tissue.

Current mosaicplasty treatments meet many of the requirements for reconstruction of osteochondral defects, including sufficient bony support, primary stability, and an intact bone-cartilage interface for a durable weight-bearing surface using a cost-effective, single-stage, arthroscopically assisted procedure. However, some problems have been encountered in the process: donor sites are limited, and the use of large grafts can cause incongruity at the recipient site, which permanently alters the biomechanics of the joint. No such donor sites that do not bear weight exist in the joint, so the morbidity ascribed to the donor sites must also be considered.

Periosteum is derived from the perichondrium in the early stages of its development, during which undifferentiated mesenchymal cells exist that can differentiate into bone under high oxygen tension or into cartilage under low oxygen tension. The potential of multidirectional differentiation can be preserved in mature periosteum tissue. Full-layer hyaline cartilage defects can be repaired by periosteum with tissue similar to cartilage. Therefore, the authors developed a practical method to reconstruct the osteochondral defects with periosteum and the subchondral bone column together, calling it mix-mosaicplasty. With this method, the initial blood supply of the periosteum was preserved, and the functional biomechanical retention of this transplanting complex was better than periosteum. In this way, subchondral bone defects can be repaired simultaneously, increasing donor sites for mosaicplasty and reducing complications to the donor sites.

When the free periosteum was cultured in vitro under high oxygen tension, all periosteum formed into bone; when cultured with lower oxygen tension, the ossification stopped and cartilage-like tissue generated. Synovial joints lack blood vessels, and the oxygen tension is low in the synovial fluids, which, when combined, are conducive to the differentiation of the stratum cells present in periosteum to chondrocytes without ossification. Transplanting the periosteum-bone plugs keeps the periosteum and subchondral bone integral, which can retain the blood supply for mesenchymal cells in periosteum and repair bone defects simultaneously. Any remaining mesenchymal cells in periosteum are capable of differentiating into multiple cell lines, including chondrocytes, which in turn repair the cartilage defects. Repair of the osteochondral defects by combining periosteum-bone plugs and osteochondral plugs can minimize complications due to donor sites in synovial joints and increase the indication for autografts. In this way, periosteum-bone plugs may be harvested through a minimal incision and used in joints other than the knee.

In the mix-mosaicplasty group, the compound defects were repaired with tissue having characteristics of cartilage. Extracellular matrix deposition was found in regenerated tissue from periosteum. The results of immunohistochemical stain for type II collagen and MRI observation support the differences between the mix-mosaicplasty and control groups.
Glycosaminoglycan assay revealed no statistical significance between the 2 groups, which may be because the transplanted column occupied a majority of the defective area. Although precise biochemical characterization of the tissue was not performed, histologic and histochemical evaluation suggests that the regenerated tissue was cartilaginous. Several limitations exist in this study, including the lack of a biomechanical test and other tests to detect hyaline cartilage and confirm tissue repair.

In a study by Van Susante et al., the periosteum-bone plug from the proximal tibia was tested to be press-fitted into the 6.5-mm circular osteochondral defective area in the medial femoral condyle. The areas of osteochondral defect were filled by a periosteum-bone plug or a bone plug containing only bone tissue with no residual cartilage, or they were left empty. Histological evaluation revealed that most bone plugs seemed to have been completely absorbed. Occasionally, chondrogenesis from the periosteum was present on the surface of the plugs. The authors believed that the cortical bone from the tibial plug may have caused the observed strong osteoclastic reaction and subsequent absorption of the graft.16

Several differences exist between the current study and that of Van Susante et al. A 6.5-mm defective area was filled with a single plug (with or without periosteum seemingly shorter than the defect produced) harvested from the proximal tibia in that study, whereas in the current study, 2 periosteum-bone plugs longer than the depth of the defective area (6 mm compared with 5 mm) and 1 osteochondral plug were press-fitted into a 6-mm defective area. By this method, the original biomechanical properties of the transplant may be better than a single shorter bone plug derived from the proximal tibia. This combined plug strategy from different regions around the knee could decrease complications ascribed to using a single donor site. The donor site for osteochondral plugs, which was filled with periosteum-bone plugs, was regenerated by fibrocartilage-like tissue in texture and color, but the margins were still apparent.

**Conclusion**

This preliminary research using large animals demonstrates the efficacy of periosteum-bone plugs and suggests that use of a combined plug strategy may substitute for osteochondral plugs so that defective osteocartilage regions can be repaired more effectively. This combined strategy would likely not increase the procedure’s morbidity and may play a beneficial role in cartilage repair.

**References**