Repair of porcine articular cartilage defect with autologous chondrocyte transplantation

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Abstract

Articular cartilage is known to have poor healing capacity after injury. Autologous chondral grafting remains the mainstay to treat well-defined, full-thickness, symptomatic cartilage defects. We demonstrated the utilization of gelatin microbeads to deliver autologous chondrocytes for in vivo cartilage generation. Chondrocytes were harvested from the left forelimbs of 12 Lee-Sung pigs. The cells were expanded in monolayer culture and then seeded onto gelatin microbeads or left in monolayer. Shortly before implantation, the cell-laden beads were mixed with collagen type I gel, while the cells in monolayer culture were collected and resuspended in culture medium. Full-thickness cartilage defects were surgically created in the weight-bearing surface of the femoral condyles of both knees, covered by periosteal patches taken from proximal tibia, and sealed with a porcine fibrin glue. In total, 48 condyles were equally allotted to experimental, control, and null groups that were filled beneath the patch with chondrocyte-laden beads in gel, chondrocytes in plain medium solution, or nothing, respectively. The repair was examined 6 months post-surgery on the basis of macroscopic appearance, histological scores based on the International Cartilage Repair Society Scale, and the proportion of characteristic chondrocytes. Tensile stress-relaxation behavior was determined from uniaxial indentation tests. The experimental group scored higher than the control group in the categories of matrix nature, cell distribution pattern, and absence of mineralization, with similar surface smoothness. Both the experimental and control groups were superior to the null group in the above-mentioned categories. Viable cell populations were equal in all groups, but the proportion of characteristic chondrocytes was highest in the experimental group. Matrix stiffness was ranked as null > native cartilage > control > experimental group. Transplanted autologous chondrocytes survive and could yield hyaline-like cartilage. The application of beads and gel for transplantation helped to retain the transferred cells in situ and maintain a better chondrocyte phenotype.

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Keywords: Autologous chondrocyte transplantation; Gelatin beads; Cartilage repair and regeneration

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Introduction

The treatment of articular cartilage injuries remains challenging. Cartilage defects usually fail to repair and, if left untreated, progress to symptomatic joint degeneration. Conservative treatment gives only symptomatic relief, while conventional surgical procedures, such as abrasive chondroplasty and penetration of subchondral bone, yield variable outcomes that usually produce fibrocartilage. Investigators have recently turned their interest to grafting defects with heterologous or autologous cartilage or to transplanting chondrocytes [7]. Surgical techniques of transplanting autologous osteochondral grafts, such as mosaic osteochondroplasty, have been introduced [3], but are limited by the deficiency of donor resources from the individual.

In principle, graft cells should be amplified to fill a defect several-fold larger, necessitating a culturing process before the transplantation [6]. Injection of cultured chondrocytes into a defect covered by a periosteal patch alone may be insufficient, and thus a defect filled with appropriate biomaterials may help to trap the injected cells, preventing loss to the macroenvironment [2]. Although not yet widely utilized clinically, surgical procedures specifically developed with the use of selected biomaterials have been introduced in North America and Europe for clinical use (Carticel, Genzyme Biosurgery, Cambridge MA, USA). The clinical outcomes are difficult to evaluate, because biopsy to obtain specimens for evaluating cartilage regeneration may cause injury to the joint [23]. Existing reports of autologous cartilage transplantation (ACT) lack extensive histological assessment [14,22], and hence animal experiments are needed for more information regarding the quality of repair tissue versus time post-ACT.

Hydrogels such as gelatin may serve to deliver in vitro cultured chondrocytes in ACT [4]. Using such a model, we tested the healing of porcine cartilage defects and investigated the histological and biomechanical properties of the in vivo generated cartilaginous tissues.

Methods

Biomaterials preparation

Gelatin powders (G-2500, Sigma, St. Louis, MO) were dissolved in 50 mM phosphate buffered saline (PBS) at 50 °C with a magnetic stirrer on a hot plate to prepare a 25% (w/v) gelatin solution. The solution (25 ml) was put into canola oil (125 ml) at 50 °C to be emulsified under stirring at 300 rpm for 30 min. The mixture was cooled to 4 °C and stirred for another 2 h. After removal of the bulk of the oil, residual oil was extracted with 300 ml of acetone. The gelatin beads were harvested by means of filtration and further washed sequentially with acetone and ethanol. Finally, the gelatin beads were fixed with 0.1% glutaraldehyde in ethanol while being stirred for 1 day. The diameter of the beads was estimated to range from 50 to 250 μm by microscopic examination. The number of chondrocytes attached on the bead surface varied with bead size.

Collagen type I gel was extracted from bovine skin (ITRI, Hsinchu, Taiwan) and dissolved in 50 mM glacial acetic acid at a concentration of 4 mg/ml. The solution was dialyzed against 1 M acetic acid at 4 °C for one day and then against water for 2 days with the water changed twice daily. After dialysis, the solution was concentrated by air-drying to 20 mg/ml before being transferred to a storage tube.

Experimental design

The experimental protocol was approved by the Institutional Animal Experiment Committee. All surgeries were carried out in the veterinary hospital operating room following standard surgical routines. Twelve Lee-Sung mini-pigs were used in the experiment; two animals expired during the study due to anaphylactic reaction to anesthetic agents and were substituted by two other pigs. The final 12 animals completing the study consisted of 5 males and 7 females, with average age of 4 ± 1 months and average weight of 11 ± 3 kg at the time of graft harvest. They were housed individually in stainless steel cages and allowed forage and water ad libitum. The environment was a sheltered outdoor space with good ventilation.

The surgical procedures were performed under general anesthesia with sterile techniques, including lint preparation and draping. Anesthesia was induced with intramuscular injection of 15 mg/kg of Zoletil 50°C (tiletamine/zolazepam, Virbac Laboratories, Carros, France) and maintained by intravenous administration of the same agent intermittently in half dosage of induction. Autologous articular cartilage was harvested from the distal radius in the stifle joints of the left foreleg. The cartilage sample was sent for processing in the lab. and cells were available for transplantation after 3 weeks. At that time the animal was operated on both knee joints of the hind legs. A full-thickness cartilage defect was created for chondrocyte transplantation on the center of the weight-bearing surface of either femoral condyle of each knee. There were a total of 48 condyles that were assigned to three groups in an incomplete block design, one-way treatment (Table 1) [15] with 16 defects in each group. These included: (1) Experimental group, in which the defects were covered with a periosteal patch and filled with cell-material composite; (2) Control group, in which the defects were covered with a periosteal patch and filled with cells suspended in plain culture medium; and (3) Null group, in which the defects were covered with a periosteal patch only.

Graft harvesting and processing

Arthroscopy of the stifle joint of the left foreleg was made through a midline longitudinal incision to harvest articular cartilage from the distal radius. Approximately 35 mg of articular cartilage was collected from each animal and was immersed immediately in DMEM.

Table 1

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E = Experimental, C = Control, N = Null.
(Dulbecco’s Modified Eagle’s Medium, HyClone, Logan UT) to be transferred for laboratory processing. The wound was closed in layers, and the animals were stabilized as they were.

The articular cartilage for grafting was processed aseptically, first rinsed with PBS, then chopped with a scalpel, and washed twice with PBS. The extracellular matrix was digested overnight using an enzyme mixture consisting of 0.2% collagenase, 100 µg/ml hyaluronidase, 200 µg/ml gentamycin, and 12.5 µg/ml amphotericin B, in DMEM. The tissue debris was removed through filtration with a 40 µm sieve, and chondrocytes in the filtrate were collected with centrifugation at 300g for 10 min. The chondrocytes were re-suspended and cultured in DMEM supplemented with 10% fetal bovine serum (HyClone, Logan UT) in a 37 °C humidified CO2 incubator.

After 4-fold replication, the cells were divided into experimental and control groups. Cells in the control group were maintained in monolayer culture. Cells in the experimental group were seeded onto the gelatin beads at a density of 1.67 x 10^6 cells/cm², and cultured in a spinner flask under steady rotation of 20 rpm to keep the beads suspended.

A week later, immediately before the implantation surgeries, cells in the control group were trypsinized, collected by centrifugation, and re-suspended in fresh DMEM at a density of 4 x 10^5 cells/ml. The cells cultured on beads in the experimental group were collected by centrifugation and then mixed with collagen type I gel and 10x DMEM at a volume ratio of 9.27:4, yielding a final cell concentration of 4 x 10^6 cells/ml. Both cell preparations were placed in syringes until implantation.

ACT surgery

Both knee joints were operated in the same surgery. An arthrotomy was made through a midline longitudinal incision and a medial parapatellar approach, with the patella dislocated laterally to expose the femoral condyles. To create the chondral defect, a 7 mm diameter trephine was used to punch the cartilage manually. Full-thickness cartilage within this circular area was peeled off from the subchondral bone, which was spared from injury. During the operation, no bleeding was observed from the surface of exposed subchondral bone. The periosteum was stripped off from the proximal tibia to fashion two round patches slightly larger than the defects. Each defect was covered with a patch, which was secured onto the adjacent cartilage by eight evenly distributed sutures of absorbable suture material (4-0 Dexon, Sherwood, Davis and Geck, St. Louis MO), with the cambium layer facing the bone. In the experimental and control groups, laboratory made fibrin-glue from porcine serum was applied around the patch to seal the defect space, and chondrocyte-laden gel was delivered by injection to full filling, indicated by slight distension of the patch, with a volume of approximately 0.4 ml. The patella was reduced, and the wound was closed in layers. The animal was stabilized in the cage, allowing free motion and weight bearing on the grafted joints. One week after the surgery, healing of the wounds was checked before the animal was released from the cage and settled into fenced space.

Examination of the grafted defects

Six months after the transplantation, the animals were euthanized by over-dose injection of Pentobarbital to retrieve the grafted femoral condyles. Morphology of the graft sites was recorded. Each of the original grafted areas was dissected along the frontal plane to examine both sides of the regenerated tissue attached to adjacent native cartilage and the subchondral bone. The specimens were paraffin embedded, sectioned, and processed for routine hematoxylin-eosin (HE) staining and immunohistological staining for collagen type II (Chemicon, Temecula CA).

The regenerated cartilage was scored on the International Cartilage Repair Society (ICRS) Visual Histological Assessment Scale [11]. All specimens were evaluated on the basis of one parameter at a time, and the highest and lowest scores assigned to the best and worst results of all of the 48 specimens. Scores were not combined but analyzed separately for each parameter.

To be defined as hyaline cartilage, the ground substance of the matrix had to be homogeneous without fibrous texture with HE staining and contain predominantly collagen type II as indicated by immunohistological staining, with cells demonstrating a round morphology and aligned in short columns localized centrally in lacunae. The cellularity of the regenerated tissue was assessed and assigned a score of 0 to 3, from absence to a predominant cell population, according to the criterion in the O’Driscoll scale [20]. Specimens were reviewed and scored in a blinded manner and evaluated by a group discussion among the examiners. Scores of individual parameters were compared among the three groups of animals and evaluated statistically by the Kruskal–Wallis rank test, setting confidence interval at 0.95 for significance. In the case of a significant difference (p < 0.05), multiple comparisons were performed using paired groups with the Wilcoxon rank test.

Biomechanical evaluation was performed on specimens taken from animal No. 11: specimens from right lateral, left lateral, and right medial condyles represented the experimental, control, and null group, respectively. For comparison, a specimen of native cartilage from the same animal was tested. The tensile stress-relaxation property of each sample was determined with an unconfined uniaxial indentation test [12,16] using a custom-designed device. Briefly, the thickness of the test specimen was measured, and the specimen was then mounted on an adjustable stage with precise height control. The stage was raised by a pre-determined amount to apply a 30% strain on the cartilage surface against a 2 mm diameter bold-tip probe, and the reaction force was determined as a function of time using a load cell on the base of the probe and transformed to compressive stress.

Results

All femoral condyles were retrieved successfully six months after the graft surgery, resulting in 16 condyles for each group. The experimental design presented here precluded discussion on variation on sidedness and laterality. Paired group comparisons of mean ICRS scores by individual parameters are shown in Fig. 1.

Macroscopic results

Joint contracture with limited range of motion occurred on both knees of two of the animals. Intradiscal fibrosis was common in all joints, especially around the repaired defect sites. In the null group, 4 defects remained as dimples with a bony floor, two were filled with whitish cartilaginous tissue with a smooth surface but thinner than adjacent native cartilage, and the other ten were filled with amorphous soft fibrous tissue that protruded into the joint. In the control group, 3 defects remained as dimples with only a thin whitish coverage over subchondral bone, four were filled with fibrous tissue as in the null group, while in 6 defects white cartilaginous tissue was present, three of which had a smooth surface and three extending up beyond the articular surface. In the other 3 condyles of this group, the defects were partially empty, and in the corners regenerated tissues grew into the joint. In the experimental group, one defect remained dimpled, while the others were found to contain white, elastic, firm cartilaginous tissue. The surface appeared smooth and continuous with surrounding normal cartilage in five of these sites, smooth but failed to merge with the adjacent tissue in three, irregular and occupying only part of the defect in three, and hypertrophic beyond the joint level in two.
For the hypertrophic tissues in three of the control and four of the experimental group, a thin cartilaginous base covered with fibrous tissue that could not be peeled off easily. Traces of the previous suture line could be found as thin streak indentations on the adjacent native cartilage; however, no residual suture material could be seen. Examples of each group are shown in Fig. 2.

Surface morphology

Surfaces of the regenerate cartilage tissue ranged from smooth to horizontal lamination to vertical fissuring to disruption with a combination of the latter two conditions. The surface of predominantly fibrous tissue was consistently irregular. Minimal cartilaginous tissue was found in the 10 defects of the null group that were filled with fibrous tissue; instead they contained merely densely packed fibers with embedded ellipsoid spindle-shaped cells that resembled fibroblasts. Sites containing cartilage covered by fibrous tissue with a distinct interface was considered smooth if the junction appeared so. However, sites containing regenerate cartilage that changed to a continuous fibrous coverage were considered fibrous, although the underlying cartilage might appear hyaline (Fig. 3). Mean scores of this parameter in both experimental and control groups were significantly higher than in the null group \( p = 0.015 \) for control group).

Matrix

The nature of the regenerate tissue ranged from hyaline cartilage to purely fibrous. The matrix of hyaline cartilage consisted of homogeneous ground substance by HE staining and was rich in collagen type II as demonstrated by immunohistochemistry (Fig. 4). The presence of collagen type II defined the hyaline cartilage from fibrocartilage and plain fibrous tissue. Generally, the more homogeneous hyaline cartilage showed higher structural integrity, which was absent in the intermingled fibrous tissue, and only prominent staining for collagen type II. For the regenerate cartilage that was covered by dense fibrous tissue, hyaline-like characteristics were detected, and the matrix was assessed as cartilage. The mean scores of matrix property were significantly higher in the experimental group than the other two groups \( p = 0.018 \) with control, and \( p = 0.001 \) with null group).

Cell distribution

Unlike native articular cartilage, where cell density was greatest near the osteochondral junction and decreased toward the surface, cells in the regenerate cartilage were more evenly distributed. Chondrocytes in the well-differentiated cartilage were aligned in a ray of 4-5 cells to form the typical palisade architecture. The regenerate cartilage, even if it was hyaline, showed shorter cell columns and even individual cells. Cell clusters of a rosette arrangement were also common and were found in 11 of the experimental, 12 of the control, and 14 of the null group. The pattern of cell distribution also showed regional variation in a given specimen; scores of this parameter were made based on the predominant pattern of the specimen. The experimental group had significantly higher mean scores than the other two groups \( p = 0.019 \) with control, and \( p = 0.0015 \) with null group).

Cell viability

Viability of the cells was indicated by a clearly delineated nucleus, regardless of cell type. Although viable, cells in fibrous tissue were not surrounded by lacunae as for normal chondrocytes and appeared spindle-shaped resembling fibroblasts. Regardless of cell type, the mean scores of cell viability did not differ significantly among the three groups. However, the cellularity
in regenerate hyaline cartilage was higher in the experimental group than the other two groups \( (p < 0.05 \text{ by Wilcoxon rank test}) \).

**Subchondral bone**

Remodeling of the subchondral bone was noted in all groups to varying extents, ranging from increased cellular infiltration to solid osseous callus (Fig. 3a and b). The mean score of the experimental group was significantly higher than that of the null group \( (p = 0.008) \), but did not differ from the control group. In specimens with regeneration of hyaline and fibrocartilage, the osteochondral junction showed the characteristic tidemark pattern of articular cartilage.

**Cartilage mineralization**

Cartilage mineralization is evaluated as yes or no on the ICRS scale. Mineralization within the regenerate tissue was seen as isolated spots or being continuous with the subchondral mineralization. It was noted in 4 of the experimental, 10 of the control, and 13 of the null group. Regardless of group, specimens with mineralization had significantly lower mean matrix scores \( (p < 0.05 \text{ by Wilcoxon rank test}) \) than those in the control groups.
Fig. 3. Macroscopic and histological analyses of femoral condyles in the experimental group at 6 months post-operation. (a) In the lateral condyle in the left knee of animal No. 13, HE stain showed that the fibrous tissue was distinguishable from the smooth surface of underlying cartilage with a narrow gap (arrows). Inset: Cross-section of the transplanted site showed that the repaired cartilage had about two-thirds the thickness of neighboring native cartilage and was covered by fibrous tissue. The subchondral bone underwent remodeling. (b) In the lateral condyle in right knee of animal No. 12, HE staining showed that the cartilage surface changed gradually to a fibrous cap without a distinct interface (arrows). Inset: Cross-section of the transplanted site showed that the regenerate cartilage was also covered with a fibrous cap; however, the subchondral bone depicted normal cancellous characteristics.

without. Scores of this parameter correlated well with the matrix score, with a Pearson coefficient of 0.63, consistent with the observation that cartilage mineralization was not seen in homogeneous hyaline cartilage, but was quite common in fibrocartilage and fibrous tissue.
Fig. 4. Histological and immunohistological analyses of repaired tissue in femoral condyle cartilage defects in control, null, and experimental groups. Upper row: Histological sections (HE stain, bar = 500 μm) of the selected specimens from those examined in Fig. 2. From the left, they are: right lateral (control), right medial (null), and left lateral (experimental) condyles. The control specimen was composed of cartilaginous tissue intermingled with thick fibrous structures, and the null specimen consisted of merely a thin layer of fibrous tissue. In the experimental specimen, a predominantly homogeneous hyaline cartilage was formed, which however did not adhere well to the neighboring native cartilage. Lower row: Immunohistological staining (bar = 100 μm) for collagen type II of specimens corresponding to those shown on the top. In the experimental specimen, the repaired tissue consisted of thick collagen type II fibers (dark-brown staining, arrow), which were absent in the null specimen.

Mechanical properties

At 30% strain, all specimens showed a biphasic stress-relaxation pattern with time. Stress rose rapidly in the initial 7-9 s with maximal values of 8.92, 4.31, 7.50, and 5.28 MPa in the control, experimental, null, and native cartilage groups, respectively, representing the viscoelastic stiffness of the specimens. The stress peak was followed by a slow decay to reach final equilibrium in the next 30 min (Fig. 5). At 20 min after strain application, the stress on these four specimens dropped to a certain level, and the changes thereafter were insignificant (p > 0.05 by Student's t-test). The equilibrium stress at 20–30 min was 0.36, 0.09, 1.54, and 0.93 MPa in the control, experimental, null, and native cartilage groups, respectively, and represented the time-independent tensile modulus of the solid phase in the viscoelastic material. In the experimental group, the value was close to
zero, suggesting either this was a nearly viscous material or was viscoelastic with failure of its solid phase occurring at this strain.

**Discussion**

Articular cartilage injuries heal poorly leading to serious, symptomatic consequences such as degenerative arthritis. Developing approaches to repair articular cartilage defects has therefore attracted increasing attention in recent decades, but with little success until recently. Tissue engineering is regarded as a promising approach for cartilage repair; however, it has been difficult to develop differentiated cartilaginous tissues in vitro [27]. Cartilage is composed of a unique extracellular matrix (ECM), produced and maintained by a limited number of chondrocytes, that serves both a structural function as well as a regulatory role in chondrocyte activities [25]. The major drawback of in vitro approaches may be the difficulty in mimicking the environment of native cartilage, which is critical to chondrocyte physiology. Use of bioreactors may be a solution, but the current technology is far from being ideal. An alternative approach is to use the cartilage defect as a “bioactive chamber” in situ to maintain and cultivate complete cartilage tissue [2]. In such an approach, it is necessary to use a substance capable of maintaining the viability and phenotype of autologous chondrocytes for a short period after their harvesting and prior to transplantation [8,10]. Once transplanted, the cells may be considered as being under conditions nearly identical to those of native cartilage.

Periosteal and perichondrial grafts were reported to have chondrogenic activity, particularly with their cambium layer facing into the joint [9,19]. In our study, we minimized this effect in order to critically assess outcome of the transplanted chondrocytes by placing the periosteal graft with the cambium layer in reverse orientation. However, cartilaginous tissue was found in defects of the null group, consistent with and suggesting the chondrogenic ability of the periosteum. The subchondral plates in these subjects remained intact; hence consideration of migrating marrow cells as the origin of the regenerate cartilage tissue was minimal.

A critical requirement of cartilage repair is its accurate assessment. Various “semi-quantitative” scaling systems, such as those described by Mankin [13] and O’Driscoll [20], evaluate the characteristics and quality of cartilage from histological and histochemical aspects. Those scales provide objective references to compare the gross morphology and infrastructural architecture among cartilage samples, but are not without controversy [21,26]. The ICRS scale attempts to eliminate discrimination among observers using a system based on visual patterns. Unlike other scales that sum up scores of all parameters to draw a global representation of the tissue, the six parameters in the ICRS scale are considered individually, since their relative importance is still unclear.

Cartilage tissue is easily identified by plain histology, characterized by its cell morphology and architecture. Chondrocytes are round cells that appear single or columnar and are located within lacunae. Further classification into hyaline, elastic, and fibrous cartilage depends on the predominant substances in the ECM that are more precisely defined by immunohistochemical analysis in addition to histology. As recommended by the Histological Endpoint Committee of ICRS [11], we have relied upon HE staining only for histological assessment and collagen type II immunostaining for differentiating hyaline cartilage from elastic and fibrous cartilages.

As reported in previous studies, patching the defects with periosteum may bring in cartilaginous tissue [9,18–20], as we noted here in the null group. However, the structural characteristics of these tissues were consistently inferior to the other two groups in all aspects. A possible explanation is that cartilage derived from a different origin, i.e. mature chondrocytes harvested from mature articular cartilage, may be more active in generating and organizing hyaline-like cartilage. It is also important to transfer an adequate number of cells to repair a defect. The chondrocyte cellularity in the experimental group was higher than in the other two groups, indicating that a simple aqueous suspension of cells failed to keep the transferred chondrocytes in place. Because a water-seal around the patch would be difficult to ensure and free motion of the joint after implantation surgery was allowed in this repair model, an aqueous cell-suspension would have been easily squeezed out of the defect. A viscous material is therefore more helpful to minimize cell leakage. Attachment of chondrocytes to the gelatin microbeads suspended in collagen gel appeared to prevent the cells from leaking away from the defect sites.

In healthy articular cartilage, chondrocytes display a unique architectural pattern. Loss of the normal pali-sade pattern and formation of clusters are indicative of degenerative changes [1,24]. Degeneration was a common feature of all our specimens in this aspect. Purely columnar distribution was seen in only 5 specimens in the experimental group and 1 in the control group. On the other hand, fibrous tissue formation took place within the cartilage defect when the transplanted cells and material failed to occupy it. These fibroblasts may be the result of transformation of the transplanted chondrocytes, the periosteal patch, or the migration of cells from elsewhere within the joint as part of inflammatory process. The ECM of the experimental group presented more hyaline characteristics than the control group, suggesting that the biomaterials contributed toward the
maintenance of the phenotype of the transplanted chondrocytes. The ICRS scale does not emphasize the proportion of chondrocytes in the viable cell population. However, we regard it as an important characteristic of the outcome in ACT, because of the requirement of healthy chondrocytes in the generation of normal cartilage.

The mechanical characteristics of articular cartilage are difficult to define and are influenced by many confounding factors, such as its anatomical location, the modes of testing, and specific conditions of the joint [5,16]. Although the confined compression model is generally adopted [17], it does not reflect a functional material behavior under physiological conditions. In this study, no attempt was made to determine the definite modes of testing, and specific conditions of the joint cartilage.

portion of chondrocytes in the viable cell population. maintenance of the phenotype of the transplanted chondrocytes on a hyaluronan derivative (Hyaff-11) into cartilage defects in rabbits. Biomaterials 2001;22:2417–24.


Our findings indicate that, when optimized, ACT is an effective technique to repair an articular cartilage defect in the porcine model. If successfully kept in place, transplanted chondrocytes maintain their phenotype and are capable of generating hyaline cartilage. Gelatin microbeads suspended in collagen gel are able to trap and retain the transplanted cells, and represent a potentially serviceable cell delivery vehicle for chondrocyte transplantation.

Acknowledgments

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References


