Tissue-engineering of Orthopaedic Surgery

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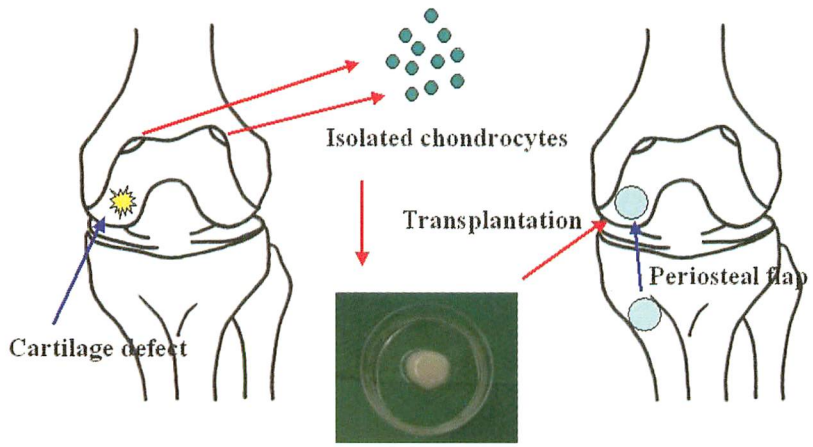
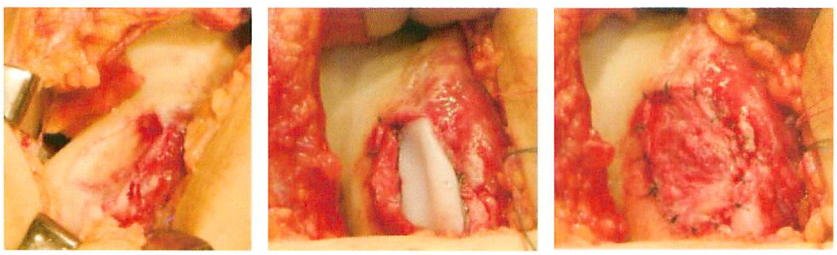
Transplantation of tissue-engineered cartilage for cartilage defect of the knee

Articular cartilage has a poor healing capacity due to its lack of vessels, nerve supply, and isolation from systemic regulation. Numerous methods have been attempted to enhance the repair of full-thickness articular cartilage defects, including abrasion arthroplasty; microfracture; transplantation of chondrocytes, perichondrium, and periosteum; and osteochondral graft. However, no known treatment has regenerated long-lasting hyaline cartilage.

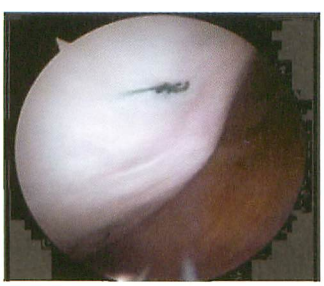
Recently, a regenerative medicine using a tissue-engineering technique for cartilage repair has been given much attention in the orthopaedic field. In 1994, Brittberg et al introduced a new cell technology in which chondrocytes expanded in monolayer culture were transplanted into the cartilage defect of the knee. As a second generation of chondrocyte transplantation, since 1996 we have been performing transplantation of tissue-engineered cartilage made ex vivo for the treatment of osteochondral defects of the joints. This signifies a concept shift from



Tissue-engineered cartilage



Cartilage defect | Transplantation | Periosteal flap



1 year after transplantation

cell transplantation to tissue transplantation made ex vivo using tissue-engineering technique.

Patients

Fifty-six knees of 56 patients (mean age: 25 y.o.) of 98 patients who had received transplantation of tissue-engineered cartilage for cartilage defects were followed up for at least 2 years. The tissue-engineered cartilage was made by cultivating autologous chondrocytes embedded in Atelocollagen gel for 3 weeks before transplantation. At 6, 12, 24 months after operation, arthroscopic, biomechanical and MRI examinations were performed. Using the Lysholm score, the clinical outcome was evaluated at the final clinical follow-up.

Results

Transplantation eliminated knee locking and reduced pain and swelling in all patients. The mean Lysholm score improved significantly. Arthroscopic assessment indicated that 50 knees had excellent or good outcomes. No problems including infection were detected, except with 11 cases of graft hypertrophy, 4 cases of partial detachment of periosteum, 1 case of partial ossification and 2 case of graft failure. Biomechanical examination also revealed that the transplants had acquired hardness similar to that of the surrounding cartilage at 12 months after operation. MRI demonstrated that the signal intensity of the grafted portion had become similar to that of normal cartilage in 77% of the cases at 24 months after operation.

Minimally invasive approach with a tissue-engineered chondral plug

Purpose

The purpose of this new approach was to evaluate the macroscopic and histological results transplanting a tissue-engineered chondral plug made of atelocollagen sponge and PLLA mesh, for the treatment of osteochondral defects.

Methods

Twelve-week-old male Japanese white rabbits were used. Fresh articular cartilage slices were taken from the humeral head, and isolated chondrocytes were embedded in atelocollagen gel which does not have antigenic portions of collagen. (2.0×10^6 cells/ml). They were seeded on the top of the atelocollagen sponge/PLLA mesh composite, and cultured for 2 weeks. The culture medium was changed every 3 days and L-ascorbic acid (50μg/ml) was added every 2 days. Culturing the composites for 2 weeks produced tissue-engineered chondral plugs. These tissue-engineered chondral plugs (4mm in diameter, 4mm in thickness) were transplanted into the osteochondral defects (4mm in diameter, 4mm in depth) in the patellar grooves of the same rabbits from which the chondrocytes had been harvested (the experimental group). In the control group, the defects were treated with the plugs without chondrocytes. The rabbits were sacrificed at 4 and 12 weeks after transplantation. The repaired tissues were evaluated macroscopically and histologically. The repaired tissue was analyzed immunohistochemically for expression of type-II collagen.

Results

Four weeks after transplantation in the experimental group, the defects were partially repaired with cartilage-like tissue with good subchondral bone formation. Twelve weeks after transplantation, the defects were repaired with hyaline cartilage-like tissue densely stained by safranin O. Well organized subchondral bone formation was also observed. In the control group, the defects were covered with only soft fibrous tissue at 4 and 12 weeks macroscopically.
Immunohistochemically, type-II collagen was detected in about 90 % of the repaired area. Histological scores in the experimental group were significantly higher than those in the control group at both 4 and 12 weeks after transplantation.

Conclusions

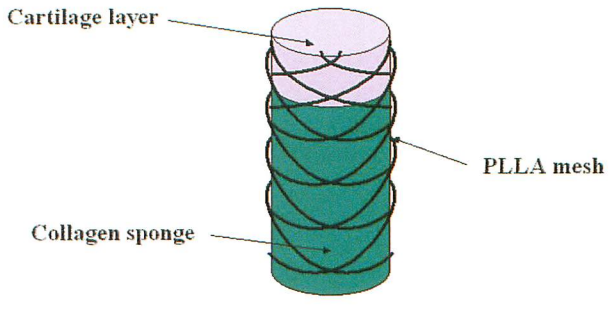
This study demonstrated that the defects treated with tissue engineered chondral plug demonstrated type-II collagen in about 90% of the repaired area.

Clinical Relevance

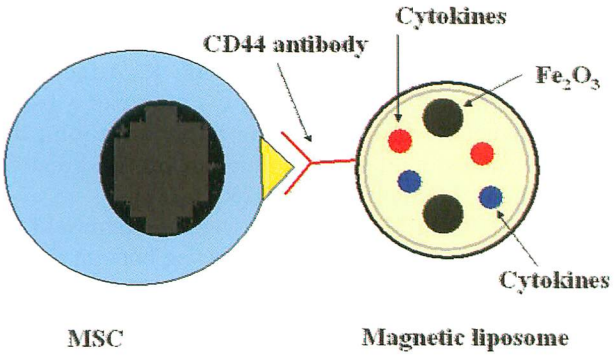
The transplantation of a tissue-engineered chondral plug will be one option for the treatment for osteochondral defects. The next step in testing our hypothesis is to evaluate the repaired tissue biomechanically and biochemically over a longer period of time.

Future direction for cartilage repair with minimally invasive tissue-engineering technique

The most optimal procedure to repair cartilage defects is not surgical treatment requiring anesthesia and hospitalization but just injection of cytokines or growth factors and cells. Our completely novel approach was to use autologous bone marrow mesenchymal stem cells attached to small-sized magnetic beads. For successful cartilage repair, it is simple to inject mesenchymal stem



Tissue-engineered chondral plug



Magnetic liposome-MSC complex

cells and effectively collect them to a specific area in the knee joint (osteochondral defect) using an external magnet force. The complex of autologous bone marrow mesenchymal stem cells and magnetic liposomes using the antibody (CD44) was created and research of the efficacy of DDS by magnetism was done as a pilot study. A full thickness cartilage defect was made in the bilateral femur condyle of a rabbit, and successfully repaired with this technique. We believe that this novel system is also effective in the treatment of brain or spinal cord injury and for malignant tumors, using natural killer cells instead of autologous bone marrow mesenchymal stem cells with our novel system.

REFERENCES

1) Ochi M, Sumen Y, Jitsuiki J. et al. Allogeneic deep frozen meniscal graft for repair of osteochondral defects in the knee joint. Arch. Orthop. Trauma Surg 1995;114:26-266.
2) Matsusaki M, Ochi M, Uchio Y, et al. Effects of basic fibroblast growth factor on proliferation and phenotype expression of chondrocytes embedded in collagen gel. Gen Pharmacol. 1998;31:759-64.
3) Kawasaki K, Ochi M, Uchio Y, et al. Hyaluronic acid enhances proliferation and chondroitin sulfate synthesis in cultured chondrocytes embedded in collagen gels. J Cell Physiol. 1999;179:142-8.
4) Uchio Y, Ochi M, Matsusaki M, et al. Human chondrocyte proliferation and matrix synthesis cultured in Atelocollagen gel. J Biomed Mater Res. 2000;50:138-43.
5) Katsube K, Ochi M, Uchio Y, et al. Repair of articular cartilage defects with cultured chondrocytes in Atelocollagen gel. Comparison with cultured chondrocytes in suspension. Arch Orthop Trauma Surg. 2000;120:121-7.
6) Ochi M, Uchio Y, Tobita M, et al. Current concepts in tissue engineering technique for repair of cartilage defect. Artif Organs. 2001;25:172-9.
7) Ochi M, Uchio Y, Kawasaki K, et al: Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br. 2002;84:571-8.
8) Nishikori T, Ochi M, Uchio Y, et al. Effects of low-intensity pulsed ultrasound on proliferation and chondroitin sulfate synthesis of cultured chondrocytes embedded in Atelocollagen gel. J Biomed Mater Res. 2002;59:201-6.
9) Adachi N, Sato K, Usas A, Ochi M, et al. Muscle derived, cell based ex vivo gene therapy for treatment of full thickness articular cartilage defects. J Rheumatol. 2002;29:1920-30.
10) Kuriwaka M, Ochi M, Uchi Y, et al. Optimum combination of monolayer and three-dimensional cultures for cartilage-like tissue engineering. Tissue Eng. 2003;9:41-9.
11) Matsuo T, Sugita T, Kubo T, Yasunaga Y, Ochi M, Murakami T. Injectable magnetic liposomes as a novel carrier of recombinant human BMP-2 for bone formation in a rat bone-defect model. J Biomed Mater Res A. 2003 15;66:747-54.
12) Murakami T, Fujimoto Y, Yasunaga Y, Ishida O, Tanaka N, Ikuta Y, Ochi M. Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. Brain Res. 2003;974:17-24.
13) Ochi M, Adachi N, Nobuto H, et al. Articular cartilage repair using tissue engineering technique--novel approach with minimally invasive procedure. Artif Organs. 2004;28:28-32.
14) Agung M, Ochi M, Adachi N, Uchio Y, Takao M, Kawasaki K. Osteochondritis dissecans of the talus treated by the transplantation of tissue-engineered cartilage. Arthroscopy. 2004 ;20:1075-80.
15) Natsu K, Ochi M, Mochizuki Y, Hachisuka H, Yanada S, Yasunaga Y. Allogeneic bone marrow-derived mesenchymal stromal cells promote the regeneration of injured skeletal muscle without differentiation into myofibers. Tissue Eng. 2004 Jul-Aug;10(7-8):1093-112.
16) Fujiwara Y, Tanaka N, Ishida O, Fujimoto Y, Murakami T, Kajihara H, Yasunaga Y, Ochi M. Intravenously injected neural progenitor cells of transgenic rats can migrate to the injured spinal cord and differentiate into neurons, astrocytes and oligodendrocytes. Neurosci Lett. 2004 19;366:287-91.
17) Adachi N, Ochi M, Uchio Y, Iwasa J, Furukawa S, Deie M. Osteochondral lesion located at the lateral femoral condyle reconstructed by the transplantation of tissue-engineered cartilage in combination with a periosteum with bone block: a case report. Knee Surg Sports Traumatol Arthrosc. 2004;12:444-7.
18) Ito Y, Tanaka N, Fujimoto Y, Yasunaga Y, Ishida O, Agung M, Ochi M.Bone formation using novel interconnected porous calcium hydroxyapatite ceramic hybridized with cultured marrow stromal stem cells derived from Green rat. J Biomed Mater Res A. 2004;69:454-61.
19) Nobuto H, Sugita T, Kubo T, Shimose S, Yasunaga Y, Murakami T, Ochi M. Evaluation of systemic chemotherapy with magnetic liposomal doxorubicin and a dipole external electromagnet. Int J Cancer. 2004;10:627-35.
20) Ito Y, Ochi M, Adachi N, et al. Repair of Osteochondral defect with tissue-engineered chon-

dral plug in a rabbit model. Arthroscopy 2005;21:1155-63.
21) Hamasaki T, Tanaka N, Ishida O, Yanada S, Kamei N, Fujiwara Y, Nishida K, Nakanishi K, Sharman P, Kawamata S, Ochi M. Characterization of labeled neural progenitor cells for magnetic targeting. Neuroreport. 2005 17;16:1641-5.
22) Nakashima Y, Deie M, Yanada S, Sharman P, Ochi M. Magnetically labeled human natural killer cells, accumulated in vitro by an external magnetic force, are effective against HOS osteosarcoma cells. Int J Oncol. 2005 ;27:965-71.
23) Nakasa T, Ishida O, Sunagawa T, Nakamae A, Yasunaga Y, Agung M, Ochi M. Prefabrication of vascularized bone graft using a combination of fibroblast growth factor-2 and vascular bundle implantation into a novel interconnected porous calcium hydroxyapatite ceramic. J Biomed Mater Res A. 2005 1;75:350-5.
24) *Adachi N, Ochi M,* Deie M, Ito Y. Transplant of mesenchymal stem cells and hydroxyapatite ceramics to treat severe osteochondral damage after septic arthritis of the knee.J Rheumatol. 2005 ;32:1615-8.
25) Yamasaki T, Deie M, Shinomiya R, Izuta Y, Yasunaga Y, Yanada S, Sharman P, Ochi M. Meniscal regeneration using tissue engineering with a scaffold derived from a rat meniscus and mesenchymal stromal cells derived from rat bone marrow. J Biomed Mater Res A. 2005;75:23-30.
26) Kajiwara R, Ishida O, Kawasaki K, Adachi N, Yasunaga Y, Ochi M. Effective repair of a fresh osteochondral defect in the rabbit knee joint by articulated joint distraction following subchondral drilling. J Orthop Res. 2005 ;23:909-15.
27) Izuta Y, Ochi M, Adachi N, Deie M, Yamasaki T, Shinomiya R. Meniscal repair using bone marrow-derived mesenchymal stem cells: experimental study using green fluorescent protein transgenic rats.Knee. 2005;12:217-23.
28) Tanaka H, Sugita T, Yasunaga Y, Shimose S, Deie M, Kubo T, Murakami T, Ochi M.Efficiency of magnetic liposomal transforming growth factor-beta 1 in the repair of articular cartilage defects in a rabbit model. J Biomed Mater Res A. 2005 ;73:255-63.
29) Hisatome T, Yasunaga Y, Yanada S, Tabata Y, Ikada Y, Ochi M.Neovascularization and bone regeneration by implantation of autologous bone marrow mononuclear cells. Biomaterials. 2005 ;26:4550-6.
30) Yanada S, Ochi M, Adachi N, et al. Effects of CD44 antibody- or RGDS peptide-Immobilized Magnetic Beads on Cell Proliferation and Chondrogenesis of Mesenchymal Stem Cells . J Biomed Mater Res Part A (in press)