

# Regenerative Medicine for Cartilage Defects

JMAJ 47(7): 307–310, 2004

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**Abstract:** The treatment of full-thickness defects of articular cartilage remains a problem for orthopedic surgeons. It has been generally accepted that once articular cartilage is injured and forms a defect, the defect cannot be repaired and bordering intact cartilage undergoes degeneration which destroys facing intact cartilage and results in osteoarthritis. Several attempts to repair articular defects with hyaline cartilage have failed. Since the clinical reports of Brittberg *et al.* in 1994, autologous chondrocyte transplantation has raised the hopes and expectations of orthopedic surgeons that a breakthrough in the repair of damaged articular cartilage is imminent. In Brittberg *et al.*'s technique, cartilage slices were obtained by arthroscopy from an unloaded area of the femoral condyle; the associated chondrocytes increased in number in a monolayer culture after enzymatic digestion, and the chondrocytes in suspension were then injected into a cartilaginous defect and covered with a flap of the periosteum. According to their report, clinical results were satisfactory and a biopsy of the graft sites showed hyaline-like cartilage repair. However, we had several reservations about their technique in regard to the culture and transplantation procedure in terms of 1) de-differentiation of chondrocytes during a long cultivation, 2) uneven distribution of the grafted chondrocytes throughout the osteochondral defects, and 3) a high risk of leakage of grafted chondrocytes from the defects. We developed a new technique that enables the shift from cell transplantation to tissue transplantation. This technique creates a new cartilage-like tissue by the tissue-engineering technique in which autologous chondrocytes were embedded in atelocollagen gel and cultivated for about 3 weeks. We carefully selected atelocollagen gel as a three-dimensional culture material from the viewpoint of safety and non-immunogenicity, since atelocollagen gel had been used clinically for the treatment of skin wrinkles in plastic surgery and dermatology. Cultivation results in the proliferation of chondrocytes and the synthesis of an extracellular matrix consisting of chondroitin sulfate and type II collagen at transplantation. By 3 weeks of cultivation, the atelocollagen gel, including chondrocytes had acquired a jelly-like hardness. We have been using our technique since gaining the approval of the ethics committee in 1996. After 3 to 4 weeks' culture of autologous chondrocytes embedded in atelocollagen gel, a tissue-engineered cartilage was transplanted into a cartilage defect and covered with a periosteal flap, which was sutured with the deep cambium layer facing the subchondral bone plate. We followed up full-thickness cartilage defects of 36 knees from 34 patients treated with our procedure over a minimum period of 2 years. Clinical, arthroscopic and biomechanical results were relatively satisfactory. We conclude that transplanting tissue-engineered cartilage made by the tissue-engineering technique can promote restoration of the cartilage of the knee.

**Key words:** Cartilage injury; Cartilage repair; Three-dimensional culture; Regenerative medicine

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This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 129, No. 3, 2003, pages 336–338).

## Introduction

With the progress in tissue engineering and molecular biology, basic research in regenerative medicine aimed at regeneration of defective tissues or organs using autologous cells or tissues and their clinical application have become widespread in recent years. For the regeneration of tissues using tissue engineering techniques, it is generally recognized that cell transplants, biocompatible materials to serve as a foothold for growth of transplanted cells (i.e., scaffold), and growth factors are usually required, and studies on regeneration of a wide variety of tissues have been extensively undertaken in the field of medical care. Regenerative medical care has thus been frequently introduced by mass media such as newspapers, periodicals, and television and widely recognized among the public at large. Knowledge of regenerative medicine is now essential to the practice of medicine.

Repair of articular cartilage, which has little or no self-repairing capacity, has been taken up as a matter to be pursued in the field of orthopedic surgery. Articular cartilage comprises chondrocytes and surrounding extracellular matrix (such as type II collagen and proteoglycans) and is devoid of blood vessels and nerve tissues. The chondrocytes are poorly proliferative and produce little extracellular matrix. Once articular cartilage is damaged, the usual tissue repair process does not proceed with a consequent lack of restoration of hyaline cartilage.

When an articular cartilage defect occurs, it gives rise over time to degeneration of the surrounding and facing intact joint cartilage, which progresses in due course to become osteoarthritis. Consequently, pain and limitation of excursion occur and functional impairment of the knee joint ensues. This constitutes a significant clinical problem. Articular cartilage injury may be encountered not only in the practice of orthopedic surgery but in other clinical settings as well in the form of osteo-

chondritis dissecans in young subjects, traumatic cartilage injury, and osteoarthritis or rheumatoid arthritis in the elderly.

Currently, there is no other choice than artificial joint replacement for reconstruction of markedly destroyed articular function due to advanced osteoarthritis or rheumatoid arthritis. For the treatment of localized articular cartilage defects, a variety of procedures has been undertaken, including the drilling method and the microfracture technique aimed at inducing marrow cells from a subchondral bone, soft tissue grafting such as periosteum-perichondrium or meniscus transplantation, and osteochondral transplantation. None of these surgical procedures has proven to provide satisfactory reconstruction of a natural, smooth cartilage with hyaline cartilage at the site of defect.

In 1994, Brittberg *et al.* reported transplantation of cultured autologous articular chondrocytes utilizing monolayer cell cultures.<sup>1)</sup> Cartilage slices obtained by arthroscopy from an unloaded region of the knee joint were trypsinized to isolate chondrocytes, which were then incubated to grow into monolayer cultures. The grown chondrocytes were dispersed in suspension and injected into a cartilaginous defect covered with a flap of the periosteum. The technique is currently in widespread use on a commercial basis mainly in Europe and the United States.

According to their report, a postoperative biopsy of the graft sites showed hyaline-like cartilage repair. The technique thus marked a major breakthrough in the repair of damaged cartilage. Several problems, however, seem to be inherent in this technique, viz. whether chondrocytes which have de-differentiated during the cultivation *in vitro* may recover their function as chondrocytes post-transplantation, whether leakage of the grafted chondrocytes in suspension may occur from the defect site, and whether the grafted chondrocytes may become evenly distributed throughout the osteochondral defect.

To resolve these problems, we have devised a

technique for tissue-engineered cartilage-like tissue grafting, namely, transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes. Based on laboratory studies, its clinical application was initiated for the first time in the world in 1996.<sup>2-7)</sup> Gratifying postoperative results have been obtained, and clinical trials are scheduled to be conducted at several university hospitals.

### Transplantation of Atelocollagen Gel-Embedded Cultured Autologous Articular Chondrocytes

The atelocollagen gel is type I collagen purified from the bovine corium and causes little or no immune responses to it because the atelocollagen is deprived of the antigenic terminal telopeptide. The gel is a remarkably safe collagen that is already in clinical use in cosmetic surgery for such purposes as eliminating skin wrinkles.

The specifics of the procedure are as follows. Cartilage specimens are obtained by arthroscopy from an intra-articular detached cartilage fragment or an unloaded region of knee joint cartilage of the patient. The specimens are sliced and trypsinized/collagenase-digested to isolate chondrocytes. The collected cells are then suspended in an atelocollagen gel and gelled, followed by incubation with a culture medium containing the patient's serum and antibiotics for 3 weeks. The embedded chondrocytes grow in the atelocollagen gel and liberate extracellular matrix to have the gel acquire a jelly-like hardness and turn into a cartilage-like tissue.

Three weeks after harvest of the autologous cartilage, the chondral defect lesion is exposed via arthrotomy, the chondrocyte-atelocollagen gel prepared *in vitro* is transplanted in the defect, and the lesion is covered by a sutured periosteal flap taken from the proximal medial tibia. One to two weeks after the transplantation, passive movement of the joint is begun. Partial weight-bearing is introduced at 4 weeks

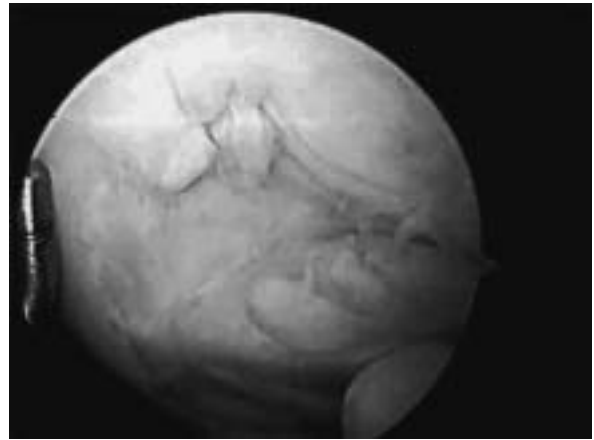


Fig. 1 Osteochondritis dissecans of the femoral medial condyle of the right knee in a 27-year-old man  
Note the cartilage defect extends as deep as the surface of subchondral bone.

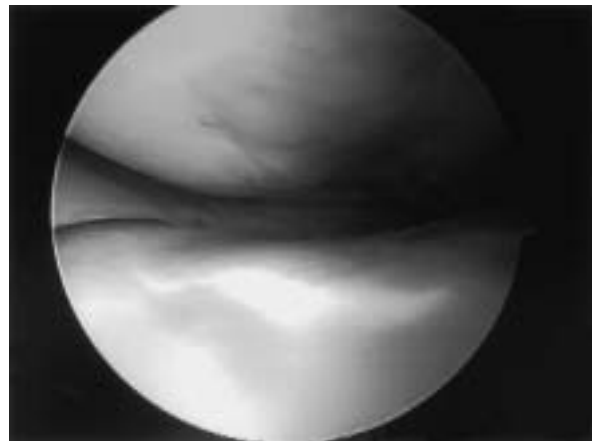


Fig. 2 Two years after transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes  
The defect is repaired with cartilage-like tissue with a smooth surface.

post-operation and is gradually increased to full weight-bearing with muscle training at 6–8 weeks post-operation.

In a laboratory study with human chondrocytes collected at an operation of artificial joint replacement, we confirmed and reported that human chondrocytes cultured in atelocollagen gel retained their three-dimensional structure and grew while maintaining their usual round shapes without incurring de-differentiation and

produced/released extracellular matrix such as type II collagen and chondroitin sulfate.

Inasmuch as chondrocytes are transplanted as embedded/cultured in the solid gel medium, there is practically no cell leakage from the periosteal sutured margin, unlike transplantation of cells in suspension. Leakage of chondrocyte transplant is thus quite unlikely even on articular movements, unless gel leakage results from significant periosteal damage; this constitutes a great advantage. The procedure is also advantageous in that it provides apparently greater uniformity of chondrocyte transplant as compared with the chondrocyte transplantation in suspension.

Treatment with this procedure has been performed on a total of 70 knees, with relatively satisfactory postoperative outcomes (Figs. 1 and 2). The patients were followed by periodic arthroscopic observations postoperatively, and the graft sites were confirmed to gradually increase in hardness to become as firm as the surrounding normal cartilage. On MRIs, the graft sites were shown over time to gain brightness close to that of surrounding normal cartilage.

The method's drawbacks are: the limited quantity of chondrocytes obtainable, the long period required for the transplanted cartilage to mature, and the two-stage operation to complete the procedure. Further spread of cultured autologous chondrocytes tissue grafting can be expected in the future upon improvement of the cultivation technique, development of a better transplant carrier supplanting the atelocollagen, and a technique enabling autologous stem cell differentiation into chondrocytes. Cooperation and joint research with other fields such as medicoengineering and molecular biology are essential for the development of the method.

## Conclusion

With the increases in the elderly population and the sports population in recent years,

opportunities to treat patients suffering from articular cartilage injury have increased. While it is not easy to repair an articular cartilage injury with the original hyaline cartilage, we devised a tissue-engineered technique for transplantation of atelocollagen gel-embedded cultured autologous articular chondrocytes and have been applying it in clinical settings. Though the postoperative follow-ups have not been long enough, satisfactory results have been obtained with this technique. Exploration of the current problems involved and various approaches to attain more satisfactory outcomes for cartilage repair are under way.

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