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Donor Cell Survival in a Fresh Osteochondral Allograft at Twenty-nine Years

A Case Report

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Investigation performed at the University of California Davis Medical Center, Sacramento, California

Fresh osteochondral allografting is a nonvascularized organ transplantation with a clinical use dating back to the early twentieth century. The procedure, in its current form, involves transplantation of a small segment of bone as a carrier with its overlying cartilage. As the bone is remodeled and revascularized by the recipient, the donor cartilage continues to function mechanically, produces matrix, and receives nutrition from the synovial fluid. The cartilage has traditionally been considered a so-called immunoprivileged tissue, avoiding the immune surveillance of the host presumably because of its paucity of vascular channels and its hypocellular matrix[1]. These qualities make fresh osteochondral allografting unique in the field of transplantation and preclude the need for systemic immunosuppression after this procedure. Although histologically normal articular cartilage has been reported in retrieved fresh osteochondral allografts, we know of no report that has definitively confirmed the specific survival of the donor cells for any length of time. Furthermore, the source of cells in retrieved transplants has not been confirmed as being from the donor or the recipient. The patient was informed that data concerning his case would be submitted for publication.

Case Report

A twenty-two-year-old man with a large defect on the articular surface of the lateral femoral condyle of the knee underwent fresh osteochondral allografting from a female donor. He recovered well from the allograft procedure and had a relatively pain-free knee for the next twenty-five years. He presented to us with knee pain on the lateral side and tricompartmental arthritis predominantly in the lateral compartment (Fig. 1). A magnetic resonance imaging scan was acquired, and it demonstrated a tear of the posterior horn of the lateral meniscus with loss of the articular cartilage thickness along the posterior aspect of the lateral femoral condyle (Fig. 2). He ultimately requested total knee arthroplasty because of the unremitting knee pain. The procedure was performed exactly twenty-nine years after the implantation of the fresh osteochondral allograft. At the time of the procedure, he had a sclerotic region on the distal aspect of the lateral femoral condyle with a small amount of remaining cartilage. No demarcation could be seen between the host bone and the allograft. Scrapings were obtained from the remaining articular cartilage along the posterior aspect of the lateral femoral condyle, and the bone resected from this region underwent histological analysis. Our hypothesis was that chondrocytes from the allograft donor remained alive and active in the patient over the twenty-nine-year interval since the procedure.

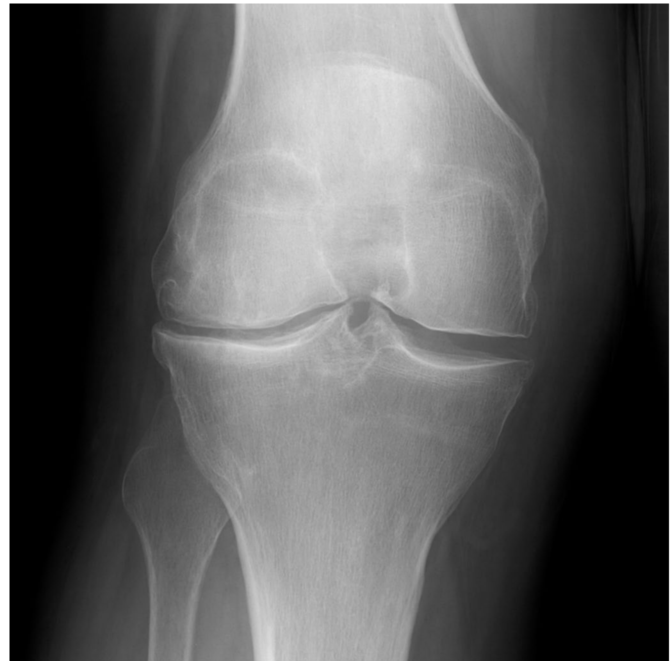

Fig. 1

Anteroposterior weight-bearing radiograph demonstrating radiographic findings of osteoarthritis, including osteophyte formation, joint space narrowing, sclerosis, and squaring of the femoral condyles.

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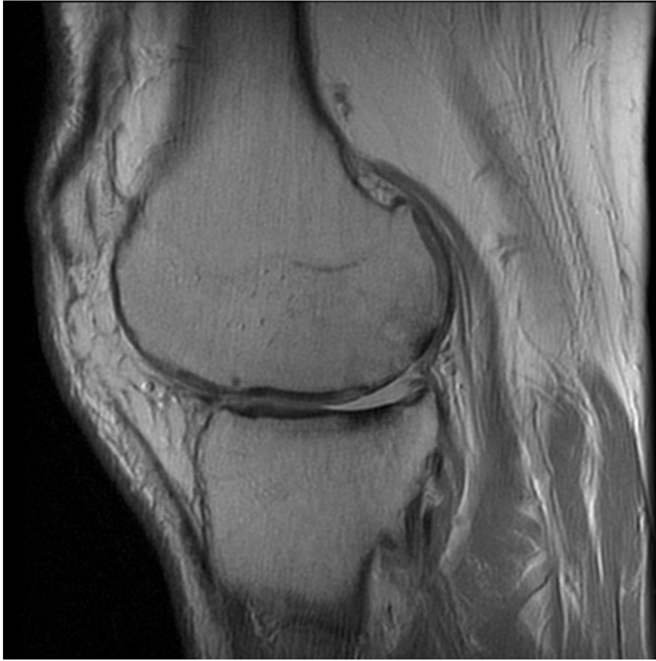

Fig. 2

Proton-density-weighted sagittal magnetic resonance imaging scan of the lateral compartment of the knee showing the absence of the posterior horn of the lateral meniscus and marked loss of the articular cartilage thickness along the posterior aspect of the lateral femoral condyle.

Analysis of Osteochondral Tissue and Remaining Articular Cartilage

Scrapings of the remaining cartilage from the posterior aspect of the lateral femoral condyle were obtained at the time of total knee arthroplasty. Fluorescence in situ hybridization (FISH) and karyotype analysis were performed on cultured chondrocytes from these scrapings. Osteochondral tissue from this region was resected during the total knee replacement. This tissue was fixed with 10% formalin, decalcified with 10% formic acid, and then held in 70% EtOH. The sections were embedded in paraffin and stained with hematoxylin and eosin.

For cytogenetic analysis, femoral cartilage tissue was minced by scalpel dissection and dissociated with collagenase (Type IV; Sigma, St. Louis, Missouri). The cells were cultured for ten days in tumor media consisting of 20% fetal bovine serum (Gemini Bio-Products, West Sacramento, California), RPMI 1640 (Invitrogen, Carlsbad, California), and 100X antibiotic-antimycotic (Invitrogen). Cells were treated with colcemid (Invitrogen) and hypotonic KCl solution and were fixed in methanol acetic acid. Chromosomes were banded with use of the standard trypsin-Giemsa method. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995)[2].

Fixed cells from cultured cartilage tissue were dropped onto glass slides and dried. Dual-color fluorescence in situ hybridization was performed on the cells with the Vysis Chro-

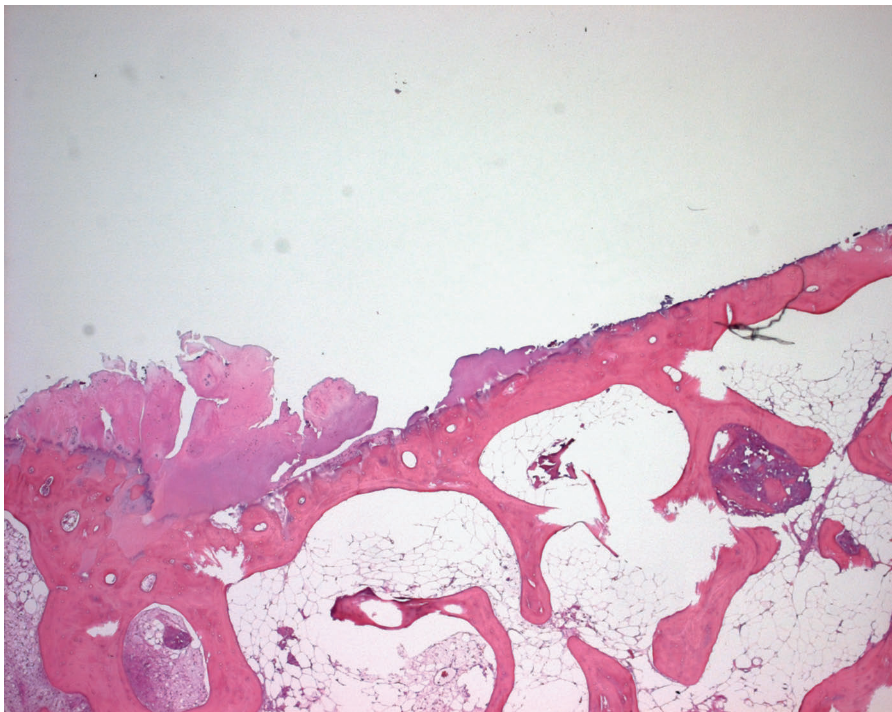

Fig. 3

Hematoxylin and eosin-stained decalcified sections from the posterior aspect of the lateral femoral condyle, demonstrating extensive loss of articular cartilage and an irregular subchondral bone surface (×100). These findings are consistent with the diagnosis of osteoarthritis.

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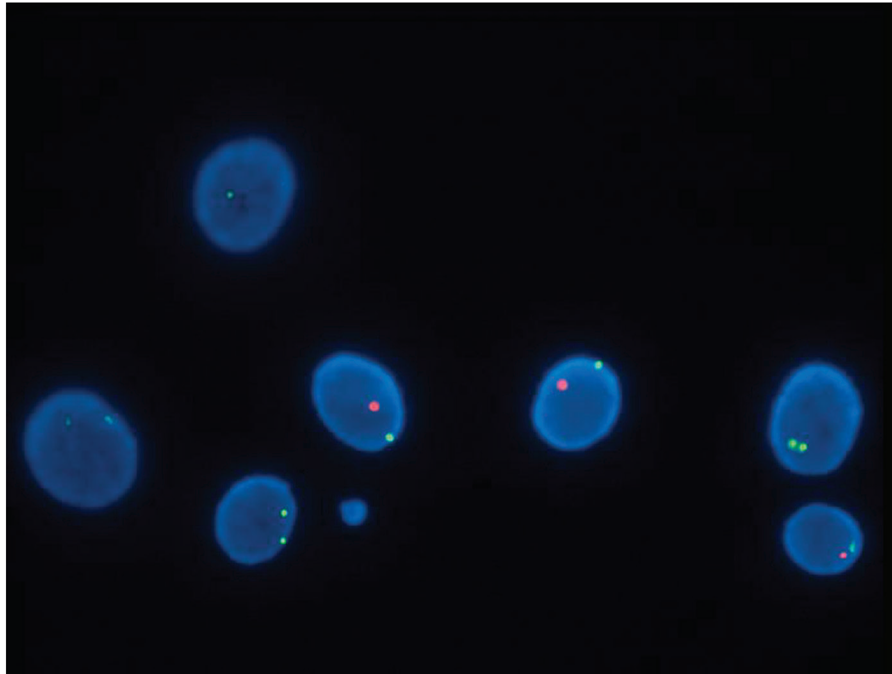

Fig. 4

Fluorescence in situ hybridization results illustrating interphase nuclei with either XX or XY sex chromosomes (×1000).

mosome Enumeration Probe (CEP) X SpectrumGreen and CEP Y SpectrumOrange probes (Vysis, Des Plaines, Illinois). These probes are specific to the centromere region of Xp11.1-q11.1 and Yp11.1-q11.1 of the X and Y chromosomes. Female cells (XX) demonstrate two green signals. Male cells (XY) demonstrate one green and one orange signal. The cells were denatured and hybridized overnight with use of a HYBrite system (Vysis). The slides were washed with 70% EtOH and then were dehydrated to 100% EtOH. Dried slides were counterstained with DAPI II (4′,6-diamidino-2-phenylindole) (125 ng/mL). A total of 206 interphase nuclei were scored.

DNA was extracted with use of Puregene reagents (Gentra Systems; Minneapolis, Minnesota) and was amplified with primers specific for the amelogenin gene on the X chromosome and the amelogenin-like sequence on the Y chromosome[3,4]. Polymerase chain reaction products were visualized in ethidium bromide-stained agarose gels after electrophoresis (data not shown).

The results of histological analysis by hematoxylin and eosin staining of the posterior aspect of the lateral femoral condyle demonstrated severe thinning of the articular cartilage with areas of complete cartilage loss and exposed subchondral bone (Fig. 3). These findings were consistent with severe osteoarthritis in this anatomical location. Gender identification by polymerase chain reaction established that the DNA extracted from the lateral femoral condyle had both X and Y-specific fragments. Since both X and Y-specific fragments were present and the polymerase chain reaction was not quantitative, it could not be determined whether the DNA was exclusively male or a mixture of male and female.

Fluorescence in situ hybridization demonstrated that 138 (67%) of the 206 interphase nuclei were XX and sixty-eight (33%) were XY (Fig. 4). Routine chromosome analysis showed nine of sixteen metaphase cells were 46,XX, and seven of sixteen metaphase cells were 46,XY.

Discussion

This report is the first, as far as we know, to confirm donor cell survival in a fresh human osteochondral allograft. More importantly, the female chondrocytes or their progeny remained intact for nearly thirty years in vivo with no systemic immunosuppression.

A number of strategies have been used to study the in vivo cell viability of fresh osteochondral grafts. Czitrom et al. used autoradiography to study the articular cartilage of allografts biopsied after transplantation[5]. They found chondrocyte viability from 37% to 99% at intervals ranging from one to six years. Although chondrocytes are considered relatively immobile cells within the matrix, the origin of these cells as donor cells was not definitively ascertained.

Convery et al. removed a fresh osteochondral allograft from a medial femoral condyle eight years after implantation[6]. They studied decalcified sections of the specimen by standard histological analysis as well as by vital staining and demonstrated a mixture of live and dead cells. Once again, the origin of the cells could not be definitively determined. McGoveran et al. analyzed a biopsy specimen obtained seventeen years after an osteoarticular distal femoral allograft replacement[7]. Standard light microscopy revealed normal cartilage thickness, a number of focally empty cartilage lacunae, and areas of

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chondrocyte clumping presumably due to chondrocyte regeneration. Examination with electron microscopy confirmed intact rough endoplasmic reticulum, mitochondria, and cellular membranes. Again, however, the cellular origin of these chondrocytes was not definitively determined.

Strategies similar to our methodology have been used to determine the survival of limbic stem cells transplanted for corneal dysfunction. Fluorescent in situ hybridization analysis was used by Shimazaki et al. to demonstrate survival of donor cells after limbic stem cell transplantation at 2.4 years after the procedure[8]. However, the patient in their study was treated with dexamethasone for three weeks and cyclosporine for six months, an important distinction from the patient in the present study in whom no immunosuppression was used.

The findings of the current report highlight the presence of a mixed population of cells after fresh osteochondral allografting. Furthermore, this report confirms the long-term survival of donor cells from a nonvascularized osteochondral allograft with no systemic immunosuppression at nearly three decades after transplantation. ■

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References

1. Langer F, Gross AE. Immunogenicity of allograft articular cartilage. J Bone Joint Surg Am. 1974;56:297-304.

2. Mitelman F. ISCN: an international system for human cytogenetic nomenclature. Basel: S. Karger; 1995.

3. Nakahori Y, Hamano K, Iwaya M, Nakagome Y. Sex identification by polymerase chain reaction using X-Y homologous primer. Am J Med Genet. 1991;39:472-3.

4. Sellathamby S, Balasubramanian P, Sivalingam S, Shaji RV, Mathews V, George B, Viswabandya A, Srivastava A, Chandy M. Developing an algorithm of informative markers for evaluation of chimerism after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2006;37:751-5.

5. Czitrom AA, Keating S, Gross AE. The viability of articular cartilage in fresh osteochondral allografts after clinical transplantation. J Bone Joint Surg Am. 1990;72:574-81.

6. Convery FR, Akeson WH, Amiel D, Meyers MH, Monosov A. Long-term survival of chondrocytes in an osteochondral articular cartilage allograft. A case report. J Bone Joint Surg Am. 1996;78:1082-8.

7. McGoveran BM, Pritzker KP, Shasha N, Price J, Gross AE. Long-term chondrocyte viability in a fresh osteochondral allograft. J Knee Surg. 2002;15:97-100.

8. Shimazaki J, Kaido M, Shinozaki N, Shimmura S, Munkhbat B, Hagihara M, Tsuji K, Tsubota K. Evidence of long-term survival of donor-derived cells after limbal allograft transplantation. Invest Ophthalmol Vis Sci. 1999;40:1664-8.

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