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The Biological Response following Autogenous Bone Grafting for Large-Volume Defects of the Knee: Index Surgery through 12 to 21 Years' Follow-up

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Abstract

Objective: This report focuses on the biological events occurring at various intervals following autogenous bone grafting of large-volume defects of the knee joint's femoral condyle secondary to osteochondritis dissecans (OCD) or osteonecrosis (ON). It was hypothesized that the autogenous bone graft would integrate and the portion exposed to the articular surface would form fibrocartilage, which would endure for years. *Methods:* Between September 29, 1987 and August 8, 1994, there were 51 patients treated with autogenous bone grafting for large-volume osteochondral defects. Twenty-five of the 51 patients were available for long-term follow-up up to 21 years. Patient follow-up was accomplished by clinical opportunity and intentional research. Videotapes were available on all index surgeries for review and comparison. All had preoperative and postoperative plain film radiographs. Long-term follow-up included MRI up to 21 years. Second-look arthroscopy and biopsy were obtained on 14 patients between 8 weeks and 20 years. *Results:* Radiological assessment showed the autogenous bone grafts integrated with the host bone. The grafts retained the physical geometry of the original placement. MRI showed soft tissue covering the grafts in all cases at long-term follow-up. Interval biopsy showed the surface covered with fibrous tissue at 8 weeks and subsequently converted to fibrocartilage with hyaline cartilage at 20 years. *Conclusion:* Autogenous bone grafting provides a matrix for large osteochondral defects that integrates with the host bone and results in a surface repair of fibrocartilage and hyaline cartilage that can endure for up to 20 years.

Keywords

articular cartilage < tissue, biopsy < research methods, histology: staining < research methods, magnetic resonance imaging < diagnostics, knee < joint involved

Introduction

This report focuses on the biological events following autogenous bone grafting of large-volume defects of the knee joint's femoral condyle secondary to osteochondritis dissecans (OCD) or osteonecrosis (ON). At the time this study was initiated, the medical literature held no prospect for sustainable cartilage repair, and the biological fate of various attempts at cartilage repair remains controversial.[1-7] The surgical treatment in the 1980s for large-volume defects of the femoral condyle was a cadaveric osteochondral allograft or total knee arthroplasty.[8,9]

At that time, physicians and patients were reluctant to consider cadaveric allografts because of the unknown risk surrounding the discovery of acquired immune deficiency syndrome (AIDS) in the early 1980s.[10] Total knee arthroplasty was an emerging technology without long-term follow-ups at that time, so patients were reluctant to sacrifice the entire joint for a localized lesion. Most cartilage repair procedures

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were directed towards the 2-dimensional superficial loss of the articular cartilage.[11] Subsequently, such procedures have been utilized for larger defects.[12-14] In recent years, autogenous osteochondral grafts were introduced for large-volume defects.[15] However, there are few long-term reports of restoration of articular surfaces regardless of the treatment method, and histological studies of such cases are exceedingly rare.

The rationale for this novel approach was based upon past experience in the treatment of OCD. It was known that fixation of an osteochondral fragment without adding an underlying bone graft may fail to unite.[16] This knowledge led to the use of adjunct autogenous bone grafting under such a lesion to ensure union. Subsequently, autogenous bone graft was used as a matrix to fill the void adjacent to a partial OCD fragment replacement. The early positive results led to wider indications for the use of autogenous bone graft to fill large-volume osteochondral defects of OCD or ON.

Precedent for neither this novel approach of arthroscopically assisted autogenous bone grafting of large osteochondral defects nor the long-term biological outcome of this procedure was known to the authors at the onset. The purpose of this study was to examine the biological fate of autogenous bone grafting of large 3-dimensional osteochondral defects of the human knee with long-term follow-up. It was hypothesized that the autogenous bone graft would integrate and the portion exposed to the articular surface would form fibrocartilage, which would endure.

Materials and Methods

This study was initiated before the advent of Institutional Review Board (IRB) jurisdiction in our community. The inclusion criteria were patients with OCD and ON of any age, with large-volume lesions. Those with superficial cartilage lesions or defects of traumatic etiology were excluded as well as those with instability, ankylosis, or severe diffuse degenerative arthritis.

Between September 29, 1987 and August 8, 1994, 51 patients were treated with autogenous bone grafting for large-volume osteochondral defects. There were 3 pathological groups: OCD with a partial fragment ($n = 14$), OCD without a fragment ($n = 12$), and ON ($n = 24$). Two patients in the ON group were iatrogenic. One was because of the use of YAG laser energy for an arthroscopic chondylar debridement, and the other was because of leukemia chemotherapy treatment including cortisone.

All surgeries were performed by one surgeon. The index surgeries included other procedures: partial meniscectomy ($n = 15$), abrasion arthroplasty ($n = 9$), chondroplasty ($n = 8$), resection osteophytes ($n = 2$), and wide synovectomy ($n = 1$). The osteochondral crater was arthroscopically cleared of all necrotic tissue, and the margins were undercut to physically secure the cancellous grafts. The surgical defects were estimated to be between 6 to 75 cm^3. When present, the backs of the hinged fragments of OCD were debrided. All chondral fragments were resected in ON. Twenty-nine percent of the 51 patients had an accompanying high tibial osteotomy when the lesion was medial and involved a large surface area and/or when there was a coexisting varus deformity. No osteotomy was performed in the OCD group when a partial fragment was replaced. Osteotomy was performed on 2 patients in the OCD group who had no fragment to replace. Forty percent of the 25 patients in the ON group had associated tibial osteotomy.

The cancellous bone donor site was the proximal tibial metaphysis except in one patient with the largest defects. This patient had bilateral massive defects so it was necessary to harvest additional cancellous graft from the same-side iliac crest. The cancellous grafts were held in place until a blood clot was formed.[17] There were 2 patients with a cortical cancellous graft taken from the Gerdy tubercle area of the tibia that was placed by open surgery. Cancellous fragments were packed around the corticocancellous graft to obliterate any space. The retained OCD fragments and the cortical cancellous grafts were held in place by screw fixation.

The postoperative care included 2 months of nonweight-bearing ambulation with crutches including intermittent active range of motion exercises. Continuous passive motion was not used in any patient. There were no postoperative infections that could have introduced a pathological tissue response.

Patient follow-up was routine on all patients through the first postoperative year as well as evaluations as necessary in the course of their care through 6 years. There was a hiatus in patient data until this formal long-term follow-up was initiated in 2005.

The subsequent clinical and radiological long-term follow-up was under Michigan State University IRB approval. Absent direct evidence, the radiological study was to serve as a means of assessing the long-term biological response of the bone graft integration and the nature of the articular surface. The long-term follow-up process for the radiological evaluation was accomplished by tracking patients via the existing office medical records and an Internet search. Some participating patients who live out of state were financially reimbursed upon request for travel, lodging, and loss of time off work by private funds. The follow-up radiological evaluations were performed without charge by the Department of Radiology at Michigan State University College of Human Medicine.

Twenty-five of the 51 patients were available for the long-term follow-up between 12 and 21 years. An electronic medical record existed for every patient. There were 7 women and 18 men. The average age at the time of the index surgery of these patients was 47 years (range, 13-82 years).

Of the 14 patients in the OCD group with partial fragment and grafting, 6 were available for long-term follow-up, and 8 were lost to follow-up. Of the 12 patients in the OCD group with grafting alone, 11 were available for long-term follow-up. One patient was reported as symptomatic but declined to participate. Of the 25 patients in the ON group with fragment resected and grafted, 8 were available for long-term follow-up. Two died, 7 were lost to follow-up, and 8 underwent a total knee replacement.

Radiological Imaging

All patients had preoperative and postoperative plain film radiographs. There were plain film radiological studies throughout the course of patient care as well as at the time of the long-term follow-up evaluations. The radiological documents included preoperative plain films in all cases taken in 5 views: bilateral standing anteroposterior (AP), standing Rosenberg view, Merchant view, supine notch view, and lateral. The long-term biological fate of the donor and host sites was documented by MRI. MRI studies were obtained on 23 patients between 12 and 21 years. One patient with a cardiac pacemaker had a CAT scan. One patient from Columbia, South America, was too elderly to travel but submitted follow-up history electronic medical record (EMR) modules and plain film x-rays.

MRI was performed at 3 T (GE Healthcare, Little Chalfont, UK). Structural imaging parameters were selected for characterization and differentiation of cartilage, subchondral and cancellous bone, and joint fluid using the following fast spin echo sequences. Imaging assessment of the joint and graft material included evaluation of 1) the presence of the subchondral bone plate, 2) the continuity of trabecular bone features between underlying native cancellous bone and graft bone, 3) the articular surface continuity over the graft and the adjacent native articular cartilage, 4) the signal characteristics of the graft bone compared to adjacent native cortical and cancellous bone, and 5) the presence of secondary degenerative changes in the neoarticular surface.

Photographic Documentation

There was arthroscopic photographic and/or video documentation in all cases, which was archived for identification of the site of the lesion and for observation of biological changes. Illustrative intraoperative findings were photographed. Surgical videotapes were saved through the end of this study to provide a means of review and clinical correlation of biopsy site identification.

Gross Pathology

Second-look arthroscopy provided gross pathology inspection on the 29 patients at variable intervals between 8 weeks to 6 years, with one opportunity for second-look arthroscopy at 20 years (Table 2). The gross pathological evidence was gathered during the scheduled screw removal, recurrent symptoms, or second-look arthroscopy granted during surgery on the other knee. The 20-year follow-up opportunity presented when the patient injured what had previously been an asymptomatic knee.

Biopsy

There were 21 postoperative biopsies in 15 patients between 8 weeks and 20 years. Accurate targeting of the biopsy site was guided by review of the prior surgery on videotape. The biopsy specimens were taken with a Jamshidi 2-mm-diameter bone marrow biopsy needle (CareFusion Corporation, San Diego, CA, USA). Routine histological preparations with hematoxylin and eosin (H&E) and Safranin O were performed.

20-Year Biopsy Histopathology

Special attention was given to the 20-year biopsy samples. They were fixed, processed, and embedded in paraffin for microtomy. Sections were stained for H&E, Safranin O, or Masson's trichrome using standard histological techniques. Sections allocated for immunohistochemical analysis were stained with the following mouse monoclonal antibodies: anti-lubricin (1:4,800 dilution; Dr. T. M. Schmid, Rush University Medical Center, Chicago, IL), anti–type II collagen (CIIC1, 1:20 dilution; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and anti–α-smooth muscle actin (SMA) (clone 1A4, 1:400 dilution; Sigma, St. Louis, MO USA). The immunohistochemical staining was performed using the Dako Autostainer (DakoCytomation, Carpinteria, CA). Deparaffinized and rehydrated sections were digested in 0.1% protease for 40 to 45 minutes to facilitate antibody penetration, followed by quenching of endogenous peroxidase with peroxidase blocking reagent (DakoCytomation) for 10 minutes. Nonspecific binding was blocked by incubation with 5% goat serum for 30 minutes. The primary antibody was applied at room temperature for 30 minutes. Negative controls were incubated with a mouse IgG and IgM cocktail (DakoCytomation) instead of the primary antibody. Peroxidase-based detection (Dako LSAB-2 System Kit, DakoCytomation) was used per the manufacturer's instructions. Briefly, sections were incubated with a biotinylated secondary antibody (goat anti-mouse IgG) for 10 to 15 minutes, followed by application of streptavidin-HRP for 10 to 15 minutes. Labeling was developed with an aminoethyl carbazole (AEC) chromogen for 10 minutes. Counterstaining was performed with Mayer's hematoxylin for 1.5 minutes, followed by application of coverslips.

Table 1. Summary of the Biological Responses

Biological Response	Time Interval	Gross Anatomy and Histology
Blood clot	Immediate	There was bleeding followed by blood clot formation over the exposed bone graft (**Figs. 1B** and **3D**).
Fibrous tissue	8 weeks	A corticocancellous graft (**Figs. 1F** and **2D**) as well as a cancellous graft (**Fig. 3G**) had similar histological appearance of integration of the bone graft below and a fibrovascular surface repair.
Fibrocartilage	10 months	There was a well-integrated white graft surface contiguous with surrounding normal cartilage (**Fig. 2G**). The histology showed dense fibrocartilage covering the surface.
	2 years	The gross appearance was white tissue soft to palpation while continuous with the surrounding normal cartilage (**Fig. 3E**). The histology showed maturing fibrocartilage.
	6 years	The arthroscopic view of the previously grafted surface showed intact white tissue moderately firm to palpation and absent any degeneration (**Fig. 5**).
Mixed mature cartilage (fibrocartilage and hyaline cartilage)	20 years	Arthroscopic view of the gross anatomy of the previously exposed cancellous bone graft showed white tissue, firm to palpation, with mild degenerative arthritic change consistent with the rest of the compartment (**Fig. 1D**). The histology of this biopsy showed mixed mature cartilage (**Fig. 1H**).
Lubricin layer on the articulating surface	20 years	Special staining showed a discrete layer of lubricin on the surface (**Fig. 1L**) as well as areas of surface fragmentation (**Suppl. Fig. S1M** and **N**).

Results

Representative Cases of Each Category and Rationale

A summary of the biological responses relative to the time interval at which the evaluation was performed is shown in Table 1. The following cases are representative of those with biological follow-up in the 3 treatment groups: OCD with fragment and graft; OCD with graft alone, either corticocancellous or all cancellous; and ON with fragment removal and graft. The representative cases are presented in the following order according to the evolution of the surgical technique. Each case provides a different time interval window for inspection of the biological response (Table 1).

The first cases were performed by arthroscopy, cancellous grafting, and internal fixation with cannulated screw fixation of the existing partial OCD fragment (**Fig. 1A**). It was observed that the exposed cancellous bone was promptly covered with a blood clot (**Fig. 1B**). At the time of screw removal, there was maturing fibrous tissue on the surface of the autogenous bone graft (**Fig. 1C**).

Early on, it was not known if a subchondral cortical bone plate would develop following large-volume cancellous bone grafting. Therefore, the surgical approach evolved to open surgery using a composite cortical cancellous graft for 2 OCD patients who had no fragment (**Fig. 2**). The donor site included the Gerdy tubercle with its convex surface to replicate the femoral condylar geometry.

Subsequently, it was learned that the cancellous grafts did develop a subchondral bone plate, so larger defects were treated arthroscopically solely with multiple fragments of a cancellous graft (**Fig. 3**). All bone grafts were recessed from the cartilaginous articular surface to be contiguous with the adjacent level of host bone.

Second-look arthroscopy at 8 weeks was accompanied by biopsies of the repaired surfaces of these cases at 8 weeks, which showed gross appearance and histomorphology similar to that observed after abrasion arthroplasty[18] (**Figs. 1F** and **2C**). These observations prompted the consideration of autogenous bone graft as a matrix for large osteochondral defects in selected cases of ON and for OCD when the fragment was absent. This rationale was supported by the recognition that fibrocartilage formed over surgically abraded exposed bone in the arthritic knee and survived for 2 years.[18] It was further reasoned that the autogenous bone grafting method would avoid the risk of disease transmission and the autogenous bone graft matrix would fill the osteochondral defect. It was anticipated that the blood clot of the surface of the graft would facilitate the formation of fibrocartilage.[17,18]

The immediate biological response was blood clot formation in and on the autogenous bone graft (**Figs. 1B** and **3B**). Gross anatomical inspection was possible between 8 weeks and 20 years. The surface over remodeled graft was contiguous with and sealed to the adjacent normal cartilage but remained soft to palpation at 8 weeks and 10 months compared to the adjacent normal articular cartilage (**Figs. 2D**and **3D**).

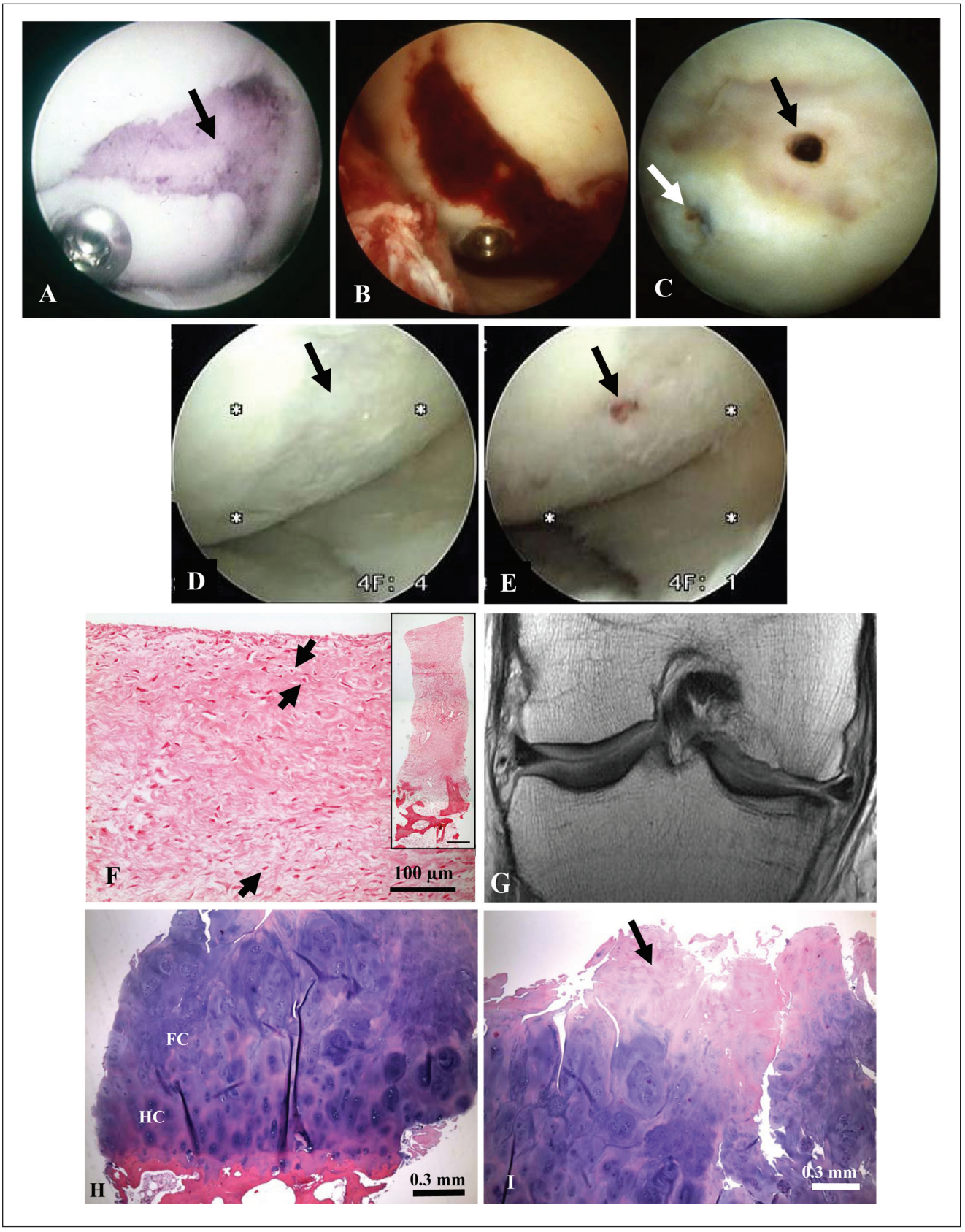

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Figure 1. (continued)

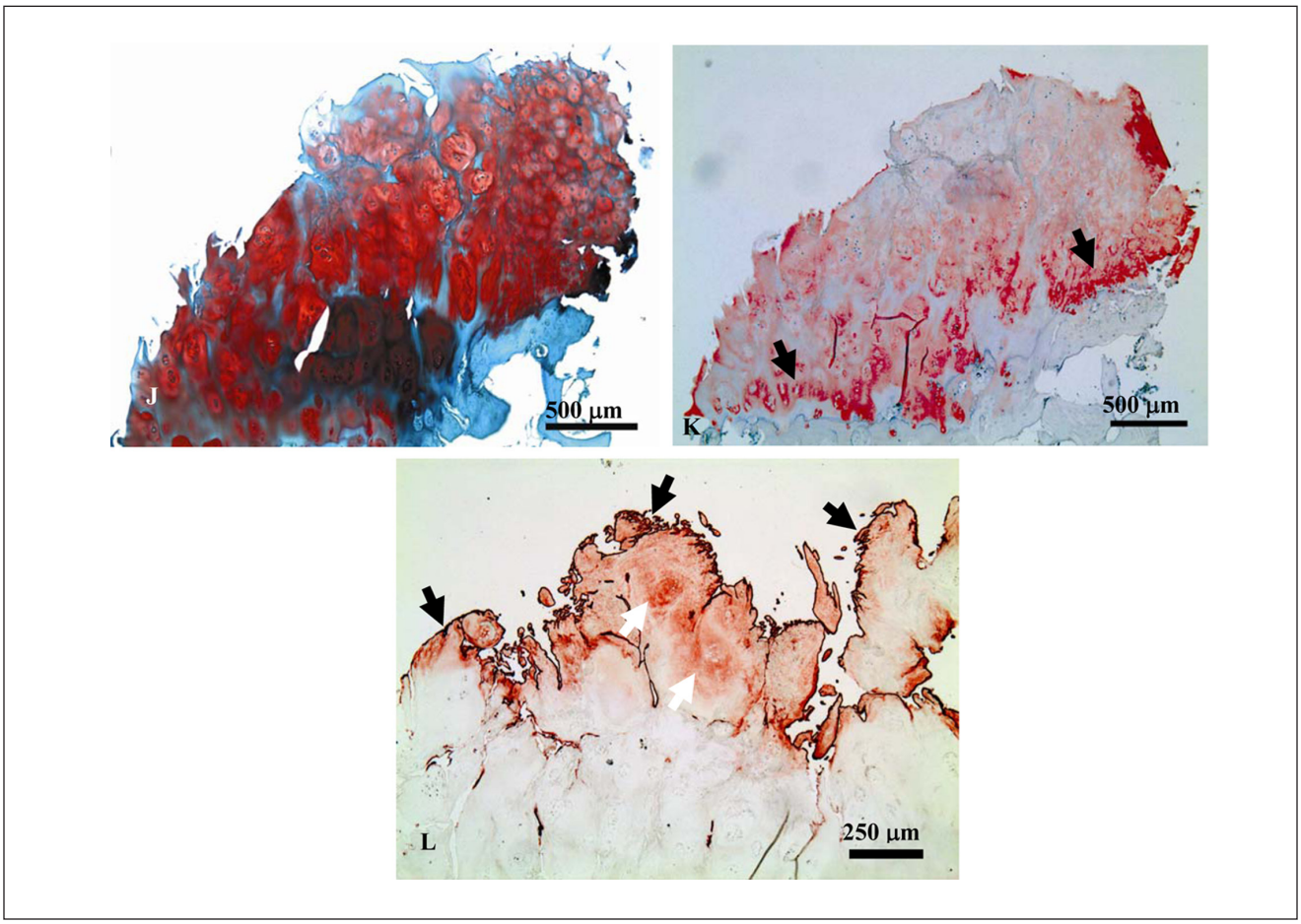

Figure 1. A representative case of a patient with OCD who had screw fixation of a partial fragment plus a cancellous bone graft. This 36-year-old man underwent arthroscopic surgery on August 4, 1987 for a large-volume osteochondral defect secondary to OCD. He had a small fragment remaining that was secured with screw fixation. An autogenous cancellous bone graft from the proximal tibial metaphysis was placed under and adjacent to the fragment. The long-term results of this patient showed the immediate postoperative biological events, followed by planned and opportunistic second-look arthroscopy and biopsy. He was asymptomatic at 19 years when MRI was taken as part of the long-term clinical follow-up on this cohort. He subsequently injured his knee that resulted in gross arthroscopic inspection at 20 years with correlated biopsy and extensive histochemical studies of the autogenous bone graft site. (**A**) Index surgery showing the partial fragment held with a screw and the adjacent exposed cancellous bone graft (arrow). It was from the site of the exposed cancellous bone (arrow) that the biopsies at 8 weeks and 20 years were taken. (**B**) Index surgery showing immediate postoperative blood clot formation over the exposed cancellous bone graft. (**C**) Eight weeks postoperatively, the arthroscopic view showed the fragment, the site of the screw removal (white arrow), and the biopsy site (black arrow). (**D**) Twenty years later, the arthroscopy showed mild, diffuse degenerative arthritis and the healed fragment and previously cancellous bone graft site (arrow). (**E**) Arthroscopic confirmation of the prior bone graft site shows that the defect of the 20-year biopsy (arrow) correlated perfectly with the prior archived videotape location of the graft. (**F**) Micrograph of the 8-week biopsy showing the fibrous tissue and fibrocartilage (rounded cells in lacunae [arrows] in a fibrous matrix) in the superficial area. Inset shows a longitudinal section of the complete biopsy. H&E stain. Additional micrographs are included in the supplementary materials. See also Suppl. Fig. S1A-C. (**G**) MRI was taken at 19 years when the patient was asymptomatic and a member of a cohort of similar patients undergoing a long-term clinical assessment. Sagittal MRI proton density images showing incorporation of the cancellous bone graft material and the osteochondral fragment can be seen in the supplementary materials. See also Suppl. Fig. S1D and E. (**H**) Photomicrograph of the 20-year biopsy. Longitudinal section through the osteochondral biopsy. FC = fibrocartilage; HC = hyaline cartilage. H&E stain. See also Suppl. Fig. S1F-K (**I**) Micrograph of the surface region of the biopsy shown in **H**, which was more eosinophilic (arrow) than the bulk of the cartilage. H&E stain. (**J**) Light micrograph of Safranin O–stained biopsy sections as seen in the cartilaginous region. The sections stained intensely for the presence of sulfated GAGs (red). Additional micrograph included in the supplementary materials. (**K**) Immunohistochemical staining of type II collagen (red chromogen) in the cartilaginous tissue at the base of the lesion, bordering the subchondral bone (arrows). Additional micrograph included in the supplementary materials. (**L**) Immunohistochemical staining results for lubricin (red chromogen). A discrete layer of lubricin on the surface of the biopsied tissue (black arrows). Lubricin staining of the extracellular matrix (white arrows) to a distance of about 200 µm below the surface was also evident. See also Suppl. Fig. S1L-N.

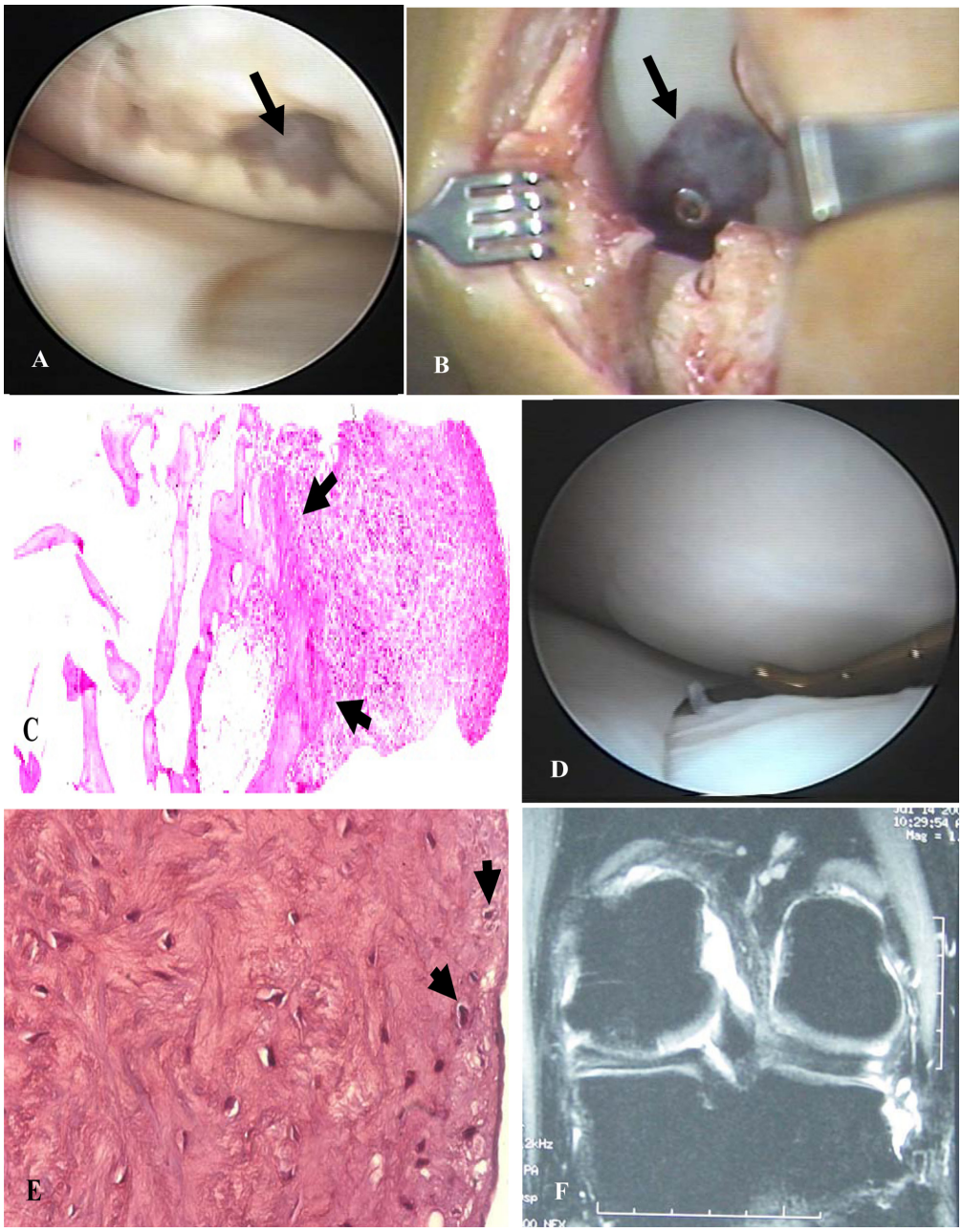

Figure 2. Representative case of a patient with a large defect filled with a corticocancellous graft. This is a 26-year-old male professional baseball player with OCD without a fragment due to previous surgical removal. A corticocancellous graft from the convex Gerdy tubercle area was placed in the defect to ensure the presence of a subchondral bone plate. Preoperative and 18-year postoperative radiographs are in Suppl. Fig. S2A, E, and F. (**A**) Arthroscopic view of the defect of the left medial femoral condyle (arrow). See also Suppl. Fig. S2B and C. (**B**) Gross appearance 8 weeks following grafting at the time of screw removal. The screw head that was placed by arthrotomy was too posterior for arthroscopic removal. Notice the red-white covering contiguous with the adjacent cartilage (arrow). (**C**) Photomicrograph of the biological status of the graft at 8 weeks shows a fibrovascular repair on the surface and integration of the bone graft below (arrows). H&E stain. Magnification, 10x. (**D**) A second-look arthroscopy at 10 months showed a well-integrated graft surface still soft to palpation. This opportunity presented during anesthesia for an opposite-side knee meniscectomy. (**E**) Photomicrograph of the 10-month biopsy. Notice the dense fibrocartilage on the surface to the right side of the illustration, with rounded cells in lacunae (arrows) in a fibrous extracellular matrix. Safranin O stain. Magnification, 200x. See also Suppl. Fig. S2D. (**F**) At 18 years postoperatively, the coronal fat-suppressed proton density image shows trabecular incorporation with continuity of the subchondral cortical bone and minimal subchondral cystic degeneration within the corticocancellous graft. There is a thin covering of tissue over the osseous component of the graft with the appearance of surrounding cartilage See also Suppl. Fig. S2G.

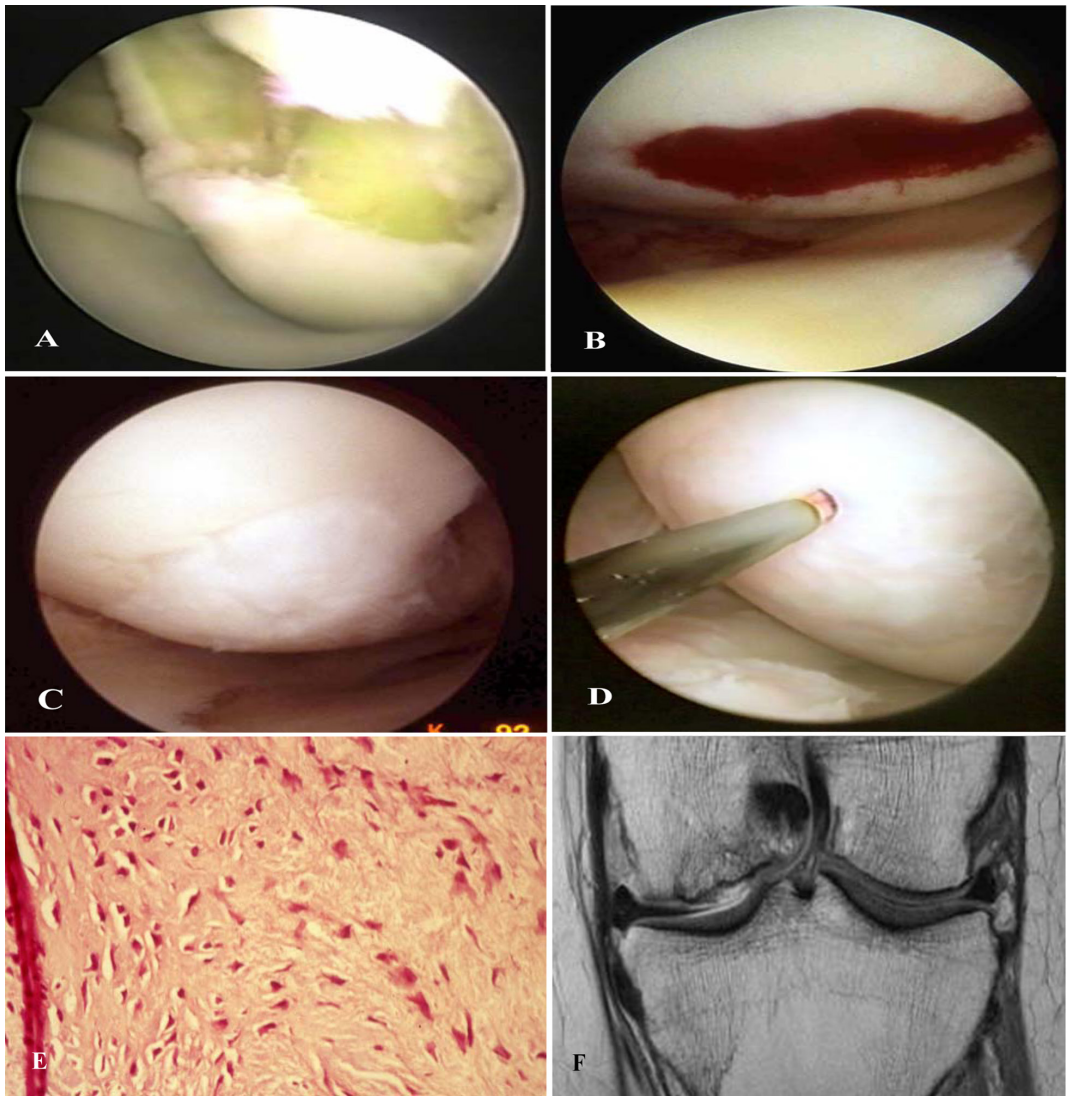

Figure 3. A representative case of those in whom the defect was filled solely with autogenous cancellous bone. A 24-year-old woman who had a large osteochondral defect after fragments of the OCD were removed by 2 previous arthroscopies. A pre-operative radiograph is in Suppl. Fig. S3A. (**A**) Arthroscopic view of the lesion after all soft tissue was debrided and the margins undercut to hold the cancellous bone graft. See also Suppl. Fig. S3B (**B**) Arthroscopic view of the grafted area immediately after blood clot formation. After time elapsed for the clot to form, the remaining blood was washed from the joint to document this biological event. (**C**) Arthroscopic view at 2 years following surgery when the patient complained of some swelling in the knee. Notice the integrity of the graft surface and continuity with the adjacent normal cartilage. (**D**) Arthroscopic view of the graft site at the time of the biopsy. The subchondral bone is seen exiting the surface at the end of the biopsy needle. (**E**) Photomicrograph of the fibrocartilaginous surface of the 2-year biopsy. Notice the lacunae surrounding many of the cells and the fibrous nature of the extracellular matrix, which are the hallmarks of fibrocartilage. Safranin O stain. Magnification, 100x. (**F**) MRI at 16 years shows an irregular pattern to the bone integration and soft tissue covering. Coronal proton density image demonstrates incorporation of the cancellous graft into the medial femoral condyle at the site of the defect. There is slight persistent depression of the articular surface at the repair site with intact but somewhat hyperintense surface tissue, likely reflecting some degree of degeneration. Additional magnetic resonance images are shown in the supplementary materials Suppl. Fig. S3C-E.

The gross appearance of the corticocancellous grafts had a similar appearance as the solely cancellous grafts after similar postoperative periods. However, in the very large lesions, the tibial and iliac crest grafts did not have sufficient volume to achieve a congruous convex articular surface at surgery. The incongruent and flat configuration remained

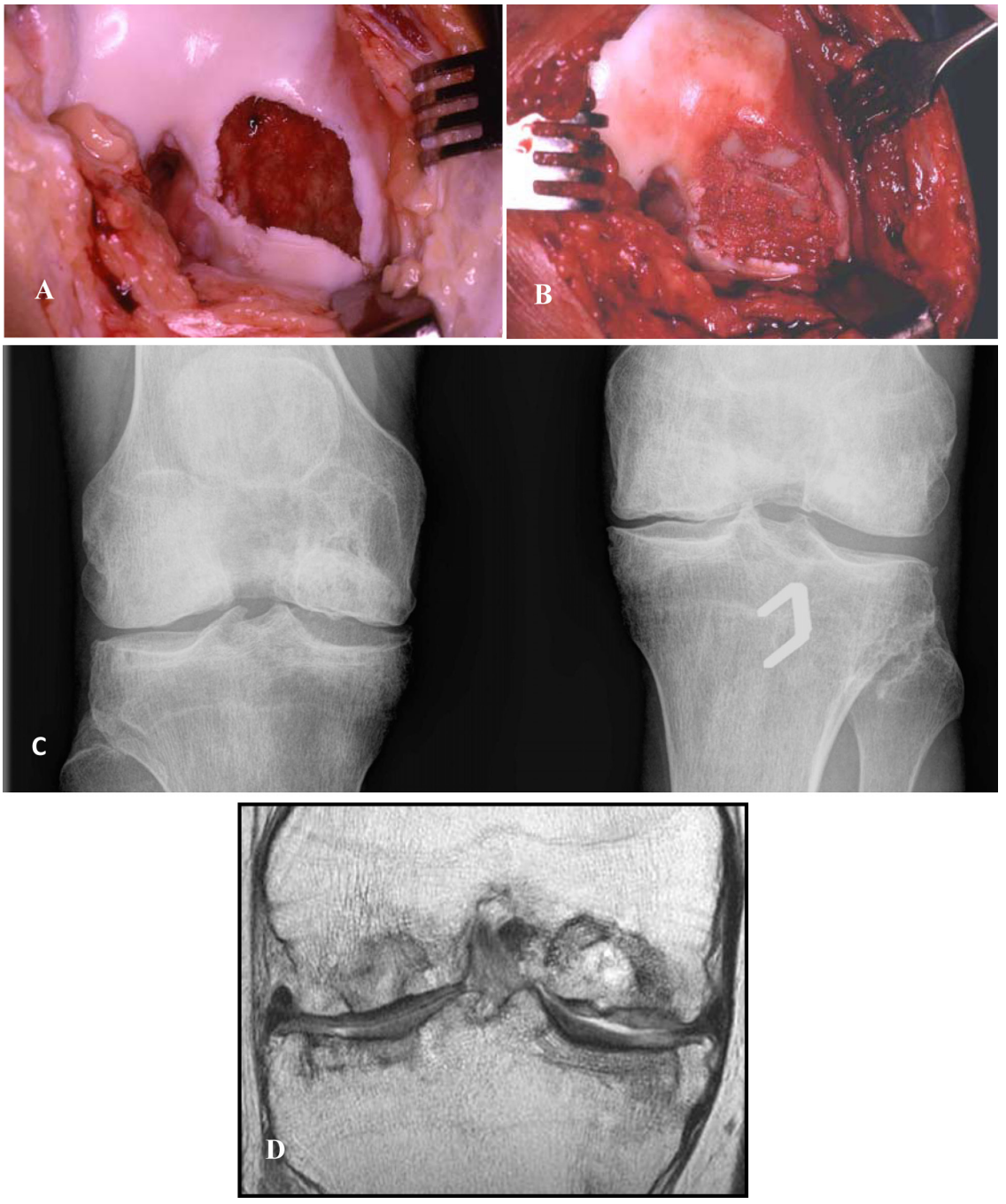

Figure 4. Representative case of a patient with a large ON defect. A 26-year-old man has massive ON of both knees and ankles secondary to chemotherapy and cortisone treatment for acute leukemia. (**A**) The right knee defect was debrided arthroscopically and then opened for bone graft from both the tibia and iliac crest because of the large size of the lesion. (**B**) The paucity of autogenous graft resulted in a flat contour to the surface, not replicating the normal convex femoral condylar geometry. (**C**) The plain film at 16 years postprocedure on the right and 13 years postprocedure of the patient aged 42 years. High tibial closing wedge osteotomies were performed on both sides due to the large-sized lesion. Medial compartmental degenerative changes are present bilaterally with mild irregularity of the articular surface and maintenance of the joint space. Subchondral mixed sclerotic and lucent regions are present in both medial femoral condyles in areas of incorporated graft material. (**D**) MRI 16 years following surgery shows the maintenance of the original flat geometry of the autogenous bone graft. Additional magnetic resonance images are shown in the supplementary materials Suppl. Fig. S4A-C.

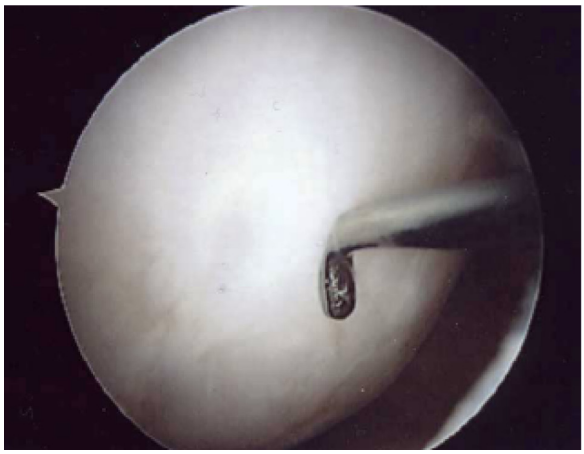

Figure 5. Representative case of a patient with ON. Second-look arthroscopy at 6 years in a medical legal case showing a healed surface, firm to probing. No biopsy was obtained at the time. This was the last arthroscopic view of the biological process during clinical care of this person as he subsequently died of natural causes.

unchanged (**Fig. 4D**). This same patient's biological response to the large bone grafts resulted in postoperative loss of range of motion due to adhesions between the graft site and the joint lining. This required 2 subsequent arthroscopic debridement procedures. Resection of the adhesions resolved the symptoms and restored motion.

A patient with an unsettled medical legal case due to iatrogenic ON had 2 subsequent arthroscopies related to continuing complaints. The second look at 6 years showed the gross anatomy to be firm to palpation and congruent with the adjacent surface without apparent pathological explanation for the symptoms (**Fig. 5**).

One patient (**Fig. 1D**) had arthroscopy at 20 years due to a recent injury. He had mild degenerative change throughout the joint as well as the surface of the previous autogenous bone graft site (**Fig. 1E**).

Second-Look Arthroscopy and Gross Pathology

There were 29 opportunities to inspect the gross anatomy of the graft site at various intervals between 8 weeks and 20 years. Both knees were involved in one patient. Three patients had 2 opportunities at different time intervals. All but one patient had the second look by arthroscopy. At 8 weeks, the grafted surface had a thin white covering and red tissue showing through (**Fig. 2B**). It was very friable to palpation. By 10 months, this grafted surface was whiter than the adjacent articular surface. It was firm to palpation (**Fig. 2D**). The same gross characteristics were there at 2 and 6 years (**Figs. 3C** and **5**).

Biopsy and Histopathology

There were 21 biopsies in 15 patients. The interval was between 8 weeks and 20 years (**Fig. 1F** and **1H**), with one opportunity at 10 months (**Suppl. Fig. S2D**) and another at 2 years (**Fig. 3E**).

Radiological Follow-up

Twenty-five of the 51 patients were available for radiological follow-up between 12 and 21 years. During the course of the long-term follow-up evaluations, it was apparent that there was a soft tissue covering seen on MRI over the area of the autogenous bone grafts (**Figs. 1G**, **2F**, **3F**, and **4D**). All donor bone sites healed (**Fig. 6**). Continuity of trabecular bone detail with a smooth transition from graft bone to native cancellous bone was consistent with graft integration. The area of the bone graft was never completely homogeneous. Bone did not grow out beyond the level of the adjacent cortex. The surface tissue overlying the graft consistently had a magnetic resonance appearance of intermediate signal intensity between that of joint fluid and meniscus, similar to that of hyaline cartilage remote from the area of grafting. Mild signal heterogeneity in the articular surface typical of degenerative change was present to varying degrees and was similar in contiguous native cartilage. There was osseous incorporation of the graft material in all groups of patients imaged including those with fixation of the native OCD fragment, those with combined corticocancellous grafts, and those with solely cancellous grafting procedures.

Histological Inspection

At 8 weeks, the cancellous grafts revealed a fibrovascular reparative tissue, approximately 3 mm in depth, overlying an active bone surface. The articular surface of the tissue, which appeared to be smooth, comprised a thin layer of cells at some locations (**Fig. 1F**). A few cells at the surface appeared rounded in lacunae, in a chondrocytic morphology. The superficial zone of the tissue (**Fig. 1F**) was hypocellular and hypovascular, relative to the underlying reparative tissue. Cells in this fibrocollagenous tissue generally appeared in fibroblast morphology (**Fig. 1F**). The surface region of the biopsy consisted of fibrous tissue with many elongated, fibroblast-like cells (**Fig. 1F**). The middle zone of the soft tissue portion of the biopsy was hypervascular, with small and large vessels distributed through a dense fibrocollagenous matrix populated with fibroblasts (**Suppl. Fig. S1A**). In the deep region of the tissue, a transition from fibrous tissue to fibrocartilage was seen, with small but notable areas of rounded cells residing in lacunae within a fibrous matrix (**Suppl. Fig. S1B**). The reparative tissue was in continuity with the underlying bone (**Suppl. Fig. S1C**), and its overall appearance was consistent with a reparative response.

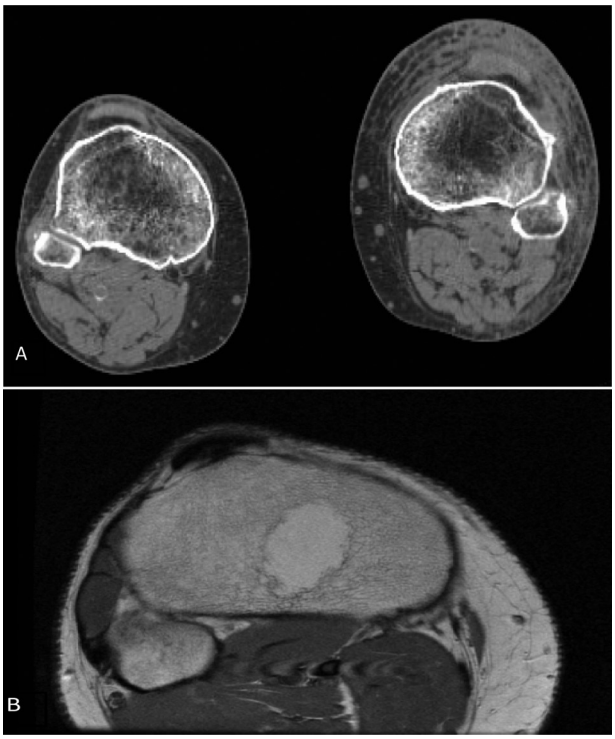

Figure 6. The bone graft donor sites healed in the proximal tibia. (**A**) The cortex formed over the site of the prior removal of the corticocancellous graft at the Gerdy tubercle as illustrated by these magnetic resonance images. (**B**) The site of the transcutaneous harvest of the tibial metaphysis shows the cortex reformed, but the small area of cancellous bone remains void in the central tibia.

Dense fibrocartilage was found in biopsies from patients at 10 months (**Fig. 2E; Suppl. Fig. S2D**) and 2 years (**Fig. 3E**). The corticocancellous grafts maintained the intact cortex, while the all-cancellous grafts had no cortical bone at 8 weeks. The cancellous grafts subsequently formed a subchondral bone plate as seen on biopsy.

The biopsy at 20 years comprised an osteochondral plug made up of the deep zone of hyaline cartilage overlying a calcified cartilage layer and subchondral bone plate (**Fig. 1H** and **1I**). Fibrocartilage was above the hyaline cartilage and extended to the surface (**Fig. 1I**). The superficial zone of the plug was irregular with many fissures and areas of fragmentation (**Fig. 1I**). There were fewer cells in the surface zone, which was more eosinophilic than the bulk of the cartilage (**Fig. 1I**, arrow). Examination of the histological sections by polarized light microscopy, to reveal the collagen organization, demonstrated the more fibrous appearance of the matrix in the fibrocartilaginous zone (**Suppl. Fig. S1F**) superficial to the hyaline cartilage at the base (**Suppl. Fig. S1G**). The polarized light microscopy also showed that collagen bundles were aligned perpendicular to the tidemark (**Suppl. Fig. S1G**). The lamellar organization of the underlying bone was also revealed by polarized light microscopy (**Suppl. Fig. S1G**).

A large portion of the section stained positive with Safranin O (**Fig. 1J**), indicating the presence of sulfated glycosaminoglycans (GAGs). Closer inspection of the tidemark region revealed that positive staining was located largely above the newly remodeled tidemark (**Suppl. Fig. S1H**).

The distribution of the staining intensities for type II collagen (**Fig. 1K**) was, in part, similar to that seen for Safranin O. Positive staining was localized in the matrix, and no intracellular retention of type II collagen was noted (**Suppl. Fig. S1I**). The negative controls did not stain for type II collagen.

Masson's trichrome staining revealed the presence of collagen in the majority of the extracellular matrix of the tissue above the tidemark (**Suppl. Fig. S1 J and K**). Of note was the fact that while the surface of the biopsy tissue showed positive staining for collagen using Masson's trichrome (**Suppl. Fig. S1 K**), very little positive staining for type II collagen was seen in the same area (**Fig. 1K**), suggesting that the collagen located at the surface was primarily type I collagen. This is consistent with the finding that the tissue was fibrocartilaginous at the surface.

Of note was the consistent finding of a thin, dense discrete layer of positive staining for lubricin on the articulating surface of the biopsy section (**Fig. 1L**). The negative

controls did not stain for lubricin. The freshly cut surfaces of the biopsy produced during trimming of the tissue did not stain for lubricin (**Suppl. Fig. S1L**), indicating that the lubricin surface layer was not edge-effect artifactual staining. The discrete layer of lubricin covered the surfaces of the crevices and fissures through the tissue (**Fig. 1L; Suppl. Fig. S1L-N**) and was on the surfaces of the fragments of the tissue (**Suppl. Fig. S1M and N**). Significant diffuse staining of the extracellular matrix was also located near the surface to variable depths ranging from 150 to 500 μm below the surface (**Fig. 1L**). Notably, no lubricin staining was seen in the deeper regions of the biopsy section. No detectable intracellular staining was observed.

Discussion

The biological fate of the autogenous bone graft matrix in this study showed integration to the host bone plus long-term survival of the newly formed articular surface. The hypothesis was confirmed by planned MRI, second-look arthroscopy, biopsy, and histomorphological inspection.

Second-look arthroscopy with biopsy at various intervals showed the early bone integration. The long-term radiological study of the patient with the very large surgical defects showed the retention of the original incongruous contour of the bone graft geometry. This observation would suggest that future bone grafts for large surface lesions should replicate the normal articular contour, perhaps with additional use of the convex surface of the Gerdy tubercle cortical cancellous graft for large defects.

The biopsies showed the articular surface to be fibrous tissue at 8 weeks and dense fibrocartilage after several months. MRI showed there was long-term maintenance of a soft tissue covering over the entire bone graft. The exact nature was not known until a 20-year second look and biopsy presented in one patient near the end of the follow-up study. This extensive histological analysis on this patient provides a rare opportunity to document the biological events following the use of an autogenous bone graft as matrix for a large-volume osteochondral defect of the knee joint femoral condyle. Clinically, the patient was asymptomatic for 20 years. MRI taken prior to the injury showed integration of the bone graft, although not completely homogeneous. Identification of the exact graft site was confirmed by review of the libraried prior surgical videotapes. The gross anatomy of the articular cartilage formation was congruent with the adjacent joint surface at 8 weeks and 20 years. The nature of the soft tissue covering of the bone graft identified by MRI was not known until the subsequent 20-year biopsy. This single case showed the soft tissue covering to consist of a mix of fibrocartilage and hyaline cartilage despite the presence of subsequent mild, diffuse degenerative arthritis as also seen in the remainder of the medial compartment. It was notable that the normal distribution of the important cartilage-lubricating molecule (lubricin) was seen on the 20-year biopsy surface. There was no intracellular staining of lubricin, suggesting the possibility that the lubricin may have been produced outside the reparative tissue and diffused from the surrounding synovial fluid rather than being produced by cells within the tissue itself. However, given the depth of some of the diffuse staining for lubricin in the extracellular matrix (up to 500 μm below the surface), it seems less likely that the molecule would have been absorbed from the joint fluid.

In retrospect, it should not have been surprising that fibrocartilage has long-term survival potential considering that the fibrocartilagenous meniscus is native to the synovial joint. The meniscus survives under pressure and shear and provides a gliding surface. Animal studies show a similar biological fate of autogenous bone grafting to large osteochondral defects.[19,20]

The surgical removal of the OCD fragment has poor outcomes.[21] Therefore, the attempts at preservation have included *in situ* drilling, replacement, screw fixation, osteochondral autografts, and allografts.[21-27] Present treatments for ON include drilling, cancellous packing, unicompartmental replacement, tibial osteotomy, and total knee arthroplasty.[27-31]

Although various biological matrixes have been proposed in recent years, autogenous bone appears to be a cost-effective, readily available matrix for large osteochondral defects.[32-34] The autogenous nature removes the risk of complications of the allograft surgery while resulting in a long-lasting biological solution for both the bone and articular surface.

The strengths of this report were the prospective design, the long-term follow-up, the high quality and consistent availability of an EMR, the archived videotapes for identification of locations of prior surgical sites for correlation with follow-up imaging and arthroscopy, and the gross and microscopic pathology provided by second-look arthroscopy with biopsy. There were pathological studies ranging from 8 weeks to 20 years supported by radiological evaluations. The magnetic resonance examinations were performed with a very high in-plane resolution of 0.31×0.42 mm in the sagittal and coronal planes and 0.27×0.36 mm in the axial plane, providing excellent delineation of cartilage features with clear discrimination of articular surface tissue from subchondral bone and articular fluid.

The weaknesses of this study were the nonrandomization and dependence upon historic controls. Gross and microscopic studies were not planned at staged intervals but were obtained when the opportunity was clinically possible. There was the absence of cartilage-specific magnetic resonance sequences. The MRI for these examinations was a higher resolution version of imaging protocols, which were considered advanced at the time our follow-up study was initiated.

Unfortunately, we did not perform quantitative imaging of cartilage in our patients. This study was contemporaneous with the development of GAG imaging with delayed gadolinium enhancement MRI and before this technique was performed at our institution.[35] Additionally, other measures of cartilage integrity, such as T2-mapping and magnetization transfer imaging, were not performed, which may have offered insight into the nature of the neoarticular tissue formed over these grafts. These methods may prove beneficial in future follow-up assessments as this novel approach to the treatment of large osteochondral defects is further refined. The clinical results will be the subject of a separate report.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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