Articular Cartilage Regeneration With Autologous Peripheral Blood Progenitor Cells and Hyaluronic Acid After Arthroscopic Subchondral Drilling: A Report of 5 Cases With Histology

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Purpose: The purpose of this study was to evaluate the quality of articular cartilage regeneration after arthroscopic subchondral drilling followed by postoperative intraarticular injections of autologous peripheral blood progenitor cells (PBPCs) in combination with hyaluronic acid (HA). **Methods:** Five patients underwent second-look arthroscopy with chondral core biopsy. These 5 patients are part of a larger pilot study in which 180 patients with International Cartilage Repair Society grade III and IV lesions of the knee joint underwent arthroscopic subchondral drilling followed by postoperative intra-articular injections. Continuous passive motion was used on the operated knee 2 hours per day for 4 weeks. Partial weight bearing was observed for the first 6 to 8 weeks. Autologous PBPCs were harvested 1 week after surgery. One week after surgery, 8 mL of the harvested PBPCs in combination with 2 mL of HA was injected intra-articularly into the operated knee. The remaining PBPCs were divided into vials and cryopreserved. A total of 5 weekly intra-articular injections were given. **Results:** Second-look arthroscopy confirmed articular cartilage regeneration, and histologic sections showed features of hyaline cartilage. Apart from the minimal discomfort of PBPC harvesting and localized pain associated with the intra-articular injections, there were no other notable adverse reactions. **Conclusions:** Articular hyaline cartilage regeneration is possible with arthroscopic subchondral drilling followed by postoperative intraarticular injections of autologous PBPCs in combination with HA. **Level of Evidence:** Level IV, therapeutic case series.

Chondral defects of the major weight-bearing joints currently pose an unresolved issue among orthopaedists. Marrow stimulation techniques, such as microfracture, have become the first-line treatment of small chondral defects of the knee because they are minimally invasive and have had proven results over the past 20 years.[1,2] These techniques stimulate marrow repair processes that produce fibrocartilage.[3,4] Recent animal studies and newer cartilage repair techniques have aimed at the regeneration of hyaline cartilage. An adjunct to marrow stimulation that achieves this goal is ideal.

Intra-articular injections of hyaluronic acid (HA) have recently proven beneficial to cartilage health and repair, illustrating the relation to increased differentiation of immature cells to chondrocytes, decreased joint inflammation, increased proteoglycan content in repair cartilage, improved histologic scores after cartilage repair, improved defect filling/incorporation af-

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ter cartilage repair, and decreased postprocedural coefficients of friction.[5-10] In addition, animal studies involving intra-articular injections of bone marrow–derived progenitor cells (BMPCs) and HA have documented histologic findings consistent with hyaline cartilage.[8,11] BMPCs used in these scenarios have shown an innate quality allowing for the migration and integration at the sites of cartilage repair.[11]

In preclinical animal studies in a goat model, the first author concluded that postoperative intra-articular injections of autologous BMPCs in combination with HA after subchondral drilling resulted in improved cartilage repair.[8] A clinical pilot study followed and involved standard marrow stimulation in the form of arthroscopic subchondral drilling and postoperative intra-articular injections of autologous peripheral blood progenitor cells (PBPCs) in combination with HA. Our objective was to assess whether the preclinical animal model could be replicated in the human knee joint. The purpose of this study was to evaluate the quality of resultant articular cartilage regeneration. We hypothesized that articular hyaline cartilage regeneration is possible with our novel approach.

METHODS

Patient Selection

Five patients underwent second-look arthroscopy with chondral core biopsy. These 5 patients are part of a larger pilot study in which 180 patients who presented with chondral defects of the knee joint were recruited. Postoperatively, the clinical course of these 5 patients presented an opportunity for a second-look arthroscopy. Two patients underwent contralateral knee surgery, and one patient had removal of a Tomofix plate and screw construct (Synthes, West Chester, PA), providing an opportune setting of anesthesia for second-look arthroscopy. One patient had recurrence of discomfort attributed to a prominent osteophyte and elected for a further arthroscopic procedure. The last patient had returned to football 18 months after articular cartilage repair and sustained a torn anterior cruciate ligament of the previously treated knee. He elected for arthroscopic reconstruction, which provided an opportunity for second-look arthroscopy. Informed consent after discussion of risks and benefits, as well as local ethics committee approval, was obtained before biopsy.

The diagnosis of chondral injury was made after clinical and radiologic evaluation. Chondral lesions were graded according to the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package.[12] The inclusion criteria were patients with ICRS grade III and IV lesions, defects of any size and number, age 18 to 60 years, deformity (lateral patella maltracking or axis correction) correctable at the time of surgery, and ligamentous instability deemed reconstructable at the same time. The exclusion criteria were patients with disease progression such that total knee arthroplasty was indicated; a history of infected knees, gross bone defects, rheumatoid arthritis, or intra-articular corticosteroids within the previous 6 weeks; and gross valgus or varus deformity not correctable during surgery.

Procedure

All surgical procedures were performed by a single surgeon (by the first author) involving standard arthroscopic techniques in the supine position without a tourniquet. Saline solution irrigation bags were chilled in an ice-water bath before use to minimize bleeding during the arthroscopic procedure. In our experience, we have had difficulty performing microfracture to the patella and areas of the plateau. For this reason, our preferred method is arthroscopic subchondral drilling modified from the principles established by Steadman et al[13] for microfracture and Pridie[14] for drilling. We begin by defining the extent of cartilage injury with a probe. A 3.5-mm full-radius shaver is used to debride loose cartilage to a stable margin; often a straight or curved arthroscopic biter is required as well. A 2-mm burr, with its guard removed, "drills" from the surface of the defect to the bone marrow, creating a conduit. The remaining area within the margin is also drilled to a depth of 5 to 10 mm. Initially, we spaced drill holes 3 to 4 mm apart. The methods have subsequently evolved so that a goal of 1 to 2 mm between drill holes is now preferred based on the results of second-look arthroscopy. It is not crucial that the subchondral drilling be performed perpendicular to the bone surface because a lesser angle of drilling capable of penetrating into the subchondral bone is sufficient. Abrasion chondroplasty up to a depth of 1 mm is performed with burring of the bony area between drill holes. The result is an extended area of bleeding bone, hence a larger surface area for the initiation of articular cartilage repair with PBPCs and HA (Fig 1). The arthroscopic portals are closed with No. 3-0 nylon suture. A mixture of 20 mL of 0.5% bupivacaine hydrochloride and epinephrine, 3 mL of 1-mg/mL

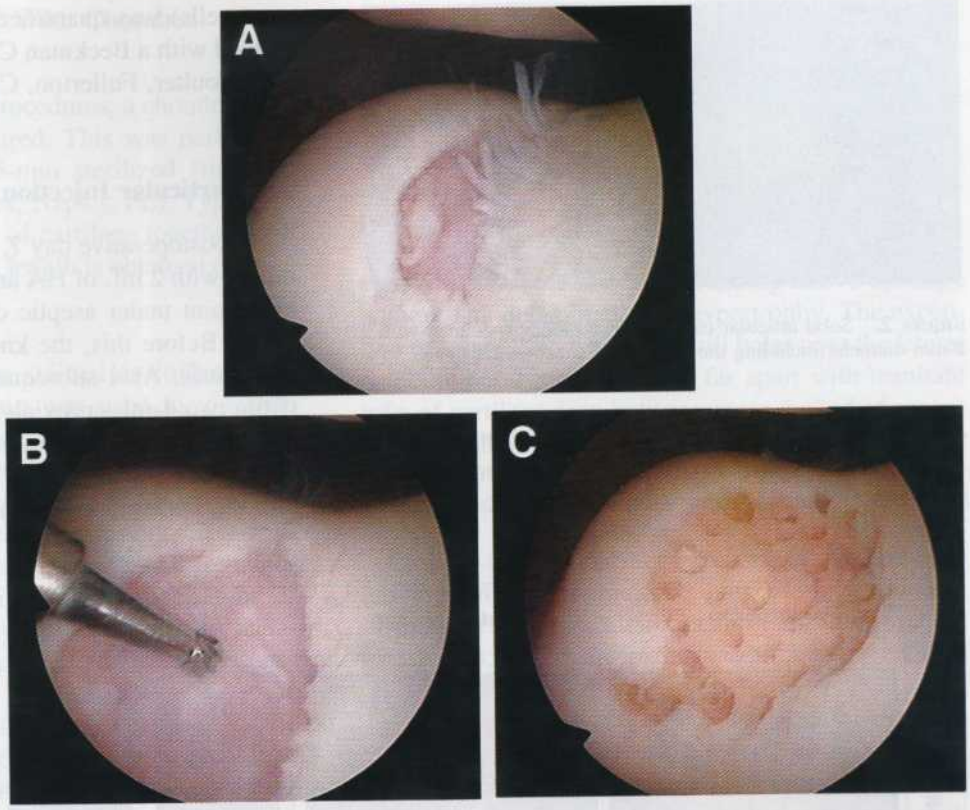

FIGURE 1. Subchondral drilling. (A) A delaminated flap tear on the medial and central trochlear area of a left knee. (B) A 2-mm burr with the guard removed allows for drilling with light suction. (C) View after debridement, subchondral drilling, and abrasion chondroplasty.

morphine, and 2 mL of HA (Hyalgan; Fidia Farmaceutici, Abano Terme, Italy) is injected into the operated knee at the end of the surgical procedure.

Postoperative Rehabilitation

Cold therapy is initiated immediately in the postanesthesia period and continued throughout the first month after surgery, including 1 hour 2 to 3 times per day. On the first postoperative day, continuous passive motion is used on the operated knee for a duration of 2 hours. This is continued daily for a period of 4 weeks. The range of motion is initially set at 0° to 30° and progresses as the clinical situation improves. Patients with subchondral drilling to the weight-bearing femorotibial joint are instructed on crutch-assisted partial weight bearing (15 to 20 kg) for the first 4 weeks. This progresses to full weight bearing in 6 to 8 weeks. Patients with drilling to the patellofemoral joint are allowed full weight bearing as tolerated with restrictions from weight bearing on stairs for the first 3 months after surgery. This is to avoid overloading the patellofemoral joint.

Neupogen Administration, Apheresis, and Cryopreservation

Human granulocyte colony–stimulating factor is a glycoprotein that regulates the production and release of functional neutrophils from the bone marrow. Neupogen contains recombinant granulocyte colony–stimulating factor and causes marked increases in peripheral blood neutrophil counts with a minor increase in monocytes within 24 hours. On postoperative days 4, 5, and 6, patients were given a morning dose of 300 μg of Neupogen (Filgrastim, Amgen, Thousand Oaks, CA) subcutaneously. On postoperative day 7, autologous PBPCs were collected by an automated cell separator (apheresis) by central venous access. Venous access was achieved through a femoral double-lumen catheter placed into the contralateral leg, under ultrasound guidance, performed by a consultant radiologist. Apheresis was performed by use of the Spectra Optia Apheresis Machine (Caridian BCT, Denver, CO). A fresh aliquot of 8 mL of PBPCs was separated for fresh intra-articular injection into the operated knee. The remaining PBPCs were cryopreserved in 10% dimethyl sulfoxide and divided into

FIGURE 2. Solid articular cartilage core biopsy specimen with a 2-mm diameter including the underlying subchondral bone.

4-mL cryovials for storage in liquid nitrogen at –196°C. Flow cytometry with $CD34^+$ (hematopoietic stem cells) and CD105+ (markers for mesenchymal stem cells) was quantified. Flow cytometry was performed with a Beckman Coulter FC500 device (Beckman Coulter, Fullerton, CA).

Intra-articular Injection

On postoperative day 7, 8 mL of the fresh PBPCs is mixed with 2 mL of HA and injected into the operated knee joint under aseptic conditions in the outpatient clinic. Before this, the knee is first aspirated for hemarthrosis. At 4 subsequent weekly intervals, 8 mL (from two 4-mL cryovials) of the frozen PBPCs were obtained from the laboratory, allowed to thaw to room temperature, mixed with 2 mL of HA, and injected into the operated knee joint.

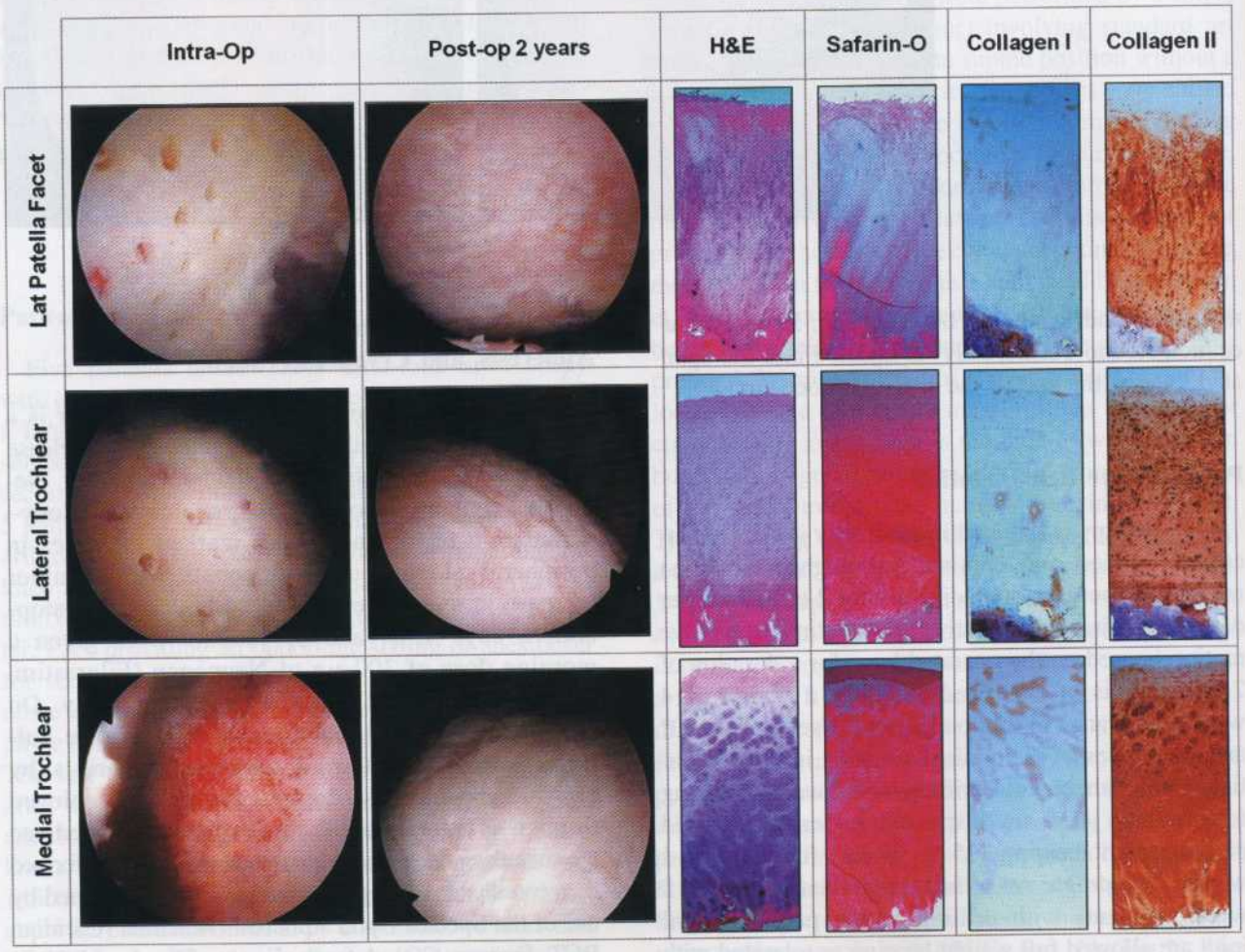

FIGURE 3. A 34-year-old woman with recurrent dislocation of her patella as an adolescent. Second-look arthroscopy from the lateral patella facet and lateral trochlear area showed tufts of cartilage forming at each individual drill hole. Histologic examination shows the red staining with safranin O representing proteoglycans and the brown staining of collagen type II diffuse throughout the regenerated tissue. Collagen type I stain is minimal and localized near the superficial layers. (Original magnification ×40.)