ARTHRITIS & RHEUMATISM
Vol. 48, No. 12, December 2003, pp 3464–3474
DOI 10.1002/art.11365

Stem Cell Therapy in a Caprine Model of Osteoarthritis

J. Mary Murphy,[1] David J. Fink,[1] Ernst B. Hunziker,[2] and Frank P. Barry[1]

Objective. **To explore the role that implanted mesenchymal stem cells may play in tissue repair or regeneration of the injured joint, by delivery of an autologous preparation of stem cells to caprine knee joints following induction of osteoarthritis (OA).**

Methods. **Adult stem cells were isolated from caprine bone marrow, expanded in culture, and transduced to express green fluorescent protein. OA was induced unilaterally in the knee joint of donor animals by complete excision of the medial meniscus and resection of the anterior cruciate ligament. After 6 weeks, a single dose of 10 million autologous cells suspended in a dilute solution of sodium hyaluronan was delivered to the injured knee by direct intraarticular injection. Control animals received sodium hyaluronan alone.**

Results. **In cell-treated joints, there was evidence of marked regeneration of the medial meniscus, and implanted cells were detected in the newly formed tissue. Degeneration of the articular cartilage, osteophytic remodeling, and subchondral sclerosis were reduced in cell-treated joints compared with joints treated with vehicle alone without cells. There was no evidence of repair of the ligament in any of the joints.**

Conclusion. **Local delivery of adult mesenchymal stem cells to injured joints stimulates regeneration of meniscal tissue and retards the progressive destruction normally seen in this model of OA.**

Mesenchymal stem cells (MSCs) have the capacity to differentiate into a variety of connective tissue cells (1–4) including bone, cartilage, tendon, muscle, and adipose tissue (3,5–10). These cells may be isolated from bone marrow with ease and expanded in culture through many generations, while retaining their capacity to differentiate when exposed to appropriate signals. The isolation of these cells from adult tissues raises opportunities for the development of novel cellular therapies without the ethical considerations associated with the use of embryonic stem cells. Multipotent cells have been isolated from various mesenchymal tissues in adults, including skeletal muscle, fat, and synovial membrane (11–13) as well as hematopoietic (14), neural (15), and hepatic (16) tissues. Because of their multipotentiality and capacity for self-renewal, adult stem cells may represent units of active regeneration of tissues damaged as a result of trauma or disease (14). In certain degenerative diseases such as osteoarthritis (OA), stem cells are depleted and have reduced proliferative capacity and reduced ability to differentiate (17). The systemic or local delivery of stem cells to these individuals may therefore enhance repair or inhibit the progressive loss of joint tissue.

OA is characterized by degeneration of the articular cartilage, with loss of matrix, fibrillation, formation of fissures, and ultimately complete loss of the cartilage surface. Other articular tissues are also affected, including the subchondral bone, ligaments, joint capsule, synovial membrane, and periarticular muscles (18,19). Although OA affects a large proportion of the population, there are few, if any, effective therapies available today that alter the pathobiologic course of the disease (20).

The objective of this study was to determine if delivery of stem cells to the knee following traumatic injury would enhance repair of damaged tissue or impede the progression to OA that is the usual consequence of such injuries (21). There are several examples of the use of stem cells for articular cartilage repair (22,23). There are also reports describing the use of these cells for the repair of segmental bone defects in long bones (24). However, there are no reports describing the use of these cellular therapies in trauma-induced OA. In general, the cells have been delivered to either cartilage or bone using a 3-dimensional scaffold that is fixed to the defect site, usually by means of an open

[1]J. Mary Murphy, PhD, David J. Fink, PhD, Frank P. Barry, PhD: Osiris Therapeutics, Baltimore, Maryland; [2]Ernst B. Hunziker, MD: M. E. Müller Institute for Biomechanics, University of Bern, Bern, Switzerland.

Address correspondence and reprint requests to Frank P. Barry, PhD, Osiris Therapeutics Inc., 2001 Aliceanna Street, Baltimore, MD 21231. E-mail: fbarry@osiristx.com.

Submitted for publication February 4, 2003; accepted in revised form August 28, 2003.

surgical procedure. There are many issues associated with the selection of the scaffold material, including its ability to support cell viability and differentiation and its retention and degradation in situ.

In the present study, we used a simpler, scaffold-free approach in which the cells were delivered as a suspension by direct intraarticular injection. The suspension was prepared in a dilute solution of sodium hyaluronan, which is commonly used for the treatment of OA (25) and also has the effect of increasing the chondrogenic activity of MSCs (26). There was evidence of cell engraftment in several tissues in the joint and a marked regeneration of the excised meniscus. In addition, there was evidence of a reduction in OA progression in the cell-treated joints. We suggest that the enhanced repair response results from interaction between the implanted cells and fibroblasts derived from the host synovium at the site of injury.

MATERIALS AND METHODS

Preparation of caprine MSCs. A heparinized bone marrow aspirate (3–7 ml) was obtained from the iliac crest of castrated male Western Cross goats. The aspirate was washed with medium (Dulbecco's modified Eagle's medium [DMEM]–low glucose; Hyclone, Logan, UT) containing 1% antibiotic–antimycotic (Gibco, Grand Island, NY), and centrifuged. The precipitated cells were then suspended in medium with 10% fetal bovine serum (FBS; Hyclone) at a final density of 1.4–1.6 $\times$ 10^6 cells/ml. Cells were seeded on T-185 flasks and maintained at 37°C with 95% humidity and 5% CO_2 in the same medium. After 5 days, red blood cells were washed off with phosphate buffered saline (PBS) and fresh medium was added. Colonies of adherent cells formed within 9 days and the colonies were trypsinized from the flasks when the colonies covered 60–90% of the plate. The cells were cryopreserved at the end of primary culture.

Differentiation assays. Chondrogenesis was performed with modifications of the pellet culture system described previously (7,9), using dexamethasone at 10 n*M* (Sigma, St. Louis, MO) and 10 ng/ml recombinant human transforming growth factor β3 (Oncogene Research Products, San Diego, CA). After 14 days in culture, pellets were harvested and fixed in 10% formalin, and cut sections were stained with Safranin O and with an antibody specific for type II collagen (27). For osteogenic differentiation, the cells were cultured in 10 n*M* dexamethasone, 10 n*M* β-glycerophosphate, 50 μM ascorbic acid 2-phosphate, 100 n*M* prostaglandin E_2, and 5% FBS (6,28). To evaluate the differentiation potential of the cells in ectopically implanted scaffolds, MSCs were loaded onto hydroxyapatite/tricalcium phosphate cubes (3 $\times$ 3 $\times$ 3 mm; Zimmer, Warsaw, IN) at a density of 5 $\times$ 10^6 cells/ml, and implanted subcutaneously on the dorsal surface of nude mice (5). The cubes were removed 6 weeks later and sections were stained with hematoxylin and modified Mallory aniline blue for the presence of bone and cartilage.

Retroviral vector construction and virus production. Caprine MSCs were transduced to express the *Aequorea Victoria* enhanced green fluorescent protein (eGFP) using the retroviral vector pOT24. The construction of this vector has been described previously (29). Briefly, the GFP-1 gene from pEGFP-1 (Clontech, Palo Alto, CA) was cloned into the pJM573neo retroviral vector (30,31). Vector supernatants containing different pseudotyped vector particles were generated using the gibbon ape leukemia virus envelope containing the PG13 packaging cell line (32). Retroviral supernatants containing the GFP vector were produced as follows: plasmid pOT24 was transfected into GP&E86 ecotropic producer cells (American Type Culture Collection [ATCC] CRL-9642) (33) using DOTAP (Boehringer-Mannheim, Indianapolis, IN) as directed by the manufacturer. The transfected cells were grown at 37°C, 5% CO_2, 90% humidity in DMEM–high glucose medium supplemented with 10% heat-inactivated FBS, 1% penicillin–streptomycin, and 0.5 mg/ml protamine sulfate–G418 (Sigma) as a selective agent.

Cultures were grown to 70% confluence, the medium was replaced with fresh retroviral medium (without G418), and the cells were incubated at 32°C for 2 days. The ecotropic retrovirus was used to transduce the gibbon ape leukemia virus envelope containing the PG13 retroviral producer cells (ATCC CRL-9078). Transduction was performed using the centrifugal transduction procedure outlined below, followed by selection with G418 (0.5 mg/ml). The culture medium containing the retroviral vectors was collected, filtered through a 0.45-μm filter, and stored at −80°C.

Transduction and expansion of caprine MSCs. Primary frozen cultures of caprine MSCs were thawed and plated in T-75 flasks at 500,000 cells/flask with DMEM–high glucose containing 1% antibiotic–antimycotic and 10% FBS. After 1 day, the culture medium was aspirated and 15 ml of retroviral supernatant, containing 8 mg/ml polybrene (Sigma), was added to each flask. Culture medium with polybrene only was used for mock-transduced cultures. Following transduction for 24 hours, the vector-containing medium was aspirated and a second round of transduction was performed with fresh retroviral supernatant. Selection of transduced cells with G418 (1.0 mg/ml) was performed for 7–10 days until confluence was reached. The selected cells were trypsinized and expanded to the end of passage 2. A sample of the cells was washed with PBS containing 2% weight/volume bovine serum albumin and 0.1% w/v sodium azide for assessment of eGFP using fluorescence-activated cell sorter (FACS) analysis. Intrinsic fluorescence from the GFP-transduced MSCs was compared with that from untransduced controls. All cells were resuspended in FACS buffer containing 1% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) immediately before analysis. Cells were analyzed by collecting 10,000 events on a Becton-Dickinson Vantage instrument using CellQuest software (Becton-Dickinson, Franklin Lakes, NJ).

Surgical protocol. The Institutional Animal Care and Use Committee approved all procedures used. Castrated male Western Cross goats (weighing 70–112 kg, ages 29–34 months; n = 24), confirmed to be free of Q fever, brucellosis, and caprine arthritis encephalitis, were used. Animals were treated with butorphenol (0.05–0.10 mg/kg) and diazepam (0.1–0.2 mg/kg) by intravenous injection and with an equal mixture of ketamine (100 mg/ml) and diazepam (5 gm/ml) at a dose of 0.5 ml/kg as

Table 1. Study design*

Group	No.	Treatment	Postsurgery termination, weeks
1 (control)	3	Vehicle injection 6 weeks postsurgery	12
2 (test)	6	GFP-transduced MSC/vehicle injection 6 weeks postsurgery	12
3 (control)	6	Vehicle injection 6 weeks postsurgery	26
4 (test)	9	GFP-transduced MSC/vehicle injection 6 weeks postsurgery	26

* GFP = green fluorescent protein; MSC = mesenchymal stem cell.

anesthetic. Intraoperative anesthesia was maintained with isoflurane (~1.5%) in oxygen delivered by endotracheal tube.

For combined anterior cruciate ligament (ACL) transection and medial meniscectomy, a lateral parapatellar skin incision was made beginning at a level 2 cm proximal to the patella and extending to the level of the tibial plateau. Subcutaneous tissue was incised, and the lateral fascia was separated from the joint capsule for ~1 cm in either direction away from the incision. The lateral aspect of the vastus lateralis and the joint capsule were incised and the patella was luxated medially to expose the trochlear groove and medial and lateral condyles of the distal femur.

ACL removal was performed by first excising its attachment on the medial aspect of the lateral femoral condyle. The proximal attachment was brought forward and the entire ligament was excised from its tibial attachment. The stifle was moved in a drawer test to ensure that the entire cruciate ligament had been excised. The medial meniscus was removed by sharp excision. The caudal horn of the meniscus was grasped with a hemostat and its axial (lateral) attachment was excised from its tibial attachment. Working from caudal to lateral, then cranial, the meniscus was excised from its attachments until it was completely removed. ACL excision was carried out first, followed by meniscectomy.

Study design. Animals were randomized into 4 groups as described in Table 1 and Figure 1. The control groups were not different from the test groups with respect to age and weight, but the test animals underwent a bone marrow aspiration for cell preparation 2 weeks prior to surgery. OA was induced unilaterally in the knee joint of donor animals by complete excision of the medial meniscus and resection of the ACL as described above, and after a recovery period of 3 weeks, all animals were exercised once daily by having them run on a hard surface for a distance of 90 meters. At all other times, animals were allowed free movement in an unconfined environment. Control animals received an injection of vehicle alone in the operated joint 6 weeks after surgery. This consisted of 5 ml sodium hyaluronan (Hylartin-V; Pharmacia & Upjohn, Peapack, NJ) at a concentration of 4 mg/ml. Test animals received a single intraarticular injection of 10×10^6 autologous GFP-transduced MSCs as a suspension in the vehicle at 6 weeks after surgery.

Intraarticular injection of MSCs. Frozen cells were thawed rapidly at 37°C, washed with culture medium and PBS, centrifuged, and resuspended in Hylartin solution at a density of 2×10^6 cells/ml. Goats were anesthetized, intubated, and placed in dorsal recumbency. Five milliliters of the cell suspension was injected into the medial compartment of the operated joint after aspiration of synovial fluid. An 18-gauge needle was inserted posterior to the medial edge of the patellar ligament, through the triangle formed by the epicondyle of the femur, the meniscal/tibial plateau, and the notch formed by their junction. Following injection, the joint was repeatedly flexed and extended for dispersal of the suspension throughout the intraarticular space.

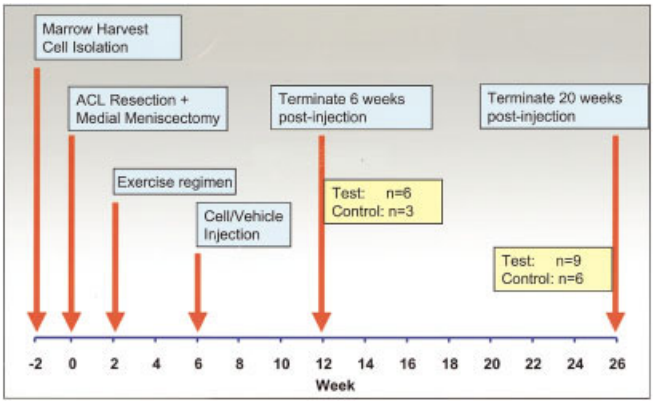

Figure 1. Study design. Experimental plan used to evaluate the effect of delivery of autologous stem cells to the knee following meniscectomy and resection of the anterior cruciate ligament (ACL) for induction of osteoarthritis. Color figure can be viewed in the online issue, which is available at http://www.arthritisrheum.org.

Histochemical analysis of bone and cartilage. Immediately after the animals were killed, the distal head of the femur and the proximal tibial plateau were removed and fixed in 10% formalin. The medial condyles and the anterior portion of the tibial plateau were dehydrated through a series of increasing ethanol concentrations and embedded in methyl methacrylate. Serial sagittal sections were cut using a diamond

Table 2. Histologic grading scheme

Parameter, grade	Description
Articular cartilage structure	
0–10	Ref. 35*
Reduction of articular cartilage matrix staining	
0	None
1	Mild
2	Moderate
3	Severe
Presence of osteophytes	
0	None
1	Cartilage/connective tissue
2	Mainly cartilage/some bone formation
3	Mainly bone formation
Subchondral bone plate thickening	
0	None
1	Mild
2	Moderate
3	Severe

* 0 = normal; 10 = complete loss to subchondral bone.

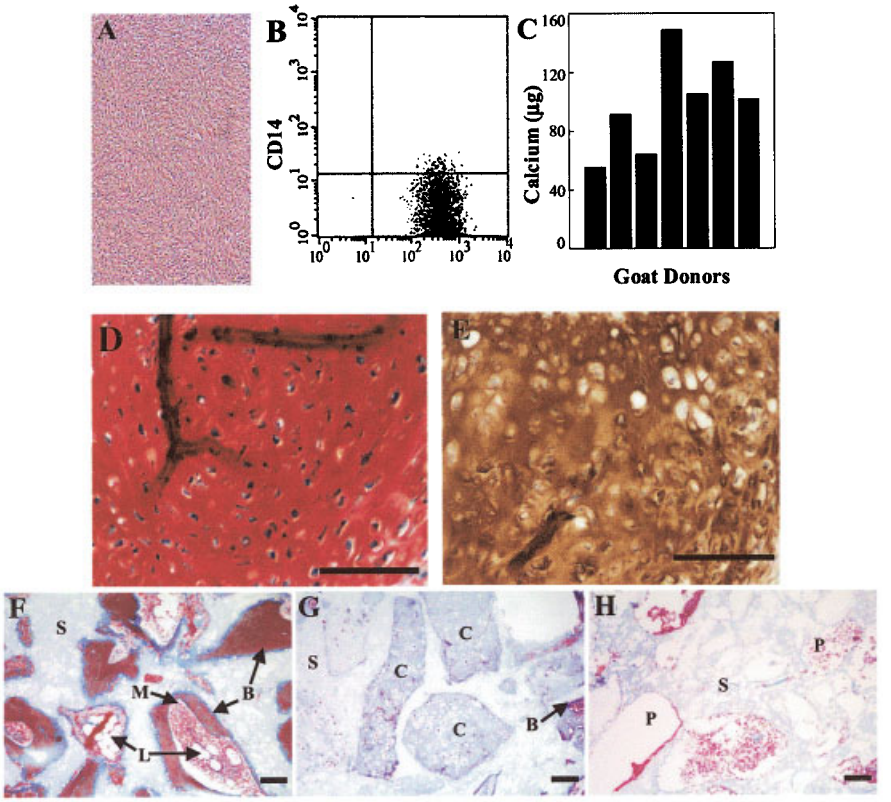

Figure 2. Isolation and characterization of mesenchymal stem cells (MSCs) from goat marrow. Adherent cells from bone marrow aspirates grew as a monolayer in passage 1 culture **(A)** (original magnification × 40) and were uniformly SH4+/CD14− by fluorescence-activated cell sorter analysis **(B)**. Goat MSCs deposited calcium in osteogenic cultures in vitro **(C)** and were capable of elaborating a proteoglycan-rich **(D)** and type II collagen–positive **(E)** extracellular matrix in chondrogenic pellet cultures in vitro. Goat MSCs loaded on a hydroxyapatite/tricalcium phosphate scaffold (S) and implanted subcutaneously in nude SCID mice showed differentiation into bone (B), fat (L), marrow (M), and cartilage (C) after 6 weeks in vivo **(F** and **G),** while control, unloaded scaffolds showed no evidence of mesenchymal differentiation and the pores (P) were empty or infiltrated with host hematopoietic cells **(H)**. Bar = 100 μM.

saw (Leco, St. Joseph, MN) from the tissues of both the operated and contralateral (unoperated) joints. The sections were glued onto plexiglass object holders, milled to a thickness of ~100 μm with a Polycut E apparatus (Reichert-Jung, Nussloch, Germany), and polished and surface-stained with McNeil's tetrachrome/toluidine blue O/basic fuchsine (34). Sections from each condyle and tibial plateau (a minimum of 3) were graded by a blinded assessor based on the following parameters: 1) articular cartilage structure, 2) reduction of articular cartilage matrix staining, 3) presence of osteophytes, and 4) subchondral bone plate thickening. The articular cartilage structure was graded on a scale ranging from 0 (normal) to 10 (complete loss to subchondral bone) as described previously (35). The remaining parameters were scored 0–3 (as described in Table 2).

Histochemical analysis of meniscal tissue. Neomeniscal tissue from the posterior horn in the medial compartment was fixed in 10% buffered formalin and embedded in paraffin. Sulfated glycosaminoglycan was visualized by staining with toluidine blue for 5 minutes at 60°C and with 0.1% Safranin O for 5 minutes at room temperature. Type I collagen was detected using monoclonal antibody I-8H5 at 0.2 μg/ml (Oncogene, San Diego, CA) and type II collagen using monoclonal antibody C4F6 (27) at 0.5 μg/ml. Sections were stained using the EnvisionSystem autostainer (Dako, Carpinteria, CA). Incubation with the primary antibody was for 30 minutes. Visualization was performed with diaminobenzidine and counterstaining with hematoxylin.

Statistical analysis. Comparisons between the histologic scores for each group were made using analysis of variance. A P value of less than 0.05 was significant.

RESULTS

Characterization of caprine MSCs. MSCs were isolated from bone marrow aspirates obtained from the

superior iliac crest of adult male goats and expanded to form confluent cultures of adherent cells with a fibroblastic morphology (Figure 2A). The cells were homogeneously SH4+/CD14− (Figure 2B) and CD44+/CD34− (3). The capacity of the cells to differentiate into chondrocytes and osteocytes was demonstrated in vitro (Figures 2C–E), and in vivo when implanted subcutaneously in nude mice (Figures 2F and G). The efficiency of transduction was 62.3 ± 6.5% (mean ± SD). Transduction of human MSCs with eGFP had no effect on the differentiation potential of the cells, and transgene expression was maintained after induction along the osteogenic, adipogenic, and chondrogenic pathways (36).

Clinical observations. In this study we assessed the capacity of MSCs to impact OA progression in goats following trauma to the joint caused by unilateral medial meniscectomy and transection of the ACL. All animals were mobile 1–2 hours after surgery and there were no instances of infection. No immobilization or splinting of the joints was used and animals were bearing weight on the operated joint within 3–5 days. Animals tolerated the cell injection well, and there was no evidence of local inflammation, immobilization, or unloading of the joint resulting from the cell treatment.

The effect of delivery of autologous GFP-transduced MSCs on the joint pathology was assessed by macroscopic evaluation. Appearance of meniscal-like repair tissue, or neomeniscus, was observed in association with the posterior medial compartment of cell-treated knees at 6 weeks after injection (Figures 3A–C). This tissue was hyaline in nature and appeared to provide a bearing surface for the tibial and femoral condyles. Immunohistochemical staining of the neomeniscus indicated a dense, type I collagen–containing network surrounding cells with fibroblastic morphology and some areas containing cells of rounded morphology surrounded by a type II collagen–positive matrix (results not shown). There was also evidence of vascularization in the neomeniscus close to the point of synovial attachment. This tissue was not observed in control (hyaluronan-treated) animals at this time point.

Twenty weeks after delivery of cells there was again evidence of repair tissue associated with the posterior medial compartment in 7 of 9 treated joints. The tissue was organized, generally detached from both femoral and tibial surfaces, and extended into the articulating region between these areas (Figures 3D and E). Functional entheses with the tibial bone were formed with repair of the meniscal insertional ligaments (Figure 3E, arrow). Control joints at this time showed evidence of disorganized repair tissue at the same site, which was generally attached to the proximal tibia, and there was little evidence of extension between the articulating surfaces (result not shown). There was no evidence of repair of the severed ACL in any of the joints, regardless of treatment.

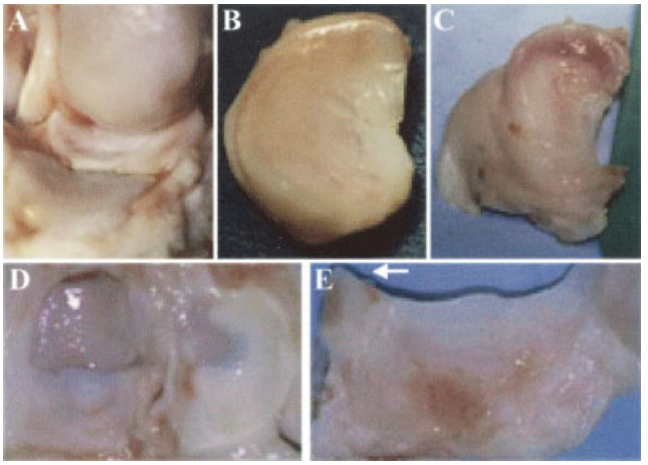

Figure 3. Appearance of regenerated medial meniscus after intraarticular treatment with autologous mesenchymal stem cells (MSCs). The cell treatment resulted in the formation of tissue in the posterior compartment at 12 weeks after complete medial meniscectomy. The excised tissues (**B** and **C**) inserted between the distal head of the femur and proximal tibial plateau (**A**) are shown. Regenerated hyaline-like meniscal tissue is also evident at 20 weeks after MSC injection (**D** and **E**). The regenerated meniscal horn protected the posterior tibial plateau (**D**) and had reformed a functional enthesis with the tibial bone via the meniscal insertional ligament (**arrow** in **E**).

Protection from OA damage. Transection of the ACL and complete medial meniscectomy in the caprine knee resulted in the development of lesions characteristic of OA (37–41), including 1) large areas of erosion of the articular cartilage on the femoral condyle and tibial plateau, 2) formation of periarticular osteophytes, and 3) changes to the trabecular organization of the subchondral bone. We carried out a histologic assessment of the medial femoral condyle from all animals to determine if there were differences between the cell-treated and control groups. In the control (vehicle-treated) joints, there was substantial fibrillation of the articular surface with loss of extracellular matrix, as well as large areas of osteophytic remodeling (Figures 4A–F) as compared with the contralateral (unoperated) joints (Figure 4Q). In the cell-treated joints, the degree of cartilage destruction, osteophyte formation, and subchondral sclerosis were all reduced compared with that in the control joints (Figures 4G–N). In 4 of the 6 cell-treated joints (Figures 4G–N), there was less dam-

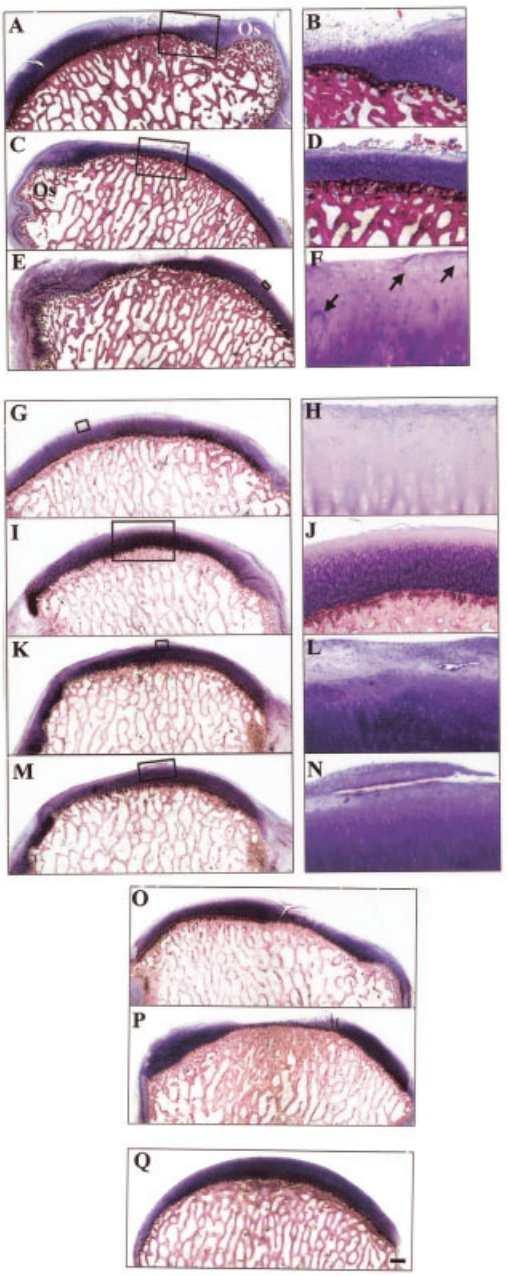

Figure 4. Microscopic analysis of the medial femoral condyle. Goat knee joints were subjected to total medial meniscectomy and anterior cruciate ligament resection, which was followed 6 weeks later by an intraarticular injection of hyaluronan **(A–F)** or mesenchymal stem cells (MSCs) resuspended in hyaluronan **(G–P)**. Osteoarthritic changes such as proteoglycan depletion (indicated by a reduction in surface staining), severe fibrillation, and loss of cartilage, and osteophytes (Os) and bone remodeling were evident in vehicle-treated joints **(A–E)**. Large chondrocyte clones **(arrows)** were evident in areas distant to the primary lesion **(F)**. Boxed areas in **A, C,** and **E** are shown as expanded images in **B, D,** and **F,** respectively. In joints that demonstrated evidence of meniscal regeneration after MSC application **(G–N)**, changes to the cartilage and bone were much less severe. Mild surface roughening **(H)** and proteoglycan depletion in the surface zone **(H–N)** were evident. Proliferation of cells at the cartilage surface was also seen **(L** and **N)**. Boxed areas in **G, I, K,** and **M** are shown as expanded images in **H, J, L,** and **N,** respectively. Protection from severe osteoarthritic changes was not evident in cell-treated joints in areas where meniscal regeneration did not occur. In a contralateral (unoperated) control joint from the same goat as in **E,** the appearance of a normal condyle can be seen **(Q)**. Bar = 1 mm.

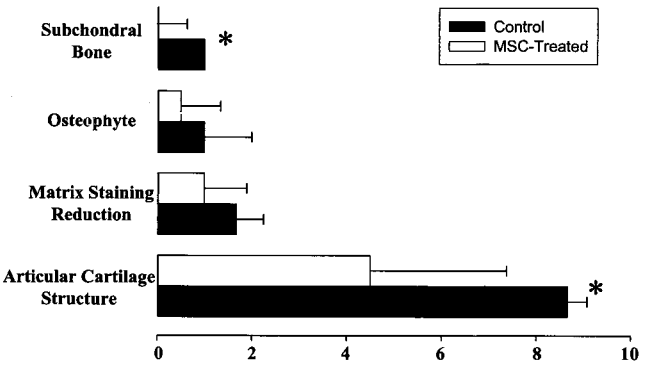

Figure 5. Histologic grading of the joint. Changes to the middle medial condyle after 6 weeks of exposure to the mesenchymal stem cells (MSC) were independently assessed with regard to articular cartilage structure, reduction of articular cartilage matrix staining, the presence of osteophytes, and subchondral bone plate thickening. Scores were compared using analysis of variance. $* = P < 0.05$ between groups. Bars show the mean and SD.

age to the articular surface and less evidence of osteophytic changes, indicating that there was some degree of protection provided to the condyle. In the remaining 2 cell-treated joints (Figures 4O and P), there was less evidence of protection of the condyle. In these joints, there was little evidence of formation of neomeniscus.

Reduction of articular cartilage matrix staining, changes in osteophyte formation, and subchondral bone plate thickening were graded as described in Table 2 (35). Histologic scores for all parameters were closer to normal in the middle medial condyle of cell-treated joints 6 weeks after injection, and treatment had a significant effect on maintenance of the articular cartilage structure and subchondral bone plate thickening (Figure 5). Twenty weeks after cell injection, there were significant OA lesions in both the cell-treated and control joints, despite the presence of neomeniscal tissue in the treated joints. It is likely that the cumulative effect of the abnormal load imposed as a result of the severed ACL resulted in progressive cartilage damage that was not prevented by repair of the meniscus alone.

MSC engraftment. Fluorescence microscopy of sections of neomeniscal tissue indicated that the GFP-transduced implanted cells were associated with the regenerated tissue (Figures 6A–D). The cells were generally associated with the surface (Figures 6B and C) of the tissue and, in some cases, were also detected in the interior (Figure 6D). Immunohistochemical staining indicated a dense, type I collagen–rich fibrillar network populated with cells with a fibroblastic morphology. In addition, there were regions containing cells with a rounded morphology that were type II collagen positive (Figures 6E and F). Twenty weeks after injection, the regenerated meniscus had large areas positive for proteoglycan and type II collagen with the typical appearance of fibrocartilage (Figures 6G–J).

Cell engraftment in the injured joint was evaluated in a separate experiment by delivery of GFP-transduced cells by intraarticular injection into goat knee joints 12 weeks following ACL resection or complete medial meniscectomy. Cells were also injected into the noninjured contralateral joints. Seven days after injection, labeled cells were detected in the synovial fluid and synovial fluid lavage whether or not OA was present. These cells were viable and proliferated when seeded in tissue culture flasks (results not shown). In addition, the injected cells colonized and integrated into surface layers of soft tissues within the joint, including the synovial lining, fat pad, and lateral meniscus. In some of these experiments, sodium hyaluronan was used as the vehicle and, in other cases, PBS was used, but the nature of the vehicle had no effect on cell engraftment. Likewise, the pattern of distribution of injected cells was the same whether ACL transection or meniscectomy was used.

The synovial capsule, fat pad, and lateral meniscus showed a high incidence of cell engraftment (Figures 7A and B). In some cases, cell engraftment was detected on the epiligament, or sheath, of the posterior cruciate ligament and the extensor digitorum longus. Analysis of articular cartilage from the middle regions of the medial and lateral femoral condyles and from the medial and lateral unprotected tibial plateaus showed no cell engraftment on either the intact or the fibrillated cartilage. Small, fluorescently bright bodies were found that were similar in size and fluorescence intensity to red blood cells. GFP-positive cells were found in some areas of synovial lining on both the intracondylar aspect and the outer aspect of the lateral and medial condyles (Figure 7C).

DISCUSSION

This study evaluates the utility of stem cell therapy for delaying the progression of arthritic lesions that occur following joint injury. Adult MSCs were delivered by intraarticular injection as a suspension without the use of a solid biomatrix 6 weeks after total medial meniscectomy and resection of the ACL. The involvement of injected GFP-transduced cells with the development of appreciable neomeniscal tissue in treated joints was associated with protection against degenera-

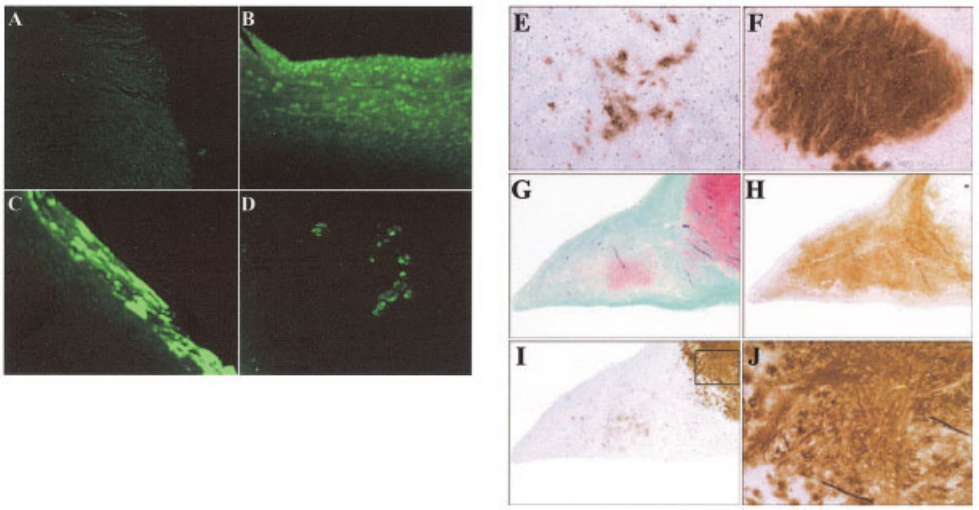

Figure 6. Microscopic analysis of regenerated meniscal tissue. Green fluorescent protein (GFP)–positive cells were detected primarily at the surface **(B** and **C)** and also in the center **(D)** of neomeniscal tissue 6 weeks after injection of GFP-transduced mesenchymal stem cells. Neomeniscal tissue not exposed to the joint environment was used as a negative control **(A)**. Immunohistochemical staining of the posterior meniscal-like tissue indicated a dense, cellular, type I collagen positive fibrous network (results not shown) with small areas of more rounded cells that were type II collagen positive **(E** and **F)**. By 20 weeks after injection, the neomeniscus had areas of Safranin O–positive proteoglycan and type II collagen **(G** and **I,** respectively) in a type I collagen background **(H)**. Further analysis of the type II collagen staining (boxed area of **I)** showed the typical appearance of fibrocartilage **(J)**. (Original magnification × 200 in **A–D**; × 20 in **G–I**; × 100 in **E**, **F**, and **J**.)

tive changes in these cell-treated joints 6 weeks after treatment. The biphasic ultrastructure of meniscus and articular cartilage is critical for load distribution, a smooth low-friction gliding surface, and resilience to compression in the complex biomechanics of the knee joint (42). Data on the incidence of OA after meniscal injury highlight the importance of restoring a functional meniscus as soon as possible after injury. Even after repair, isolated meniscal rupture has been associated with a 10-fold increase in the risk of development of arthrosis, and meniscectomy doubles this risk in a joint with intact ligaments (21). Ligament rupture associated with meniscal injury further increases this risk, and the fact that reconstruction of the ACL was not performed in this study may explain the continuing degradation of treated joints that occurred at the 26-week time point.

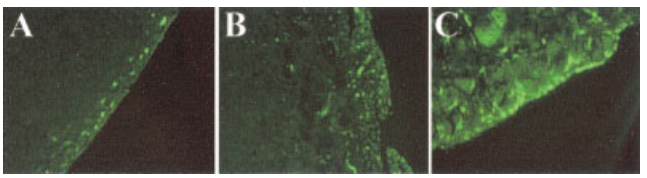

Figure 7. Retention of cells in the joint. Green fluorescent protein–transduced mesenchymal stem cells, injected into normal (noninjured) joints or into joints 12 weeks after medial meniscectomy or anterior cruciate ligament resection, were found to be integrated into the cell layers lining joint structures such as the meniscus **(A)**, synovial capsule **(B)**, and periosteum on the medial aspect of the medial condyle **(C)** 1 week after injection.

Several surgical and tissue-engineering approaches are currently in use for the repair of meniscal tissue. These have involved the use of a broad variety of materials, including small intestine submucosa (43), devitalized tissue (44) or collagen scaffolds (45), or the placement of meniscal allograft tissue derived from cadavers (46,47). These approaches are specifically designed for replacement of damaged meniscal tissue but may have no general effect on surrounding joint tissues. Human and animal studies have shown a regenerative response to meniscectomy (48–50). This regeneration, although minimal, is enhanced in the case of human studies by preservation of the meniscal rim (50). MSCs may contribute to and enhance this normal repair process.

Some augmentation of natural repair by MSCs loaded on a collagen sponge in a rabbit partial meniscectomy model has been described (51). Activation of mesenchymal cells is an important and perhaps rate-

limiting step in the formation of granulation tissue during wound healing (52). Hyaluronan also contributes to the granulation phase of both fetal and adult wound healing (53,54) and stimulates the migration and mitosis of mesenchymal and epithelial cells (55,56). MSC-based repair in the presence of hyaluronan may therefore accelerate and amplify the natural repair process of recruiting these cells to the site of tissue repair or regeneration, and may contribute to the formation of new meniscus after meniscectomy. Meniscal regeneration after partial meniscectomy in a rabbit model was enhanced by 5 injections of hyaluronan (57). Because the cells used in the present study were retrovirally transduced to express GFP, it is conceivable that expressed GFP or the vector used for the transduction may have affected the outcome. However, transduction of the cells did not affect their capacity to proliferate, and engraftment of the transduced cells in the neomeniscus occurred without evidence of an immune response at this site or elsewhere in the joint.

Several tissue-engineering approaches have been used for the repair of joint lesions. For example, the fixation of implanted chondrocytes beneath a sutured flap of ectopic tissue, such as periosteum, has been widely used for the treatment of cartilage defects (58). Other approaches have centered on the use of cells loaded on a scaffold and delivered to a lesion site. These methods are applicable to the repair of focal defects of defined dimensions, but not to the treatment of complex lesions that cover a large surface area of the joint and that may be associated with severe and progressive inflammatory conditions such as OA. Multipotent cells have been isolated from the surface zone of articular cartilage (59) and MSCs have the capacity to repair fibrillated cartilage in vitro (60). It is attractive to hypothesize that MSCs could have a role in cartilage protection by a direct resurfacing of the articular cartilage or act to preserve subchondral or trabecular bone structure–associated mechanical integrity of the joint. Early bone changes have been associated with development of OA and may precede changes to the articular cartilage (61,62). MSCs injected into the knee joint did not bind to normal or fibrillated articular cartilage in vivo, but we cannot exclude the hypothesis that the cells may act to mediate OA progression by an effect other than that seen on meniscal regeneration.

This study suggests that there may be a therapeutic benefit associated with intraarticular injection of stem cells following traumatic injury to the knee. The longer term effect of this may be a reduction or delay in the progression to OA. This is a scaffold-free method for cell delivery and is therefore unencumbered by the complexities associated with placement of a solid cell construct. Other cell-based approaches are possible that might enhance the effects observed in this study. For example, direct implantation of meniscal cells, chondrocytes, or stem cells, perhaps in combination with delivery of appropriate mitogens or growth factors to stimulate host cell proliferation, may lead to a sustained therapeutic effect. Alternative approaches might also involve the delivery of cell-binding or cytotactic factors to enhance the local progenitor cell population, leading ultimately to the reversal of the degradative process. Finally, because of the close integration of the implanted cells with host tissues, use of genetically modified cells to deliver therapeutic genes to the site of injury in the joint may also enhance the observed effects.

ACKNOWLEDGMENTS

We thank Stephanie Oppenheimer, Jerry Skwarek, Mike Ponticiello, Karl Kavalkovich, and Don Simonetti for their expert technical assistance, and Drs. Alastair Mackay and Arnold Caplan for critical review of the manuscript.

REFERENCES

1. Friedenstein AJ. Precursor cells of mechanocytes. Int Rev Cytol 1976;47:327–59.
2. Owen M, Friedenstein AJ. Stromal stem cells: marrow-derived osteogenic precursors. In: Evered D, Hartnett S, editors. Cell and molecular biology of vertebrate hard tissues. Chichester, UK: Wiley; 1988. p.42–60.
3. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
4. Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci U S A 2001;98:7841–5.
5. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. Bone 1992;13:81–8.
6. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 1997;64:295–312.
7. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. Tissue Engineering 1998;4:415–28.
8. Young RG, Butler DL, Weberm W, Caplan AI, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 1998;16:406–13.
9. Barry F, Boynton RE, Liu B, Murphy JM. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Exp Cell Res 2001;268:189–200.
10. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002;105:93–8.
11. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent

mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928–42.
12. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001;189:54–63.
13. Deasy BM, Jankowski RJ, Huard J. Muscle-derived stem cells: characterization and potential for cell-mediated therapy. Blood Cells Mol Dis 2001;27:924–33.
14. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science 2000;287:1442–6.
15. Gage FH. Mammalian neural stem cells. Science 2000;287:1433–8.
16. Alison M, Sarraf C. Hepatic stem cells. J Hepatol 1998;29:676–82.
17. Murphy JM, Dixon K, Beck S, Fabian DF, Feldman A, Barry FP. Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. Arthritis Rheum 2002;46:704–13.
18. Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997;350:503–8.
19. Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect 1998;47:487–504.
20. Felson DT, Lawrence RC, Hochberg MC, McAlindon T, Dieppe PA, Minor MA, et al. Osteoarthritis: new insights. Part 2: treatment approaches. Ann Intern Med 2000;133:726–37.
21. Gillquist J, Messner K. Anterior cruciate ligament reconstruction and the long-term incidence of gonarthrosis. Sports Med 1999;27: 143–56.
22. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994;76: 579–92.
23. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002;10:199–206.
24. Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S. Mesenchymal stem cells in osteobiology and applied bone regeneration. Clin Orthop 1998;355:S247–56.
25. Rydell N, Balazs EA. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of osteoarthritis and on granulation tissue formation. Clin Orthop 1971;80:25–32.
26. Kavalkovich KW, Boynton RE, Murphy JM, Barry F. Chondrogenic differentiation of human mesenchymal stem cells within an alginate layer culture system in vitro. Cell Develop Biol—Animal 2002; 38:457–66.
27. Srinivas GR, Chichester CO, Barrach HJ, Pillai V, Matoney AL. Production of type II collagen specific monoclonal antibodies. Immunol Invest 1994;23:85–98.
28. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 1997;64:278–94.
29. Mosca JD, Hendricks JK, Buyaner D, Davis-Sproul J, Chuang LC, Majumdar MK, et al. Mesenchymal stem cells as vehicles for gene delivery. Clin Orthop 2000;379:S71–90.
30. Keller G, Paige C, Gilboa E, Wagner EF. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent hematopoietic precursors. Nature 1985;318:149–54.
31. Ghattas IR, Sanes JR, Majors JE. The encephalomyocarditis virus internal ribosome entry site allows efficient coexpression of two genes from a recombinant provirus in cultured cells and in embryos. Mol Cell Biol 1991;11:5848–59.
32. Miller AD, Garcia JV, von Suhr N, Lynch CM, Wilson C, Eiden MV. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. J Virol 1991;65:2220–4.
33. Markowitz D, Goff S, Bank A. A safe packaging line for gene transfer: separating viral genes on two different plasmids. J Virol 1988;62:1120–4.
34. Hunziker EB. Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. Osteoarthritis Cartilage 2001; 9:22–32.
35. Carlson CS, Guilak F, Vail TP, Gardin JF, Kraus VB. Synovial fluid biomarker levels predict articular cartilage damage following complete medial meniscectomy in the canine knee. J Orthop Res 2002;20:92–100.
36. Lee K, Majumdar MK, Buyaner D, Hendricks JK, Pittenger MF, Mosca JD. Human mesenchymal stem cells maintain transgene expression during expansion and differentiation. Mol Ther 2001; 3:857–66.
37. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis 1973;32:387–8.
38. Altman RD, Tenenbaum J, Latta L, Riskin W, Blanco LN, Howell DS. Biomechanical and biochemical properties of dog cartilage in experimentally induced osteoarthritis. Ann Rheum Dis 1984;43: 83–90.
39. Rorvik AM, Teige J. Unstable stifles without clinical or radiographic osteoarthritis in young goats: an experimental study. Acta Vet Scand 1996;37:265–72.
40. Little C, Smith S, Ghosh P, Bellenger C. Histomorphological and immunohistochemical evaluation of joint changes in a model of osteoarthritis induced by lateral meniscectomy in sheep. J Rheumatol 1997;11:2199–209.
41. Smith MM, Little CB, Rodgers K, Ghosh P. Animal models used for the evaluation of anti-osteoarthritis drugs. Pathol Biol 1997; 45:313–20.
42. Rangger C, Kathrein A, Klestil T, Glotzer W. Partial meniscectomy and osteoarthritis: implications for treatment of athletes. Sports Med 1997;23:61–8.
43. Gastel JA, Muirhead WR, Lifrak JT, Fadale PD, Hulstyn MJ, Labrador DP. Meniscal tissue regeneration using a collagenous biomaterial derived from porcine small intestine submucosa. Arthroscopy 2001;17:151–9.
44. Peretti GM, Caruso EM, Randolph MA, Zaleske DJ. Meniscal repair using engineered tissue. J Orthop Res 2001;19:278–85.
45. Rodkey WG, Steadman JR, Li ST. A clinical study of collagen meniscus implants to restore the injured meniscus. Clin Orthop 1999;367:S281–92.
46. Jackson DW, Whelan J, Simon TM. Cell survival after transplantation of fresh meniscal allografts: DNA probe analysis in a goat model. J Sports Med 1993;21:540–50.
47. Rodeo SA. Meniscal allografts: where do we stand? Am J Sports Med 2001;29:246–61.
48. Lutfi AM. Morphological changes in the articular cartilage after meniscectomy: an experimental study in the monkey. J Bone Joint Surg Br 1975;57:525–8.
49. Elmer RM, Moskowitz RW, Frankel VH. Meniscal regeneration and postmeniscectomy degenerative joint disease. Clin Orthop 1977;124:304–10.
50. Burr DB, Radin EL. Meniscal function and the importance of meniscal regeneration in preventing late medical compartment osteoarthrosis. Clin Orthop 1982;171:121–6.
51. Walsh CJ, Goodman D, Caplan AI, Goldberg VM. Meniscus regeneration in a rabbit partial meniscectomy model. Tissue Eng 1999;5:327–37.
52. McClain SA, Simon M, Jones E, Nandi A, Gailit JO, Tonnesen MG, et al. Mesenchymal cell activation is the rate-limiting step of granulation tissue induction. Am J Pathol 1996;149:1257–70.
53. Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. Wound Repair 1999;7:79–89.
54. Dostal GH, Gamelli RL. Fetal wound healing. Surg Gynecol Obstet 1993;176:299–306.
55. McCarty MF. Glucosamine for wound healing. Med Hypotheses 1996;47:273–5.
56. Murashita T, Nakayama Y, Hirano T, Ohashi S. Acceleration of

granulation tissue ingrowth by hyaluronic acid in artificial skin. Br J Plast Surg 1996;49:58–63.
57. Kobayashi K, Amiel M, Harwood FL, Healey RM, Sonoda M, Moriya H, et al. The long-term effects of hyaluronan during development of osteoarthritis following partial meniscectomy in a rabbit model. Osteoarthritis Cartilage 2000:8:359–65.
58. Brittberg M, Tallheden T, Sjogren-Jansson B, Lindahl A, Peterson L. Autologous chondrocytes used for articular cartilage repair: an update. Clin Orthop 2001;391:S337–48.
59. Archer CW, Redman S, Bishop J, Bowyer S, Dowthwaite GP. The identification and characterization of articular cartilage progenitor cells. Transactions of the 48th Annual Meeting of the Orthopaedic Res Society S009; 2002.
60. Kavalkovich KW, Murphy JM, Barry FP. Adhesion of mesenchymal stem cells to fibrillated osteoarthritic cartilage [abstract]. Osteoarthritis Cartilage 2000;8 Suppl B:S24.
61. Li B, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or OA. J Bone Miner Res 1997;12:641–51.
62. Dequeker J, Mokassa L, Aerssens J, Boonen S. Bone density and local growth factors in generalized OA. Microsc Res Tech 1997; 137:358–71.