TABLE E-1 Histological Tissue Quality Score (Ishida Score)*

Category	Points
Reparative tissues with bonding	
Bilaterally bonds with surrounding meniscus	2
Partially bonds with surrounding meniscus	1
No bond with surrounding meniscus	0
Existence of fibrochondrocytes	
Fibrochondrocytes exist diffusely in the reparative tissues	2
Fibrochondrocytes are localized in the reparative tissues	1
No fibrochondrocytes in the reparative tissues	0
Staining with safranin O	
Densely stained with safranin O	2
Faintly stained with safranin O	1
Not stained with safranin O	0

*Reproduced, with modification, from Ishida K, Kuroda R, Miwa M., Tabata Y, Hokugo A, Kawamoto T, Sasaki K, Doita M, Kurosaka M. The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. Tissue Eng. 2007;13:1103-12. Reprinted with permission.

Appendix 1 Expanded Materials and Methods

Tissue Harvesting and Mesenchymal Stem Cell Preparation

Synovial tissue from the rabbit donor knee was minced and digested for three hours at 37°C with type-V collagenase (0.2%; Sigma-Aldrich, St. Louis, Missouri) and passed through a 40-μm filter. Nucleated cells from the bone marrow were isolated on a density gradient (Ficoll-Paque; Pharmacia, Piscataway, New Jersey)[20]. Cells were then cultured in complete medium (αMEM [minimum essential medium], 16.5% FBS [fetal bovine serum], 2 mM/mL L-glutamine, 100 U/mL penicillin-streptomycin; Invitrogen, Carlsbad, California) for three to four days.

Cells were then replated at 100 cells/cm^2, cultured for fourteen days, and then frozen at –80°C as passage 1. Vials of the frozen cells were then rapidly thawed in a water bath at 37°C, plated in a 150-cm^2 dish, and harvested after three to four days (passage 2). Then the cells were replated once again at 100 cells/cm^2, cultured for fourteen days, and collected (passage 3).

At passage 3, synovial tissue-derived cells were resuspended in phosphate-buffered saline solution (PBS) and a fluorescent lipophilic tracer, CM-DiI (Invitrogen), was added at 5 μl/mL in PBS in order to facilitate localization of implanted cells at the time of meniscal harvest. After incubation for twenty minutes at 37°C with 5% humidified CO_2, the cells were centrifuged and washed twice with PBS. CM-DiI-labeled cells were prepared for implantation with use of a hemocytometer at a concentration of 2×10^6 cells/50 μL in PBS[33].

In Vitro Differentiation Assay

For adipogenesis, synovial tissue-derived cells were cultured to approximately 70% confluency and the medium was then changed to adipogenic medium consisting of complete medium supplemented with 0.5 μM dexamethasone (Sigma-Aldrich), 0.5 mM isobutylmethylxanthine (Sigma-Aldrich), and 50 μM indomethacin for an additional fourteen days. The adipogenic cultures were fixed in 4% paraformaldehyde and then stained with fresh oil-red-O solution.

For calcification, cells were cultured to approximately 70% confluency and the medium was changed to calcification medium consisting of complete medium supplemented with 10^{-9} M dexamethasone, 20 mM β-glycerol phosphate, and 50 μg/mL ascorbate-2-phosphate (Sigma-Aldrich) for an additional twenty-one days. These dishes were then stained with 0.5% alizarin-red solution.

For chondrogenesis, cells were cultured to approximately 70% confluency and 250,000 cells were placed in a 15-mL polypropylene tube (Becton Dickinson, Franklin Lakes, New Jersey) and centrifuged at 450 g for ten minutes. The resulting pellets were cultured for twenty-one days in chondrogenic medium consisting of high-glucose Dulbecco modified Eagle medium (Invitrogen) supplemented with 500 ng/mL bone

morphogenetic protein-2 (R&D Systems, Minneapolis, Minnesota), 10 ng/mL transforming growth factor-β3 (R&D Systems), 10^{-7} M dexamethasone (Sigma-Aldrich), 50 μg/mL ascorbate-2-phosphate, 40 μg/mL proline, 100 μg/mL pyruvate, and 50 mg/mL ITS+Premix (Becton Dickinson). For microscopy, the pellets were embedded in paraffin, cut into 5-μm sections, and stained with safranin O.

Tissue Quantity Analysis

The quantity of tissue regeneration was evaluated with use of random tissue sections obtained from the central portion of the meniscal defect as shown in Figure 1. In order to define the original defect, horizontal lines were drawn connecting the superior and inferior surfaces of the meniscus at the site of the original 1.5-mm biopsy. Tissue regeneration was evaluated within the region bounded by the above lines and the bordering native meniscal tissue. The area of the original defect (defect; D) and the area occupied by regenerated tissue inside the defect (regenerated tissue; R) were calculated with use of Photoshop CS3 software (Adobe Systems, San Jose, California) (Fig. 1). Tissue regeneration is expressed as the ratio of regenerated tissue area to the entire defect area (Regeneration Ratio = R/D). The ideal quantity of regeneration would result in a value of 1, and incomplete regeneration would result in a value of <1.

Immunohistochemistry for Type-I and Type-II Collagen

Sections from Group-B menisci were washed with PBS and blocked with PBS containing 5% normal horse serum for twenty minutes at room temperature. Sections were incubated for one hour at room temperature with a mouse monoclonal antibody against type-I collagen (clone I-8H5, 1:500 dilution; MP Biomedicals; Solon, Ohio) or type-II collagen (clone II-4C11, 1:500 dilution; MP Biomedicals). The sections were washed three times and incubated with anti-mouse fluorescent antibody (Alexa Fluor 594, 1:500 dilution; Invitrogen). Nuclei were counterstained with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) for ten minutes.