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APPENDIX F
Feasibility Study Publications

U.S. Feasibility Study – Five to Six Year Follow-up

Tissue-Engineered Collagen Meniscus Implants: 5- to 6-Year Feasibility Study Results

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Purpose: In this feasibility study, a 5- to 6-year clinical follow-up evaluation was conducted on 8 patients who had undergone reconstruction of 1 injured medial meniscus with a tissue-engineered collagen meniscus implant. The hypothesis was that these patients would show significant clinical improvement over their preoperative status and would have maintained their status determined at the 2-year follow-up evaluation. **Type of Study:** Prospective longitudinal feasibility study follow-up evaluation. **Methods:** Eight patients underwent arthroscopic placement of a collagen meniscus implant by a single surgeon to reconstruct and restore the irreparably damaged medial meniscus of 1 knee. All patients returned for clinical, radiographic, magnetic resonance imaging, and arthroscopic examinations an average of 5.8 years (range, 5.5–6.3 y) after collagen meniscus implant placement. **Results:** Lysholm scores improved significantly ($P = .045$) from 75 preoperatively to 88 at most recent follow-up evaluation. Average Tegner activity scores improved significantly ($P = .001$) from 3 to 6. Patient self-assessment improved significantly ($P = .046$) from 2.4 to 1.9 (1 = normal, 4 = severely abnormal). Pain scores improved from 23 to 11 (0 = no pain, 100 = worst pain). Imaging studies confirmed that the chondral surfaces of the medial compartment had not degenerated further since the placement of the implant 5.8 years earlier. Relook arthroscopy with direct measurement of the newly generated tissue revealed 69% defect filling. Histologic assessment of tissue biopsy specimens from 3 patients showed the presence of fibrocartilage with a uniform extracellular matrix. **Conclusions:** The meniscus-like tissue that developed after collagen meniscus implant placement has maintained its structure and functioned without negative effects for more than 5 years. The hypothesis was affirmed that these patients were improved significantly compared with their preoperative status and unchanged compared with 2-year evaluations. **Level of Evidence:** Level IV. **Key Words:** Meniscus—Collagen meniscus implants—Meniscus reconstruction—Tissue engineering—Tissue-engineered scaffolds.

Numerous studies have documented the importance of the menisci to the health of the knee joint.¹⁻⁸ Replacement of the damaged or lost portion of the meniscus cartilage would seem an appropriate approach to prevent or minimize the progressive de-

generative joint disease that may develop as a sequela. Many different materials have been evaluated for replacement of the meniscus, including artificial materials, autogenous tissue, and allograft tissue.⁹⁻¹⁷ The longer-term results of using these various materials and tissues generally have not proven successful or remain uncertain.¹⁸⁻²⁰ Recent studies also have raised the distinct possibilities of disease transmission²¹ and immunologic reactions^{20,22} with use of allograft meniscus tissue, but other reports make clear the advantage of initial pain relief provided by meniscus allografts.²³⁻²⁶ Therefore, we used tissue engineering techniques to develop a collagen meniscus implant that served as a scaffold to support generation of new meniscus-like tissue rather than attempting to replace the damaged meniscus by artificial means.

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The collagen meniscus implant was tested extensively *in vitro* and in laboratory animal trials.²⁷⁻³² An initial phase I clinical feasibility study was completed successfully.³³ Based on that study, the collagen meniscus implant was modified and improved for use in a phase II feasibility trial in which patients were followed-up for 2 years.³⁴

The purpose of this prospective study was to conduct a 5- to 6-year serial follow-up evaluation on those patients who had undergone reconstruction of 1 injured medial meniscus in the phase II feasibility clinical trial of the collagen meniscus implant. We wanted to determine if the newly generated tissue³⁴ had persisted within the original meniscus defect and remained functional. Furthermore, we wanted to ensure that no detrimental effects had been produced by the implant or the newly generated tissue over the 5 to 6 years. The hypothesis of this present study was that these patients would have clinical outcomes better than their preoperative status, and that they would have maintained or improved their status determined 2 years after surgical placement of the tissue-engineered collagen meniscus implant without experiencing negative effects.

METHODS

Techniques for the formulation and fabrication of the tissue-engineered collagen meniscus implant (ReGen Biologics, Inc., Franklin Lakes, NJ) used in this study have been reported in detail previously.^{31,32,34,35} Briefly, the collagen meniscus implants are fabricated from type I collagen derived from U.S.-origin bovine Achilles tendons. After the tendon tissue is trimmed and minced, the type I collagen fibers are purified by using various chemical treatments to remove noncollagenous materials and lipids. Next, the purified collagen fibers are swelled in hyaluronic acid and chondroitin sulfate, and then homogenized. The swollen collagen fibers plus the glycosaminoglycans are coprecipitated by the addition of ammonium hydroxide. The precipitated fibers are dehydrated, manually oriented in a mold, lyophilized, and chemically cross-linked. Finally, terminal sterilization is performed by γ irradiation.^{31,32,34,35}

U.S. Food and Drug Administration and local institutional review board approvals were obtained before commencing this follow-up study. Written informed consent was obtained from all patients. The original phase II feasibility study³⁴ was open to men and women ages 18 to 50 years who had an irreparable injury or previous partial loss of their medial meniscus.

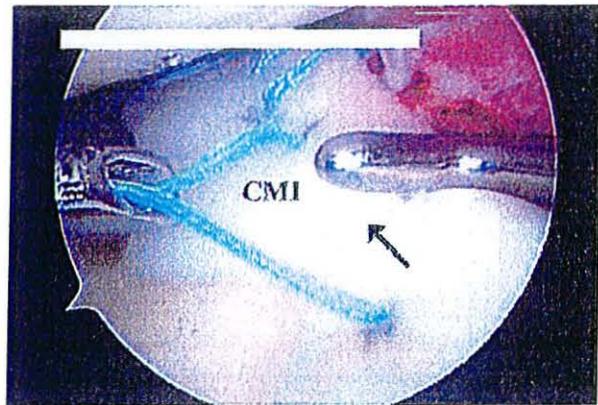


FIGURE 1. The collagen meniscus implant (CMI) has been inserted into the lesion and is being sutured into place using an inside-out technique. The arrow points to the interface between the native meniscus remnant and the implant.

The study included patients with acute or chronic injuries that resulted in the loss of at least one third of the native meniscus but who had an intact meniscus rim of at least 1 mm or greater. It also required that the involved knee be stable ligamentously or stabilized at the time of the index surgery—the surgical procedure to place the collagen meniscus implant. Patients were excluded if they had total meniscus loss, grade IV (full thickness) chondral defects, varus axial malalignment, suffered from inflammatory or systemic disease, had known collagen allergies, were diagnosed with autoimmune disease, or were pregnant. Because this was a clinical feasibility study, Food and Drug Administration guidelines for the study precluded randomization and a concurrent cohort of control patients.

The technique for arthroscopic placement of the collagen meniscus implant has been described previously.^{31,34} Briefly, a partial meniscectomy is performed to remove only damaged or pathologic tissue and to ensure smooth, even margins of the debrided defect. The remainder of the native meniscus is left intact. A specially designed arthroscopic measuring device is used to determine the length and width of the meniscus defect as well as the total dimensions of the involved meniscus to the nearest millimeter. Based on these measurements, the percent of meniscus loss can be calculated. On the surgical field, the collagen meniscus implant then is trimmed to fit the lesion. The implant is delivered into the joint through a cannula, and then it is manipulated into the prepared lesion. Fixation of the implant to the host meniscus rim is with size 2-0 nonabsorbable sutures using an inside-out technique (Fig 1). Horizontal mattress sutures are

used in the anterior and posterior margins, and vertical mattress sutures (4 to 5 mm apart) are used along the body rim.

Between December 1995 and July 1996, the 8 patients in this present study underwent arthroscopic placement of the collagen meniscus implant by a single surgeon to reconstruct and restore the irreparably damaged medial meniscus of 1 knee.³⁴ No concomitant intra-articular procedures (e.g., ligament reconstruction) were performed. By chance, all patients enrolled in the study were men. The average age of the patients was 40 years (range, 24–49 y). Seven patients had 1 or more prior medial meniscectomies (range, 1–5), and 1 patient had an acute irreparable medial meniscus injury. Results of that initial evaluation have been reported elsewhere.³⁴

For the present follow-up study, all 8 patients (100%) returned an average of 5.8 years (range, 5.5–6.3 y) after placement of the collagen meniscus implant. The average patient age was 46 years (range, 30–55 y) at the most recent follow-up evaluation. Patients underwent clinical, radiographic, magnetic resonance imaging, and arthroscopic examinations. Pain (visual analog scale), Lysholm, Tegner, and self-assessment (from the International Knee Documentation Committee form³⁶) scores were determined and compared with scores at index surgery and 1 and 2 years after implantation.

Radiographic and magnetic resonance images were evaluated by the same independent radiologist and compared with the original preoperative and the 2-year images using the same criteria.³⁴ All postoperative magnetic resonance images were from the same unit using identical scanning protocols. Scanning sequences included proton-density sagittal images, dual-echo coronal images, and proton-density fat saturation sagittal and axial images. Radiographic examination included standing anteroposterior, flexed posteroanterior, lateral, and bilateral long standing (~51 in, 130 cm) views. On the long standing radiographs, a straight line drawn from the center of the femoral head to the center of the tibiotarsal joint was used to determine the mechanical axis of the knee joint. Medial joint heights were measured on the long standing radiographs using electronic digital calipers by a single individual who was blinded to patient identification and surgical status.

At arthroscopy the amount of the original meniscus defect remaining filled by newly generated meniscus-like tissue was determined with physical measurements and by comparison with video images of the index surgery and the first relook procedures. Physical

measurements were made using the same arthroscopic measuring device that had been used during the index surgery. For example, if the original implant was 50-mm long and 7-mm wide, then it covered an area of 350 mm². If the newly generated tissue was measured and determined to cover 300 mm², then the original defect was calculated to remain 86% filled. A single surgeon performed all arthroscopic procedures.

Biopsy examination of the new tissue was not a requirement for patient participation in this present study; however, 3 patients requested biopsy examination for personal knowledge enhancement. The biopsy specimens were obtained with a 14-gauge biopsy needle and were fixed in formalin. The biopsy needle was inserted parallel to the tibial plateau and oriented perpendicular to and through what was observed to be approximately the center of the new tissue. The biopsy specimen was the full width of the meniscus in an effort to obtain tissue from the interface of the new and native meniscus tissues. The core biopsy specimen was embedded in paraffin and 6- μ m thick sections were cut in the longitudinal plane. The sections were stained with H&E, Masson trichrome, or phosphotungstic acid hematoxylin, and examined by an orthopedic pathologist.

Statistical Analysis

All scores were recorded before surgery, at 12 and 24 months after surgery, and at the most recent follow-up evaluation, an average of 5.8 years after the index surgery. The Lysholm score and Tegner activity score improvements at the most recent follow-up evaluation were compared with the preoperative scores using the paired-samples *t* test. Time-related improvement in the Lysholm score and the Tegner activity score was assessed using 1-way repeated-measures analysis of variance with within-subjects contrasts. Patient self-assessment scores were compared using the Wilcoxon nonparametric test. Visual analog scale pain scores were compared using paired *t* tests.

Statistical analyses were performed using SPSS (version 10.1; SPSS Inc., Chicago, IL), SAS (version 6.12; SAS Institute Inc., Cary, NC), and nQuery Advisor (version 4.0; Statistical Solutions, Saugus, MA) software packages. All reported *P* values are 2-tailed, with an α level of .05 indicating statistical significance.

RESULTS

Clinical examination at the most recent follow-up evaluation revealed normal physical findings in all 8

TABLE 1. Lysholm Scores

Patient Number	At Time of Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery	5.8 Years After Index Surgery
21001	94	95	95	87
21002	88	100	95	95
21003	80	100	90	95
21005	52	86	79	74
21009	55	85	89	89
21010	72	73	89	76
21011	97	82	99	95
21012	64	94	96	94
Average	75	89*	91*	88*

*Significantly improved from index surgery.

patients. No patient was observed to have any symptoms or signs suggestive of meniscus derangement such as clicking, locking, medial joint line pain, decreased range of motion, or effusions. The McMurray test was negative in all 8 patients. No complications related to the implant were reported.

Table 1 and Fig 2 show the Lysholm score data. There was significant improvement in the Lysholm scores at the most recent follow-up evaluation (at time of index surgery, 75 [SD = 17.3]; 5.8 years after index surgery, 88 [SD = 8.7]; $P = .045$). There was significant time-related improvement in the Lysholm score ($F = 7.00$; $P = .016$), with within-subject contrasts showing significant differences between preoperative scores and all follow-up times (12 months, $P = .050$; 24 months, $P = .004$; 5.8 years, $P = .045$), and no differences between follow-up times ($P > .05$). Two patients had slightly lower scores at the most recent follow-up evaluation compared with preoperative status.

Table 2 and Fig 3 show the Tegner activity score data. There was significant improvement in the Tegner scores at the most recent follow-up evaluation (at time of index surgery, 3 [SD = 1.3]; 5.8 years after index surgery, 6 [SD = 1.4]; $P = .001$). There was significant time-related improvement in the Tegner scores ($F = 7.40$; $P = .005$), with within-subject contrasts showing significant differences between preoperative scores and the 24-month follow-up scores ($P = .021$) and the 5.8-year follow-up scores ($P = .001$), with no differences between follow-up times ($P > .05$). All patients showed an improvement in Tegner scores over time.

Patient self-assessment scores (Table 3) showed significant improvement from preoperative scores (2.4 [SD = .5]) compared with 24 months (1.6 [SD = .5];

$P = .034$) and 5.8 years (1.9 [SD = .4]; $P = .046$). There was no significant difference between preoperative scores and 12-month scores (1.8 [SD = .5]; $P = .059$). There were no differences between follow-up times ($P > .05$). No patient was worse at the most recent follow-up evaluation compared with their preoperative status.

Visual analog scale pain scores, as shown in Table 4, showed improvement from preoperative scores (23 [SD = 11.4]) compared with 12-month scores (7 [SD = 3.2]; $P = .008$) and 24-month scores (2 [SD = 1.9]; $P = .002$). There was no significant difference between preoperative scores and 5.8-year scores (11 [SD = 17.8]; $P = .095$). There was a significant difference between 12-month and 24-month scores ($P = .006$). There were no differences between 12-month and 5.8-year scores ($P = .179$) or between 24-month and 5.8-year scores ($P = .592$). One patient reported increased pain at 5.8 years compared with his preoperative status.

Radiographs taken at an average of 5.8 years after placement of the collagen meniscus implant confirmed that the medial compartment bone surfaces appeared to be protected from further detectable degeneration compared with preoperative and 2-year examinations. Based on the long-standing

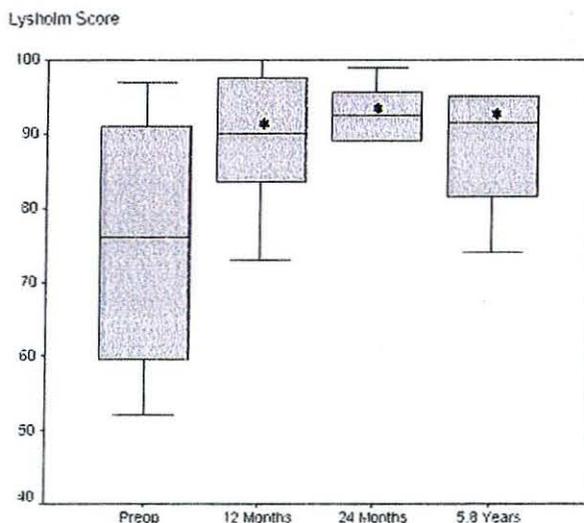


FIGURE 2. Graphic display of Lysholm scores over time. Lysholm scores and Tegner activity scores (Fig 3) are shown as box plots that are summary plots based on the median, quartiles, and extreme values. The box represents the interquartile range, which contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values. A line across the box indicates the median. *Significant increase ($P < .05$) compared with the preoperative value.

TABLE 2. Tegner Activity Scale

Patient Number	Before Initial Meniscus Injury	At Time of Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery	5.8 Years After Index Surgery
21001	9	5	5	7	7
21002	5	4	4	5	6
21003	7	5	5	4	7
21005	7	3	7	5	4
21009	9	2	5	8	8
21010	10	3	3	4	6
21011	6	1	2	3	4
21012	6	4	6	6	6
Average	7.4	3.4	4.6*	5.3*	6.0*

*Significantly improved from index surgery.

radiographs, it was determined that the mechanical axis of 1 patient had migrated 2-mm medially compared with his preoperative status, but for all other patients there were no differences measured for the mechanical axes. Joint height measurements revealed that 3 joints had decreased by less than .5 mm, 3 joints were unchanged, and 2 joints had increased heights of less than .5 mm. Magnetic resonance images revealed that the appearance of the newly generated tissue had continued to mature between 2 years and 5.8 years after implant, becoming more well defined and smoothly marginated with a decrease of previous intermediate to high intrasubstance signal to low or no signal, similar to mature fibrocartilage of native meniscus tissue. The new tissue was indistinguishable from the native meniscus tissue, and the interface between the new

tissue and the native meniscus tissue no longer could be resolved. The adjacent hyaline articular chondral surfaces showed little or no change compared with preoperative or 2-year magnetic resonance image examinations, and there was no progression in magnetic resonance image features of chondral degeneration or surface breakdown. Likewise, no bony changes were noted on magnetic resonance imaging.

Another relook arthroscopy was performed to assess the status of the newly generated meniscus-like tissue as well as the condition of the adjacent chondral surfaces compared with the observations made during the initial relook procedure performed at 6 or 12 months. Arthroscopic observations indicated that the newly grown tissue appeared grossly meniscus-like, and it was as good as and sometimes better than at the time of the earlier relook arthroscopies based on visual comparisons of photographs

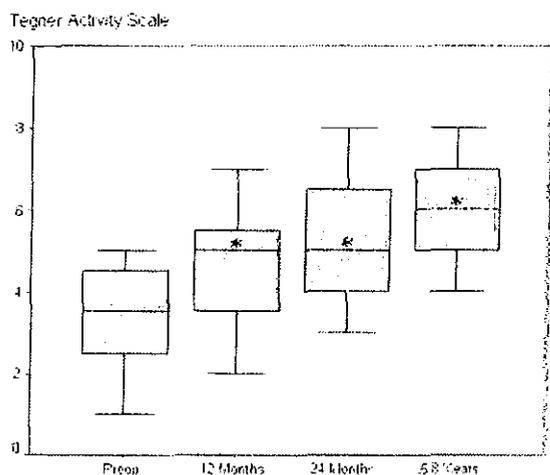


FIGURE 3. Graphic display of Tegner activity scores over time. A description of the box plots is included in the Fig 2 legend.

TABLE 3. Patient Assessment

Patient Number	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery	5.8 Years After Index Surgery
21001	2	1	1	2
21002	2	2	1	1
21003	3	1	1	2
21005	3	2	2	2
21009	2	2	2	2
21010	3	2	2	2
21011	2	2	2	2
21012	2	2	2	2
Average	2.4	1.8	1.6*	1.9*

NOTE. Self-assessment scoring system; 1 = normal; 2 = nearly normal; 3 = abnormal; 4 = severely abnormal.

*Significantly improved from index surgery.

TABLE 4. Pain

Patient Number	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery	5.8 Years After Index Surgery
21001	28	10	0	15
21002	26	10	3	2
21003	11	6	0	0
21005	28	4	4	53
21009	34	4	0	6
21010	33	10	0	9
21011	1	10	4	2
21012	23	3	3	0
Average	23	7*	2*	11

NOTE. Pain visual analog scale based on 100-mm scale for activities of daily living.

*Significantly improved from index surgery.

and video recordings. When probed, the tissue was indistinguishable from native meniscus, it was supple, and it did not give the impression of having shrunk. This new tissue also appeared to be harmless to the adjacent chondral surfaces of the medial compartments because no further degenerative changes were noted in this group of patients (Figs 4 and 5). Table 5 shows the status of the chondral surfaces at the index and subsequent relook surgeries. By using Outerbridge grades, we observed that 3 patients had improved chondral surfaces, 3 patients remained unchanged, and 2 patients decreased by 1 grade at an average of 5.8 years. Based on physical measurements as described and visual observations, the original meniscus defects that had been determined to be 77% filled with new tissue at

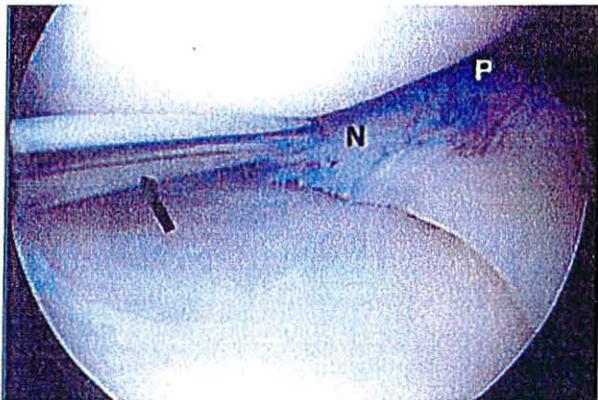


FIGURE 4. An intraoperative view at the 6-month relook. A biopsy needle (arrow) can be seen penetrating the newly generated tissue (N) that fills the defect almost completely. Notice the synovial pannus (P) that covers the new tissue.

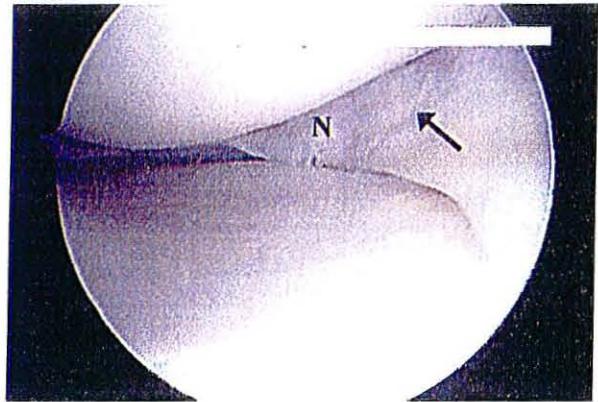


FIGURE 5. An intraoperative view of the same patient shown in Fig 4 at the relook 6.3 years after placement of the collagen meniscus implant. The defect remains completely filled with new tissue (N), and the chondral surfaces are unchanged since the 6-month relook. The interface (arrow) between the new and the native tissue is barely distinguishable.

the initial relook arthroscopy (either 6 or 12 months after implant placement),³⁴ still were filled 69% at 5.8 years after placement of the collagen meniscus implant (Table 6). By adding the amount of filled defect to the amount of meniscus remaining at the time of index surgery, this group of 8 patients had 81% of their normal meniscus (range, 66% to 98%). The percent of meniscus gain compared with the index remnant (the quotient of the percent of new tissue divided by the percent of remaining meniscus at index surgery) averaged 170% (range, 27% to 340%). No negative findings, such as damage to the chondral surfaces or exuberant tissue growth, attributable to the implant were observed.

Histologic assessment of the newly generated meniscus-like tissue in the 3 patients who had biopsy examination of their new tissue showed the presence of fibrocartilage (Fig 6). Histochemical stains revealed a uniform extracellular matrix. The trichrome stain confirmed the collagenous nature of the tissue. Excess fibrin was not identified with the phosphotungstic acid hematoxylin stain. The cells had the appearance of normal meniscus fibrochondrocytes, and no inflammatory infiltrates were observed. The meniscus-like nature of the tissue was consistent in all 3 biopsy specimens. Unlike the initial biopsy specimens obtained from all patients at 6 or 12 months, there was no evidence of remnants of the collagen meniscus implant in the 3 biopsy specimens in the present study. There was no indication of any infection, inflammation, or immune response in any of the biopsy specimens examined.

TABLE 5. Chondral Surface Status Based on Outerbridge Grades

Patient Number	Time of First Relook Arthroscopy	Time of Second Relook Arthroscopy	Outerbridge Grade at Index Surgery	Outerbridge Grade at First Relook Arthroscopy	Outerbridge Grade at Second Relook Arthroscopy
21001	6 mo	69 mo	III	II	II
21002	6 mo	71 mo	II	II	II
21003	6 mo	75 mo	I	I	I
21005	6 mo	72 mo	II	II	I
21009	6 mo	70 mo	II	II	III
21010	6 mo	67 mo	I	I	II
21011	12 mo	70 mo	I	I	I
21012	12 mo	66 mo	I	Normal	Normal

DISCUSSION

The main goal of meniscus replacement is to re-establish normal joint load transmission and distribution to prevent articular cartilage degeneration that is observed frequently after meniscectomy.^{18,19,37-39} The principal challenge is to find a substitute that will survive and function within the joint over time.^{19,37,38} The most logical approach would be to use a replacement meniscus, presumably from a human donor. Although medial meniscus allografts have proven successful initially, especially for providing pain relief, the long-term results remain uncertain.^{18-20,22-26} An immune response against meniscus allografts has been documented, and the investigators of those reports speculate that this immune reaction could affect healing, incorporation, and revascularization of the grafts.^{20,22} The immune effects on long-term clinical outcomes remain under study.²² The possibility of

disease transmission through meniscus allografts also is a concern for their use.^{19,21} Whether or not the allograft meniscus can protect the knee from progressive degenerative arthritis has not been determined.^{18,23,24} More long-term data are needed to help ensure the safety and efficacy of meniscus allografts.^{18-20,22-24}

We report the use of a tissue-engineered scaffold designed to permit the body to generate its own replacement tissue, perhaps obviating some of the less-desirable characteristics of allograft tissue. This same tissue-engineering scaffold principle has been used successfully and extensively to regenerate new bone to fill skeletal defects as well as to regenerate other connective tissues.⁴⁰⁻⁴³ The tissue-engineered collagen meniscus implant supports generation of new tissue that seems to function, in this group of 8 feasibility patients, similarly to native meniscus tissue.

TABLE 6. Meniscus Loss and Regeneration

Patient Number	Age at Index Surgery (y)	Acute or Chronic	Percent Meniscus Remaining at Index Surgery	Percent Defect Filling at Initial Relook Arthroscopy	Percent Defect Filling at 5.8-Year Relook	Percent of Meniscus at 5.8 Years Compared With Normal*	Percent Meniscus Gained† Compared With Index Surgery
21001	47	Chronic (3)	65	75	50	83	27
21002	47	Chronic (2)	50	65	70	85	70
21003	49	Acute	60	95	95	98	63
21005	24	Chronic (5)	15	85	60‡	66	340
21009	38	Chronic (4)	25	90	60‡	70	180
21010	25	Chronic (3)	20	70	60‡	68	240
21011	41	Chronic (4)	20	100	95	96	380
21012	49	Chronic (1)	50	40	60	80	60
Average			38	77	69	81	170

NOTE. Number in parentheses is the number of previous partial meniscectomies.

*Sum of the remaining meniscus at index surgery plus the percent of defect filled.

†Quotient of the percent of new tissue divided by the percent of remaining meniscus at index surgery.

‡After debridement of some central margin fraying.

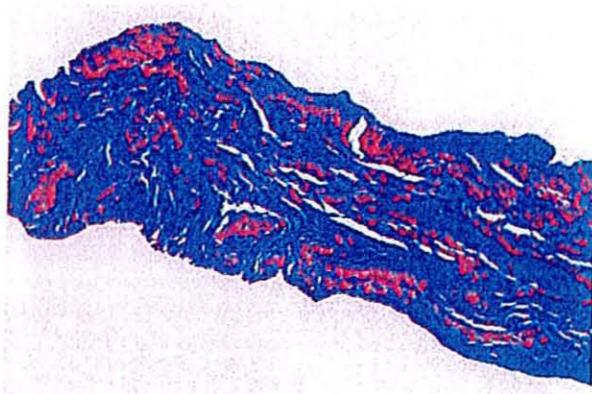


FIGURE 6. A biopsy specimen obtained 6.3 years after placement of the collagen meniscus implant (from the patient shown in Figs 4 and 5) showing fibrocartilaginous tissue that is meniscus-like in appearance. The original magnification is 25 \times . The stain is Masson trichrome.

However, because there was no control cohort, we cannot state conclusively that the new tissue is chondroprotective of the adjacent articular cartilage surfaces. Nonetheless, we did not observe any detrimental effects in the medial compartment suggestive of negative or further degenerative changes over an average of 5.8 years.

The collagen meniscus implant was developed as a tissue-engineering approach to reconstruct and restore irreparably damaged or lost meniscus tissue. Numerous animal studies²⁷⁻³² yielded encouraging results that supported obtaining regulatory approval for human studies.^{33,34} An initial phase I feasibility study³³ provided valuable information that guided structural changes in the implant and improved surgical techniques that led to a phase II feasibility study.³⁴ The 5.5 to 6.3 years of follow-up evaluation of that phase II feasibility study is the subject of this present article.

We have followed-up prospectively all 8 patients for an average of 5.8 years after they underwent reconstruction of their partially lost medial meniscus. All patients had meniscus reconstruction with the same type of collagen meniscus implant. All aspects of this study were performed under a U.S. Food and Drug Administration Investigational Device Exemption. This study is unique in that it is prospective, 5 to 6 years in duration, had 100% participation in the follow-up evaluation, and all patients underwent 2 separate arthroscopic relook procedures 4 to 5 years apart to document the usefulness of the investigational device and the durability of the new tissue.

In the earlier phase II study,³⁴ we observed that the

patients continued to improve in all clinical outcomes from 1 to 2 years after the index surgery. The present longitudinal study confirmed that the clinical outcomes statistically were unchanged from the 2-year assessment to the 5.8-year evaluation. Especially noteworthy is that the Tegner activity score continued to improve from 5 to 6 during this nearly 4-year period, and patients are performing sports, work, and other activities at their desired level despite increasing age (30–55 y). This finding, in conjunction with other longitudinal observations, confirms that the new tissue is durable and has remained functionally meniscus-like for more than 5 years in this study group. Thus, the hypothesis of this study is affirmed.

Not all patients improved in every parameter measured. Two patients had slightly decreased Lysholm scores at the most recent follow-up evaluation, but they had the 2 highest Lysholm scores before the index surgery (94 and 97). Although no patient rated his knee lower at the most recent follow-up evaluation, 4 patients remained the same compared with their status before index surgery. These 4 patients had rated their knees nearly normal before receiving the collagen meniscus implant, and they maintained that status. All 4 of these patients were chronic and had undergone an average of 3 prior partial meniscectomies; hence, it is unlikely that these patients would have completely normal knees in the future. One patient reported increased pain at the most recent evaluation. This patient had sustained a work-related injury to his involved knee 1 year earlier, and he still was in litigation for compensation.

Unlike meniscus allografts that are used to replace the entire meniscus,^{18,20,25,26,39,44} the collagen meniscus implant is designed to replace the damaged or missing portion of the meniscus. As such, it is not necessary to remove normal meniscus tissue to place the collagen meniscus implant. When meniscus allograft procedures are performed, frequently it is necessary to remove a significant amount of normal meniscus tissue to fit the allograft into the joint.^{18,20,25,26,39} Graft sizing is also a major consideration with meniscus allografts for optimal mechanical function and ultimate success.¹⁸⁻²⁰ For the collagen meniscus implant, the device is trimmed on the surgical field to fit the meniscus defect, thus eliminating the need to have a variety of device sizes available. Another advantage of the collagen meniscus implant is the minimal risk for human disease transmission because the device is made of U.S.-origin bovine-derived collagen.

There is a paucity of published objective data on long-term outcomes of meniscus allograft transplan-

tation. Observations in the published reports include persistent pain²⁶ and progressive shrinkage with increased density and stiffness of the allograft tissue over time.^{18,20,25,44} Based on actual physical measurements at arthroscopy, we determined that the amount of new meniscus-like tissue from the collagen implant filling the defects decreased slightly from 77% at the initial relook arthroscopy (6 or 12 months after implantation) to 69% at an average of 70 months (5.8 y) after implantation. Nonetheless, this group of patients had an average of 81% of normal meniscus at 5.8 years, and the amount of increase compared with the index remnant was an average of 170%. Probing of the tissue under direct arthroscopic observation revealed that it was supple and meniscus-like without any impression of shrinkage or stiffening. By way of video recording comparison with the initial relook procedure, we concluded that the slightly decreased volume of tissue likely was caused by some wear at the central margin of the restored meniscus. We remain uncertain of the significance of the loss of this central meniscus tissue.

Various materials, both natural and synthetic, have been evaluated to replace the injured meniscus.⁹⁻¹⁷ To our knowledge, other than human meniscus allografts, none of these materials has advanced to human clinical use. Because of the limited success or other major shortcomings of those efforts, a tissue-engineered device was chosen as a scaffold to generate new meniscus-like tissue.

Messner¹⁹ stated that all menisci are likely to be individually shaped, and joints typically undergo remodeling after partial or complete meniscus loss so that even the original meniscus might not fit its native joint after a period of time. These size and shape variations add to the complexity of selecting a meniscus allograft or autogenous tissue that adequately fits the joint and provides optimal biomechanical function. The collagen meniscus implant used in the present study has the advantage that it is trimmed to fit the meniscus defect, and then it conforms to the shape of the joint in which it is placed.³⁴ New tissue then replaces the implant over time and assumes the shape of the scaffold. Hence, joint size and shape are not critical issues, and that may help explain the longevity and durability of the meniscus-like tissue that has been documented in the present study.

Arnoczky³⁷ pointed out that the concept of tissue engineering holds excellent potential for the generation of new tissues, especially for the meniscus, and may be useful to enhance and optimize growth of new meniscus-like tissue. In the present study we have

followed this approach by using the collagen meniscus implant as a regeneration scaffold to grow new tissue to fill meniscus defects. In another article, Arnoczky³⁸ stated that a meniscus replacement device and any regenerated tissue should be chondroprotective, restore normal meniscus kinematics within the joint, provide pain relief, and have no deleterious effects on surrounding tissue. Arnoczky³⁸ also stated that the tissue-engineered device must be able to integrate with the host tissue.

We have shown in this group of 8 patients that the collagen meniscus implant generally meets the criteria advocated by Arnoczky³⁸ and maintains them through more than 5 years. For example, imaging studies as well as direct observation with relook arthroscopy confirmed that the chondral surfaces of the medial compartment appeared protected, or at least they had not degenerated further since the placement of the implant 5.8 years earlier. The significant ($P < .05$) increasing physical activity levels (Tegner scores) of the 8 patients in this study attest to the fact that general knee function, and hence presumably the meniscus kinematics, had been reestablished after placement of the implant and maintained for more than 5 years. This present study also documented significant ($P < .05$) improvements in the Lysholm and self-assessment scores of these patients from time of the index surgery through the most recent follow-up evaluation. No adverse effects from placement of the collagen meniscus implant were observed, either in the early postoperative period or during the later evaluations. Finally, magnetic resonance imaging studies as well as direct arthroscopic visualization confirmed the excellent integration between the host meniscus rim and the new meniscus-like tissue. Hence, we have shown that this tissue-engineered collagen meniscus implant was an acceptable approach to generate functional tissue in meniscus defects in this group of 8 patients. Furthermore, it is the only device of its type that has progressed to human clinical use.

The most prominent limitation of this present study was that the sample size was small, consisting of only 8 patients. However, it was designed as a feasibility study approved by the U.S. Food and Drug Administration for its stated purpose. Additionally, there was 100% longitudinal follow-up evaluation and patient participation throughout all aspects of the study. Because of the guidelines of the U.S. Food and Drug Administration for this feasibility study, there was no concurrent control cohort against which the collagen meniscus implant could be measured directly. Nonetheless, 7 of 8 patients had undergone between 1 and

5 prior partial meniscectomies before the implant procedure, and all 7 patients had clinical problems referable to their meniscus deficiencies at the time of index surgery. In essence, these patients served as their own controls. Unlike in our animal studies³² we were unable to determine the mechanical properties of the new tissue from these clinical patients. Although such information would be desirable, we did confirm that the new tissue survived and still was present after 5.8 years.

A large randomized multicenter clinical trial involving approximately 300 patients in the United States is nearing completion. This multicenter study compares the collagen meniscus implant with standard partial meniscectomy. This study will allow the comparison of outcomes of treatment with this tissue-engineered device with the natural history outcomes of partial meniscectomy alone. That study will provide a level of evidence I, whereas the present study is a level of evidence IV.

Although the advantage of the collagen meniscus implant, as opposed to partial meniscectomy alone, in limiting the progression of degenerative joint disease has not been proven definitely in this feasibility study of 8 patients, the results of this series provide evidence that a collagen meniscus implant-based, tissue-engineered meniscus structure can survive within the joint. The presence of a meniscus replacement tissue that remains in place for 5.8 years and does not cause any untoward effects in the knee joint, permits return of physical activity, and has the histologic characteristics of normal meniscus tissue lends strong support to the concept that a collagen meniscus implant can be used to replace irreparable or removed meniscus tissue.

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U.S. Feasibility Study – Minimum 24 Month Follow-up

A Clinical Study of Collagen Meniscus Implants to Restore the Injured Meniscus

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The meniscus performs critical functions within the knee, and its loss frequently leads to osteoarthritis and irreversible joint damage. Because prosthetic replacement of the meniscus has proven ineffective, the authors used tissue engineering techniques to develop a resorbable collagen scaffold (collagen meniscus implant) that supports ingrowth of new tissue and eventual regeneration of the lost meniscus. Eight patients underwent arthroscopic placement the collagen meniscus implant to reconstruct and restore the irreparably damaged medial meniscus of one knee. Seven patients had one or more prior meniscectomies, and one patient had an acute meniscus injury. Patients were observed with frequent clinical, serologic, radiographic, and magnetic resonance imaging examinations for at least 24 months (range, 24–32 months). All patients underwent relook arthroscopy and biopsy of the implant regenerated tissue at either 6 or 12 months after implantation. All patients improved clinically from preoperatively to 1 and 2 years postoperatively based on pain,

Lysholm scores, Tegner activity scale, and self assessment. Relook arthroscopy revealed tissue regeneration in all patients with apparent preservation of the joint surfaces based on visual observations. Histologic analysis confirmed new fibrocartilage matrix formation. Radiographs confirmed no progression of degenerative joint disease. The collagen meniscus implant is implantable, biocompatible, resorbable, and supports new tissue regeneration as it is resorbed. This tissue seems to function similar to meniscus tissue by protecting the chondral surfaces.

List of Abbreviations Used

GAG	Glycosaminoglycans
ELISA	Enzyme-linked immunosorbent assay

The meniscus cartilage of the knee originally was thought to be functionless remains of an unnecessary leg muscle.^{27,36} Total meniscectomies were performed for many years based on the assumption there were no adverse effects from removal of the menisci.²⁷ There even was speculation that removal of the meniscus cartilage would result in satisfactory regeneration of a new structure.²⁷ Some years later Fairbank⁸ documented radiographically that degenerative joint disease and osteoarthritis frequently followed meniscectomy. In recent years, many studies have documented the extreme importance of the menisci to the health of the

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knee.^{1,2,4-7,9,10,14,15,17,19,20,22-25,28,29,40} The menisci play many important roles, including load bearing, load or force distribution across the knee joint, joint stability, joint lubrication, and proprioception.^{1,2,5,9,14,15,19,20,22,24,40} It now is evident that the menisci are integral components in the complex biomechanics of the knee joint.

It also has become evident in recent years that injuries to the menisci have a significant impact on the healthcare system. For example, it is estimated that approximately 1,500,000 arthroscopic surgical procedures of the knee are performed each year. (Personal communication, Research Department, American Academy of Orthopaedic Surgeons, 1998). Of this number, 850,000, or more than 1/2 of all knee arthroscopies, are related to the meniscus cartilage of the knee. It is apparent that many of the patients who lose a significant portion of their meniscus cartilage will suffer various derangements of the knee. There is loss of stability of the joint, loss of joint lubrication, a concentration of mechanical forces on the underlying articular cartilage of the femur and tibia, and in many patients, a progressive degenerative process that leads to osteoarthritis and possibly the need for total joint replacement. Because most of these meniscus injuries are unsuitable for repair with sutures or other tissue attachment devices, replacement of the damaged or lost meniscus cartilage would seem an appropriate approach to prevent or minimize the progressive degenerative joint disease. Many different materials have been evaluated for prostheses of the meniscus, including artificial materials, autogenous tissue, and allograft tissue.^{3,12,13,16,21,37-39,42} The results of using these various materials and tissues generally have not proven successful. Therefore, the authors' used tissue engineering to help identify a method to regenerate new meniscus tissue rather than attempting to replace it by artificial means.

Tissue engineering is a relatively new discipline that recently has received attention.²⁶ Tissue engineering has provided a fundamental understanding and technology that has per-

mitted the development of structures derived from biologic tissues to treat various maladies.¹¹ Bioresorbable collagen matrices are one example of innovative new devices that have resulted from the discipline of tissue engineering. These collagen matrix materials have many positive features for use in preservation and restoration of meniscus tissue, including a controlled rate of resorption based on the degree of crosslinking, processing of the collagen can minimize potential immune responses, and the extremely complex biochemical composition of the normal meniscus can be closely approximated during the production process. If such a material could serve successfully as a scaffold for regeneration of new tissue, then many of the previously reported negative effects of losing the meniscus cartilage might be prevented or at least minimized.

After studying the collagen meniscus implant extensively for more than 7 years in vitro and in laboratory trials,^{21,31-34,41} an initial Phase I clinical feasibility study was completed successfully.³⁵ Based on that study, the collagen meniscus implant was modified and improved for use in the present Phase II clinical trial. The purpose of the present study was to determine the safety of the implant and its potential efficacy. Specifically, the objectives of this study were to determine whether the scaffold was arthroscopically implantable in patients, to assure that the patients would recover without complications, to determine whether the implant and any associated new tissue would remain mechanically stable, and to confirm tissue regeneration in patients similar to that which had been observed in previous animal studies³²⁻³⁴ and the first human study.³⁵

MATERIALS AND METHODS

The collagen meniscus implant (ReGen Biologics, Inc, Redwood City, CA) used in this Phase II feasibility study was of the same chemical composition as that used in the previously reported Phase I study.³⁵ Only the physical size and shape were altered so that the

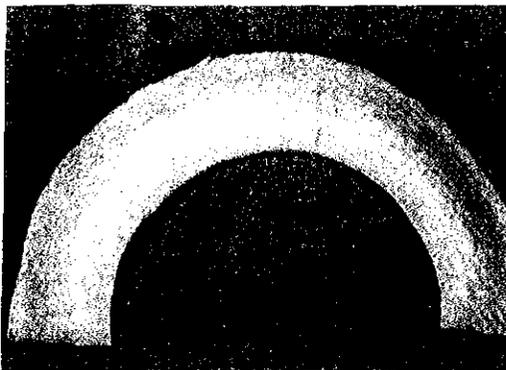


Fig 1. The collagen meniscus implant that was used in this study is shown. The outer circumference is approximately 70 mm long, it is 8 mm wide, and it is 4 mm thick at the peripheral margin.

implant more closely approximated a normal human medial meniscus (Fig 1). Techniques for the formulation and fabrication of the collagen meniscus implant have been reported in detail previously.^{18,30,35} Briefly, the collagen meniscus implants are fabricated from Type I collagen from bovine Achilles tendons. The tendon tissue is trimmed and minced and then washed copiously with tap water to remove blood residue and water soluble materials. The Type I collagen fibers are purified using various chemical treatments, including water, salt, acid, base, and organic solvent extractions to remove noncollagenous materials and lipids. The isolated Type I collagen fibers then are analyzed for purity. The purified collagen fibers were swollen in the presence of equal quantities of hyaluronic acid and chondroitin sulfate and homogenized. After addition of GAG, the swollen collagen and GAG fibers were coprecipitated by the addition of ammonium hydroxide. The purified collagen fibers then were dehydrated, manually oriented in a mold, lyophilized, and chemically crosslinked with formaldehyde. After additional processing terminal sterilization was done by gamma irradiation.

United States Food and Drug Administration and local institutional review board approvals were obtained before commencing

this Phase II clinical feasibility study with the collagen meniscus implant. Written informed consent was obtained from all patients before placement of the implant. The study was open to men and women ages 18 to 50 years who had irreparable injury or previous loss of their medial meniscus. The study included patients with acute injuries and patients with chronic injuries. The study also required that the involved knee be ligamentously stable or stabilized at the time of the index surgery, the surgical procedure done to place the collagen meniscus implant. Patients were excluded if they had Grade IV (full thickness) chondral defects, suffered from inflammatory or systemic disease, had known collagen allergies, were diagnosed with autoimmune disease, or were pregnant. Because this was a clinical feasibility study, there was no randomization and there was no cohort of control patients.

Eight patients were enrolled in this study. Although this study was open to either gender, by happenstance the first eight patients who qualified for inclusion into the study were men. The patients who were enrolled in the study ranged in age from 24 to 49 years with an average age of 40 years. One patient had an acutely displaced bucket handle tear of his meniscus, and the remaining seven patients had chronic meniscus injuries. The patients with chronic injuries had undergone from one to five previous surgeries on the involved medial meniscus. The mechanism of the original injury in seven of the patients was sports related, and one patient had sustained an injury on the job. No patients in this study required ligamentous stabilization before the index surgical procedure to place the collagen meniscus implant. Two patients had undergone microfracture for chondral defects at least 1 year before the index operation, and two additional patients had undergone microfracture for chondral defects on weight-bearing surfaces 8 to 12 weeks before the index surgery for placement of the collagen meniscus implant.

All patients underwent thorough physical examination and had baseline radiographs and

was removed and discarded. The sutures then were tied over the capsule in a standard manner. Closure of all incisions and portal wounds was done using routine techniques.

Immediately postoperatively, patients' knees were placed in a brace locked in full extension. This locked extension brace was maintained for 6 weeks, but the patients removed the brace three to four times per day to perform self assisted passive range of motion (ROM) exercises. Typically, each patient did at least 500 repetitions three times a day. During the first 4 weeks, the ROM was limited from 0° to 60°, then it was increased to 0° to 90° for weeks 5 and 6. After the sixth week, the brace was unlocked, and patients wore the brace for comfort as desired. At the same time the patients also started unlimited active and passive ROM exercises. During the initial 6 weeks, patients were nonweightbearing while using crutches to walk. Patients were allowed to stand with the knee loaded in an axial position. After the sixth week, patients were allowed full weightbearing, but they were encouraged to use one or both crutches for at least 2 more weeks until they were able to walk without a limp. After the sixth week, rehabilitation exercises progressed on a weekly basis until the patient had returned to full, unrestricted activity at 6 months after collagen meniscus implant placement.

All patients were observed closely with clinical and MRI examination and blood samples to monitor for the presence of humoral or cell mediated responses to the collagen meniscus implant. Clinical followup and blood collection were done at 1, 6, 12, 26, and 52 weeks. Magnetic resonance imaging examinations were done at 6, 12, 26 and 52 weeks. Clinical evaluations included thorough physical and orthopaedic examinations, Lysholm scores, Tegner activity scales, self assessment and pain evaluation during activities of daily living. Pain was measured on a 100 mm visual analog scale, with 0 being no pain and 100 being the worst pain.

All eight patients completed the study. All patients agreed to return for a relook ar-

throscopy and biopsy of the newly regenerated tissue. Six of the patients underwent relook arthroscopy and biopsy at 6 months after the index surgery, and the remaining two patients underwent these procedures at 1 year after implantation. All patients also returned for long term followup. At the last examination, the minimum followup was 2 years with a range of 24 months to 32 months.

At the time of relook arthroscopy and biopsy, video and photographic documentation were made in all patients. If excessive scar tissue was observed, it was removed, but no other procedures were performed at the time of the relook arthroscopies. The biopsy of the newly regenerated tissue was performed using a 15 gauge modified Menghini biopsy needle (Boston Scientific Corporation, Watertown, MA) (Fig 3). The location of the biopsy site was based on the appearance of the new tissue and a comparison with the video documentation made at the time of the index surgery. Each biopsy was taken to include new tissue, host meniscus rim and adjacent capsule. All biopsy specimens were read and interpreted by an independent pathologist.



Fig 3. An intraoperative view taken at the time of the 6 month relook procedure. The modified Menghini biopsy needle (M) has penetrated the new regenerated tissue. The arrow points to the anterior interface between the native meniscus and the new regenerated tissue.

magnetic resonance imaging (MRI) of the involved knee before the index surgery. Each patient also filled out extensive medical questionnaires regarding the medical history of the involved knee. All patients entering the study also agreed to undergo relook arthroscopy and biopsy to assess the extent of tissue regeneration and the overall status of the involved knee. All patients also had blood drawn before placement of the collagen meniscus implant to serve as baseline for ELISA testing for the presence of humoral antibodies to collagen and for lymphocyte proliferation assay tests to assess for any cell mediated immune response to the implant.

The collagen meniscus implant was placed using routine arthroscopic surgical procedures. All surgical procedures were performed by the senior author (JRS). Three portals were made about the knee, one for outflow and two as working portals. The damaged meniscus tissue was debrided only until healthy tissue was reached. In those cases where the debridement did not reach the red zone of the meniscus, a microfracture awl (Linvatec Corporation, Largo, FL) was used to perforate the host meniscus rim until a bleeding bed could be assured. A special Teflon measuring device (ReGen Biologics, Inc) developed for this procedure was used to measure the exact size of the defect. The collagen meniscus implant then could be measured and trimmed to the correct size on the sterile field of the operating environment. In these eight patients, the percent of meniscus loss averaged 62% with a range of 35% to 85%. The measured length of the defect averaged 42.5 mm with a range of 27 to 55 mm.

A posterior medial incision was made approximately 3 cm in length parallel and just posterior to the medial collateral ligament directly over the joint line so that the inside out meniscus repair needles could be captured and the sutures tied over the capsule. The implant procedure was done by initially placing a suture approximately midway from anterior to posterior of the lesion with the arms of the suture going over and under the meniscus. This suture

served as a loop or a lasso which would hold the implant in place while the permanent sutures were being placed. A specially designed introducer containing the collagen meniscus implant was inserted through the ipsilateral portal and guided through the lasso suture. A plunger then was used to push the implant out of the delivery device, and simultaneously the lasso suture was tightened around the implant. When positioning was deemed satisfactory, the implant was sutured to the host meniscus rim using standard inside out techniques with zone specific meniscus repair cannulae (Linvatec Corporation). Sutures were placed approximately 4 to 5 mm apart. In the initial part of this study, absorbable 2-0 sutures were used. In the second half of this study the preference was 2-0 nonabsorbable braided sutures. Sutures were placed in a vertical mattress pattern around the rim of the meniscus remnant, and a horizontal pattern was used in the anterior and posterior horns. Typically, eight to 10 sutures were used to secure the implant in place (Fig 2). When all of the securing sutures had been placed, the lasso suture



Fig 2. An intraoperative view showing the collagen meniscus implant being sutured to the host meniscus rim. The zone specific cannula (Z) in the foreground is used to pass the needles and suture. The arrow points to the dark temporary lasso suture that is used to stabilize the implant while it is being sutured into place.

The serial MRI scans all were made on the same MR imaging machine. T1 weighted axial and coronal images and T2 weighted gradient echo contrast axial, sagittal, and coronal images also were made. Fast spin echo and fat suppression techniques were used. Intravenous gadolinium enhancement also was used in all patients. All MRI scans were read by an independent radiologist.

The serologic testing was done by an independent laboratory. An ELISA was used to detect any humoral antibodies that may have developed in response to the presence of the collagen meniscus implant. These sera were tested at different dilutions and compared with those of a positive control rabbit serum that was run parallel to the clinical samples. The lymphocyte proliferation assay test was done to compare with known mitogens (pokeweed, phytohemagglutinin, and Con-A) and specific recall antigens (streptokinase, tetanus toxoid, and *Candida albicans*) as well as an established antigen that had been produced in rabbits in response to the collagen meniscus implant.

RESULTS

All eight patients have remained in the study for greater than 2 years. There were no significant complications attributed to the collagen meniscus implant. One patient underwent additional relook arthroscopy at 9 months after implant placement because of excessive scar tissue formation. That patient responded fully to the joint debridement without additional consequence.

Clinically, all patients returned to activities of daily living by 3 months and were fully active by 6 months. The Lysholm score improved from preoperatively (before index surgery) to 1 year after implantation in seven of eight patients. By 2 years, all eight patients had higher Lysholm scores than before the index surgery. For the Tegner activity scale, at 1 year after the index surgery to place the collagen meniscus implant, four patients had improved results and four had unchanged results from

the preoperative evaluation. By 2 years, seven patients had higher Tegner scores and one patient had a lower score compared with preoperatively. For patient self assessment at 1 year, four patients had improved results and four patients results remained the same from their preoperative assessment. Of the four patients whose results remained unchanged, all had assessed their knees as nearly normal preoperatively. By 2 years, five patients thought their knees had improved from before the index surgery, and the other three patients continued to rate their knees as nearly normal. For pain during activities of daily living, seven of eight patients had improved results from before index surgery to 1 year postoperatively, then their results improved additionally or remained stable from 1 year to 2 years postoperatively. One patient's pain worsened slightly from preoperatively to 1 year postoperative, but his pain then improved from 1 to 2 years postoperatively without additional treatment. Tables 1 through 3 summarize these scores and values.

Preoperative radiographs were compared with postoperative radiographs at 1 and 2 years. The postoperative radiographs revealed no significant progression of Fairbank changes,⁸ nor was there any noteworthy change in joint space or in axial alignment based on long standing radiographic films.

The serial MRI scans through 1 year were

TABLE 1. Lysholm Scores

Patient Number	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery
21001	94	95	100
21002	88	100	95
21003	80	100	100
21005	52	86	79
21009	55	85	89
21010	72	73	89
21011	97	82	99
21012	64	94	96

TABLE 2. Tegner Activity Scale

Patient Number	Before Injury	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery
21001	9	5	5	7
21002	5	4	4	5
21003	7	5	5	4
21005	7	3	7	5
21009	9	3	5	8
21010	10	3	3	4
21011	6	1	2	3
21012	6	4	6	6

compared. The earliest postoperative MRI scan (6 weeks) revealed almost uniformly that the implant and new tissue complex were somewhat smaller than would be expected of the normal medial meniscus. However, for the remainder of the series of MRI scans, the size of the complex did not change with time. There was no apparent shrinkage or significant loss of the new tissue. Furthermore, there consistently was a decreasing signal intensity with time that suggested an ongoing maturation process of the newly regenerated tissue. This maturation process seemed to be still actively

underway at 1 year after placement of the collagen meniscus implant.

Six of eight patients underwent relook arthroscopy at 6 months. Grossly, there was new tissue regeneration present in all patients. The newly regenerated tissue showed a variable degree of maturity similar to what was reported previously for the Phase I study.³⁵ This new tissue had a stable interface with the host meniscus rim when probed, and no patient had any significant fragmentation of the implant and new tissue complex. There were no negative findings at the time of arthroscopy. There was no indication of wear particles, synovitis, inflammation, or abrasion to the articular surfaces. Two patients underwent relook arthroscopy and biopsy at 12 months after implantation. In these patients, the tissue had a more mature appearance than it did at 6 months (Fig 4). The new tissue again had a stable interface with the host meniscus rim, and there was gross evidence of new tissue regeneration in both patients. One patient did have some fragmentation of the posterior horn. The portion that fragmented had been covered by a superior flap of meniscus tissue that had been left intact at the time of the index surgery. No negative findings were observed in the patients undergoing relook arthroscopy at 12

TABLE 3. Patient Assessment and Pain

Patient Number	Patient Self Assessment			Pain Visual Analog Scale		
	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery	Before Index Surgery	12 Months After Index Surgery	24 Months After Index Surgery
21001	2	1	1	28	10	0
21002	2	2	1	26	10	3
21003	3	1	1	11	6	0
21005	3	2	2	28	4	4
21009	2	2	2	34	4	0
21010	3	2	2	33	10	0
21011	2	2	2	1	10	4
21012	2	2	2	23	3	3

Self Assessment: 1 = Normal; 2 = Nearly Normal; 3 = Abnormal
Pain Visual Analog Scale Based on 100 mm Scale for Activities of Daily Living



Fig 4. Arrows point to the new regenerated tissue at the time of a 12 month arthroscopic relook. New tissue has completely replaced the collagen meniscus implant. The arthroscopic probe (P) is at the anterior interface between the native meniscus and the new regenerated tissue.

months. For all patients, the average size of the meniscus loss at the time of index surgery was 62% based on measurements of the defects. The average filling of the defect at time of relook arthroscopy was 77% based on measurements and the surgeon's estimates. Those findings are summarized in Table 4.

One patient who had undergone relook arthroscopy at 6 months returned at 32 months because of a painful plica. Arthroscopy was performed, so it was possible to observe the

regenerated meniscus tissue. The tissue appeared to be of the same size without any shrinkage or fragmentation compared with the 6 month relook arthroscopy (Fig 5). The chondral surfaces remained unchanged and without damage or degeneration. The patient refused additional biopsy of the regenerated tissue.

Histologically, biopsy specimens showed that the collagen meniscus implant was progressively invaded and replaced by cells similar to meniscus fibrochondrocytes with pro-

TABLE 4. Meniscus Loss and Regeneration

Patient Number	Age at Index Surgery	Acute or Chronic	Percent Meniscus Deficit at Index Surgery	Percent Defect Filling at Relook Arthroscopy	Time of Relook Arthroscopy
21001	47	Chronic	35	75	6 months
21002	47	Chronic	50	65	6 months
21003	49	Acute	40	95	6 months
21005	24	Chronic	85	85	6 months
21009	38	Chronic	75	90	6 months
21010	25	Chronic	80	70	6 months
21011	41	Chronic	80	100	12 months
21012	49	Chronic	50	40	12 months



Fig 5. An intraoperative view 32 months after placement of the collagen meniscus implant showing the same patient as in Figure 3. The new tissue has retained its size and shape, and the chondral surfaces remain in excellent condition. The interface between the native meniscus and new tissue is now nearly indistinguishable.

duction of new matrix in all patients. No inflammatory cells or other histologic evidence of immunologic reactions were observed in any of the biopsy specimens. Likewise, there was no indication of any infection in any of the patients examined. This new tissue was becoming more dense and organized in most patients, but it ranged in maturity from chondroid to dense fibrocartilage (Fig 6). The 12 month biopsies showed excellent new matrix formation. This matrix was becoming dense and starting to take on a more fibrocartilaginous appearance. There still were some remnants of the collagen scaffold in the biopsy specimens obtained at 12 months. Again, there were no adverse effects observed, including no inflammatory or immune response, no hypervascularity, and no indication of infection.

On immunology testing, the ELISA assay revealed that there was no significant increase in antibodies to the implant in any of the patients at any of the time points. There was no humoral response. The lymphocyte proliferation assay test showed the response to all mitogens was not altered in the presence of the implant. The only recall antigen to respond was streptokinase, and then only at 12 weeks.

The most critical test was the response to the implant material because it would have showed any potential hypersensitivity to the implant. Three patients had a small increased response to the implant at 12 weeks, but at 26 and 52 weeks all patients' cells were responding as they did before implant, suggesting that hypersensitivity to the implant is not an issue.

DISCUSSION

The discipline of tissue engineering remains in its infancy, but it is clear that it holds great promise.^{11,26} The authors think that tissue engineering is the future for the repair, preservation, restoration, and regeneration of many different musculoskeletal tissues that are damaged beyond repair with current techniques.

The authors developed a bioresorbable collagen matrix to serve as a scaffold or a template into which new tissue can grow to regenerate damaged structures. In previous studies the authors have described the formulation of this material^{18,35} and described the *in vitro* and *in vivo* laboratory studies.^{21,31-34} This collagen scaffold has been developed to provide a suitable physical and chemical environment

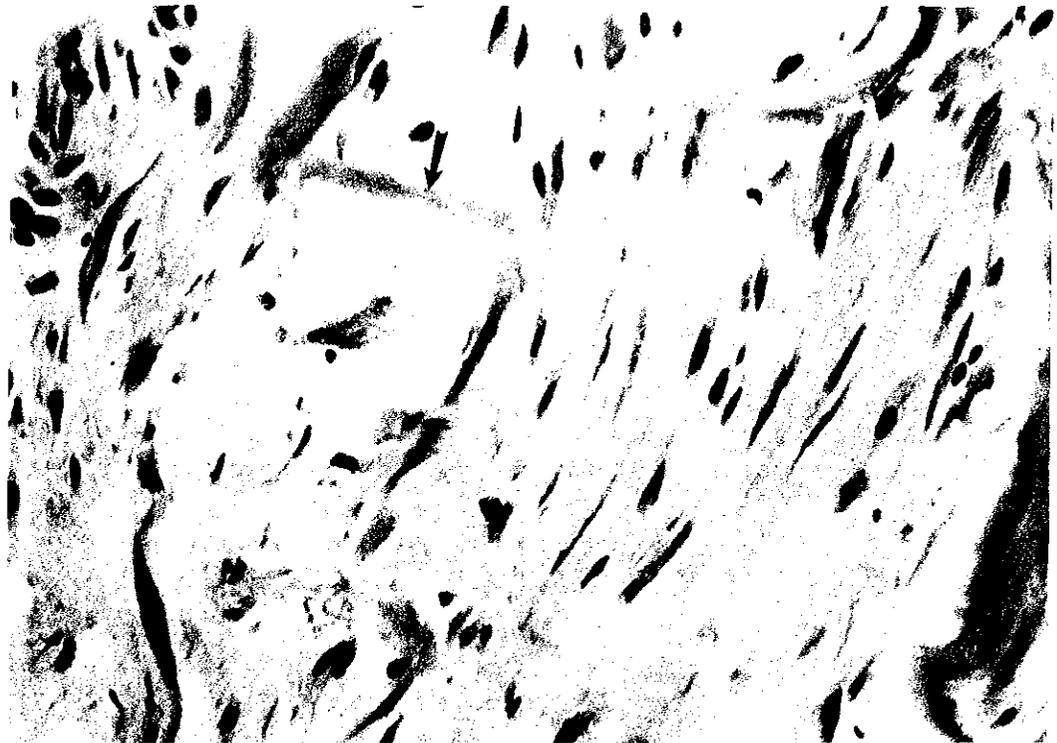


Fig 6. Histologic appearance of a 6 month biopsy specimen. The new tissue is taking on fibrocartilaginous-like characteristics with the new collagen starting to become organized. The arrow points to a remnant of the collagen meniscus implant. (Stain, hematoxylin and eosin; original magnification, $\times 100$.)

for cellular ingrowth and matrix production.¹⁸ This material has been shown in numerous animal studies³²⁻³⁴ and in one previous human clinical feasibility study³⁵ to be nonimmunogenic and free of wear particles.

On the basis of an initial human clinical feasibility study, the authors made numerous changes in the physical structure of the collagen meniscus implant, then carried out the present Phase II clinical feasibility study. This study did not include concomitant controls, so the progress of these patients only can be compared with their preoperative status. Likewise, to the authors' knowledge there are no other regeneration templates comparable with this collagen meniscus implant to which one can compare these results.

Although meniscus allografts have become more common in their clinical use, the colla-

gen meniscus implant has different indications and goals. Whereas meniscus allografts are used as a prosthetic replacement for a completely lost or removed meniscus, the collagen meniscus implant is designed as a regeneration template so that the body's own tissue will grow into it. Furthermore, the collagen meniscus implant is trimmed to fit an existing defect in the meniscus cartilage, and removal of otherwise competent meniscus tissue is aggressively avoided.

In this study, it was confirmed that cells have the ability to grow into the collagen meniscus implant, establish themselves, and produce new matrix. Furthermore, the serial MRI scans and the relook arthroscopies and biopsies done at 6 and 12 months confirm that this tissue has an ongoing maturation process, but it still seems to be active at 1 year after im-

plantation. This finding suggests that it may take multiple years before this newly regenerated tissue has converted to dense fibrocartilage characteristic of the normal meniscus. Although the MRI scans done at 6 weeks showed that the implant and new tissue complex was smaller than the normal meniscus, the size of the complex did not change after 6 weeks through 1 year. There were no significant adverse events reported in patients in the present study. Similarly, the histology did not reveal any negative findings that would lead one to conclude that this collagen scaffold material and the newly regenerated tissue are other than safe and compatible.

Overall these findings have confirmed that the collagen meniscus implant is implantable, biocompatible, has the ability to support new tissue regeneration, and seems to be safe for the described use. Not unexpectedly, there was notable variation of the biologic response between patients. Some patients had significantly more tissue regeneration than others. Long term survival of this new tissue is unknown at present.

Based on these generally positive results, the authors have obtained regulatory permission to commence a multicenter clinical trial to assess the efficacy of the collagen meniscus implant additionally. The multicenter trial received regulatory approval to commence, and patient enrollment began early in 1997. The multicenter trial uses similar inclusion and exclusion criteria as described above. However, the multicenter trial is prospective and randomized. When patients agree to participate in this study, they choose an envelope that indicates whether they receive the collagen meniscus implant or whether they are a control patient and receive the current standard of care for meniscus injuries (partial meniscectomy and debridement). This study is designed so that 1/2 of the individuals in this study will receive the collagen meniscus implant and the other 1/2 will serve as controls. Furthermore, this study is separated into two parts to examine separately those patients who have had no prior surgery on the involved meniscus com-

pared with those who have had one, two, or three prior surgeries on the involved meniscus. This study currently is underway at 15 sites in the United States and will include 288 patients. Results will be forthcoming.

The authors think that the collagen meniscus implant has shown the use and biocompatibility necessary for this specific tissue engineering application, and it supports new tissue regeneration and ingrowth as the collagen scaffold material is resorbed by the body. Importantly, this newly regenerated tissue seems to function in a biomechanical manner similar to normal meniscus tissue in that the chondral surfaces of the joint seem to be protected up to 32 months after placement of the collagen meniscus implant. The collagen meniscus implant seems to be safe for clinical use based on the current study and the previous Phase I study.³⁵ No serious adverse effects attributable to the collagen scaffold have been reported.

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APPENDIX G
Publications from Europe

Histology and Ultrastructure of a Tissue-Engineered Collagen Meniscus Before and After Implantation

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Abstract: The collagen meniscus implant (CMI) is a tissue-engineering technique designed to stimulate regeneration of meniscus-like tissue in cases of irreparable tears or previous meniscectomy. CMI morphology was investigated before and after implantation by light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). In a case series biopsy specimens were harvested from four patients who underwent a second arthroscopic look 6 months after placement of the CMI. CMI sections appeared composed of parallel connective laminae of 10–30 μm , connected by smaller bundles (5–10 μm). This connective network formed lacunae with diameters between 40 and 60 μm . At greater magnification, the walls of the lacunae demonstrated tightly packed and randomly distributed collagen fibrils, with diameters ranging from 73 to 439 nm. In the biopsy specimens, the lacunae were filled with connective tissue that contained newly formed vessels and fibroblast-like cells, presenting an abundant rough endoplasmic reticulum and several mitochondria. In the extracellular matrix, the collagen fibrils showed uniform diameters (126 nm \pm 32 nm). The original structure of CMI was still recognizable, and no inflammatory cells were detected within the implant. The morphological findings of this case series demonstrate that CMI provides a three-dimensional scaffold suitable for colonization by precursor cells and vessels and leading to the formation of a fully functional tissue. © 2005 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 74B: 808–816, 2005

Keywords: collagen; scaffolds; porous; extracellular matrix; tissue engineering

INTRODUCTION

Degenerative joint changes may often follow meniscectomy and many patients complain of knee pain after this procedure.^{1–6} Different open and arthroscopic techniques have thus been described for repairing meniscal tears.^{7–10} However, some lesions are difficult to treat because of their location and shape, and also because tissue quality might not permit a stable repair, as in degenerative lesion. Meniscus allografts can be useful for total meniscectomies, but this invasive procedure is technically demanding and carries potential risks of transmissible diseases.¹¹

The collagen meniscus implant (CMI) (ReGen Biologics, Inc., Franklin Lakes, NJ) is a tissue-engineering technique, described in 1992, designed for stimulating regeneration of meniscus-like tissue.¹² This method has been adopted for patients who underwent partial meniscectomy or presented with irreparable meniscus tears.^{13–16}

CMI is composed of Type I collagen derived from bovine Achilles tendon and enriched with glycosaminoglycans (GAGs), including chondroitin sulfate and hyaluronic acid, in order to stimulate cellular ingrowth. It is processed chemically and physically to remove molecular antigens and noncollagenous materials.^{15,17} The shape is similar to the human meniscus and the materials used are biocompatible (Figure 1).^{13–16}

Preliminary clinical results showed a significant improvement of symptoms in eight of eight treated patients with a follow-up of about 6 years.^{13,14} Human biopsy specimens harvested 1 year after implantation showed cellular colonization and tissue ingrowth within the scaffold. Light microscopy observations demonstrated newly formed fibrocartilage with dense, well-organized collagen bundles.^{13,14,16}

However, there are no published ultrastructural data regarding CMI before and after implantation in humans. In the present case series the objective was to report pre- and postoperative findings observed by light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). It was hypothesized that the newly formed tissue would have morphological characteristic similar to native meniscus tissue.

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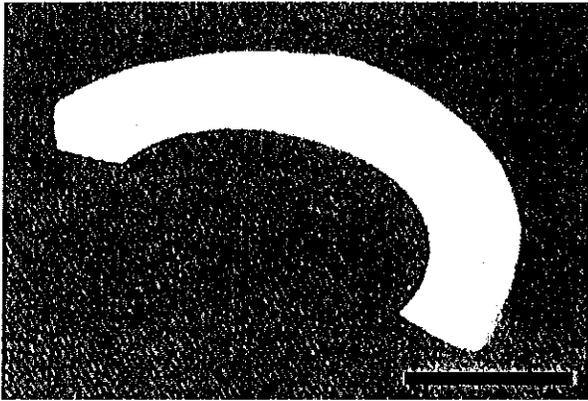


Figure 1. Collagen meniscus implant (CMI). The semicircular shape and triangular section are similar to the human meniscus (Bar = 15mm).

MATERIALS AND METHODS

CMI was performed on four patients, affected by traumatic irreparable tears of the posterior horn of the medial meniscus. All the procedures were carried out arthroscopically according to the surgical technique described by Rodkey and co-workers (Figure 2).^{13,16} Patients' ages ranged from 24 to 50 years, with an average of 38 years. The meniscus tear was the sole intrarticular lesion detected, and the chondral surfaces of the medial compartment were intact.

CMI samples were collected at the time of surgery from residual portions of the scaffolds implanted in these patients. Biopsy specimens were harvested 6 months after implantation from the same patients, at the time of a second arthroscopic look, performed for evaluating the implant evolution. No patients complained of pain or other symptoms in the operated knee. Written informed consent was obtained for performing both arthroscopy and biopsy.

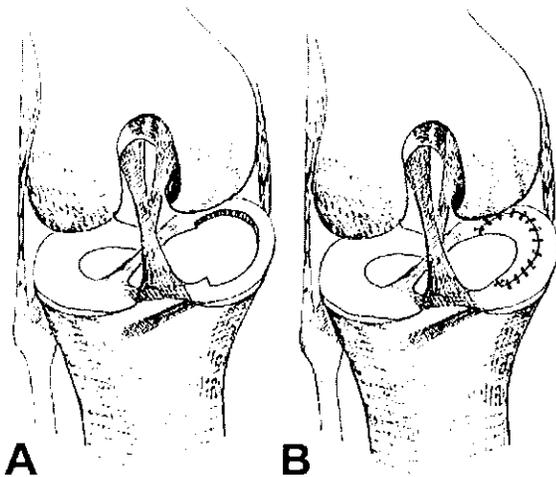


Figure 2. Diagram of CMI procedure. (A) Partial medial meniscectomy with preservation of the peripheral portion. (B) CMI suture to the meniscal stump.

TABLE I. The Lysholm Score and Tegner Activity Scale Increased in all Operated Knees During the 6-Month Period Following CMI

Patient Number	Age at Surgery	Before Surgery		6 Months after Surgery	
		Lysholm Scale	Tegner Activity Scale	Lysholm Scale	Tegner Activity Scale
1	24	70	3	100	5
2	36	68	2	95	5
3	42	70	2	98	4
4	50	41	2	82	4
Mean	38	62.25	2.25	93.75	4.5

All knees were evaluated before CMI and at the time of biopsy with the use of the Lysholm II score and Tegner activity scale. The biopsies were performed with an 18G Temno device (Allegiance Healthcare Corp., McGaw Park, IL), routinely used for prostate biopsies. This device minimized trauma to the implant–new tissue complex. Biopsy specimens measured 8 mm in length and 0.7 mm in diameter.

All samples were immediately fixed in 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 7.4) for 6 h at 4°C. Subsequently, they were subdivided in three different groups.

Light Microscopy. Specimens were dehydrated in ascending grades of ethanol and then embedded in paraffin. They were sectioned at a 5- μ m thickness with a Reichert Ultracut S ultratome (Leica, Vienna, Austria) and then stained with hematoxylin and eosin. Histological evaluation was performed with light microscopy (Nikon Eclipse E600 microscope, Nikon, Tokyo, Japan).

Scanning Electron Microscopy. Specimens were post-fixed in a solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide for 3 h. Slices were washed in pH 7.2 phosphate-buffered saline (PBS), dehydrated in ascending grades of ethanol and subjected to critical-point drying in CO₂. Dried slices were mounted on standard stubs, gold-coated in an Emitech K550 sputter coater (Emitech Products Inc., Houston, TX) and then observed on a Philips XL-30 SEM-FEG microscope (FEI, Eindhoven, Netherlands) fitted with a 1424 \times 968 pixel frame store for direct digital imaging. Collagen fibril diameters before and after implantation were compared by measuring 1000 fibrils on 40 SEM images. The diameter of collagen fibrils was determined by a digital ruler (AnalySIS, Soft Imaging System, Munster, Germany) and divided into 25 diameter classes, each corresponding to a 14-nm interval.

Transmission Electron Microscopy. Specimens were post-fixed for 2 h with 1% osmium tetroxide in 0.1M Na-cacodylate buffer (pH 7.2) at 4°C. After standard dehydration

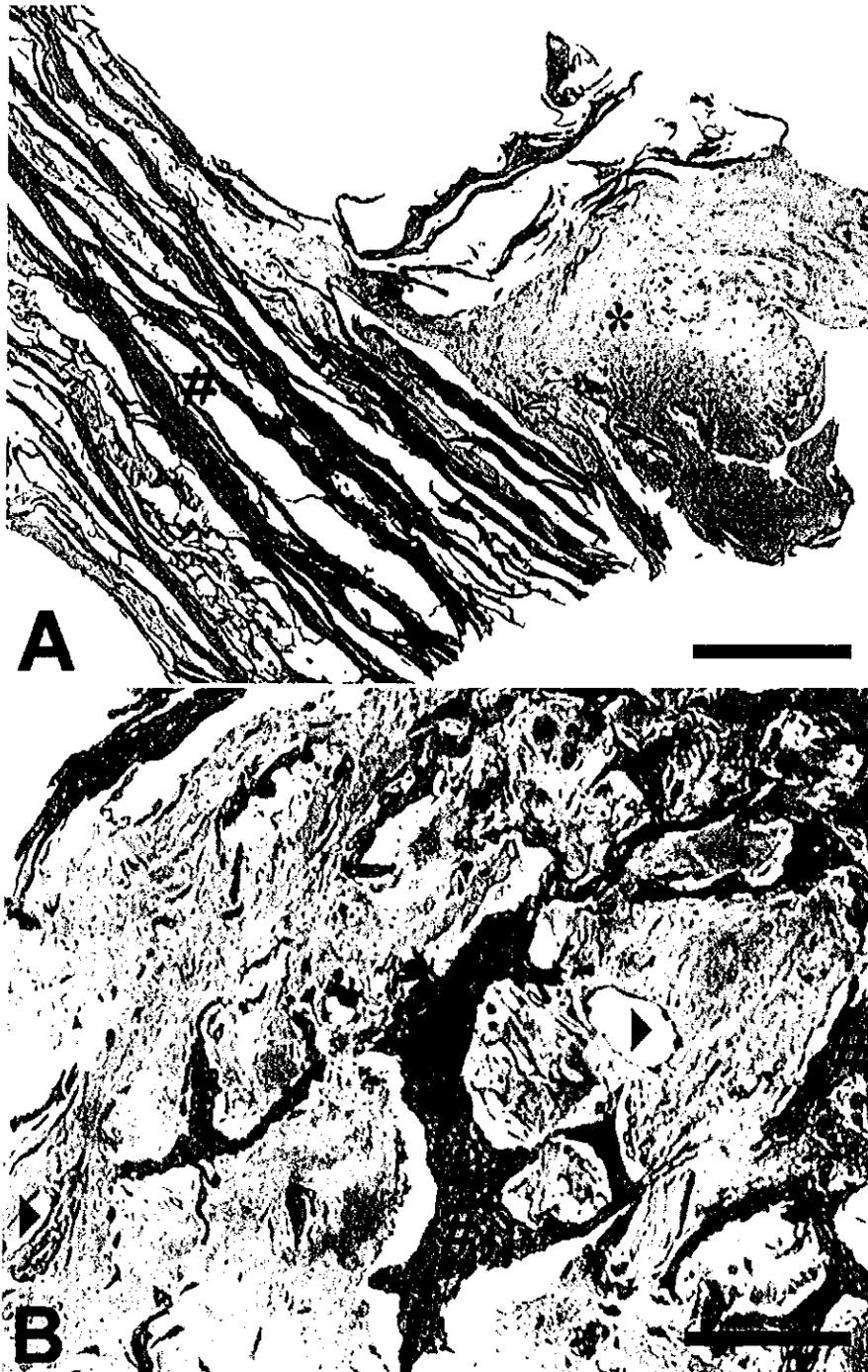


Figure 3. Light microscopy of the implant stained with hematoxylin and eosin. (A) The CMI (number sign) is partially invaded from posterior meniscus tissue (asterisk). A more compact scaffold is evident (bar = 500 μm). (B) The CMI scaffold is clearly evident (number sign). Connective tissue inside the lacunae and new vessels (triangles) are evident (bar = 40 μm).

in ethanol series, specimens were embedded in Epon 812. They were sectioned to 60-nm-thick ultrathin sections with an ultramicrotome (RMC MTXL ultramicrotome, Boeckeler Instruments, Tucson, AZ) fitted with a diamond knife.

The ultrathin sections were collected on copper grids, stained with uranyl citrate and lead acetate, and observed with TEM (1010 EX electron microscope, Jeol, Tokyo, Japan).

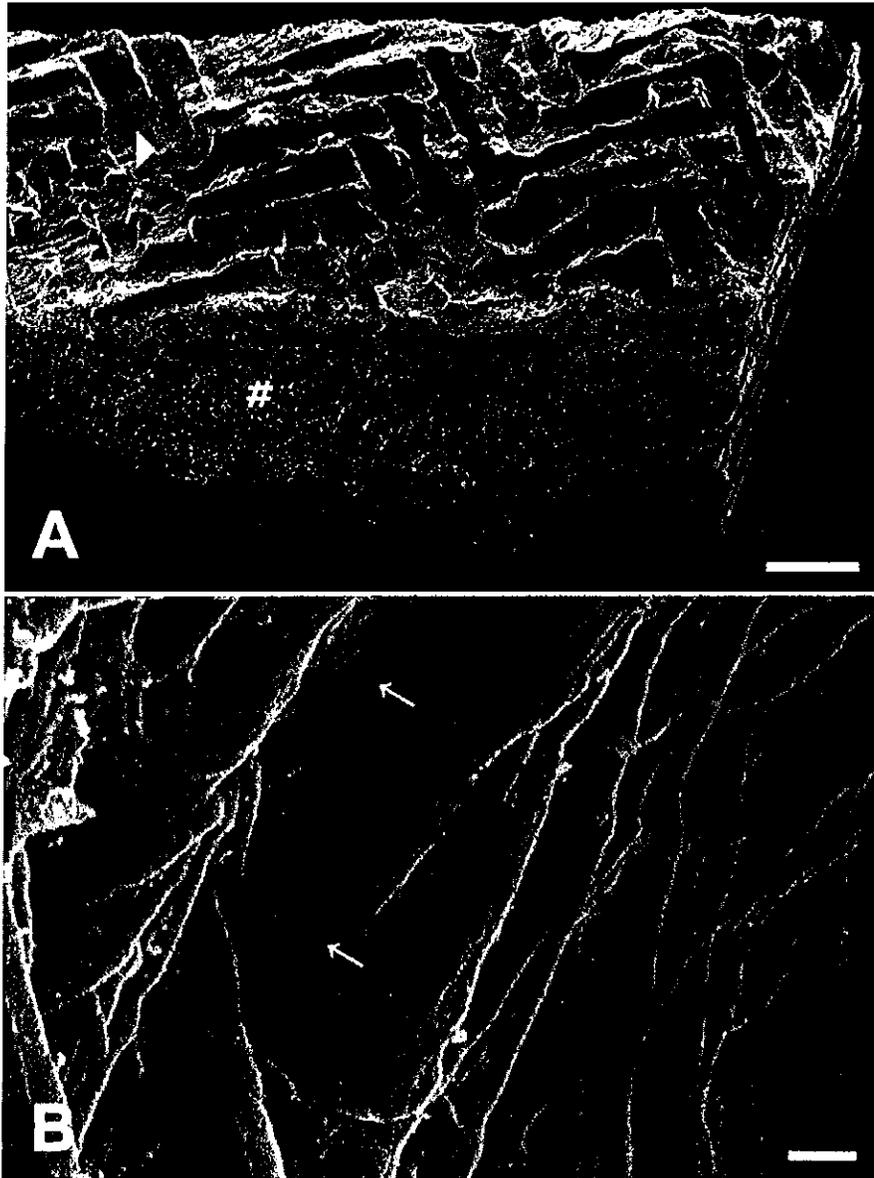


Figure 4. Scanning electron microscopy of the CMI. (A) On the top surface (triangle) some regular cristae are interposed with herringbone grooves that are about 70 μm wide. The lateral surface (number sign) shows lacunae 60–90 μm wide, formed by collagen laminae interconnected by thinner fibrils (bar = 250 μm). (B) The fibrils of the lacunae wall exhibit a random distribution with diameters varying from 73 to 439 μm . A 67-nm period (arrows) can be observed (bar = 700 nm).

RESULTS

Clinical and Arthroscopic Observation

No complications occurred in the postoperative period. All patients returned to activities of daily living by 3 months and were fully active at 6 months. The Lysholm score and Tegner activity scale increased in all operated knees during the 6-month period following CMI (Table I).

At second arthroscopic look, regeneration of meniscal-like tissue with healing of the implant to the capsule and to the residual meniscal stump was observed in all knees. Only one

implant showed a small area of fragmentation that did not require any debridement. There were no signs of synovitis or joint damage, with intact chondral surfaces of the medial compartment.

Light Microscopy

Six months after implantation, the multilamellar structure typical of CMI is less evident owing to tissue invasion inside the lacunae. The more dense appearance of the implant might also result from mechanical compaction caused by compressive forces acting on the knee joint

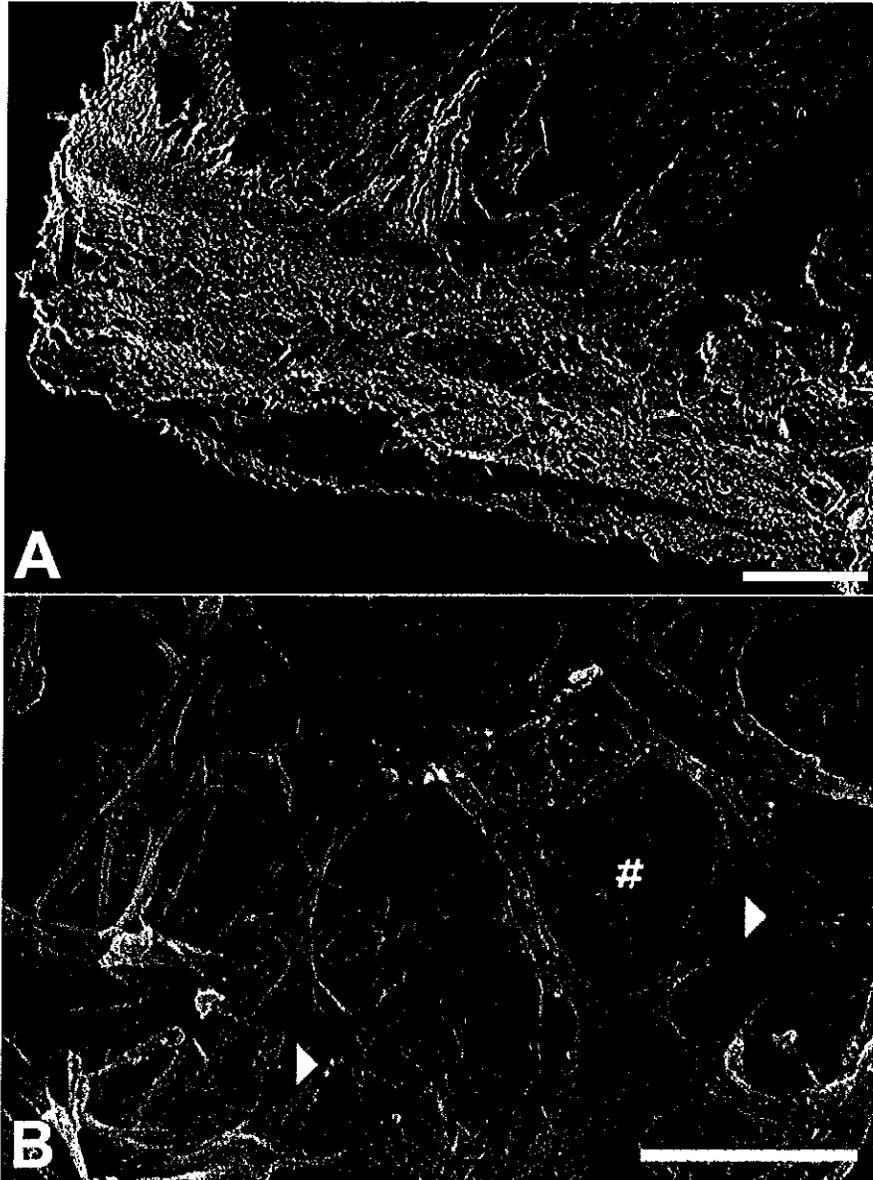


Figure 5. Scanning electron microscopy of the implant. (A) The multilamellar structure of CMI scaffold is readily recognizable (bar = 100 μm). (B) The new collagen fibrils (arrows) are readily recognizable by their small diameter in contrast with the larger and flattened fibrils of the scaffold (number sign) (bar = 5 μm).

[Figure 3(A)]. The architecture of the implanted CMI was preserved and the scaffold was still well recognizable, in contrast with previous *in vivo* studies reporting extensive scaffold resorption at 6 weeks in pigs.¹⁸ The lacunae were filled by connective tissue, where many cells, either spindle-shaped or roundish, were surrounded by newly formed extracellular matrix and blood vessels [Figure 3(B)]. No phagocytes or macrophages were observed.

Scanning Electron Microscopy

The CMI is a semicircular scaffold in which three surfaces are recognizable: upper, lower, and lateral. The upper and lower surfaces appeared composed of dense connective tissue in which cristae and grooves could be observed. The cristae were 500 μm long and appeared in a herringbone

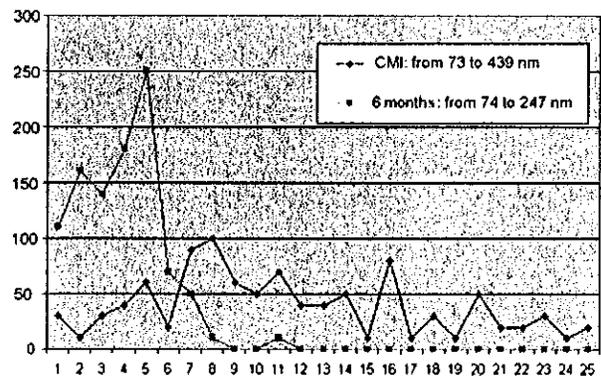


Figure 6. The scaffold fibrils show a multimodal distribution with diameters ranging from 73 to 439 nm (mean, 234 ± 89 nm). The newly synthesized fibrils demonstrate a broad distribution with diameters between 74 and 247 nm with a mean of 126 ± 32 nm.

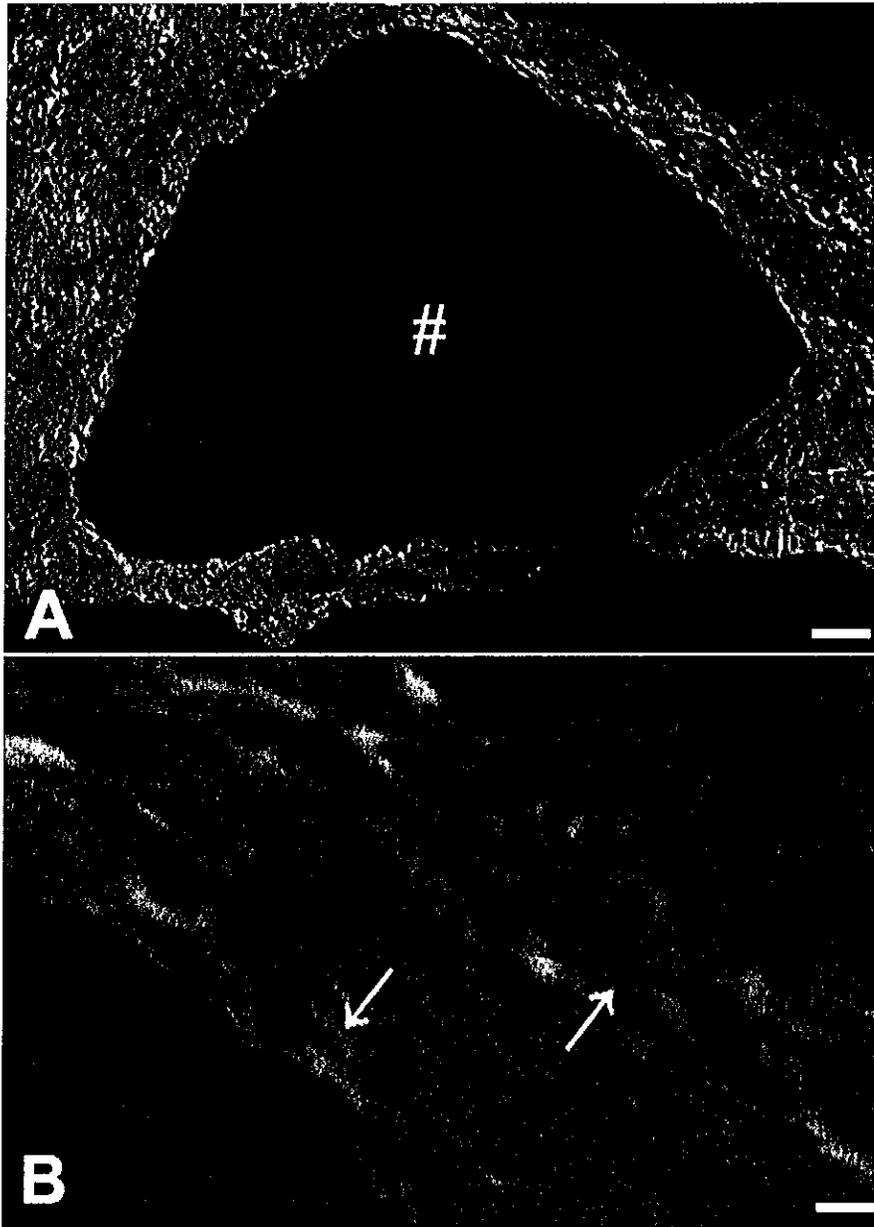


Figure 7. Transmission electron microscopy of the CMI. (A) Empty scaffold lacunae (number sign) are formed by collagen walls (bar = 2 μm). (B) Collagen fibrils are tightly packed and difficult to resolve with this technique. Their 67-nm period (arrows) is, however, evident (bar = 500 nm).

pattern with 80- μm wide grooves [Figure 4(A)]. The lateral surface of CMI contained lacunae, with diameters from 60 to 90 μm . The lacunae were formed by stratified connective layers in which smaller (5–10 μm) connective bundles could be recognized [Figure 4(A)]. At higher magnification, the walls of the lacunae appeared composed of a randomly arranged fibrillar network. The fibrils were tightly packed and their diameters varied from 73 to 439 nm. The collagen fibrils presented the typical 67-nm period [Figure 4(B)].

In the biopsy specimens, the multilamellar structure of CMI was still evident, even though the lacunae were less

recognizable in comparison with the preoperative samples [Figure 5(A)]. The native connective network of the scaffold was clearly distinguishable from the newly synthesized fibrils, owing to the larger and less uniform diameters [Figure 5(B)].

Based upon measurements performed at SEM, the scaffold fibrils showed a great variability in diameter, ranging from 73 to 439 nm (mean, 234 ± 89 nm), with a multimodal distribution. Conversely, the newly synthesized fibrils showed a broad distribution with diameters ranging from 74 to 247 nm with a mean of 126 ± 32 nm (Figure 6).

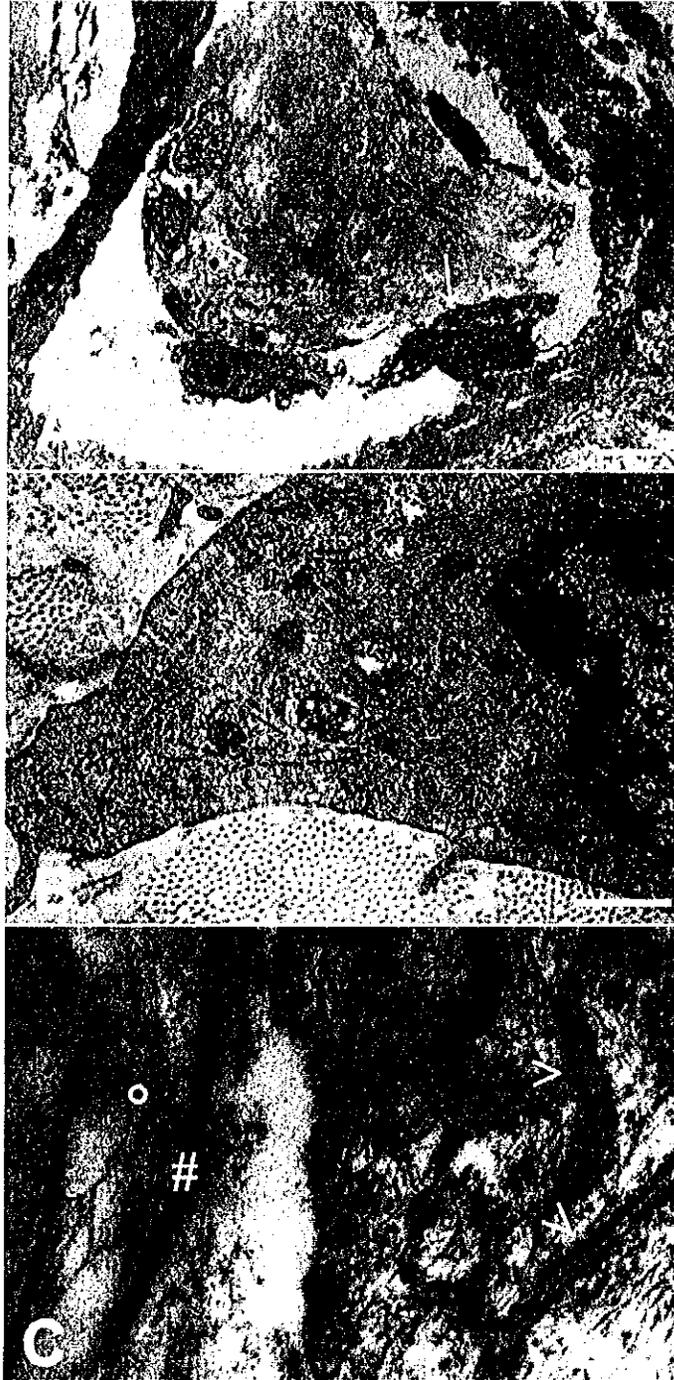


Figure 8. Transmission electron microscopy of the implant. (A) Several fibroblast like cells (arrows) with euchromatic nucleus are present within the lacunae (bar = 5 μm). (B) The cellular cytoplasm shows rough endoplasmic reticulum, mitochondria, cisternae, and abundant vesicles. Near the cell wall some vesicles (triangles) are pouring out proteins into the extracellular matrix. The matrix is composed of parallel fibrils of regular diameters (bar = 450 nm). (C) Rough endoplasmic reticulum is noted in the cell cytoplasm (pointers). Evident in the extracellular matrix is the typical 67-nm period of collagen fibrils (number sign). The matrix adjacent to the collagen fibrils appears composed of irregular filamentous material (circles) (bar = 200 nm).

Transmission Electron Microscopy

In the scaffold, no cells or cellular debris were evident inside the lacunae [Figure 7(A)]. The walls of the lacunae appeared

composed of amorphous material, in which the typical 67-nm period of collagen fibrils was often recognizable [Figure 7(B)]. After implantation, the lacunae were filled by fibroblast-like cells, presenting large nuclei with a poorly con-

denser nuclear chromatin. The cells were surrounded by new collagen matrix that was separated from the native scaffold by an empty space [Figure 8(A)]. An abundant rough endoplasmic reticulum, several mitochondria, cisternae, and numerous vesicles were present inside the cytoplasm. Most of the vesicles were adjacent to the cytoplasmic membrane; some of them were pouring out their contents into the extracellular space [Figure 8(B)]. Pseudopodia were also evident, showing a close relationship with collagen bundles. Similar to the SEM observation, the newly synthesized fibrils presented uniform diameters [Figure 8(B)]. At higher magnification, filamentous material was visible between collagen fibrils [Figure 8(C)].

DISCUSSION

The collagen meniscus implant is composed of a three-dimensional collagen network, derived from bovine Achilles tendons and processed to achieve adequate biocompatibility and shape for human implantation.¹³⁻¹⁷ In accordance with previous studies, no adverse events occurred in this series of patients after CMI. A general improvement in the clinical status was observed postoperatively, but this trend might also be related to partial meniscectomy and not only to CMI. However, a recent report highlighted the effectiveness of CMI in controlling knee pain with respect to simple meniscectomy.¹⁴ Even though the followup is too short for demonstrating a chondroprotective role of CMI, there was no damage to the opposing cartilage surfaces 6 months after implantation.

On light microscopy, the CMI has lacunae formed by large, parallel connective laminae that are connected by smaller fibers.^{13,15-17} This structure is very similar to the *in vivo* conditions, and matrix synthesis can be enhanced by a porous scaffold. Indeed, research has been mainly directed to the production of porous meniscus scaffolds, derived not only from collagen,^{12-17,19} but also from synthetics, such as polyurethanes.²⁰⁻²³

The present findings on biopsies, performed 6 months after CMI implantation, are consistent with light-microscopy observations of other authors.^{13,15,16} The connective framework of the scaffold is still evident in the biopsy specimens. The invasion of the lacunae by vessels, fibroblast-like cells and connective tissue matrix, as well as the absence of phagocytes and macrophages confirm the biocompatibility of CMI material.

The dense upper and lower surfaces of the scaffold, with their herringbone cristae, are clearly evident at SEM. Such arrangement, created by the manufacturing process,^{15,17} offers sufficient mechanical strength to resist compressive and shear stresses, and prevents cell migration outside the scaffold in contrast with the porous, multilamellar structure of the lateral surface and inner transverse sections that are designed for tissue invasion.

The collagen network of the scaffold is composed of fibrils of variable diameters. This broad distribution is actually quite distinctive for tendons and has been reported in a range of

different animals,^{24,25} whereas the newly synthesized collagen fibrils observed in the 6-month biopsies have more uniform diameters and show a tendency to organize in bundles. This pattern resembles the normal meniscus ultrastructure,²⁶ even though the dimensions of the biopsies do not allow us to draw conclusions about the general architecture of the collagen network.

TEM observation allowed a more detailed study of tissue ingrowth inside the lacunae. The cells show an intense metabolic activity, demonstrated by the poorly condensed nuclear chromatin, the cytoplasmic organuli, and the exocytosis vesicles. The pseudopodia organize the bundles of collagen fibrils in a three-dimensional network.²⁷ These features, as well as the elongated shape, are characteristic of fibroblast-like cells.²⁸ Nonetheless, these precursor cells are of unknown origin. Other authors^{12,14,16} speculate that the cells come primarily from the synovium, but currently no definitive data are available to confirm the cell source.

The pericellular filamentous material, the mesh-like pattern of the fibrillar network, the presence of fibroblast-like cells, and the lack of organization in chondrons demonstrate that the tissue is still undergoing a maturation process.

CONCLUSIONS

CMI is a tissue-engineering technique designed to prevent degenerative joint changes caused by meniscectomy. Morphological findings of this case series demonstrate that the collagen scaffold is still evident 6 months after implantation and does not elicit any inflammatory reaction. Histological and ultrastructural evidence of tissue ingrowth support the hypothesis that CMI possesses tissue-conductive properties for regeneration of meniscus-like tissue. The short followup of these four patients does not allow us to confirm its clinical effectiveness in the long term to prevent osteoarthritis. Further morphological studies designed to clarify the final evolution of these implants are now under way.

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Short-term evaluation of collagen meniscus implants by MRI and morphological analysis

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Abstract Meniscectomy can lead to degenerative joint changes in the knee. Collagen meniscus implantation is a tissue engineering technique designed to stimulate regeneration of meniscal tissue in case of irreparable tears or previous meniscectomy. The implant is composed of type I collagen derived from bovine Achilles tendon and enriched with glycosaminoglycans. Previous clinical trials demonstrated satisfactory medium-term results in patients who received a collagen meniscus implant (CMI). In this study, CMI structure was analysed by light microscopy and scanning electronic microscopy (SEM). The same morphological studies were performed on two implant biopsies, obtained from two patients who underwent a second arthroscopic look six months after implantation. The evolution of the implant was also investigated by magnetic resonance imaging, 6 and 12 months postoperatively. CMI presented a multilamellar structure, with inner lacunae allowing tissue

ingrowth. The lamellae were made of collagen fibrils, randomly oriented and preserving the typical 64-nm period. At second arthroscopic look, the implant appeared in continuity to the native residual meniscus and parameniscus, and showed good consistency and stability at probing. The biopsy specimens demonstrated invasion of the scaffold by connective tissue and blood vessels. The newly synthesised collagen fibrils were clearly distinguishable from the scaffold ones. No phagocytomacrophagic cells nor inflammatory reactions were observed inside the implant. MRI findings confirmed CMI biocompatibility and highlighted the evolution of the integration process with time. The data achieved in this study support the hypothesis that CMI stimulates regeneration of meniscal-like tissue, which could prevent the development of degenerative changes after meniscectomy.

Key words Collagen • Meniscus • Tissue engineering

Introduction

For many years meniscectomy has been commonly performed in the conviction that it would not imply any joint damage [1]. It was also believed that some regeneration of meniscal tissue could occur after its resection [2]. However, during the last decades several authors have demonstrated

that degenerative joint disease is a common sequela of meniscectomy [3–8].

Meniscal repair has been advocated for preserving normal joint kinematics [9–11], but in some cases it is not feasible. Meniscal allografts can be useful in case of total meniscectomy, but these procedures are invasive and technically demanding, and carry potential risks of transmissible diseases [12].

An alternative therapeutic option is represented by collagen meniscus implantation, a tissue engineering technique described in 1992 [13]. The technique has been applied on patients who had undergone meniscectomy or presented irreparable meniscal tears, with satisfactory clinical results at medium term [14, 15]. The rationale of this technique is represented by the possibility of enhancing regeneration of meniscal-like tissue.

In this study we evaluated the collagen meniscus implant (CMI) by magnetic resonance imaging (MRI), light microscopy and scanning electron microscopy (SEM) before and after implantation, in order to assess tissue regeneration in the site of the implant.

Materials and methods

Collagen meniscus implantation is a surgical technique applied at our Institution for the management of previous meniscectomy as well as for irreparable meniscal tears. The technique employs a collagen meniscus implant (CMI; ReGen Biologics, Franklin Lake, NJ, USA), composed of type I collagen derived from bovine Achilles tendon and enriched with glycosaminoglycans (GAGs), including chondroitin sulfate and hyaluronic acid, in order to stimulate cellular ingrowth. It is processed chemically and physically to remove molecular antigens and non-collagenous materials. The shape is similar to that of the normal meniscus (Fig. 1) and the materials used are biocompatible [16].

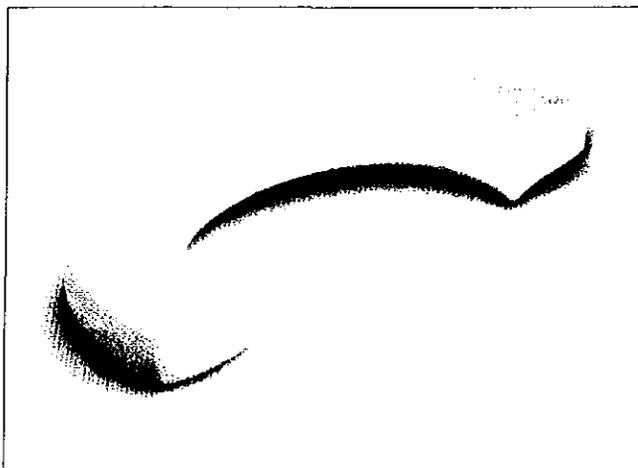


Fig. 1 Collagen meniscus implant (CMI). The semicircular shape and triangular section are evident

The surgical procedure is performed arthroscopically and CMI is sutured to the residual meniscal stump and to the parameniscus using inside-out technique [14] with non-absorbable stitches.

CMI samples were studied before implantation by light microscopy and SEM. Postoperatively, biopsy specimens were taken 6 months after implantation on two patients who underwent a second arthroscopic look. Written informed consent was obtained from both patients. Biopsy was performed with an 18G Temno

device (Allergiance Healthcare, McGaw Park, IL, USA), normally used for prostate biopsies.

All the samples were immediately fixed in 2.5% paraformaldehyde, 2% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) for 6 hours at 4° C, and then washed in the same buffer.

For light microscopy, the specimens were dehydrated in ascending grades of alcohol and propyleneoxide, and then embedded in paraffin. The sections obtained with a microtome (Reichert-Jung, 2030 MOT) were collected on slides, hematoxylin-eosin stained, observed at light microscopy (Nikon Eclipse E600) and photographed (Polaroid DMC).

To obtain a three-dimensional image by SEM, specimens were post-fixed in a solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide for 3 hours, changing the solution twice. Slices were washed in phosphate buffered saline (PBS) pH 7.2, dehydrated in an increasing series of ethanol, and subjected to critical point drying with CO₂.

Dried slices were mounted on stubs, gold-coated with a Emitech K550 sputter coater fitted with an Emitech K150 thickness monitor and then observed at SEM (Philips SEM-FEG XL-30 microscope).

MRI was performed 6 months after implantation (just before the second arthroscopic look) and at 12 months. T1- and T2-weighted fast spin echo (FSE), fat-suppressed MR images (Piker Marconi 1.5 tesla) were used to study the evolution of the implant.

Results

Preoperative findings

At light microscopy, the scaffold of the implant appeared as a porous structure, in which the lacunae were radially oriented. These lacunae have a diameter ranging from 40 to 60 μ m and are limited by large, parallel connective bundles (10–20 μ m) connected by smaller (5–10 μ m) connective fibres. No cellular debris or cells were detected in any sections (Fig. 2).

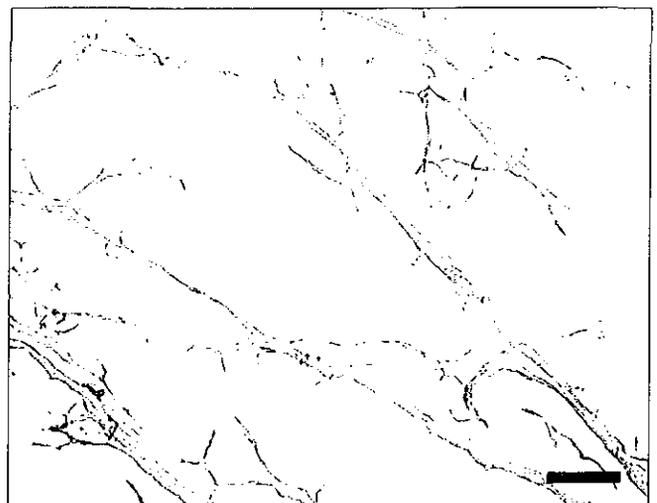


Fig. 2 Light micrograph of CMI section showing the lacunar structure with collagen bundles delimitating acellular spaces. Hematoxylin-eosin stain. Bar, 25 μ m

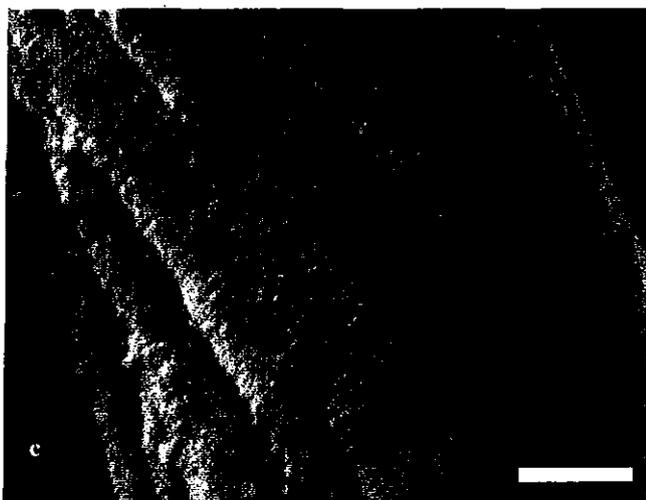
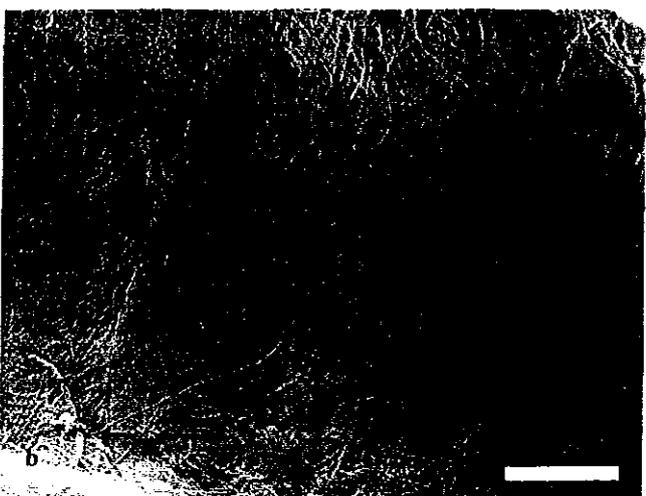
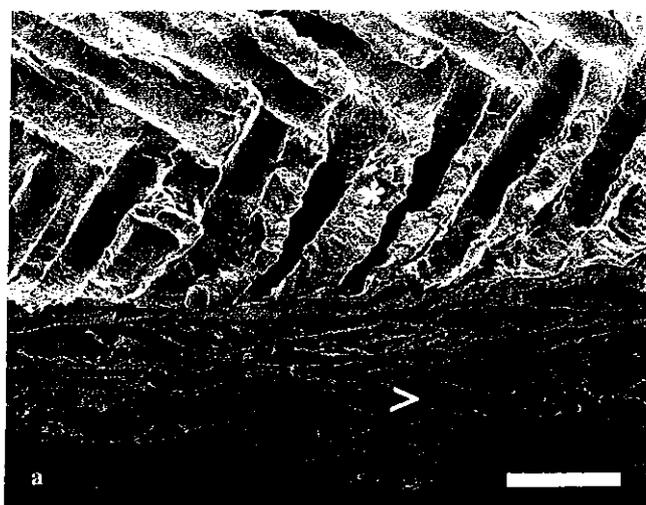


Fig. 3a-c Scanning electron microscopy. a Superior plate of CMI (*) presenting collagen cristae: the herringbone pattern is evident. Free lateral edge (>) with lacunar structure (bar, 200 μ m). b Higher magnification of the superior surface demonstrating randomly oriented fibrils (bar, 10 μ m). c Collagen fibrils with the typical 64-nm period (bar, 450 μ m)

SEM observations showed the triangular section of the semicircular scaffold, in which three different surfaces were distinguished: the superior and inferior plates delimitating the inner portion of the implant, and the free lateral edge (Fig. 3a). On the superior and inferior surfaces, the scaffold presented collagen cristae (500 μ m long and 45 μ m high) disposed in a herringbone pattern (Fig. 3a). At higher magnification, the surfaces showed a randomly oriented fibrillar network (Fig. 3b). In the lateral edge, lacunae of variable diameter (range, 60–90 μ m), limited by connective laminar planes, were visible (Fig. 3a). The laminar planes were made of remnants of collagen fibrils (diameter range, 75–439 nm), randomly oriented and strictly packed next to each other, with a regular 64-nm period (Fig. 3c). The lacunae of the lateral edge were in continuity with identical lacunae constituting the inner portion of the scaffold.

Postoperative findings

Macroscopic examination in occasion of the second arthroscopic look demonstrated healing of the implant to the native residual meniscus and to the parameniscus (Fig. 4a,b). Consistency similar to fibrocartilage and implant stability were verified by probing.

The original architecture of the implanted CMI was well preserved in the bioptic specimens. At light microscopy, the lacunae appeared inhabited by connective tissue in which cells and blood vessels were recognized. The cellular population was composed by spindle-shaped as well as roundish elements; no phagocytomacrophagic cells were present. Some blood vessels were delimited exclusively by a continuous endothelial wall, while in some others a tunica media was detected (Fig. 5).

The multilamellar structure of CMI was still evident at SEM observation, but the lacunae appeared reduced in width (Fig. 6a). At higher magnification, the native collagen fibrils were surrounded by newly synthesised collagen fibrils that were clearly distinguishable because of their smaller and more uniform diameter (range, 75–150 nm) (Fig. 6b).

At six months, MRI demonstrated the presence of a triangular meniscal-like shaped structure in continuity with the parameniscus, at the site of implant in both knees. On T2-weighted scans, the signal intensity of the matrix was high and showed a non-homogeneous pattern (Fig. 7a). Scattered spots of signal absence were present near the capsular surface, owing to the presence of non-absorbable sutures. T1-weighted scans did not allow clear evaluation of the margins of the implant from the adjacent tissues.

At 12 months, shape and dimensions of the implants were unchanged, but the signal on T2-weighted images was more homogeneous and less intense with respect to the 6-month images (Fig. 7b). The aspect was more similar to that of normal meniscal fibrocartilage.

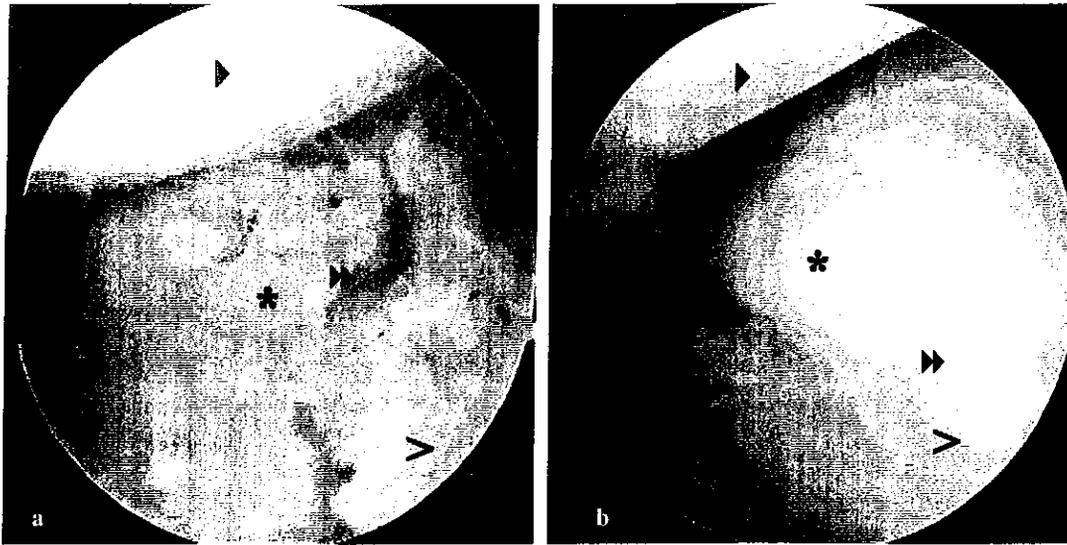


Fig. 4a, b *Implanted CMI. a* Final arthroscopic view of CMI after implantation. *b* Appearance of the implant at six months. *, implant; >, residual native meniscus; ▶, femoral condyle; ▶, non-absorbable suture

Fig. 5 *Light micrograph of CMI section stained with toluidine blue. Six months after implantation, the scaffold (>) appears invaded by newly synthesised connective tissue. *, Blood vessel; bar, 40 μ m*

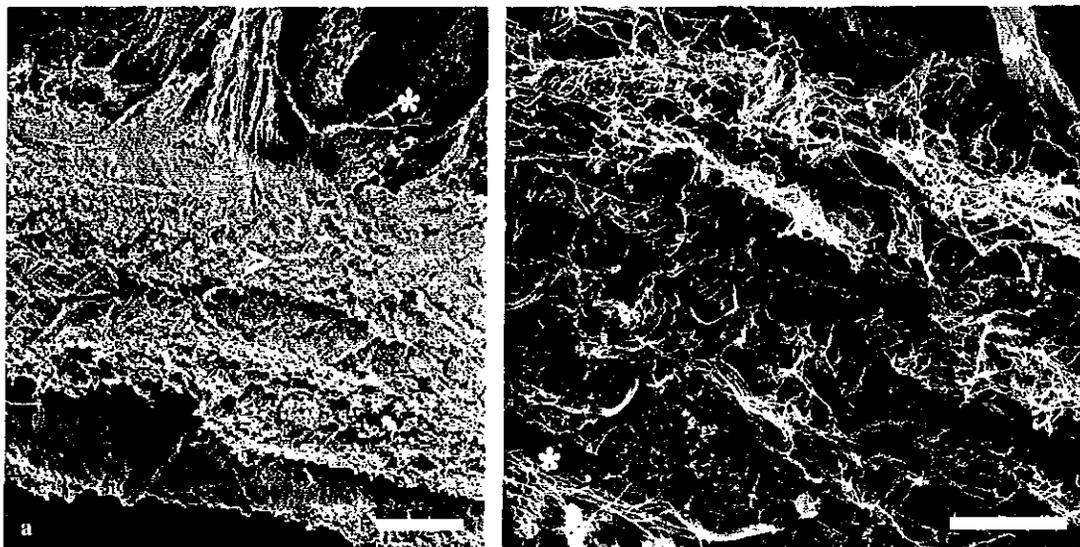
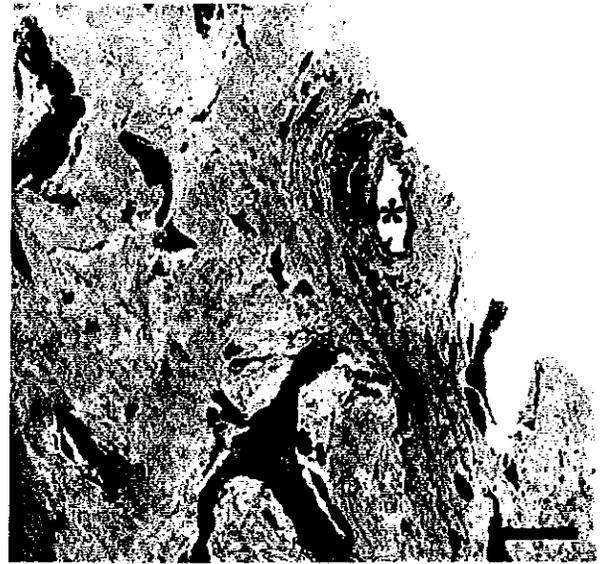


Fig. 6a, b *Scanning electron micrographs. a* Multilamellar structure of the implant: reduction in width of the lacunae with scaffold invasion by connective tissue. *, Superior plate; >, inner portion of the implant; bar, 50 μ m. *b* Newly synthesised collagen fibrils presenting uniform diameter, clearly distinguishable from the (*) collagen scaffold. Bar, 5 μ m

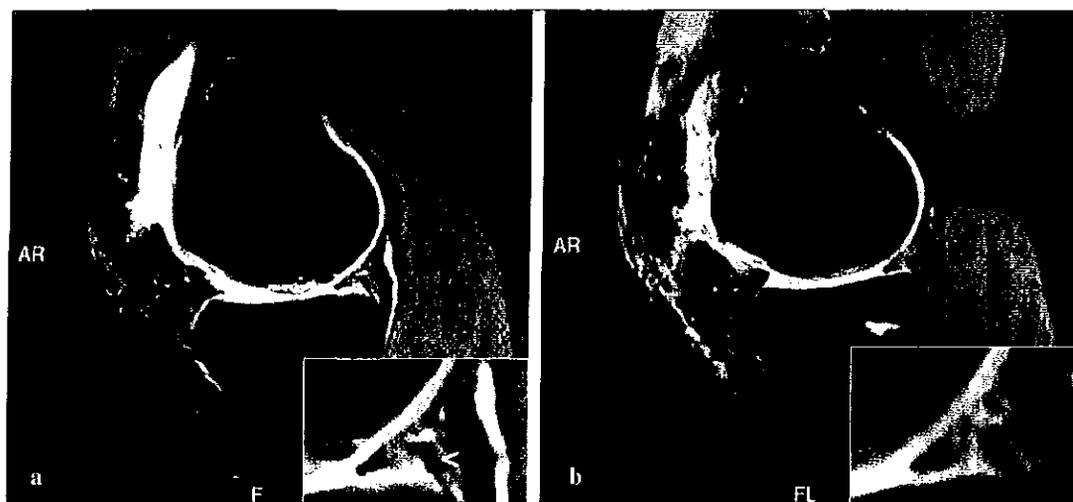


Fig. 7a, b T2-weighted FSE fat-suppressed MR images. a Six months postoperatively, the implant shows a non-homogeneous signal and appears in continuity with the parameniscus. *Inset*, close-up of the implant. <, Non-absorbable suture. b Control at one year: the signal appears more homogeneous

Discussion

During the last decade, tissue engineering techniques have become popular for the possibility of replacing tissues without any capability of intrinsic repair or regeneration after damage. The collagen meniscus implant (CMI) is designed for the management of irreparable meniscal tears or previous meniscectomy, in order to prevent degenerative joint changes in the knee [14].

The scaffold of CMI is made of a tridimensional collagen network, reproducing the shape of a normal meniscus. The lacunar framework of the scaffold as well as the presence of collagen and GAGs stimulate cellular ingrowth and invasion by blood vessels. Moreover, it provides an adequate environment for a correct fibrillogenesis [17]. The superior and inferior surfaces, presenting a more dense structure provided by manufacture processing, act as barriers against uncontrolled cell migration. In the scaffold, the collagen fibrils show a wide range of diameters and a random distribution, as a result of the preparation techniques, but they maintain the characteristic 64-nm period.

After implantation, the tridimensional structure of the scaffold is modified, even though the original architecture is still evident. As demonstrated with light microscopy and SEM, the lacunae appear reduced in width: this shape modification is probably related to the effect of repeated loading with weight bearing. No sign of scaffold resorption was detected microscopically on the biopsy specimens.

The lacunae were inhabited by fibroblast-like cells, actively synthesising collagen fibrils and extracellular matrix. Two types of collagen fibrils were present; the newly synthesised fibrils were distinguishable for their uniform diameter.

The presence of different types of blood vessels testified tissue vitality. The absence of phagocytomacrophagic cells and the normal appearance of the joint at MRI supported the biocompatibility of CMI.

CMI can be nicely visualised after implantation with MRI, using T2-weighted FSE, fat-suppressed sequences, which enhance the contrast between the implant, chondral surfaces and synovial fluid. T1-weighted images did not consent to achieve a good definition of these structures and thus are not considered effective for monitoring the evolution of the implant.

In this study, MRI demonstrated integration of the implant with the native meniscal tissue and the parameniscus. The non-homogenous signal detected at six months is likely related to the scaffold invasion by newly formed connective tissue. The more homogeneous and intense signal at 12 months reflects an evolution of the integration process, with preservation of implant shape and dimensions. These changes might reflect initial resorption of the scaffold or further organization of new meniscal tissue. Unfortunately, biopsy specimens were not harvested at this time and these hypotheses cannot be confirmed by histological findings.

In conclusion, collagen meniscal implantation is a tissue engineering technique designed to prevent degenerative joint changes subsequent to meniscectomy. The follow-up of our patients does not allow us to determine its effectiveness in the long term, but good clinical results were reported by other authors [14, 15, 18]. Morphological findings of this study demonstrate CMI biocompatibility and capability to stimulate regeneration of meniscal-like tissue. MRI evaluation of the implant should be performed using dedicated scans, able to provide good definition of the integration process.

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APPENDIX H
Risks Associated with CS versus Predicates

COMPARISON OF COMPLICATIONS WITH PREDICATES

Complications and Potential Risks	Collagen Scaffold (ReGen)	Restore (DePuy)	Fistula Plug (Cook Biotech)	Surgisis /Stratasis (Cook Biotech)	CuffPatch (Organogenesis)	TissueMend (TEI)	ZCR Patch, Endurance, Permacol, Pelvicol (TSL)	Peri-Guard (Synovis)
Infection		X*†	X*	X†			X†	
Abscess		X†	X*	X†			X†	
Wound drainage / incisional dehiscence/ Op site blister		X†			X†	X†	X†	X†
Inflammation / Swelling / Redness / Pain / Fever / Granuloma tissue/ Cyst/ Synovitis		X*†	X*	X†	X†	X†	X†	
Sterile Effusion		X*†			X†			
Seroma/Hematoma Formation			X*				X†	
Induration			X*				X†	
Allergic reaction		X*	X*					
Immunologic reaction		X*†						
Adhesion / Agglutination		X*					X†	
Fistula Formation			X*	X†			X†	
Device Stretch / Fracture / Tear/ Instability		X*					X†	X†
Device Migration / Extrusion			X*		X†		X†	X†
Delayed or failed incorporation / inadequate healing / Recurrence of Defect		X*†	X*	X†	X†	X†	X†	X†
Tissue necrosis							X†	
Restricted Freedom of Movement / Stiffness		X*†						

Prolonged Post-op Rehab		X*						
Patient non-compliance with rehab		X*						
General surgical risks such as neurological, cardiac or respiratory deficit		X*						
Death		X†						

*From Product Labeling
†From MAUDE Database

APPENDIX I
Complications in CS and Predicates



APPENDIX J
Clinical Benefit of the CS Device



