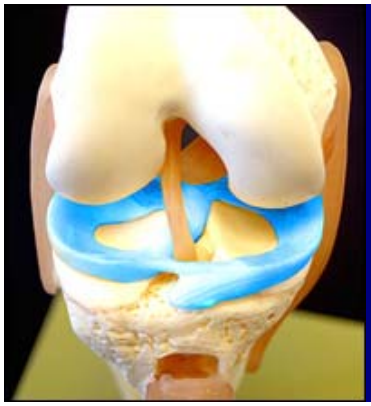

2010 Meniscus Transplantation Study Group

Thursday, March 11, 2010 1:30 – 3:30 PM

Windsor Court Hotel, Gallery Room

300 Gravier Street, New Orleans, LA 70130

At the 2010 AAOS Annual Meeting

Meeting Agenda:

INTRODUCTION:

Kevin R. Stone, MD, Chairman ***Long-term Survival of Concurrent Meniscus Allograft Transplantation and Articular Cartilage Repair: A Prospective 2 – 12 Year Follow-up Evaluation***	***1:30 – 1:40*** ***2 min discussion***
Brian Cole, MD, MBA, Moderator	***1:43 – 1:53*** ***2 min discussion***

PRESENTATIONS:

Proximal Tibial Osteotomy and Meniscal Transplantation: A Biomechanical Analysis Presented by Geoffrey S. Van Thiel, MD, MBA	***1:56 – 2:06*** ***5 min discussion***
A new Arthroscopically assisted All-Inside Technique for Lateral Meniscal Allograft Transplantation: a cadaveric study Presented by Philippe Hardy, MD, PhD	***2:12 – 2:22*** ***5 min discussion***
All-inside Medial Meniscus Allograft Transplantation vs. Medial Meniscectomy: Prospective Clinical, Radiological and MRI Study With a Mean 3-Year Follow-Up Presented by Stefano Zaffagnini, MD	***2:28 – 2:38*** ***5 min discussion***
Effects of Serial Sectioning and Repair of Radial Tears in the Lateral Meniscus Presented by Geoffrey S. Van Thiel, MD, MBA	***2:44 – 2:54*** ***5 min discussion***
Reconstruction of the Anterior Cruciate Ligament with Autologous Fibroblasts or Mesenchymal Cells Seeded on a Type I/III Collagen Membrane Presented by Stephen P. Abelow, MD	***3:00 – 3:10*** ***5 min discussion***
Industry Feedback: Sterilization, Pricing, Availability and Market Size for Meniscus Allografts	***3:16 – 3:30*** ***5 min discussion***

Long-Term Survival of Concurrent Meniscus Allograft Transplantation and Articular Cartilage Repair: A Prospective 12-Year Follow-Up Evaluation

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INTRODUCTION:

Loss of the meniscus generates increased forces on the knee cartilage and other joint structures and increases the risk of articular cartilage degeneration and development of arthritis [4,9]. The appropriate treatment for loss of the meniscus with unicompatmental knee arthrosis remains controversial[3,5]: with common treatment being osteotomy, unicompartmental (UNI) or total knee arthroplasty (TKA). Biologic treatment options, including meniscus allograft transplantation and articular cartilage repair, can potentially slow the progression of arthritis without limiting a patient's option for joint arthroplasty in the future.

METHODS:

One hundred nineteen meniscus allograft transplantations were performed in 115 patients with severe articular cartilage damage. All patients underwent an informed consent process as approved by an independent Institutional Review Board. Study inclusion criteria consisted of irreparable injury of the meniscus or loss of the meniscus with pain and Outerbridge (OB) Grade III or IV changes in the respective compartment and knee range-of-motion of at least 90°. Microfracture was used to treat articular cartilage damage if the defect area was small (≤25 mm^2), if it was located far posterior, or if it was directly under the meniscus allograft transplant on the tibial side. Articular cartilage paste grafting[7] was used to treat accessible defects > 25 mm^2. Patients consented to clinical examinations with subjective patient evaluations pre-operatively and at 2-, 3-, 5-, 7-, and 10-year post-operative intervals. IKDC, WOMAC, and Tegner Index scoring methods were used to follow pain, activity, and function. Tegner Index represents the ratio of current Tegner score as compared with highest pre-injury Tegner score[6]. Procedure failure was defined as removal of allograft without revision, or progression to knee arthroplasty (TKA or UNI)[2,8]. Analysis of overall patient survival was achieved by the Kaplan-Meier (KM) survival analysis method. Multivariate analysis using the Cox proportional hazards model was carried out to assess the effect of confounding variables on allograft survival. Secondary analysis of patient reported subjective outcomes data was accomplished using the Wilcoxon rank-sum test for 2 independent non-parametric samples. Significance level was set at $\alpha < 0.05$. Results are presented as mean ± standard deviation. Ninety-five percent confidence intervals, where given, are presented in brackets. Subjective patient outcomes were evaluated in cases with a minimum 2-year follow-up[1] (N = 101).

RESULTS:

Eighty-three (69.7%) patients were male and 32 (30.3%) female. Eighty-five (71.4%) cases were medial and 34 (28.6%) cases were lateral. Mean age at time of surgery was 46.9 years (range, 14.1-73.2 years). Twenty-two cases were classified intraoperatively as OB grade III (18.5%) and 97 cases were classified as OB grade IV (81.5%).Patients underwent an average of 5 concomitant procedures (range, 1-9 procedures). Average follow-up was 5.8 years (range, 2.1 months – 12.3 years). Forty-seven percent of cases required at least one subsequent non-failure related surgery. Kaplan-Meier estimated mean survival time was 9.93 ± 0.40 years (Figure 1). Twenty-five of the original 119 procedures failed (20.1%) with a mean failure time of 4.7 years (range, 2.1 months -10.4 years); 18 of these cases progressed to knee arthroplasty. There was no significant difference in the number of concomitant procedures between those cases that failed (5.32 ± 1.55 procedures) and those that did not (4.95 ± 1.74 procedures), ($p = 0.333$). Patients experienced significant improvements from baseline in subjective outcome measures of pain, activity, and function over the course of follow-up ($p < 0.05$), with exception the 7-year Tegner Index score* (Figure 2). Procedure survival was not affected by sex, severity of cartilage damage, axial alignment, degree of joint space narrowing, or medial vs lateral allograft.

Figure 1. Mean Survival Distribution

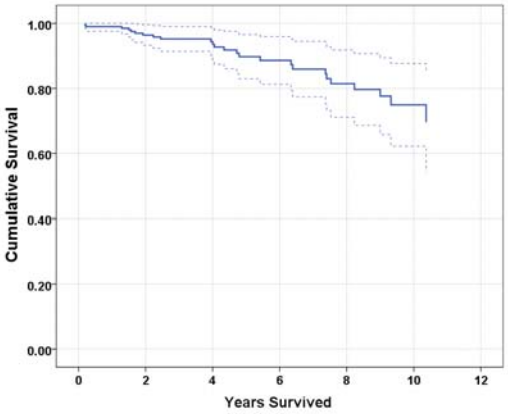
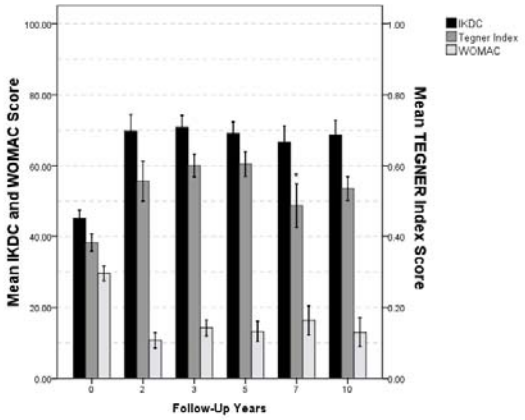


Figure 2. Subjective Outcome Measures



DISCUSSION:

Severe arthritis is often considered a contraindication for meniscus allograft transplantation. However, in this study of meniscus replacement combined with an articular cartilage repair in a heterogeneous patient population, improvements in pain, activity, and function occurred independent of the classic contraindications of age, severity of arthritis, joint space narrowing, and axial alignment. Due to concomitant procedures performed, it is difficult to narrow in on the isolated effect of the meniscus allograft. We speculate that these combined procedures may produce a soft-tissue interpositional arthroplasty, which accounts for some of the improvement. Meniscus allograft transplantation, when performed with articular cartilage repair, need not be limited to young patients with minimal articular cartilage damage as demonstrated by the results of this study, which representing the longest and largest evaluation of its kind. Biologic joint reconstruction, rather than bionic (artificial) replacement, may be an appropriate first step for many people with knee joint arthritis.

REFERENCES:

1. Cole (2006); 2. Farr (2007); 3. Gioe (2007); 4. Lohmander (2007); 5. Pennington (2003); 6. Rodkey (2008);7. Stone (2006); 8. Verdonk (2006); 9. Zielinska (2006)

Proximal Tibial Osteotomy and Meniscal Transplantation: A Biomechanical Analysis

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Introduction

Meniscectomy is a common procedure that has been shown to biomechanically increase tibial contact force with a decreased tibial contact area. Thus, meniscal transplantation has recently experienced increased clinical applicability.

It is often recommended to perform a high tibial osteotomy (HTO) in the setting of a varus-aligned knee prior to performing a meniscal transplantation. The HTO is advocated to unload the medial compartment, improve overall joint mechanics, and potentially improve the longevity of the meniscal transplant tissue. However, it is not known if an HTO prior to meniscal transplantation is necessary to improve joint contact forces.

The purpose of this study was to determine the optimal alignment and clarify the biomechanical effects that an HTO has on a meniscal transplant. We hypothesize that an HTO will further improve the medial compartment contact pressures in the context of a meniscal transplantation. This scenario has not been well studied, and has the potential to translate into improved clinical outcomes.

Methods

1. 6 cadaver knees available for testing
2. Radiographs were taken to determine anatomic alignment
3. Knees were dissected down to capsule and placed in a Taylor Spatial Frame (Smith and Nephew, Memphis, TN) (Figure 1)
4. Valgus osteotomy was completed
5. Tekscan (Boston, MA) sensors were placed sub-meniscal in both the medial and lateral compartments
6. Knees were mounted in an MTS machine in extension (Figure 1)
7. Photo analysis was used to determine approximately 5º of anatomic valgus - defined as mechanical "neutral"
8. Neutral alignment was confirmed by loading the knee so that 60% of the pressure was medial and 40% was lateral
9. The knee was then loaded to 800 N from 6º varus to 8º valgus for a meniscal intact, meniscectomized, and transplanted state
10. Meniscal transplant was done using a bone slot/trough technique with the original meniscus re-implanted
11. Total pressure and peak pressures were recorded and an ANOVA analysis with post-hoc testing was used to determine significance

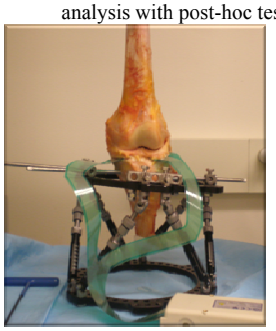


Figure 1 – Testing Set-up – Tibia in Taylor Spatial Frame with femur in MTS machine and Tekscan Sensors placed sub-meniscal. Real time pressure measurements were taken for the medial and lateral compartments.

Results

The table below illustrates the peak contact pressures in the medial compartment. The intact and transplanted states have significantly lower peak stresses at neutral and all degrees of valgus than the meniscectomized condition ($p<0.05$). Figures 2, 3 and 4 illustrate the total and peak pressures in the medial compartment and the combined values (medial and lateral).

Condition	Intact	Deficient	Transplant	ANOVA
6 Varus	27.85 ±4.09	34.79 ±4.86	31.13 ±3.81	P < 0.05
3 Varus	23.90 ±4.21	33.23 ±4.19	27.54 ±4.56	P < 0.01
Neutral	19.49 ±2.51	27.62 ±2.11	21.58 ±4.90	P < 0.01
3 Valgus	9.59 ±5.27	19.26 ±4.76	12.92 ±5.15	P < 0.01
6 Valgus	8.62 ±7.08	13.34 ±9.59	9.18 ±8.99	P < 0.05
8 Valgus	4.88 ±7.92	10.07 ±11.39	3.14 ±5.19	P < 0.05
Neutral – Slope	16.63 ±2.66	25.93 ±5.42	18.31 ±4.54	P < 0.01
3 Valgus – Slope	13.71 ±4.04	21.60 ±5.86	12.19 ±5.44	P < 0.01
6 Valgus - Slope	7.93 ±9.93	13.39 ±11.36	7.35 ±7.78	P < 0.05

Figure 2 - Medial Compartment Peak Pressures: Significant Decrease from Neutral to 3º Valgus (red box)

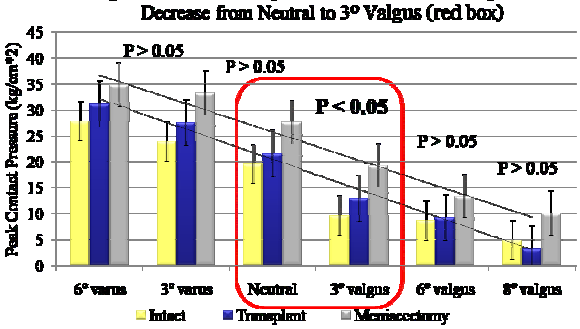
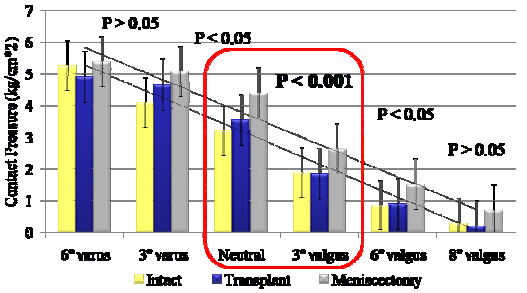


Figure 3 - Medial Compartment Total Pressures: Large Decrease from Neutral to 3º Valgus (red box)



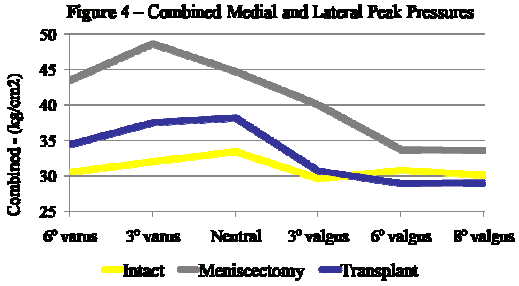
Discussion

This study confirms the concept that a valgus producing high tibial osteotomy improves the medial compartment environment in the context of a meniscal transplant. Peak and total contact pressures were significantly decreased with an HTO at almost all angulations. Furthermore, figures 2 and 3 illustrate a significant decrease in medial pressures from neutral to 3º of mechanical valgus. This fact, is also suggested in the combined peak pressure values for the intact and transplanted state (figure 4). There was no significant change in contact pressures with a 3º increase in posterior slope that is often experienced with an HTO.

These biomechanical results suggest a couple conclusions:

- An HTO will improve the medial pressure profile in addition to a meniscal transplant.
- Valgus re-alignment improves knee stresses for a varus knee.
- The increased slope associated with a high tibial osteotomy does not affect the pressures of the medial compartment.
- The medial compartment of a neutrally aligned knee significantly benefits from correction to 3º mechanical valgus (red box – Figures 2 and 3)
- Combined peak pressures in meniscal intact knees appear to reach an "ideal" value at 3º of mechanical valgus.

Figure 4 – Combined Medial and Lateral Peak Pressures



A new Arthroscopically assisted All-Inside Technique for Lateral Meniscal Allograft Transplantation: a cadaveric study

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92100 Boulogne France

Introduction

The efficacy of meniscal transplantation was demonstrated at intermediate-term studies with high levels of patient satisfaction. The meniscus can be implanted with the use of either an open or an arthroscopically assisted technique. Although results of both methods have been reported to be similar, arthroscopic techniques are routinely used. Whatever the technique, the meniscus should be fixed in an anatomical position. Bone anchoring of the anterior and posterior horns is essential regarding meniscal function. Although it is technically easier to secure the graft to soft tissue alone, research has indicated that load transmission is superior when the graft is secured to the bone. The most common methods use either bone plugs or bone bridge. These techniques are technically highly demanding. Besides this difficulty, bone tunnel have their specific morbidity. We performed a cadaveric study on feasibility of a new arthroscopic lateral meniscal allograft technique with bone fixation and without bone tunnel.

Material and Method

Twelve cadaveric specimens were used for this study. For each specimen, one knee was dedicated to receive the graft, the other one was used to assess meniscal positioning and as a donor site for another specimen. The technique was performed with cadaver associated 2 by 2, with similar sizing in order to have the best adapted lateral meniscal allograft. The donor knee was disjointed to take a picture of the lateral meniscus positioning before it was removed and implanted on the other cadaver. Second, an arthroscopic lateral meniscectomy was performed on every knees elected to receive a graft.. The procedure was an all-inside arthroscopically assisted technique using three approaches: antero-medial, antero-lateral and an accessory lateral portal. The meniscal horns were fixed on the tibial plateau by an Arthrex Swivel-Lock™ (Naples Florida) suture anchor. The meniscal wall was fixed to the articular capsule by all-inside (Meniscal Cinch TM Arthrex Florida) and out-in techniques. The knee which received the meniscal allograft was disjoint too. A picture was taken of the lateral meniscal allograft positioning. A statistical analysis was performed to compare the meniscal allograft to the native meniscus.

Results

Eleven implantations were performed. One failed because of a too short meniscal allograft. On a standard tibial plateau (75mm x 40 mm), the meniscal allograft posterior horn was measured at a mean distance of 4,6mm to the native posterior horn. This difference was not significant (p>0, 05)

Discussion / Conclusion

This original technique doesn't use any bone plug nor bone tunnels. In case of simultaneous ligament reconstruction, this technique doesn't interfere with ligament fixation. This procedure is easier than the others, but needs a learning curve.
This original technique of meniscal allograft implantation is feasible, with poor morbidity, reproducible and allows a satisfactory meniscal posterior horn positioning.

All-inside Medial Meniscus Allograft Transplantation vs. Medial Meniscectomy: Prospective clinical, radiological and MRI study with a mean 3 year follow-up.

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Introduction: It has been well demonstrated that removal of the meniscus leads to progressive degenerative arthritis of the knee at long-term. In literature meniscal allograft transplantation (MAT) has been performed as a potential biological solution for symptomatic meniscus-deficient young patients (1). Many retrospective analysis of MAT, even at long-term follow-up (FU), has been performed (2). In our series the prospective design and the presence of a control group improves the quality of the study.

Purpose: The purpose of this study is to prospectively compare the short to intermediate-term results of all-inside medial meniscal allograft transplantations to total/subtotal medial meniscectomies in order to evaluate the outcome of our technique and to verify the real effects of this biological implant in preventing degenerative joint disease.

Methods: From 2005 to 2008 we performed 30 medial meniscal allograft transplantations (without bone plugs fixation and using an all-inside arthroscopic technique) in young symptomatic patients who had undergone previously a total/subtotal arthroscopic medial meniscectomy (group A). We decided to create a control group of 30 totally medial meniscectomised patients (group B).

For both groups the exclusion criteria were: advanced arthrosis, history of knee infection, malalignment, instability, arthrofibrosis, age more than 50 years. All patients were prospectively evaluated clinically (IKDC, Tegner, Lysholm, Womac, VAS and SF-36 scores) and radiologically (X-ray and MRI) preoperatively and at final FU.

Result: For group A all scores improved: subjective IKDC from 60 ± 14.91 to 65.3 ± 17,98; objective IKDC class from 2A, 20B and 8C to 5A, 15B and 8C; Tegner from 5.8 ± 2.7 to 6.5 ± 2.88; Lysholm from 68.83 ± 21.12 to 85.5 ±18.29 ; Womac from 85 ± 7.7 to 88.96 ± 9.96; VAS from 5.2 ± 2.4 to 1.7 ± 1.8; SF-36 from 85.5 ± 1.91 to 88,9 ± 1.82. The X-Ray control has been showed the mean medial compartment narrowing of 0.75 mm respect of the controlateral one. MRI controls showed a pretty good signal of implants and in one case the meniscus was dislocated with no clinical correlation.

For group B all scores improved: subjective IKDC from 72.5 ± 5 to 76 ± 4.69; objective IKDC class from 20B and 10C to 2A, 18B and 10C; Tegner from 4.15 ± 2.44 to 4.75 ± 1.25; Lysholm from 53.25 ± 20.07; Womac from 86.17 ± 7.7 to 88.16 ± 9.56; VAS from 4.9 ± 2.95 to 1.8 ± 2.17; SF-36 from 88.5 ± 1.91 to 87,25 ± 4.57. The X-Ray control has been showed the mean medial compartment narrowing of 0.88 mm respect of the controlateral one. MRI controls often showed a sub-chondral bone hedema (a sign of initial cartilage degeneration).

Discussion: Based on available results up to 3 year mean FU we did not find statistically significant differences between our two groups, even a positive trend of group A respect to group B was detectable. This was expected because of the type of treatment performed (biological procedure) and of the short-term FU. Long-term data are necessary to proof the benefits of All-inside MAT.

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2. Verdonk PC, Verstraete KL, Almqvist KF, De Cuyper K, Veys EM, Verbruggen G, Verdonk R. Meniscal allograft transplantation: long-term clinical results with radiological and magnetic resonance imaging correlations. Knee Surg Sports Traumatol Arthrosc. 2006 Aug;14(8):694-706.

Effects of Serial Sectioning and Repair of Radial Tears in the Lateral Meniscus

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Samuel McArthur MD (Rush University Medical Center - Dept. of Orthopedic Surgery, Chicago, IL)
Geoffrey S. Van Thiel MD, **MBA** (Rush University Medical Center - Dept. of Orthopedic Surgery, Chicago, IL)
Jim Kercher MD, Rush University Medical Center, Division of Sports Medicine
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Elizabeth Shewman MS (Rush University Medical Center - Dept. of Orthopedic Surgery, Chicago, IL)
Vincent M. Wang, PhD (Rush University Medical Center, Dept. of Orthopedic Surgery, Chicago, IL)
Brian J. Cole MD, MBA (Midwest Orthopaedics at Rush, Chicago, IL)

Objectives:
Radial meniscal tears are a difficult condition to treat with both long and short term implications. The longitudinal orientation of peripheral meniscal fibers transmit hoop stresses and are the structural foundation for a functioning meniscus. Transection of these fibers could theoretically render a meniscus non-functional with significant clinical sequelae for the patient. We hypothesize that sequential radial sectioning of the lateral meniscus will increase contact pressures, and repair with both an all inside as well as an inside out technique will restore meniscal function.

Methods:
Ten paired cadaver knees were dissected down to the capsule. Each tibia was placed in a Taylor Spatial Frame and the femur was affixed in a custom made jig. A vertical osteotomy of the lateral femoral condyle was performed for access to the lateral compartment1. Tekscan sensors were placed submeniscal in both compartments. For each condition, the knees were loaded at 800 N in both extension and 60 degrees of flexion in an MTS machine. The osteotomy site was opened and sequential radial sections were made in the lateral meniscus just posterior to the popliteal hiatus (Figure 1). Complete sectioning was followed by a pair matched repair using either an all-inside or an inside-out technique. Each condition was loaded two times to ensure reproducibility and during repair subset testing, the knees were cycled 50 times to test structural integrity.

Conditions tested:
- Intact lateral meniscus
- 50%, 75%, and 100% radial width incision
- Repaired lateral meniscus
- Complete lateral meniscectomy

Results:
ANOVA testing was completed for all data (Figure 1). Overall, there were no significant differences in contact area or pressure with radial sectioning up to 75% of the meniscal width. However, there was a significant change from 75% to 100% ($p<.05$). The two repair constructs showed improved pressure profiles when compared to the sectioned state, but no differences between each other

Conclusions:
Biomechanical data regarding radial meniscal tears in the literature is limited. Radial meniscal tears that violate the periphery do benefit from repair with either an all inside or inside-out repair at time zero. This study is also relevant for surgeons performing partial meniscectomies. Violation of the meniscal periphery results in a pressure profile identical to a total meniscectomy. Repair at time zero approximates the intact condition.

References:
1. Dienst, Greis, Ellis, Bachus, Burks. Effect of Lateral Meniscal Allograft Sizing on Contact Mechanics of the Lateral Tibial Plateau Am J Sports Med 2007 35: 34.

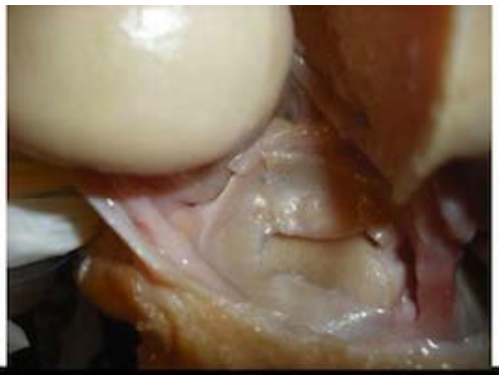

Figure 1 - Data Table and Repair
Image 1: Inside-out repair following 100% radial width section;
Table 1: Contact area and contact pressure in extension and flexion

		Intact vs 50%	Intact vs 75%	Intact vs 100%	Intact vs Repair	100% vs Meniscectomy	100% vs Repair	75% vs 100%
Extension	Contact Area	-0.03 p>0.05	-0.02 p>0.05	-0.48 p<0.001	-0.27 p<0.01	-0.01 p>0.05	0.21 p<0.05	-0.46 p<0.001
	Contact Pressure	-0.18 p>0.05	-0.53 p>0.05	3.91 p<0.001	0.85 p>0.05	1.93 p>0.05	-3.06 p<0.01	4.44 p<0.001
Flexion	Contact Area	-0.04 p>0.05	-0.04 p>0.05	-0.41 p<0.001	-0.32 p<0.001	-0.08 p>0.05	0.18 p<0.05	-0.37 p<0.001
	Contact Pressure	-0.18 p>0.05	-0.31 p>0.05	1.81 p<0.05	0.32 p>0.05	2.4 p<0.01	-1.49 p<0.05	2.13 p<0.01

Reconstruction of the Anterior Cruciate Ligament with Autologous Fibroblasts or Mesenchymal Cells Seeded on a Type I/III Collagen Membrane

Director: Pedro Guillen. MD, Clinica CEMTRO, Madrid, Spain
Presenter: Stephen P Abelow, MD, Clinica CEMTRO, Madrid, Spain; Lake Tahoe Sports Medicine Center, South Lake Tahoe, CA; Clinica CEMTRO, Madrid, Spain
Researchers: Prof Pedro Guillen, MD, Stephen P Abelow, MD, Elena Rodriguez-Inigo, PhD, Juan Manuel Lopez-Alcorocho, PhD, Marta Guillen, MD. PhD, Isabel Guillen, MD, PhD; Clinica CEMTRO, Madrid, Spain.
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Hypothesis:
A cell-based therapy method has been developed to reconstruct the ACL focused on the treatment with autologous fibroblasts extracted from ACL and mesenchymal cells extracted from adipose tissue seeded on a type I/III collagen membrane. The cell-seeded membrane is applied to the site of the torn ACL. The study is being conducted in a Merino sheep model.

Purpose:
1. To examine the use of cell types for autologous transplantation: fibroblasts or mesenchymal cells.
2. To examine the use of new biomaterials combined with cultured cells promoting the ACL repair by generating a new tissue with adequate biomechanical properties.

Pilot Study:
Starting in 2007, ACL biopsies from 62 patients who underwent ACL surgery or other lesions of the knee have been processed in our laboratory. (median age 32 years; range 15-74 years).

Lesions were classified as **Acute** (elapsed time form ACL rupture to surgery was shorter than 1 month) **Chronic** (elapsed time from ACL rupture was longer than 1 month) and **Normal** (ACL biopsies taken from patients who had a knee surgery other than ACL reconstruction).

The cells were isolated by enzymatic digestion of the ACL samples.

Among the 62 samples, primary culture could only be established in 43 of them.

Initial results show:
1) The elapsed time between obtaining of the sample and its enzyme treatment could not be more than 2 days.
2) The sample size seems to be important and should be big enough to render at least 20,000 cells.
3) Primary culture is more difficult in older than younger patients

In 19 cases the primary culture could not be established. The characteristics of these cases and samples were:
1) 13 samples were processed at 12 days (mean time) after their harvesting.
2) 4 cases the number of isolated cells was lower than 20,000
3) 2 cases the patients were 74 years old.

The growth rate of cultured cells was estimated as the ratio between the number of viable cells of the first passage and the initial number of cells. We have observed that the growth rate and age of the patient were inversely proportional. This appears to be only a trend because the negative correlation was not statistically significant (probably due to the low number of patients included so far) ($R= -0.226$; $P=0.146$).

Cell growth rate was greater in the acute ACL, followed by normal ACL and finally the chronic ACL (not statistically significant, see above).

In randomly selected patients histological study of these membranes have been carried out. The histological study showed that the fibroblasts are included on the membrane.

Our results indicate that it is possible to isolate and culture fibroblasts from ACL and after reaching the appropriate number including them on a collagen membrane. This study is ongoing.

Reconstruction of the Anterior Cruciate Ligament with Autologous Fibroblasts or Mesenchymal Cells Seeded on a Type I/III Collagen Membrane (contd)

Animal Study Objectives:

1) To study if treatment of ruptured ACL with autologous fibroblasts and mesenchymal cells seeded on type I/III collagen membranes improves the regeneration of ACL in comparison with non-treated ACL.
2) To compare the results in regeneration of ACL derived from the cell types used in the treatment: autologous fibroblasts (obtained from ACL) or mesenchymal cells (obtained from adipose tissue).
3) To estimate the appropriate number of viable cells seeded in a type I/III collagen membrane necessary for treatment of ACL (5 million vs. 10 million fibroblasts).

Animal model:

Merino Sheep

Sample size (to date):

To date 12 Merino sheep have been implanted

-Group 1 (4 animals): Treated with 5 million autologous fibroblasts

-Group 2 (4 animals) Treated with 10 million autologous fibroblasts

-Group 3 (4 animals): Treated with mesenchymal cells isolated from adipose tissue.

-The opposite knee serves as a control.
-The animals will be sacrificed.

Methods:

1). Isolation of fibroblasts and mesenchymal cell isolation: primary cultures.

-ACL and adipose tissue samples placed in a sterile medium

-Samples digested with collagenase and cultured.

-The number of viable cells is estimated in a Neubauer chamber by trypan blue exclusion method.

-Cells are seeded in culture flasks at 37^0C with 95% relative humidity and 5% CO_2 pressure.

2). Cell culture expansion.

Histological study

-Histologic analysis by H&E and Masson's trichrome stain.
-Electron microscopy of all samples.

Molecular Study/ Genetic Study

-Total RNA form the cells will be isolated and quantified using the Nanodrop system and transcribe to cDNA with inverse transcriptase of the aviar myeloblastosis virus (AMV)
-Study expression of type I collagen, type III collagen, SOX 9, and Tenascin-C genes by RT-PCR using UPL (universal probe library) probes compared with glyceraldehyde 3-phosplate dehydrogenase as housekeeping gene.
-Gene expression will be analyzed in all specimens
-In the collagen membrane seeded with mesenchymal cells, molecular analysis will be performed to study if mesenchymal cells express the molecular markers.

Assessment of Effectiveness

The effectiveness of the treatment will be determined by the histological study and by the molecular analysis (genotypic & phenotypic features of the cells after treatment) as well as biomechanical testing. All histology and molecular studies will be performed in a blinded fashion.